



DR/4000 SPECTROPHOTOMETER PROCEDURES MANUAL



HACH COMPANY TRADEMARKS

AccuGrow®	H2O University™	Pond In Pillow™
AccuVac®	H2OU™	PourRite®
AccuVer™	Hach Logo®	PrepTab™
AccuVial™	Hach One®	ProNetic™
Add-A-Test™	Hach Oval®	Pump Colorimeter™
AgriTrak™	Hach.com™	QuanTab®
AluVer®	HachLink™	Rapid Liquid™
AmVer™	Hawkeye The Hach Guy™	RapidSilver™
APA 6000™	HexaVer®	Ratio™
AquaChek™	HgEx™	RoVer®
AquaTrend®	HydraVer®	sension™
BariVer®	ICE-PIC™	Simply Accurate SM
BODTrak™	IncuTrol®	SINGLET™
BoroTrace™	Just Add Water™	SofChek™
BoroVer®	LeadTrak®	SoilSYS™
C. Moore Green™	M-ColiBlue24®	SP 510™
CA 610™	ManVer®	Specv™
CalVer®	MolyVer®	StablCal®
ChromaVer®	Mug-O-Meter®	StannaVer®
ColorQuik®	NetSketcher™	SteriChek™
CoolTrak®	NitraVer®	StillVer®
CuVer®	NitriVer®	SulfaVer®
CyaniVer®	NTrak®	Surface Scatter®
Digesdahl®	OASIS™	TanniVer®
DithiVer®	On Site Analysis.	TenSette®
Dr. F. Fluent™	Results You Can Trust SM	Test 'N Tube™
Dr. H. Tueau™	OptiQuant™	TestYES! SM
DR/Check™	OriFlow™	TitraStir®
EC 310™	OxyVer™	TitraVer®
FerroMo®	PathoScreen™	ToxTrak™
FerroVer®	PbEx®	UniVer®
FerroZine®	PermaChem®	VIScreen™
FilterTrak™ 660	PhosVer®	Voluette®
Formula 2533™	Pocket Colorimeter™	WasteAway™
Formula 2589™	Pocket Pal™	ZincoVer®
Gelex®	Pocket Turbidimeter™	

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SECTION 1 PROCEDURES 79

ALACHLOR in Water

Immunoassay Method

ALUMINUM

Method 8012	Aluminon Method	Powder Pillows	(0 to 0.800 mg/L)
Method 8326	Eriochrome Cyanine R Method	Powder Pillows	(0 to 0.250 mg/L Al ³⁺)
	Chromazurol S Method	UniCell™ Vials	(0 to 0.50 mg/L Al ³⁺)

AMMONIUM

Salicylate Method	UniCell™ Vials	LR (0.00 to 1.50 mg/L NH ₄ ⁺)
Salicylate Method	UniCell™ Vials	HR (0.0 to 45.0 mg/L NH ₄ ⁺)

ARSENIC

✓ Method 8013	Silver Diethyldithiocarbamate Method	(0 to 0.200 mg/L)
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ATRAZINE in Water

Immunoassay Method

BARIUM

Method 8014	Turbidimetric Method	Powder Pillows or AccuVac® Ampuls	(0 to 100 mg/L)
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BENZOTRIAZOLE or TOLYLTRIAZOLE

Method 8079	UV Photolysis Method	Powder Pillows	(0 to 16.0 mg/L)
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BORON

Method 10061	Azomethine-H Method	Powder Pillows	LR (0 to 1.50 mg/L as B)
Method 8015	Carmine Method	Powder Pillows	(0 to 14.0 mg/L)

BROMINE

Method 8016	DPD Method	Powder Pillows or AccuVac® Ampuls	(0 to 4.50 mg/L)
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CADMIUM

Method 8017	Dithizone Method	Powder Pillows	(0 to 80.0 µg/L)
	Cadion Method	UniCell™ Vials	(0 to 0.30 mg/L Free Cd)

CHLORAMINE, Mono, Low Range

Method 10171	Indophenol method	LR (0–4.50 mg/L Cl ₂)
Method 10172	Indophenol method	HR (0–10.0 mg/L Cl ₂)

CHLORIDE

Method 8113	Mercuric Thiocyanate Method	(0 to 25.00 mg/L Cl ⁻)
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CHLORINE, Free

✓ Method 8021	DPD Method	Powder Pillows or AccuVac® Ampuls	(0 to 2.00 mg/L)
Method 10102	DPD Method	Test 'N Tube™ Vials	(0 to 5.00 mg/L)

CHLORINE, Free

Method 10069	DPD method	UHR (0.1–10.0 mg/L as Cl ₂)
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Powder Pillow	Method 10069	CHLORINE, Total	
✓ Method 8370	DPD Method		ULR (0 to 500 µg/L as Cl ₂)
✓ Method 10014	DPD Method		ULR (0 to 500 µg/L as Cl ₂)
✓ Method 8167	DPD Method	Powder Pillows or AccuVac® Ampuls	(0 to 2.00 mg/L)
Method 10101	DPD Method	Test 'N Tube™ Vials	(0 to 5.00 mg/L)

CHLORINE, Total

Method 10070	DPD method		UHR (0.1–10.0 mg/L as Cl ₂)
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Powder Pillow Method 10070 CHLORINE DIOXIDE

Amaranth Method			LR (20 to 500 µg/L)
Method 8065	Chlorophenol Red Method		LR (0 to 1.00 mg/L)
Method 10126	DPD Method	Powder Pillows and AccuVac® Ampuls	(0 to 5.00 mg/L)
Method 8345	Direct Reading Method		MR (0 to 50 mg/L)
Method 8138	Direct Reading Method		HR (0 to 1000 mg/L)

CHLOROPHYLL-A

Acetone Extraction Method

CHROMIUM, Hexavalent

✓ Method 8023	1,5-Diphenylcarbohydrazide Method	Powder Pillows or AccuVac® Ampuls	(0 to 0.700 mg/L Cr ⁶⁺)
	1,5-Diphenylcarbohydrazide Method	UniCell™ Vials	(0 to 1.00 mg/L Cr ⁶⁺)

CHROMIUM, Hexavalent, in soil

Method 10051	1,5-Diphenylcarbohydrazide Method		(0 to 0.700 mg/L Cr ⁶⁺)
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CHROMIUM, Total

Method 8024	Alkaline Hypobromite Oxidation Method	Powder Pillows	(0 to 0.700 mg/L)
	1,5-Diphenylcarbohydrazide Method	UniCell™ Vials	(0 to 1.00 mg/L Total Cr)

CHROMIUM, Trivalent

Method 8069	Colorimetric Method		(0 to 20.0 g/L Cr ³⁺)
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COBALT

Method 8078	1-(2-Pyridylazo)-2-Naphthol (PAN) Method	Powder Pillows	(0 to 2.00 mg/L)
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COLOR

Method 10048	ADMI Weighted Ordinate Method		(0 to 250 units Pt-Co)
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COLOR, Gardner

Method 10105	ASTM Method D 6166-97		(1 to 18 Gardner Color Units)
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COLOR, Tristimulus Values and Chromaticity Coordinates

Method 10103	ASTM Method E 308-95		
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COLOR, True and Apparent

Method 8025	Platinum-Cobalt Standard Method		(0 to 500 units)
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COLOR, Yellowness Index

Method 10104	ASTM Method E 313-96		
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COPPER

✓ Method 8506 and Method 8026	Bicinchoninate Method	Powder Pillows or AccuVac® Ampuls	(0 to 5.000 mg/L)
Method 8143	Porphyrin Method	Powder Pillows	(0 to 210.0 µg/L)
	Bathocuproine Method	UniCell™ Vials	(0 to 6.00 mg/L)

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CYANIDE

Method 8027 Pyridine-Pyrazalone Method Powder Pillows (0 to 0.240 mg/L CN⁻)
Cyanogen Chloride UniCell™ Vials (0 to 0.50 mg/L CN⁻)

DETERGENTS, Anionic (Surfactants)

Method 8028 Crystal Violet Method (0 to 0.275 mg/L)

FLUORIDE

✓ Method 8029 SPADNS Method Reagent Solution or AccuVac® Ampuls (0 to 2.00 mg/L F⁻)

FORMALDEHYDE

Method 8110 MBTH Method Powder Pillows (0 to 500 µg/L)

HARDNESS

Method 8030 Calcium and Magnesium; Calmagite Colorimetric Method (0 to 4.00 mg/L Ca and Mg as CaCO₃)

HARDNESS, Total

Method 8374 Calcium and Magnesium;
Chlorophosphonazo Colorimetric Method ULR (0 to 1,000 µg/L Ca & Mg as CaCO₃)

HYDRAZINE

Method 8141 p-Dimethylaminobenzaldehyde Method Reagent Solution or AccuVac® Ampuls (0 to 600.0 µg/L)

IODINE

Method 8031 DPD Method Powder Pillows or AccuVac® Ampuls (0 to 7.00 mg/L)

IRON

Method 8147 FerroZine Method (0 to 1.400 mg/L)

IRON, Ferrous

Method 8146 1,10 Phenanthroline Method Powder Pillows or AccuVac® Ampuls (0 to 3.000 mg/L)

IRON, Total

Method 8365 FerroMo Method Powder Pillows (0 to 1.800 mg/L)

✓ Method 8008 FerroVer Method Powder Pillows or AccuVac® Ampuls (0 to 3.000 mg/L)

Method 8112 TPTZ Method Powder Pillows or AccuVac® Ampuls (0 to 1.800 mg/L)

1, 10-Phenanthroline Method UniCell™ Vials (0 to 5.00 mg/L)

LEAD

✓ Method 8033 Dithizone Method (0 to 300 µg/L)

Method 8317 LeadTrak Fast Column Extraction Method (0 to 150 µg/L)

PAR Method UniCell™ Vials (0 to 2.00 mg/L free Pb)

MANGANESE

Method 8149 PAN Method Powder Pillows LR (0 to 0.700 mg/L)

✓ Method 8034 Periodate Oxidation Method HR (0 to 20.0 mg/L)

MERCURY

Method 10065 Cold Vapor Mercury Preconcentration Method (0.1 to 2.5 µg/L)

METOLACHLOR in Water

Immunoassay Method

MOLYBDENUM, Molybdate

Method 8036 Mercaptoacetic Acid Method Powder Pillows or AccuVac® Ampuls HR (0 to 50.0 mg/L)

Method 8169 Ternary Complex Method Powder Pillows LR (0 to 3.00 mg/L)

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NICKEL

Method 8150	1-(2 Pyridylazo)-2-Naphthol (PAN)	Powder Pillows	(0 to 1.000 mg/L)
✓ Method 8037	Heptoxime Method	Powder Pillows	(0 to 1.80 mg/L Ni)
	Dimethylglyoxime Method	UniCell™ Vials	(0 to 6.00 mg/L)

NITRATE

Method 8192	Cadmium Reduction Method	Powder Pillows	LR (0 to 0.50 mg/L NO ₃ ⁻ -N)
Method 8171	Cadmium Reduction Method	Powder Pillows or AccuVac® Ampuls	MR (0 to 5.0 mg/L NO ₃ ⁻ -N)
Method 8039	Cadmium Reduction Method	Powder Pillows or AccuVac® Ampuls	HR (0 to 30.0 mg/L NO ₃ ⁻ -N)
Method 10020	Chromotropic Acid Method	Test 'N Tube™ Vials	HR (0 to 30.0 mg/L NO ₃ ⁻ -N)
	UniCell™ Vials		(0.0 to 13.5 mg/L NO ₃ ⁻ -N)

NITRITE

✓ Method 8507	Diazotization Method	Powder Pillows or AccuVac® Ampuls	LR (0 to 0.300 mg/L NO ₂ ⁻ -N)
Method 10019	Diazotization Method	Test 'N Tube™ Vials	LR (0 to 0.500 mg/L NO ₂ ⁻ -N)
Method 8153	Ferrous Sulfate Method	Powder Pillows	HR (0 to 250 mg/L NO ₂ ⁻ -N)
	Diazotization Method	UniCell™ Vials	(0 to 0.600 mg/L NO ₂ ⁻ -N)

NITROGEN, Ammonia

✓ Method 8038	Nessler Method		(0 to 2.500 mg/L NH ₃ -N)
Method 8155	Salicylate Method	Powder Pillows	(0 to 0.80 mg/L NH ₃ -N)
Method 10031	Salicylate Method	Test 'N Tube™ Vials	HR (0 to 50.0 mg/L NH ₃ -N)
Method 10023	Salicylate Method	Test 'N Tube™ Vials	LR (0 to 2.500 mg/L NH ₃ -N)

NITROGEN, Nitrate

Method 10049	UV Direct Reading Method		(0.0 to 10.2 mg/L NO ₃ ⁻ -N)
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NITROGEN, Total

Method 10071	Persulfate Digestion Method	Test 'N Tube™ Vials	(0.0 to 25.0 mg/L N)
✓ Method 10072	Persulfate Digestion Method	Test 'N Tube™ Vials	HR (10 to 150 mg/L N)
	Persulfate Digestion Method	UniCell™ Vials	(0 to 40.0 mg/L N or TN _p)

NITROGEN, Total Inorganic

Method 10021	Titanium Trichloride Reduction Method	Test 'N Tube™ Vials	(0 to 25.0 mg/L N)
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NITROGEN, Total Kjeldahl

Method 8075	Nessler Method (Digestion Required)		(0 to 150.0 mg/L)
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ORGANIC CARBON, Total

Method 10129	Direct Method		LR (0.0 to 20.0 mg/L C)
Method 10173	Direct Method		MR (15 to 150 mg/L C)

ORGANIC CARBON, Total

Method 10128	Direct Method		HR (100 to 700 mg/L C)
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ORGANIC CONSTITUENTS, UV Absorbing (UV-254)

Method 10054	Direct Reading Method		
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OXYGEN, Dissolved

Method 8316	Indigo Carmine Method	AccuVac® Ampuls	LR (0 to 1000 µg/L O ₂)
Method 8166	HRDO Method		HR (0 to 15.0 mg/L O ₂)
Method 8333	Ultra High Range Method		UHR (0 to 40.0 mg/L O ₂)

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OXYGEN DEMAND, Chemical

Method 10067	Manganese III Reactor Digestion Method (without chloride removal)	(30-1000 mg/L)
Method 10067	Manganese III Digestion Method (with optional chloride removal)	(20 to 1000 mg/L COD)
Method 8000	Reactor Digestion Method	(0 to 40.0 mg/L COD)
✓ Method 8000	Reactor Digestion Method	(0 to 150.0 mg/L COD)
✓ Method 8000	Reactor Digestion Method	(0 to 1500 and 0 to 15,000 mg/L COD)
✓ Method 8000	Reactor Digestion Method	(For all ranges)

OXYGEN SCAVENGERS

Method 8140	Iron Reduction Method for Oxygen Scavengers	0-600 µg/L carbohydrazide; 0-500 µg/L DEHA; 0-1000 µg/L hydroquinone; 0-1500 µg/L iso-ascorbic acid; 0-1000 µg/L methylethyl ketoxime (MEKO)
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OZONE

Method 8311	Indigo Method	AccuVac® Ampuls	(0 to 0.25 mg/L, 0 to 0.75 mg/L or 0 to 1.50 mg/L O ₃)
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PCB

(Polychlorinated Biphenyls)

Method 10050	Immunoassay Method
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PHENOLS

✓ Method 8047	4-Aminoantipyrine Method	(0 to 0.200 mg/L)
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PHOSPHONATES

Method 8007	Persulfate UV Oxidation Method	Powder Pillows	(0-2.50 to 0-125 mg/L)
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PHOSPHORUS, Acid Hydrolyzable

Method 8180	PhosVer 3 with Acid Hydrolysis	(0.00 to 5.00 mg/L PO ₄ ³⁻)
	Test 'N Tube™ Vials	(0.00 to 1.60 mg/L P)

PHOSPHORUS, Reactive (Orthophosphate)

Method 8178	Amino Acid Method		(0 to 30.00 mg/L PO ₄ ³⁻)
Method 8114	Molybdovanadate Method	Reagent Solution or AccuVac® Ampuls	(0 to 45.00 mg/L PO ₄ ³⁻)
Method 8114	Molybdovanadate Method	Test 'N Tube™ Vials	HR (0.0 to 100.0 mg/L PO ₄ ³⁻)
✓ Method 8048	PhosVer 3 (Ascorbic Acid) Method	Powder Pillows or AccuVac® Ampuls	(0 to 2.500 mg/L PO ₄ ³⁻)
✓ Method 8048	PhosVer 3 Method		(0.00 to 5.00 mg/L PO ₄ ³⁻)
	Test 'N Tube™ Vials		(0.00 to 1.60 mg/L P)
	Ascorbic Acid Method	UniCell™ Vials	(0.0 to 15.0 mg/L PO ₄ ³⁻)
	Ascorbic Acid Method	UniCell™ Vials	(0.0 to 60.0 mg/L PO ₄ ³⁻)

PHOSPHORUS, Total

✓ Method 8190	PhosVer 3 with Acid Persulfate Digestion	(0.00 to 3.50 mg/L PO ₄ ³⁻)
	Test 'N Tube™ Vials	(0.00 to 1.10 mg/L P)
Method 10127	Molybdovanadate Method	with Acid Persulfate Digestion
	Test 'N Tube™ Vials	HR (0.0 to 100.0 mg/L PO ₄ ³⁻)

PHOSPHORUS, Total, Digestion

✓ Method 8190	Acid Persulfate Digestion Method		(0 to 0.800 mg/L)
	Ascorbic Acid with Acid Persulfate Digestion Method	UniCell™ Vials	(0.0 to 15.0 mg/L PO ₄ ³⁻)
	Ascorbic Acid Method	UniCell™ Vials	(0.0 to 60.0 mg/L PO ₄ ³⁻)

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POTASSIUM

Method 8049 Tetraphenylborate Method (0 to 7.0 mg/L)

QUATERNARY AMMONIUM COMPOUNDS

Method 8337 Direct Binary Complex Method Powder Pillows (0 to 5.00 mg/L as CTAB)

SELENIUM

Method 8194 Diaminobenzidine Method (0 to 1.000 mg/L)

SILICA

Method 8282 Heteropoly Blue Method ULR (0 to 1000.0 µg/L as SiO₂)

Method 8186 Heteropoly Blue Method LR (0 to 1.600 mg/L as SiO₂)

Method 8185 Silicomolybdate Method HR (0 to 100.0 mg/L)

SILVER

Method 8120 Colorimetric Method Powder Pillows (0 to 0.700 mg/L)

SULFATE

✓ Method 8051 SulfaVer 4 Method Powder Pillows or AccuVac® Ampuls (0 to 70.0 mg/L)

Turbidimetric Method UniCell™ Vials LR (40 to 150 mg/L SO₄²⁻)

Turbidimetric Method UniCell™ Vials HR (150 to 900 mg/L SO₄²⁻)

SULFIDE

✓ Method 8131 Methylene Blue Method (0 to 800 µg/L)

SULFITE

Colorimetric Method (0 to 5.00 mg/L)

TANNIN and LIGNIN

Method 8193 Tyrosine Method (0 to 9.00 mg/L)

TRIHALOMETHANES

Method 10132 THM Plus™ Water Bath Method (0–200 ppb as Chloroform)

THM Plus™ TRIHALOMETHANES

Method 10140 THM Reactor Method Powder Pillows (0–200 ppb as Chloroform)

TOXICITY

Method 10017 ToxTrak Method (0 to 100% Inhibition)

TPH

Method 10050 Immunoassay Method (10 and 100 ppm TPH thresholds)

TURBIDITY

Method 10047 Attenuated Radiation Method (Direct Reading) (0 to 5000 Formazin Attenuation Units)

VOLATILE ACIDS

Method 8196 Esterification Method (0 to 2800 mg/L)

ZINC

✓ Method 8009 Zincon Method (0 to 3.000 mg/L)

PAR UniCell™ Vials (0 to 6.00 mg/L Zn)

INTRODUCTION

This manual is divided into five sections:

SECTION 1 CHEMICAL ANALYSIS INFORMATION on page 17

This section applies to all the procedures. It provides background information and reference/review material for the technician or chemist. Commonly used techniques are explained in detail.

SECTION 1 SAMPLE PRETREATMENT on page 45

This section provides a brief overview of sample pretreatment and three digestion procedures. Two are USEPA digestions. The Hach Digesdahl method is also included.

SECTION 1 WASTE MANAGEMENT AND SAFETY on page 63

This contains information on waste management, regulations, waste disposal and resources on waste management. The Safety portion covers reading a Material Safety Data Sheet and general safety guidelines.

SECTION 1 GENERAL INFORMATION on page 73

This section provides information needed for ordering, shipping and returning of items.

SECTION 1 PROCEDURES on page 79

This contains step-by-step illustrated instructions for measuring over 120 parameters. The steps also include helpful notes. Each procedure contains information on sample collection, storage and preservation, accuracy checks, possible interferences, summary of method and a list of the reagents and apparatus necessary to run the test.

<p>Before performing analysis using these procedures, read the <i>DR/4000 Instrument Manual</i> to learn about the instrument's features and how it operates.</p>
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SAMPLE PROCEDURE EXPLAINED

Procedure Identification Number
✓ Denotes USEPA accepted and approval

Procedure Name

Type of samples analyzed, any USEPA approval, digestion or distillation requirements, estimated detection limit

Name of method used

Range with units of measure

Illustration of procedure steps and instrument keystrokes required

Additional information that may be applicable

Reference for method used

Reference for EPA approval

Procedure step

Instrument Display

Keystrokes required

HACH **DR/4000** **PROCEDURE** **SULFIDE**

✓ Method 8131 **Methylene Blue Method***
(0-800 µg/L)

Scope and Application: For testing total sulfides, h²s, hs- and certain metal sulfides in groundwater, wastewater brines and seawater; USEPA accepted for reporting wastewater analysis **

* Adapted from *Standard Methods for the Examination of Water and Wastewater*
** Procedure is equivalent to USEPA method 376.2 and Standard Method 4500-S²-D for wastewater.

1. Press the soft key under **HACH PROGRAM**.
Select the stored reagent number 3500 (S²-) by pressing 3500 with the numeric keys.
Press: **ENTER**

2. The display will show:
HACH PROGRAM: 3500 Sulfide
The wavelength (λ), **665 nm**, is automatically selected.

3. Measure 25 mL of sample into a sample cell. This will be the prepared sample.
Note: For turbid samples, see **INTERFERENCES** (following these steps) for pretreatment instructions.

4. Measure 25 mL of deionized water into a second sample cell (the blank).
Note: Excessive agitation will cause loss of sulfide. Use a pipet to minimize sulfide loss.

5. Add 1.0 mL of Sulfide 1 Reagent to each cell. Swirl to mix.
Note: Use the calibrated 1-mL dropper.

6. Add 1.0 mL of Sulfide 2 Reagent to each cell. Immediately swirl to mix.
Note: A pink color will develop, then the solution will turn blue if sulfide is present.

7. Press the soft key under **START TIMER**.
A 5-minute reaction period will begin.

8. When the timer beeps, place the blank in the cell holder. Close the light shield.

Sulfide_None_Other_MLB_ENG_4000.fm

SULFIDE
Page 1 of 4

Procedure Name (for easy reference)

Page

SULFIDE, continued



11. Press the soft key under **ZERO**.

The display will show:

0 µg/L S²⁻

Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



10. Place the prepared sample in the cell holder. Close the light shield. Results in µg/L sulfide (or chosen units) will be displayed.

Note: Some sulfide loss may occur if dilution is necessary.

Interferences

Table 1 Interfering Substances and Suggested Treatments

Interfering Substances	Interference Levels and Treatments
Strong reducing substances (sulfite, thiosulfate and hydrosulfite)	Interfere by reducing the blue color or preventing its development
Sulfide, high levels	High concentrations of sulfide may inhibit full color development and require sample dilution. Some sulfide loss may occur when sample is diluted.
Turbidity	For turbid samples, prepare a sulfide-free blank as follows. Use it in place of the deionized water blank in the procedure. <ol style="list-style-type: none"> 1. Measure 25 mL of sample into a 50-mL erlenmeyer flask. 2. Add Bromine Water dropwise with constant swirling until a permanent yellow color just appears. 3. Add Phenol Solution dropwise until the yellow color just disappears. Use this solution in Step 4 in place of deionized water.

Sample Collection, Storage and Preservation

Collect samples in clean plastic or glass bottles. Fill completely and cap tightly. Avoid excessive agitation or prolonged exposure to air. Analyze sample immediately.

SULFIDE
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Levels of common sample substances or conditions that will cause inaccurate results

Specific sampling, storage, and preservation information

Expected performance of the method

Concise explanation of the method

Safety reminder

Specific information on managing wastes generated by the method

SULFIDE, continued

Method Performance

Precision
Standard: 400 µg/L S²⁻

Program	95% Confidence Limits
3500	399–401 µg/L S ²⁻

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
3500	2 µg/L

For more information on derivation and use of Hach's estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, appendix B, see Section 1.5.1.

Sensitivity
Program Number: 3500

Portion of Curve	Δ Abs	Δ Concentration
3500	0.010	4.9 µg/L

See Section 1.5.3 *Sensitivity Explained* for more information.

Determining Soluble Sulfides

Determine soluble sulfides by centrifuging the sample in completely filled, capped tubes and analyzing the supernatant. Insoluble sulfides are then estimated by subtracting the soluble sulfide concentration from the total sulfide result.

Summary of Method

Hydrogen sulfide and acid-soluble metal sulfides react with N,N=dimethyl-p-phenylenediamine sulfate to form methylene blue. The intensity of the blue color is proportional to the sulfide concentration.

High sulfide levels in oil field waters may be determined after proper dilution.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the Material Safety Data Sheet for information specific to the reagents used. For additional information, refer to Section 3.

Pollution Prevention and Waste Management

Sulfide 2 reagent contains potassium dichromate. The final solution will contain hexavalent chromium (D007) at a concentration regulated as a hazardous waste by Federal RCRA. Please see Section 3 for further information on proper disposal of these materials.

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SULFIDE
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Lists of reagents
and standards
required for the
procedure

Amount of reagents
and apparatus needed
to perform the procedure

Items needed
to perform
the procedure
that are not
included with
the instrument

Supplemental
reagents and
apparatus
mentioned in
notes or after
the procedure

Use this phone
number to
obtain technical
assistance

SULFIDE, continued

REQUIRED REAGENTS AND STANDARDS

Sulfide Reagent Set (100 tests) 22445-00
Includes: (2) 1816-32, (2) 1817-32

Description	Quantity Required per test	Unit	Cat No.
Sulfide 1 Reagent	2 mL	100 mL MDB	1816-32
Sulfide 2 Reagent	2 mL	100 mL MDB	1817-32
Water, deionized	25 mL	4 liters	272-56

REQUIRED EQUIPMENT AND SUPPLIES

Cylinder, graduated, 25 mL	1	each	508-40
or Pipet, volumetric, Class A, 25 mL	1	each	14515-40
DR/4000 1-inch Cell Adapter	1	each	48190-00
Pipet Filler, safety bulb	1	each	14651-00

OPTIONAL REAGENTS AND STANDARDS

Bromine Water, 30 g/L	29 mL	2211-20
Phenol Solution, 30 g/L	29 mL	2112-20

OPTIONAL EQUIPMENT AND SUPPLIES

DR/4000 Carousel Module Kit	each	48070-02
DR/4000 Flow Cell Module Kit, 1-inch	each	48070-04
DR/4000 Flow Cell Module Kit, 1-cm	each	48070-05
DR/4000 Sipper Module Kit, 1-inch	each	48090-03
Dropper, for 1 oz. bottle	each	2258-00
Flask, Erlenmeyer, 50 mL	each	505-41



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SECTION 1 CHEMICAL ANALYSIS INFORMATION

1.1 Abbreviations and Conversions

1.1.1 Abbreviations

The following abbreviations are used throughout the procedure section:

°C	degree(s) Celsius (Centigrade)
°F	degree(s) Fahrenheit
ACS	American Chemical Society reagent purity
APHA Standard Methods	<i>Standard Methods for the Examination of Water and Wastewater</i> , published jointly by the American Public Health Association (APHA), the American Water Works Association (AWWA), and the Water Environment Federation (WEF). Order from Hach requesting Cat. No. 22708-00 or from the Publication Office of the American Public Health Association. This book is the standard reference work for water analysis. Many procedures contained in this manual are based on <i>Standard Methods</i> .
AV	AccuVac
Bicn	bicinchoninate
conc	concentrated
DB	dropping bottle
CFR	Code of Federal Regulations
EDL	Estimated detection limit
F&T	free and total
FTU	Formazin Turbidity Units. Turbidity unit of measure based on a Formazin stock suspension.
FV	FerroVer
FZ	FerroZine
g	grams

gr/gal	grains per gallon (1 gr/gal = 17.12 mg/L)
HR	high range
kg/ha	kilograms per hectare
Lbs/Ac	pounds per acre
LR	low range
MDL	Method detection limit
MDB	marked dropping bottle
mg/L	milligrams per liter (ppm)
µg/L	micrograms per liter (ppb)
mL	(milliliter)-approximately the same as a cubic centimeter (cc) or 1/1000 of a liter.
MR	medium range
NIPDWR	National Interim Primary Drinking Water Regulations
NPDES	National Pollutant Discharge Elimination System
P	phosphorus
PCB	Poly-chlorinated biphenyl
PV	PhosVer
SCDB	self-contained dropping bottle
TPH	Total petroleum hydrocarbons
TPTZ	(2,4,6-tri(2-Pyridyl)-1, 3, 5-Triazine)
USEPA	United States Environmental Protection Agency
ULR	ultra low range

SECTION 1, continued

1.1.2 Converting Chemical Species

Species conversion factors for many of the commonly used units of measure have been pre-programmed into the DR/4000 to simplify calculations. Conversions are method specific and can be accessed through the **OPTIONS** soft key when performing a method. Conversions and conversion factors are listed below.

Nitrogen

Nitrite (NO_2) = Nitrogen (N) x 3.28
Nitrate (NO_3) = Nitrogen (N) x 4.42
Ammonia (NH_3) = Nitrogen (N) x 1.22
Ammonium (NH_4) = Nitrogen (N) x 1.29

Phosphate

Phosphorus (P) = Phosphate (PO_4) x 0.326
Phosphorus Pentoxide (P_2O_5) = Phosphate (PO_4) x 0.75

Table 1 Hardness Conversion Factors

Units of Measure	mg/L CaCO_3	British gr/gal (Imperial) CaCO_3	American gr/gal (US) CaCO_3	French parts/100,000 CaCO_3	German parts/ 100,000 CaO	meq/L*	g/L CaO	lb./cu ft CaCO_3
mg/L CaCO_3	1.0	0.07	0.058	0.1	0.056	0.02	5.6×10^{-4}	6.23×10^{-5}
English gr/gal CaCO_3	14.3	1.0	0.83	1.43	0.83	0.286	8.0×10^{-3}	8.91×10^{-4}
US gr/gal CaCO_3	17.1	1.2	1.0	1.72	0.96	0.343	9.66×10^{-3}	1.07×10^{-3}
Fr. p/100,000 CaCO_3	10.0	0.7	0.58	1.0	0.56	0.2	5.6×10^{-3}	6.23×10^{-4}
Ger. p/100,000 CaO	17.9	1.25	1.04	1.79	1.0	0.358	1.0×10^{-2}	1.12×10^{-3}
meq/L	50.0	3.5	2.9	5.0	2.8	1.0	2.8×10^{-2}	3.11×10^{-3}
g/L CaO	1,790.0	125.0	104.2	179.0	100.0	35.8	1.0	0.112
lb./cu ft CaCO_3	16,100.0	1,123.0	935.0	1,610.0	900.0	321.0	9.0	1.0

* or 'epm/L' or 'mval/L' N.B. 1 meq/L = N/1000

Dissolved Oxygen

The following table lists the mg/L dissolved oxygen in water at saturation for various temperatures and atmospheric pressures. The table was formulated in a laboratory using pure water; thus, the values given should be considered as only approximations when estimating the oxygen content of a particular body of surface water.

SECTION 1, continued

Table 2 Dissolved Oxygen Saturation In Water

		Pressure in Millimeters and Inches Hg							
		mm							
		775	760	750	725	700	675	650	625
Temp		inches							
		30.51	29.92	29.53	28.45	27.56	26.57	25.59	24.61
°F	°C								
32.0	0	14.9	14.6	14.4	13.9	13.5	12.9	12.5	12.0
33.8	1	14.5	14.2	14.1	13.6	13.1	12.6	12.2	11.7
35.6	2	14.1	13.9	13.7	13.2	12.9	12.3	11.8	11.4
37.4	3	13.8	13.5	13.3	12.9	12.4	12.0	11.5	11.1
39.2	4	13.4	13.2	13.0	12.5	12.1	11.7	11.2	10.8
41.0	5	13.1	12.8	12.6	12.2	11.8	11.4	10.9	10.5
42.8	6	12.7	12.5	12.3	11.9	11.5	11.1	10.7	10.3
44.6	7	12.4	12.2	12.0	11.6	11.2	10.8	10.4	10.0
46.4	8	12.1	11.9	11.7	11.3	10.9	10.5	10.1	9.8
48.2	9	11.8	11.6	11.5	11.1	10.7	10.3	9.9	9.5
50.0	10	11.6	11.3	11.2	10.8	10.4	10.1	9.7	9.3
51.8	11	11.3	11.1	10.9	10.6	10.2	9.8	9.5	9.1
53.6	12	11.1	10.8	10.7	10.3	10.0	9.6	9.2	8.9
55.4	13	10.8	10.6	10.5	10.1	9.8	9.4	9.1	8.7
57.2	14	10.6	10.4	10.2	9.9	9.5	9.2	8.9	8.5
59.0	15	10.4	10.2	10.0	9.7	9.3	9.0	8.7	8.3
60.8	16	10.1	9.9	9.8	9.5	9.1	8.8	8.5	8.1
62.6	17	9.9	9.7	9.6	9.3	9.0	8.6	8.3	8.0
64.4	18	9.7	9.5	9.4	9.1	8.8	8.4	8.1	7.8
66.2	19	9.5	9.3	9.2	8.9	8.6	8.3	8.0	7.6
68.0	20	9.3	9.2	9.1	8.7	8.4	8.1	7.8	7.5
69.8	21	9.2	9.0	8.9	8.6	8.3	8.0	7.7	7.4
71.6	22	9.0	8.8	8.7	8.4	8.1	7.8	7.5	7.2
73.4	23	8.8	8.7	8.5	8.2	8.0	7.7	7.4	7.1
75.2	24	8.7	8.5	8.4	8.1	7.8	7.5	7.2	7.0
77.0	25	8.5	8.4	8.3	8.0	7.7	7.4	7.1	6.8
78.8	26	8.4	8.2	8.1	7.8	7.6	7.3	7.0	6.7
80.6	27	8.2	8.1	8.0	7.7	7.4	7.1	6.9	6.6
82.4	28	8.1	7.9	7.8	7.6	7.3	7.0	6.7	6.5
84.2	29	7.9	7.8	7.7	7.4	7.2	6.9	6.6	6.4
86.0	30	7.8	7.7	7.6	7.3	7.0	6.8	6.5	6.2
87.8	31	7.7	7.5	7.4	7.2	6.9	6.7	6.4	6.1
89.6	32	7.6	7.4	7.3	7.0	6.8	6.6	6.3	6.0
91.4	33	7.4	7.3	7.2	6.9	6.7	6.4	6.2	5.9
93.2	34	7.3	7.2	7.1	6.8	6.6	6.3	6.1	5.8
95.0	35	7.2	7.1	7.0	6.7	6.5	6.2	6.0	5.7
96.8	36	7.1	7.0	6.9	6.6	6.4	6.1	5.9	5.6
98.6	37	7.0	6.8	6.7	6.5	6.3	6.0	5.8	5.6

SECTION 1, continued

Table 2 Dissolved Oxygen Saturation In Water (Continued)

		Pressure in Millimeters and Inches Hg							
		mm							
		775	760	750	725	700	675	650	625
Temp		inches							
		30.51	29.92	29.53	28.45	27.56	26.57	25.59	24.61
°F	°C								
100.4	38	6.9	6.7	6.6	6.4	6.2	5.9	5.7	5.5
102.2	39	6.8	6.6	6.5	6.3	6.1	5.8	5.6	5.4
104.0	40	6.7	6.5	6.4	6.2	6.0	5.7	5.5	5.3
105.8	41	6.6	6.4	6.3	6.1	5.9	5.6	5.4	5.2
107.6	42	6.5	6.3	6.2	6.0	5.8	5.6	5.3	5.1
109.4	43	6.4	6.2	6.1	5.9	5.7	5.5	5.2	5.0
111.2	44	6.3	6.1	6.0	5.8	5.6	5.4	5.2	4.9
113.0	45	6.2	6.0	5.9	5.7	5.5	5.3	5.1	4.8
114.8	46	6.1	5.9	5.9	5.6	5.4	5.2	5.4	4.8
116.6	47	6.0	5.9	5.8	5.6	5.3	5.1	4.8	4.7
118.4	48	5.9	5.8	5.7	5.5	5.3	5.0	4.8	4.6
120.2	49	5.8	5.7	5.6	5.4	5.2	5.0	4.7	4.5
122.0	50	5.7	5.6	5.5	5.3	5.1	4.9	4.7	4.4

1.2 Analytical Techniques

1.2.1 Sample Collection, Preservation and Storage

Correct sampling and storage are critical for accurate testing. For greatest accuracy, thoroughly clean sampling devices and containers to prevent carry-over from previous samples. Preserve the sample properly; each procedure has information about sample preservation.

1.2.1.1 Collecting Water Samples

Obtain the best sample by careful collection. In general, collect samples near the center of the vessel or duct and below the surface. Use only clean containers (bottles, beakers). Rinse the container several times first with the water to be sampled.

Take samples as close as possible to the source of the supply. This reduces the influence the distribution system has on the sample. Let the water run long enough to flush the system. Fill sample containers slowly with a gentle stream to avoid turbulence and air bubbles. Collect water samples from wells after the pump has run long enough to deliver water representative of the ground water feeding the well.

It is hard to obtain a truly representative sample when collecting surface water samples. Obtain best results by testing several samples. Use samples taken at different times from several locations and depths. The results can be used to establish patterns for that particular body of water.

Generally, as little time as possible should elapse between collecting the sample and analyzing it.

Depending on the test, special precautions in handling the sample may be necessary. This prevents natural interferences such as organic growth or loss or gain of dissolved gases. Each procedure describes sample preservatives and storage techniques for samples that are held for testing.

1.2.1.2 Acid-Washing Bottles

If a procedure suggests acid washing, use the following procedure:

- a. Clean the glassware or plasticware with laboratory detergent.
- b. Rinse well with tap water.
- c. Rinse with a 1:1 Hydrochloric Acid Solution or 1:1 Nitric Acid Solution. The nitric acid rinse is important for testing for lead.
- d. Rinse well with deionized water. Up to 12–15 rinses may be necessary if chromium is being determined.
- e. Air dry.

Use chromic acid or chromium-free substitutes to remove organic deposits from glass containers. Rinse containers thoroughly with water to remove traces of chromium.

Wash glassware for phosphate determinations with phosphate-free detergents and acid wash with 1:1 HCl. Thoroughly rinse the glassware with distilled water. For ammonia and Kjeldahl nitrogen, rinse with ammonia-free water.

1.2.1.3 Storage and Preservation

- The least expensive containers are polypropylene or polyethylene.
- The best and most expensive containers are quartz or TFE (tetrafluoroethylene, Teflon).
- Avoid soft glass containers when testing for metals in the microgram-per-liter range.
- Store samples for silver determination in light-absorbing containers.

Avoid contaminating the sample with metals from containers, distilled water or membrane filters. Thoroughly clean sample containers as described under Acid Washing Bottles.

Preservation slows the chemical and biological changes that continue after collection. These changes may change the amount of a chemical species available for analysis. Normally, analyze the samples as soon as possible after collection, especially when the analyte concentration is expected to be low. This also reduces the chance for error and minimizes labor.

Preservation methods include pH control, chemical addition, refrigeration and freezing. Section 3 gives the recommended preservation for various substances. It also suggests an appropriate container type and the maximum recommended holding times for properly preserved samples.

Preserve aluminum, cadmium, chromium, cobalt, copper, iron, lead, nickel, potassium, silver and zinc samples for at least 24 hours by adding one Nitric Acid

SECTION 1, continued

Solution Pillow 1:1 (Cat. No. 2540-98) per liter of sample. Check the pH with pH indicator paper or a pH meter to assure the pH is 2 or less. Add additional pillows if necessary. Adjust the sample pH prior to analysis by adding an equal number of Sodium Carbonate Anhydrous Powder Pillows (Cat. No. 179-98). Or raise the pH to 4.5 with Sodium Hydroxide Standard Solution, 1 N or 5 N.

Table 3 Required Containers, Preservation Techniques and Holding Times*

Parameter No./Name	Container**	Preservation***,****	Holding Time*****
Table 1A-Bacterial Tests:			
1–4. Coliform, fecal and total	P, G	Cool, 4 °C, 0.008% Na ₂ S ₂ O ₃ *****	6 hours
5. Fecal streptococci	P, G	Cool, 4 °C, 0.008% Na ₂ S ₂ O ₃ ⁶	6 hours
Table 1B-Inorganic Tests:			
1. Acidity	P, G	Cool, 4 °C	14 days
2. Alkalinity	P, G	Cool, 4 °C	14 days
4. Ammonia	P, G	Cool, 4 °C, H ₂ SO ₄ to pH<2	28 days
9. Biochemical oxygen demand	P, G	Cool, 4 °C	48 hours
10. Boron	PFTE or quartz	HNO ₃ to pH<2	6 months
11. Bromide	P, G	None required	28 days
14. Biochemical oxygen demand, carbonaceous	P, G	Cool, 4 °C	48 hours
15. Chemical oxygen demand	P, G	Cool, 4 °C, H ₂ SO ₄ to pH<2	28 days
16. Chloride	P, G	None required	28 days
17. Chlorine, total residual	P, G	None required.	Analyze immediately
21. Color	P, G	Cool, 4 °C	48 hours
23-24. Cyanide, total and amenable to chlorination	P, G	Cool, 4 °C, NaOH to pH >12, 0.6 g ascorbic acid ⁶	14 days*****
25. Fluoride	P	None required	28 days
27. Hardness	P, G	HNO ₃ to pH<2, H ₂ SO ₄ to pH<2	6 months
28. Hydrogen ion (pH)	P, G	None required	Analyze immediately
31,43. Kjeldahl and organic nitrogen	P, G	Cool, 4 °C, H ₂ SO ₄ to pH <2	28 days
Metals:*****			
18. Chromium VI	P, G	Cool, 4 °C	24 hours
35. Mercury	P, G	HNO ₃ to pH<2	6 months
3, 5–8, 12, 13, 19, 20, 22, 26, 29, 30, 32-34, 36, 37, 45, 47, 51, 52, 58-60, 62, 63, 70–72, 74, 75. Metals, except boron, chromium VI and mercury***** ⁹	P, G	HNO ₃ to pH<2	6 months
38. Nitrate	P, G	Cool, 4 °C	48 hours
39. Nitrate-nitrite	P, G	Cool, 4 °C, H ₂ SO ₄ to pH<2	28 days
40. Nitrite	P, G	Cool, 4 °C	48 hours
41. Oil and grease	G	Cool, 4 °C, HCl or H ₂ SO ₄ to pH<2	28 days
42. Organic carbon	P, G	Cool, 4 °C, HCl or H ₂ SO ₄ or H ₃ PO ₄ to pH<2	28 days
44. Orthophosphate	P, G	Filter immediately, cool, 4 °C	48 hours
46. Oxygen, Dissolved, Probe	G bottle and top	None required	Analyze immediately
47. Winkler	G bottle and top	Fix on site and store in dark	8 hours
48. Phenols	G only	Cool, 4 °C, H ₂ SO ₄ to pH<2	28 days

SECTION 1, continued

Table 3 Required Containers, Preservation Techniques and Holding Times* (Continued)

Parameter No./Name	Container**	Preservation***,****	Holding Time*****
49. Phosphorus (elemental)	G	Cool, 4 °C	48 hours
50. Phosphorus, total	P, G	Cool, 4 °C, H ₂ SO ₄ to pH<2	28 days
53. Residue, total	P, G	Cool, 4 °C	7 days
54. Residue, filterable	P, G	Cool, 4 °C	7 days
55. Residue, nonfilterable (TSS)	P, G	Cool, 4 °C	7 days
56. Residue, settleable	P, G	Cool, 4 °C	48 hours
57. Residue, volatile	P, G	Cool, 4 °C	7 days
61. Silica	PFTE or quartz	Cool, 4 °C	28 days
64. Specific conductance	P, G	Cool, 4 °C	28 days
65. Sulfate	P, G	Cool, 4 °C	28 days
66. Sulfide	P, G	Cool, 4 °C, add zinc acetate plus sodium hydroxide to pH>9.	7 days
67. Sulfite	P, G	None required	Analyze immediately
68. Surfactants	P, G	Cool, 4 °C	48 hours
69. Temperature	P, G	None required	Analyze immediately
73. Turbidity	P, G	Cool, 4 °C	48 hours

* This table was adapted from Table II published in the Federal Register, July 1, 1997, 40 CFR, Part 136.3, pages 26-28. Organic tests are not included.

** Polyethylene (P) or glass (G).

*** Sample preservation should be performed immediately upon sample collection. For composite chemical samples each aliquot should be preserved at the time of collection. When use of automated sampler makes it impossible to preserve each aliquot, then chemical samples may be preserved by maintaining at 4 °C until compositing and sample splitting is completed.

**** When any sample is to be shipped by common carrier or sent through United States Mails, it must comply with the Department of Transportation Hazardous Material Regulations (49 CFR Part 172). The person offering such material for transportation is responsible for ensuring such compliance. For the preservation requirements of Table II, the Office of Hazardous Materials, Materials Transportation Bureau, Department of Transportation has determined that Hazardous Materials Regulations do not apply to the following materials: hydrochloric acid (HCl) in water solutions at concentrations of 0.04% by weight or less (pH about 1.96 or greater); nitric acid (HNO₃) in water solutions at concentrations of 0.15% by weight or less (pH about 1.62 or greater); sulfuric acid (H₂SO₄) in water solutions at concentrations of 0.35% by weight or less (about pH 1.15 or greater); and sodium hydroxide (NaOH) in water solutions at concentrations of 0.080% by weight or less (pH about 12.30 or less).

***** Samples should be analyzed as soon as possible after collection. The times listed are the maximum times that samples may be held before analysis and still be considered valid. Samples may be held for longer periods only if the permittee, or monitoring laboratory, has data on file to show that the specific types of samples under study are stable for the longer time, and has received a variance from the Regional Administrator under §136.3(e). Some samples may not be stable for the maximum time period given in the table. A permittee, or monitoring laboratory, is obligated to hold the sample for a shorter time if knowledge exists to show that this is necessary to maintain sample stability. See §136.3(e) for details. The term "analyze immediately" usually means within 15 minutes or less after sample collection.

***** Should only be used in the presence of residual chlorine.

***** Maximum holding time is 24 hours when sulfide is present. Optionally all samples may be tested with lead acetate paper before pH adjustments in order to determine if sulfide is present. If sulfide is present, it can be removed by the addition of cadmium nitrate powder until a negative spot test is obtained. The sample is filtered and then NaOH is added to a pH of 12.

***** Samples should be filtered immediately on site before adding preservative for dissolved metals.

***** Numbers refer to parameter numbers in 40 CFR, Part 136.3, table 1B.

1.2.2 Correcting for Volume Additions

If you use a large volume of preservative, correct for the volume of preservative added. This accounts for dilution due to the acid added to preserve the sample and the base used to adjust the pH to the range of the procedure. This correction is made as follows:

1. Determine the total volume of initial sample, the volume of acid added and base added, and the final volume of the sample.
2. Divide the total volume by the initial volume.
3. Multiply the test result by this factor.

An example:

A one-liter sample was preserved with 2 mL of nitric acid. It was neutralized with 5 mL of 6 N sodium hydroxide. The result of the analysis procedure was 10.00 mg/L. What is the volume correction factor and correct result?

1. Total volume = 1000 mL + 2 mL + 5 mL = 1007
2. $\frac{1007}{1000} = 1.007 = \text{volume correction factor}$
3. $10.00 \text{ mg/L} \times 1.007 = 10.07 \text{ mg/L} = \text{correct result}$

The addition of a Sodium Carbonate Anhydrous Powder Pillow does not need to be corrected for.

1.2.3 Boiling Aids

Boiling is necessary in some procedures. Using a boiling aid such as boiling chips (Cat. No. 14835-31) may reduce bumping. Bumping is caused by the sudden, almost explosive conversion of water to steam as it is heated. Avoid bumping; it may cause sample loss or injury.

Make sure the boiling aids will not contaminate the sample. Do not use boiling aids (except glass beads) more than once. Loosely covering the sample during boiling will prevent splashing, reduce the chances of contamination and minimize sample loss.

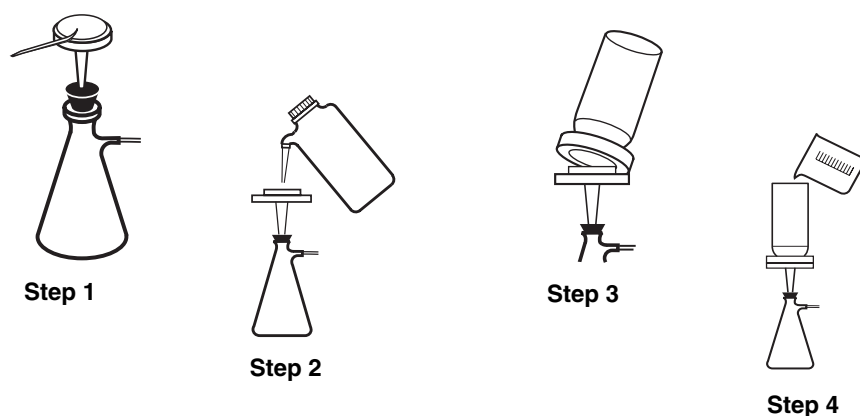
1.2.4 Sample Filtration

Filtration separates particles from the sample. Filtration uses a medium, usually filter paper, to retain particles but pass solution. This is especially helpful when sample turbidity interferes with analysis. Two general methods of filtration are gravity and vacuum. Gravity filtration uses gravity to pull the sample through the filter paper. Vacuum filtration uses suction and gravity to move the sample through the filter. An aspirator or vacuum pump creates the suction. Vacuum filtration is faster than gravity filtration. Proceed as follows (see *Figure 1*):

1. Place a filter paper into the filter holder.
2. Place the filter holder assembly in the filtering flask. Wet the filter with deionized water to ensure adhesion to the holder.
3. Position the funnel housing on the filter holder assembly.
4. While applying a vacuum to the filtering flask, transfer the sample to the filtering apparatus.

5. Slowly release the vacuum from the filtering flask and transfer the solution from the filter flask to another container.

Figure 1 Vacuum Filtration



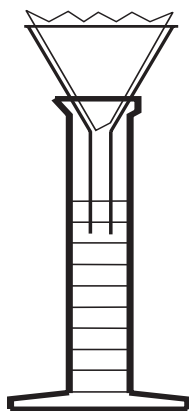
REQUIRED APPARATUS

Description	Unit	Cat. No.
Filter Discs, glass, fiber 47-mm	100/pkg	2530-00
Filter Holder, membrane	each	13529-00
Flask, filter, 500-mL	each	546-49
Pump, vacuum, hand operated	each	14283-00
<i>or</i>		
Pump, vacuum, portable, 115 VAC	each	14697-00
Pump, vacuum, portable, 220 VAC	each	14697-02

Many of the procedures in this manual use gravity filtration. The only labware required are filter paper, a conical funnel and a receiving flask. This labware is included under Optional Equipment and Supplies at the end of a procedure. Gravity filtration is better for retaining fine particles. For faster filtering, add solution until the filter paper cone is three-fourths filled. Never fill the cone completely. Proceed as follows (see *Figure 2*):

1. Place a filter paper into the funnel.
2. Wet the filter with deionized water to ensure adhesion to the funnel.
3. Place the funnel into an erlenmeyer flask or graduated cylinder.
4. Pour the sample into the funnel.

Figure 2 Gravity Filtration



REQUIRED APPARATUS

Description	Unit	Cat No.
Cylinder, graduated, 100-mL	each.....	508-42
Funnel, poly, 65-mm	each.....	1083-67
Filter Paper, 12.5-cm	each.....	1894-57
Flask, Erlenmeyer, 125-mL.....	each.....	505-43

Testing for metals requires acid and heat to pretreat the sample. Since these conditions destroy filter paper, vacuum filtration with glass fiber filter discs is recommended. Also, glass filter discs, unlike paper, do not retain colored species.

1.2.5 Temperature Considerations

For best results, most tests in this manual should be performed with sample temperatures between 20 °C (68 °F) and 25 °C (77 °F). If a test requires closer temperature control, notes in the procedure will indicate this.

1.2.6 Sample Dilution Techniques

Most tests use 10- and 25-mL volumes. In some tests, however, the color developed in the sample may be too intense to be measured. Unexpected colors may develop in other tests. In both cases, dilute the sample to determine if interfering substances are present.

To dilute the sample, pipet the chosen sample portion into a clean, graduated cylinder (or volumetric flask for more accurate work). Fill the cylinder (or flask) to the desired volume with deionized water. Mix well. Use the diluted sample when running the test.

To help with dilutions, *Table 4* shows the amount of sample used, the amount of deionized water used to bring the volume up to 25 mL, and the multiplication factor.

The concentration of the sample is equal to the diluted sample reading multiplied by the multiplication factor.

An example: A 2.5-mL sample was diluted with 22.5 mL of deionized water. The result was 0.35 mg/L. What is the concentration of the sample?

Table 4 Sample Dilutions

Sample Volume (mL)	Deionized water used to bring the volume to 25 mL (mL)	Multiplication Factor
25.0	0.0	1
12.5	12.5	2
10.0*	15.0	2.5
5.0*	20.0	5
2.5*	22.5	10
1.0*	24.0	25
0.250*	24.75	100

* For sample sizes of 10 mL or less, a pipet should be used to measure the sample into the graduated cylinder or volumetric flask.

$$0.35 \times 10 = 3.5 \text{ mg/L}$$

More accurate dilutions can be done with a pipet and a 100-mL volumetric flask (see *Table 5* for more information). Pipet the sample and dilute to volume with deionized water. Swirl to mix.

Table 5 Multiplication Factors For Diluting to 100 mL

Sample Volume (mL)	Multiplication Factor
1	100
2	50
5	20
10	10
25	4
50	2

Sample Dilution and Interfering Substances

Sample dilution may influence the level at which a substance may interfere. The effect of the interferences decreases as the dilution increases. In other words, higher levels of an interfering substance can be present if the sample is diluted.

An example: Copper does not interfere at or below 100 mg/L for a 25-mL sample in a procedure. If the sample volume is diluted with an equal volume of water, what is the level at which copper will not interfere?

$$\frac{\text{Total volume}}{\text{Sample volume}} = \text{Dilution factor}$$

$$\frac{25}{12.5} = 2$$

$$100 \times 2 = 200 \text{ mg/L}$$

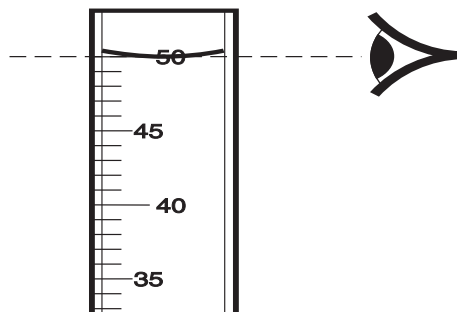
Thus, the level at which copper will not interfere in the sample is at or below 200 mg/L.

SECTION 1, continued

1.2.7 Using Pipets and Graduated Cylinders

When small sample quantities are used, the accuracy of measurements is important. *Figure 3* illustrates the proper way to read the sample level or the meniscus formed when the liquid wets the cylinder or pipet walls.

Figure 3 **Reading the Meniscus**



Rinse the pipet or cylinder two or three times with the sample to be tested before filling. Use a pipet filler or pipet bulb to draw the sample into the pipet. Never pipet chemical reagent solutions or samples by mouth. When filling a pipet, keep the tip of the pipet below the surface of the sample as the sample is drawn into the pipet.

Serological pipets have marks that indicate the volume of liquid delivered by the pipet. The marks may extend to the tip of the pipet or may be only on the straight portion of the tube. Fill serological pipets to the zero mark and discharge the sample by allowing the sample to drain until the meniscus is level with the desired mark. If the serological pipet has marks extended to the tip of the pipet, the sample must be blown out of the tip for accurate sample measurements.

Volumetric (transfer) pipets have a bulb in the middle and a single ring above the bulb to indicate the volume of liquid when it is filled to the mark. To discharge a volumetric pipet, hold the tip of the pipet at a slight angle against the container wall and drain. Do not attempt to discharge sample or reagent remaining in the tip of the pipet after draining. Volumetric pipets are designed to retain a small amount of sample in the pipet tip.

If sample drops stay on the walls of the pipet, the pipet is dirty and is not delivering the correct amount of sample. Wash the pipet thoroughly with a laboratory detergent or cleaning solution and rinse several times with deionized water.

1.2.8 Using the TenSette Pipet

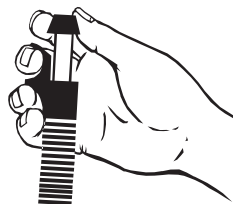
For best results use a new tip each time you pipet. After several uses, the pipet tip may retain some liquid, causing inaccurate delivery. Each pipet is supplied with 100 tips; order Hach replacement tips for best results.

Always use careful, even hand movements for best reproducibility. If the pipet does not operate smoothly, disassemble and coat the piston and retainer with high-quality stopcock grease. Also coat the metering turret lightly with grease. Refer to the *TenSette Pipet* manual.

For best pipetting accuracy, the solution and the room temperature should be 20–25 °C. Avoid holding the TenSette Pipet for a long time—the increased temperature may affect accurate delivery.

Never lay the pipet down with the liquid in the tip. Solution could leak into the pipet and cause corrosion.

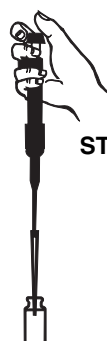
Operating the TenSette Pipet



1. Attach a clean tip by holding the pipet body in one hand and gently pressing the large end of the pipet tip onto the tapered end of the pipet. Be sure a good seal is obtained.
2. Turn the turret cap to align the desired volume with the mark on the pipet body.
3. Using a smooth motion, press down on the turret cap until it reaches the stop. Immerse the tip about 5 mm (1/4 inch) below the solution surface to avoid drawing air into the pipet. Do not insert the tip any deeper or the delivery volume may be affected.
4. While maintaining a constant pressure, allow the turret to return slowly to the extended position. A rapid return may affect the delivery volume.
5. With the turret up, take the tip out of the solution and move it to the receiving vessel. Do not press on the turret cap while moving the pipet.



STEP 3



STEP 4



STEP 5



STEP 6

6. Use the thumb and forefinger to twist the turret cap to the next higher volume position to ensure quantitative transfer of the sample. The "F" position provides full blowout.



STEP 7

7. With the tip in contact with the side of the receiving vessel, slowly and smoothly press down on the turret cap until it reaches the stop and the solution is completely discharged.

1.2.9 Mixing Water Samples

The following two methods may be helpful in tests that require mixing sample with chemicals (usually indicated by “swirl to mix” instructions).

1. When mixing sample in a square sample cell, swirl with a simple twisting motion (see *Figure 4*). Grasp the neck of the cell with the thumb and index finger of one hand. Rest the concave bottom of the cell on the tip of the index finger of the other hand. Rotate the cell quickly, first one way and then the other, to mix the sample.
2. Swirling is recommended when mixing samples in a graduated cylinder or a titration flask. Grip the cylinder (or flask) firmly with the tips of three fingers (see *Figure 5*). Hold the cylinder at a 45-degree angle and twist-out wrist. This should move the cylinder in an approximately 12-inch circle, creating enough rotation to complete the mixing in a few turns.

This swirling procedure is the most gentle and offers the least interference from the atmosphere when testing for carbon dioxide and other gases. Both methods are simple but take a bit of practice in order to obtain the best results.

Figure 4 **Swirling a Sample Cell**

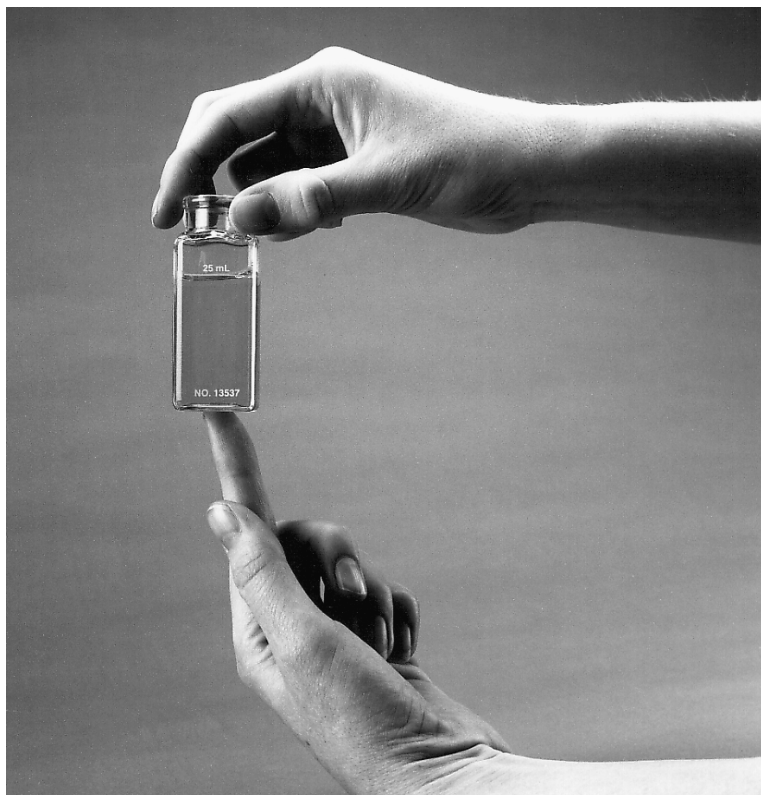
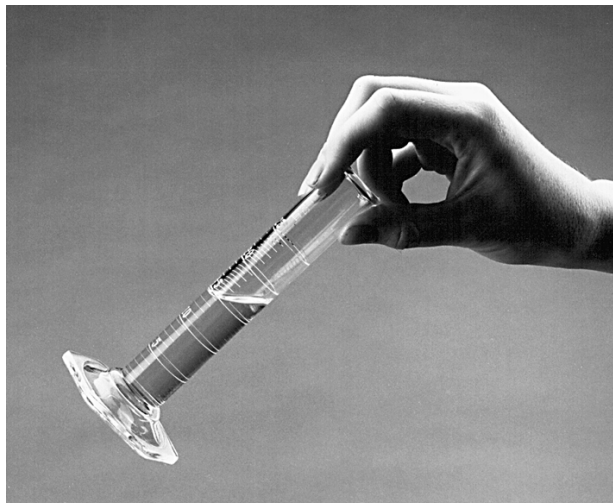


Figure 5 **Swirling a Graduated Cylinder**



1.2.10 Using DR/4000 Sample Cells

A variety of sample cells are available for use on your DR/4000 Spectrophotometer. A short review of recommended cleaning and handling procedures will help you obtain the most accurate results.

In general, the glass sample cells are intended for use in the visible spectrum of the instrument (350 to 1100 nm with the Tungsten Lamp) whereas the quartz cells can be used in the full range of the UV/VIS instrument (190 to 1100 nm). Quartz cells must be used for UV measurements.

A pair of matched 1-inch glass sample cells (unstoppered) with 10- and 25-mL fill marks are specified with the instrument. Use these 1-inch sample cells for the majority of the Hach procedures programmed in the instrument. An optional set of stoppered, matched 1-inch glass cells are also available and are required for some Hach procedures. The UV/VIS instrument also includes a matched pair of research-grade 1-cm quartz cells.

Never touch the clear sides of the research-grade cells with your fingers—skin oils will etch the windows.

Research-grade, long-path sample cells with path lengths of 5 and 10 cm are also available in both glass and quartz. These cells are sold as individual pieces and have a path length tolerance of ± 0.01 mm.

Orientation of Sample Cells

To minimize variability of measurements using a particular cell, always place it into the cell holder with the same orientation. The Hach 1-inch cells (10- and 25-mL fill marks) are matched using the two clear sides adjacent to the fill marks. Since the light beam travels from left to right, the **fill marks on the cells should always face the user**. This orientating procedure is different from the DR/2000 and DR/3000.

The research grade cells (1-cm quartz, 5- and 10-cm glass and quartz) have two clear sides opposite each other in addition to two frosted sides. Always orient these cells so that the clear sides face the left and right sides of the DR/4000 cell compartment.

In addition to proper orientation, the sides of the cells should be free of smudges, fingerprints, etc., to insure accurate readings. Wipe the sides of the cells with a soft cloth to clean the surface before taking measurements.

Care of Hach 1-Inch Sample Cells

Store sample cells in their boxes when not in use to protect them from scratching and breaking. It is good laboratory practice to empty and clean sample cells after analyses are complete—avoid leaving colored solutions in the cells for extended periods of time. Finish the cleaning procedure with a few rinses of deionized water and allow to dry. Individual procedures often recommend specific cleaning methods for special circumstances.

Care of Research-Grade (Starna) Cells

The surfaces of fully fused cells resist damage by most laboratory cleaning agents. However, strong alkalies will damage the optical surfaces and should only be used as a last resort. Both quartz and glass may absorb some metal ions onto the surface and this may require a great deal of rinsing to remove the residue. Some methods that may be used follow this paragraph.

Detergent Solutions:

Most laboratory detergents can be used at recommended concentrations. If the pH is greater than 8.5, etching may occur. Neutral detergents such as Neutracon are safer if regular cleaning is required, as in the case of protein residues.

Chromic Acid:

Use Chromic Acid as a 5–10% solution in 90% sulfuric acid or as a 5% solution in 20:80 nitric:sulfuric acid. Soak cells for up to 12 hours or overnight and rinse at least 10 times with distilled water to remove chromate ions from the cell surface. The reagent is very corrosive and should only be used by properly trained staff. Also, do not use the reagent more than once a month or etching of the cells may occur.

Chlorate-Hydrochloric Acid:

Immerse the cells in concentrated (SG 1.18) HCl and potassium chlorate added in small quantities up to about 10% with frequent agitation. This must be done in an efficient fume hood by properly trained staff.

Alcoholic Potassium Hydroxide:

Alcoholic Potassium Hydroxide may be used as a 5% solution, but it will etch the cells if used repeatedly. Use this method as a last resort.

Cleaning Procedures and Suggestions

If using a detergent, you can speed cleaning by increasing the temperature or using an ultrasonic bath. Use care when using an ultrasonic bath; if the cell touches the metal bath liner or the cell is damaged when it is put in, the ultrasonic energy may destroy the cell.

Rinsing is more efficient when using distilled water followed by ethanol or acetone. Use analytical-grade acetone.

Cells used with phosphoric acid will be slowly attacked by the sample. It is best to keep the sample in the cell the minimum of time necessary. The best way to clean cells after exposure to phosphoric acid is to immerse them in a detergent solution for a short time then rinse with distilled water.

When using cells with stoppers, leave an air gap between the sample and stopper. The force caused by pressing a stopper against the liquid in the cell can cause the windows to weaken and crack.

Store the cells in the velvet-lined boxes or the plastic and foam boxes they were shipped in. This will protect them from being scratched or broken.

1.2.11 Volume Measurement Accuracy

Sample cells supplied with the spectrophotometer are marked to indicate approximately 10 and 25 mL. In most tests filling to the mark is sufficient. In tests where volume measurements are critical, the procedure specifies the appropriate method.

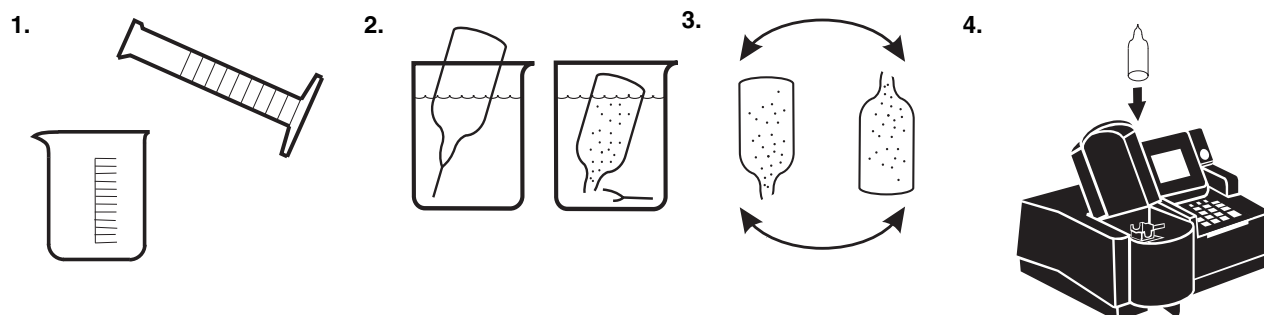
If a sample must be diluted, use a pipet for volume measurement. Accuracy is important because a slight mistake in measuring a small sample will cause a substantial error in the result. For instance, a 0.1-mL mistake in the measurement of a 1.0-mL sample produces a 10% error in the test result.

1.2.12 Using AccuVac Ampuls

AccuVac Ampuls contain pre-measured powder or liquid in optical-quality glass ampuls (see *Figure 6*).

1. Collect the sample in a beaker or other open container.
2. Place the ampule tip well below the sample surface and break the tip off (see Step 2 below) against the beaker wall. The break must be far enough below the surface to prevent air from being drawn in as the level of the sample lowers.
3. Invert the ampule several times to dissolve the reagent. Do not place your finger over the broken end; the liquid will stay in the ampule when inverted. Wipe the ampule with a towel to remove fingerprints, etc.
4. Insert the ampule into the AccuVac Adapter in the DR/4000 and read the results directly.

Figure 6 Using AccuVac Ampuls



1.2.13 Using Reagent Powder Pillows

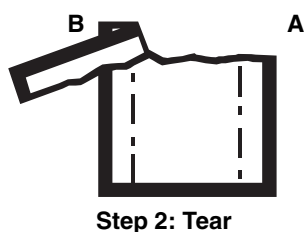
Hach uses dry powdered reagents when possible. This minimizes leakage and deterioration. Some powders are packaged in individual, pre-measured, polyethylene “powder pillows” or foil pillows called PermaChem pillows (see Section 1.2.14). Each pillow contains enough reagent for one test. Open the powder pillows with nail clippers or scissors (see *Figure 7*).

Figure 7 **Opening Powder Pillows**



1.2.14 Using PermaChem Pillows

1. Tap the pillow on a hard surface to collect the powdered reagent in the bottom.
2. Tear (or cut) across, from A to B, holding the pillow away from your face.
3. Using two hands, push both sides toward each other to form a spout.
4. Pour the pillow contents into the sample cell and continue the procedure according to the instructions.



Step 2: Tear



Step 3: Push



Step 4: Pour

1.2.15 Reagent and Standard Stability

Hach strives to make stable formulations and package them to provide maximum protection. Most chemicals and prepared reagents do not deteriorate after manufacture. However, the way they are stored and their packaging can affect how long the reagents are stable. Light, bacterial action, and absorption of moisture and gases from the atmosphere can affect shelf life. Some chemicals may react with the storage container or other chemicals.

1.2.16 Chemicals Supplied with the DR/4000

Chemicals supplied with the DR/4000 Spectrophotometer have an indefinite shelf life when stored under average room conditions, unless the package says otherwise. Product labels state any special storage conditions required. Otherwise, store reagents in a cool, dry, dark place for maximum life. It is always good practice to date chemicals when you receive them. Use older supplies first. If in doubt about the reagent shelf life, run a standard to check its effectiveness.

1.3 Interferences

Substances in the sample may interfere with a measurement. Hach describes common interferences in the test procedures. The reagent formulations eliminate many interferences. You can remove others with sample pretreatments described in the procedure.

If you get an unusual answer, a color that you don't expect, or an unusual odor or turbidity, the result may be wrong. Repeat the test on a sample diluted with deionized water (see Section 1.2.6 *Sample Dilution Techniques*). Compare the result (corrected for the dilution) with the result of the original test. If these two are not close, the original result may be wrong and you should make an additional dilution to check the second test (first dilution). Repeat this process until you get the same corrected result twice in a row.

More information about interferences and methods to overcome them is contained in Section 1.4.1 *Standard Additions* and the *General Introduction* section of APHA Standard Methods. Hach urges the analyst to obtain this book and refer to it when problems are encountered.

1.3.1 pH Interference

Many of the procedures in this manual only work within a certain pH range. Hach reagents contain buffers to adjust the pH of the typical sample to the correct pH range. However, the reagent buffer may not be strong enough for some samples. This occurs most often with highly buffered samples or samples with extreme pH.

The *Sampling Collection, Preservation and Storage* section of each procedure gives the proper pH range for the sample. Adjust the sample to the proper pH range before testing. If this information is not given, follow these steps:

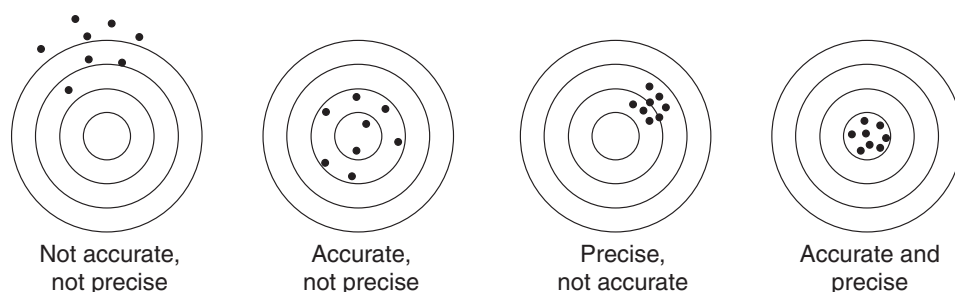
1. Measure the pH of your analyzed sample with a pH meter. For measuring K^+ or Cl^- , use pH paper.
2. Prepare a reagent blank using deionized water as the sample. Add all reagents called for in the procedure. Timer sequences, etc., may be ignored. Mix well.
3. Measure the pH of the reagent blank with a pH meter.
4. Compare the pH values of your analyzed sample with the reagent blank.

5. If there is little difference in the values of your analyzed sample and the reagent blank, then pH interference is not the problem. Follow the *Accuracy Check* given in the procedure to more clearly identify the problem.
6. If there is a large difference between the value of your analyzed sample and the reagent blank, adjust the sample pH to the value of the reagent blank. Adjust the sample pH to this same pH for all future samples before analysis. Use the appropriate acid, usually nitric acid, to lower the pH. Use the appropriate base, usually sodium hydroxide, to raise the pH. Adjust the final result for any dilution caused by adding acid or base; see Section 1.2.2 *Correcting for Volume Additions*.
7. Analyze the sample as before.
8. Some purchased standards may be very acidic and will not work directly with Hach procedures. Adjust the pH of these standards as described above. Adjust the final concentration of the standard for the dilution. The Hach standard solutions suggested in the procedures are formulated so that no pH adjustment is necessary.

1.4 Accuracy and Precision

Accuracy is the nearness of a test result to the true value. Precision is how closely repeated measurements agree with each other. Although good precision suggests good accuracy, precise results can be inaccurate. The following paragraphs describe how to improve accuracy and precision of analyses by using Standard Additions.

Figure 8 Precision and Accuracy Illustrated



One of the greatest aids is knowing what is in the sample. You don't need to know exactly what is in each sample, but be aware of substances that are likely to interfere in the analysis method you use. When using a method, it may be helpful to determine if those interferences are present.

1.4.1 Standard Additions

Standard Additions is a common technique for checking test results. Other names are “spiking” and “known additions.” The technique can test for interferences, bad reagents, faulty instruments and incorrect procedures.

Perform Standard Additions by adding a small amount of a standard solution to your sample and repeating the test. Use the same reagents, equipment, and technique. You should get about 100% recovery. If not, you have an identifiable problem.

If Standard Additions works for your test, a *Standard Additions Method* section will be in the procedure under *Accuracy Check*. Follow the detailed instructions given.

If you get about 100% recovery for each addition, everything is working properly and your results are correct.

If you don’t get about 100% recovery for each addition, a problem exists. You can tell if you have an interference. Repeat the Standard Additions using deionized water as your sample. If you get about 100% recovery for each addition, you have an interference. See Section 1.4.2 *Estimating Concentration Using Standard Additions*.

If you didn’t get good recoveries with the deionized water, use the following checklist to find the problem:

1. Check to see that you are following the procedure exactly:
 - a. Are you using the proper reagents in the proper order?
 - b. Are you waiting the necessary time for color to develop?
 - c. Are you using the correct glassware?
 - d. Is the glassware clean?
 - e. Does the test need a specific sample temperature?
 - f. Is the sample’s pH in the correct range?

Hach’s written procedure should help you to answer these questions.

2. Check the performance of your instrument. Follow the instructions in the *Service Checks* section of the *DR/4000 Instrument Manual*.
3. Check your reagents. Repeat the Standard Additions using new, fresh reagents. If your results are good, the original reagents were faulty.
4. If nothing else is wrong, the standard is almost certainly defective. Repeat the Standard Additions with a new standard.
5. If you still cannot identify the problem, you need some extra help. Please call Hach’s Technical Support Group at 800-227-4224 (U.S.A.) or 303-669-3050. A representative will be happy to help you.

1.4.2 Estimating Concentration Using Standard Additions

If you know you have an interference, you may still be able to estimate the concentration of the analyte in your sample. The following steps will help you to estimate the result:

1. Use the *Standard Additions Method* section under *Accuracy Check* in your procedure to analyze the sample.
2. When you finish, the display shows a plot of the data and a line that estimates it. It also shows a concentration in the lower left corner. It may also say “LINE?” below the plot. If it doesn’t say “LINE?” skip to Step 4.
3. “LINE?” means the data may not plot as a line or your results shows as a negative number. Check the plot on the screen carefully and answer the following questions.
 - a. Do the points seem to plot as a line? If no, you may not estimate the result. Try to use another method to analyze the sample. If yes, go to Step 3b.
 - b. If the concentration is a small negative number, there is no analyte in the sample. If the concentration is a large negative number, you will not be able to estimate a result. Try to use an alternative method to analyze your sample.

If you answered yes to 3a and you have a positive number, go to Step 4.

4. If the data plots as a line, you can estimate the result. The concentration shown in the lower left corner of the display is the estimate.

Below the plot is a number equal to r^2 . This is a measure of how well the data plot as a line. If $r^2 = 1.000$, 100% of the variation in your data is due to the standard additions you made. If $r^2 = 0.900$, 90% of the variation in your data is due to the standard additions you made. The other 10% is unexplained. “LINE?” will be displayed if r^2 is less than 0.900. In this case, the unexplained variation is large and your data may not plot as a line. Carefully checking the plot will help you decide if your data really plots as a line. If it plots as a curve, you cannot estimate a result. Remember, the smaller r^2 is, the less the chance your data plots as a line.

“LINE?” will also be displayed if the calculated concentration is less than zero. Sometimes the measurement variation will cause a small negative reading for a zero concentration. Just report zero for the concentration. A large negative number means the data doesn’t plot as a line. Use another method to analyze your sample if this occurs.

1.5 Method Performance

1.5.1 Determining the Method Detection Limit (MDL)

This method is in accordance with the USEPA definition in 40 CFR, Part 136, Appendix B in the 7-1-94 edition.

The USEPA defines the method detection limit (MDL) as the minimum concentration that can be determined with 99% confidence that the true concentration is greater than zero. Since the MDL will vary from analyst to analyst, it is important that analysts determine the MDL based on their unique operating conditions.

The procedure for determining MDL is based on replicate analyses at a concentration 1 to 5 times the estimated detection limit. The MDL value is calculated from the standard deviation of the replicate study results multiplied by the appropriate Student's *t* value for a 99% confidence interval. For this definition, the MDL does not account for variation in sample composition and can only be achieved under ideal conditions.

1. Estimate the detection limit. Use the Hach estimated detection limit (EDL) value stated in the *Method Performance* section of the analysis procedure.
2. Prepare a laboratory standard of the analyte in deionized water which is free of the analyte that is 1 to 5 times the estimated detection limit.
3. Analyze at least 7 portions of the laboratory standard and record each result.
4. Calculate the average and standard deviation (*s*) of the results.
5. Compute the MDL using the appropriate Student's *t* value (see table below) and the standard deviation value:

$$\text{MDL} = \text{Student's } t \times s$$

Number of Test Portions	Student's <i>t</i> Value
7	3.143
8	2.998
9	2.896
10	2.821

An example:

The EDL for measuring iron using the FerroZine method is 0.003 mg/L. An analyst accurately prepared 1 liter of a 0.010 mg/L (about 3x the EDL) laboratory standard by diluting a 10-mg/L iron standard in iron-free deionized water.

SECTION 1, continued

Eight portions of the standard were tested according to the FerroZine method with the following results:

Sample #	Result (mg/L)
1	0.009
2	0.010
3	0.009
4	0.010
5	0.008
6	0.011
7	0.010
9	0.009

Using a calculator program, the average concentration = 0.010 mg/L and the standard deviation (symbolized as “(s)”) = 0.0009 mg/L.

Based on the USEPA’s definition, calculate the MDL as follows:

MDL for FerroZine method = $2.998(\text{Student's } t) \times 0.0009 \text{ (s)}$

MDL = 0.003 mg/L (agrees with initial estimates)

Note: Occasionally, the calculated MDL may be very different than Hach’s estimate of the detection limit. To test how reasonable the calculated MDL is, repeat the procedure using a standard near the calculated MDL. The average result calculated for the second MDL derivation should agree with the initial calculated MDL. Refer to 40 CFR, Part 136, Appendix B (7-1-94), pages 635-637 for detailed procedures to verify the MDL determination.

Note: Run a laboratory blank containing deionized water without analyte through the test procedure to confirm that the blank measurement is less than the calculated MDL. If the blank measurement is near the calculated MDL, repeat the MDL procedure using a separate blank for analysis for each standard solution portion analyzed. Subtract the average blank measurement from each standard and use the corrected standard values to calculate the average and standard deviation used in the MDL.

1.5.2 Estimated Detection Limit

Ranges for chemical measurements have limits. The lower limit is important because it determines whether a measurement is different from zero. Many experts disagree about the definition of this detection limit, and determining it can be difficult. The Code of Federal Regulations (40 CFR, Part 136, Appendix B) provides a procedure to determine the “Method Detection Limit” or MDL. The MDL is the lowest concentration that is different from zero with a 99% level of confidence. A measurement below this MDL may be useful, but there is a greater chance that it is actually zero.

The MDL is not fixed; it varies for each reagent lot, instrument, analyst, sample type, etc. Therefore, a published MDL may be a useful guide, but is only accurate for a specific set of circumstances. Each analyst should determine a more accurate MDL for each specific sample matrix using the same equipment, reagents and standards that will routinely be used for measurements. See the section above for determining an MDL.

Hach provides a value called the Estimated Detection Limit (EDL). It is the calculated lowest average concentration in a deionized water matrix that is different from zero with a 99% level of confidence. Specifically, it is the upper

99% confidence limit for zero concentration based on the calibration data used to prepare the pre-programmed calibration curve. **Do not use the EDL as an MDL.** The conditions for MDL determination must be exactly the same as the conditions used for analysis. The EDL may be useful to the analyst as a starting point in determining an MDL or as a way to compare methods. Measurements below the EDL may also be valuable because they can show a trend, indicate the presence of analyte and/or provide statistical data. However, these values have a large uncertainty.

1.5.3 Sensitivity Explained

Hach's definition of sensitivity for the DR/4000 is the change in concentration (Δ Concentration) for a 0.010 change in absorbance (Δ Abs).

Use sensitivity when comparing different methods. For example, Hach has three DR/4000 methods for determining iron:

Iron Analysis Method	Portion of Curve	Δ Abs	Δ Concentration
FerroVer	Entire range	0.010	0.0209 mg/L
FerroZine	Entire range	0.010	0.0086 mg/L
TPTZ	0.010 Abs	0.010	0.0107 mg/L
	0.900 mg/L	0.010	0.0112 mg/L
	1.620 mg/L	0.010	0.0116 mg/L

Notice that the FerroZine method has the greatest sensitivity of the three methods because it will measure the smallest change in concentration. Also notice the three sensitivities shown for the TPTZ method. If the sensitivity changes more than 5% over the range of the test, Hach reports the sensitivity at three points: 0.010 Abs above the reagent blank, at 50% of the range maximum, and at 90% of the range maximum.

The technical definition of sensitivity comes from a calibration curve with Abs on the x-axis and concentration on the y-axis as follows:

1. If the calibration is a line, the sensitivity is the slope of the line multiplied by 0.010.
2. If the calibration is a curve, the sensitivity is the slope of the tangent line to the curve at the concentration of interest multiplied by 0.010.

1.5.4 Precision

Every measurement has some degree of uncertainty. Just as a ruler with markings of 0.1 mm leaves some doubt as to the exact length of a measurement, chemical measurements also have some degree of uncertainty. The quality of the entire calibration curve determines the precision.

Uncertainty in chemical measurements may be due to systematic errors and/or random errors. A systematic error is a mistake that is always the same for every measurement made. For example, a blank can add to each measurement for a specific compound, giving consistently high results (a positive bias). Random errors are different for every test and add either positive or negative bias. Random errors may be caused by variation in analytical technique and cause response variation. Hach chemists work hard to eliminate systematic errors in Hach procedures using Hach reagents, but response variation occurs in all chemical measurements.

Hach's precision data are an estimate of the response variation in a method. Specifically, the precision is the 95% confidence interval for the stated concentration. The precision range is an estimate of the *average* response variation and is based on multiple reagent lots and instruments used in the calibration. Therefore, it will not exactly predict the true precision range for each reagent lot, but does provide a useful estimate. Any single reading may fall outside the range, but the average of several readings should fall within the range 95 times out of 100. These values are good only for a deionized water matrix; the range can change depending on the nature of a sample matrix.

The precision table in each procedure is an easy way to check individual analytical technique to ensure high quality measurement. Test a standard of the concentration indicated in the precision statement 3 to 5 times and calculate the average measurement. If this average falls within the stated range, this indicates good analytical technique and the precision obtained is consistent with the data used to generate the preprogrammed calibration curve. If it does not fall within the range, carefully re-read the procedure, correct any possible technique errors, and repeat this check.

High-quality measurements are possible with attention to detail and technique. The precision statement provides the information required to assure high-quality measurements with a minimum of standard measurement checks.

1.5.5 Adjusting the Standard Curve

The DR/4000 has over 100 Hach programs permanently installed in memory. A program usually includes a pre-programmed calibration curve. Each curve is the result of an extensive calibration performed under ideal conditions and is normally adequate for most testing. Deviations from the curve can occur from using compromised testing reagents, defective sample cells, incorrect test procedure, incorrect technique, or other correctable causes. Interfering substances or other causes may be beyond the analyst's control.

In some situations, using the pre-programmed curve may not be convenient:

- a. Running tests where frequent calibration curve checks are required.
- b. Testing samples which give a consistent test interference.

Consider the following before adjusting the calibration curve:

1. Will future test results be improved by adjusting the curve?
2. Are interfering substances consistent in all the samples that you will test?

Any estimated detection limit, sensitivity, precision, and test range information provided with the procedure may not apply to an adjusted curve calibration.

You can adjust many of the calibration curves by following the steps found in the test procedure. Generally, you add test reagents to a blank and standard solution. Working carefully is important. After the adjustment, it is wise to run standard solutions of several concentrations to make sure the adjusted curve is satisfactory. Performing standard additions on typical samples may also help determine if the adjusted curve is acceptable.

Think of adjusting a measurement as a two-step process. First, the instrument measures the sample using the pre-programmed calibration. Second, it multiplies this measurement by an adjustment factor. The factor is the same for all concentrations. The DR/4000 will remember the factor indefinitely and will display the standard adjustment icon when it is used. You can return to the pre-programmed curve any time by selecting the Hach Program from the main menu.

1.1 Digestion

Several procedures require sample digestion. Digestion uses chemicals and heat to break down a substance into components that can be analyzed. This section has three different digestion procedures.

The Hach Digesdahl system is a process that yields a digest suitable for the determination of metals, total phosphorus and total kjeldahl nitrogen (TKN). It is rapid, convenient and the method of choice.

For USEPA reporting purposes, USEPA-approved digestions are required. USEPA presents two digestions (mild and vigorous) for metals analysis. These are much more inconvenient and time consuming compared to the Hach Digesdahl system. Other tedious digestion procedures are required for mercury, arsenic, phosphorus and TKN.

1.1.1 USEPA Mild Digestion with Hot Plate for Metals Analysis Only

1. Acidify the entire sample at the time of collection with concentrated nitric acid by adding 5 mL of acid per liter (or quart) of sample.
2. Transfer 100 mL of well-mixed sample to a beaker or flask. Add 5 mL distilled 1:1 hydrochloric acid (HCl).
3. Heat using a steam bath or hot plate until the volume has been reduced to 15-20 mL. Make certain the sample does not boil.
4. After this treatment, the sample may be filtered to remove any insoluble material.
5. Adjust the digested sample to pH 4 by drop-wise addition of 5.0 N Sodium Hydroxide Standard Solution. Mix thoroughly and check the pH after each addition.
6. Quantitatively transfer the sample with deionized water to a 100-mL volumetric flask and dilute to volume with deionized water. Continue with the procedure. This mild digestion may not suffice for all sample types. A reagent blank also should be carried through the digestion and measurement procedures.

1.1.2 USEPA Vigorous Digestion with Hot Plate for Metals Analysis Only

A vigorous digestion can be followed to ensure all organo-metallic bonds are broken.

1. Acidify the entire sample with redistilled 1:1 Nitric Acid Solution to a pH of less than two. Do not filter the sample before digestion.
2. Transfer an appropriate sample volume (see *Table 1*) into a beaker and add 3 mL of concentrated redistilled nitric acid.
3. Place the beaker on a hot plate and evaporate to near dryness, making certain the sample does not boil.
4. Cool the beaker and add another 3 mL of the concentrated redistilled nitric acid.

5. Cover the beaker with a watch glass and return it to the hot plate. Increase the temperature of the hot plate so that a gentle reflux occurs. Add additional acid, if necessary, until the digestion is complete (generally indicated when the digestate is light in color or does not change color in appearance with continued refluxing).
6. Again, evaporate to dryness (do not bake) and cool the beaker. If any residue or precipitate results from the evaporation, add redistilled 1:1 hydrochloric acid (5 mL per 100 mL of final volume). See *Table 1*.
7. Warm the beaker. Add 5 mL of 5.0 N sodium hydroxide and quantitatively transfer the sample with deionized water to a volumetric flask. See *Table 1* for the suggested final volume.
8. Adjust the sample to pH 4 by drop-wise addition of 5.0 N Sodium Hydroxide Standard Solution; mix thoroughly and check the pH after each addition. Dilute to volume with deionized water. Multiply the result by the correction factor in *Table 1*. A reagent blank also should be carried through the digestion and measurement procedures.

Table 1 Vigorous Digestion

Expected Metal Concentration	Suggested Sample Vol. for Digestion	Suggested Volume of 1:1 HCl	Suggested Final Volume After Digestion	Correction Factor
1 mg/L	50 mL	10 mL	200 mL	4
10 mg/L	5 mL	10 mL	200 mL	40
100 mg/L	1 mL	25 mL	500 mL	500

1.1.3 General Digesdahl Digestion (Not USEPA accepted)

In this procedure the sample is oxidized by a mixture of sulfuric acid and hydrogen peroxide. Digestion of a dry sample requires less than ten minutes, while aqueous samples require about 1 minute/mL. The digestion is done in a special flat-bottomed 100-mL volumetric flask. Aliquots (sample portions) are taken for analyses using colorimetric methods.

Sample and Analysis Volumes

(For liquid samples only)

Note: For the digestion of oils and solids see the table *Digestion Guidelines For Specific Sample Types* on page 59. For more information, obtain a copy of the *Digesdahl Digestion Apparatus Manual*, Cat. No. 23130-18.

1. Select the **sample amount** from the appropriate table below and digest this amount using the Digesdahl procedure.
2. For testing, use the **analysis volume** in the table that corresponds to the sample amount.
3. Pipet the **analysis volume** in the table into the specified graduated mixing cylinder. If this amount is more than 0.5 mL, adjust the pH according to pH Adjustment following the digestion procedure. Dilute to volume with deionized water. Use the diluted digest for analysis in the specific procedure.
4. Calculate the concentration of the analyte using the formula in the appropriate table. These apply to liquid samples only.

Aluminum, Aluminon (Method 8012)

Expected Al conc. (mg/L)	Sample amount (mL)	Analysis volume (mL)	Dilute to
0.1–5	40.0	20.0	50 mL
0.5–20	20.0	10.0	50 mL
2.0–80	10.0	5.00	50 mL
20–800	5.00	1.00	50 mL
200–8000	1.00	0.50	50 mL

$$\frac{A \times 5000}{B \times C} = \text{Total mg/L Al}$$

A = mg/L reading from instrument

B = mL sample amount from table

C = mL analysis volume from table

Aluminum, ECR (Method 8326)

Expected A conc. (mg/L) Al	Sample amount (mL)	Analysis volume (mL)	Dilute to
0.05–1.3	40.0	20.0	50 mL
0.2–5.5	20.0	10.0	50 mL
0.8–22	10.0	5.00	50 mL
8.0–220	5.00	1.00	50 mL
80–2200	1.00	0.50	50 mL

$$\frac{A \times 5000}{B \times C} = \text{Total mg/L Al}$$

A = mg/L reading from instrument

B = mL sample amount from table

C = mL analysis volume from table

Cadmium, Dithizone (Method 8017)

Expected Cd conc. (mg/L)	Sample amount (mL)	Analysis volume (mL)	Dilute to
0.05–2.5	40.0	20.0	250.0 mL
0.2–10	20.0	10.0	250.0 mL
1.0–40	10.0	5.00	250.0 mL
10–400	5.00	1.00	250.0 mL
100–4000	1.00	0.50	250.0 mL

$$\frac{A \times 25}{B \times C} = \text{Total mg/L Cd}$$

A = µg/L reading from instrument

B = mL sample amount from table

C = mL analysis volume from table

SECTION 1, continued

Chromium, Total (Method 8024)

Expected Cr conc. (mg/L)	Sample amount (mL)	Analysis volume (mL)	Dilute to
0.05–1.8	40.0	20.0	25.0 mL
0.20–7.5	20.0	10.0	25.0 mL
0.75–30	10.0	5.00	25.0 mL
7.5–300	5.00	1.00	25.0 mL
75–3000	1.00	0.50	25.0 mL

$$\frac{A \times 2500}{B \times C} = \text{Total mg/L Cr}$$

A = mg/L reading from instrument

B = mL sample amount from table

C = mL analysis volume from table

Cobalt (Method 8078)

Expected Co conc. (mg/L)	Sample amount (mL)	Analysis volume (mL)	Dilute to
0.1–6.0	40.0	20.0	25.0 mL
0.5–25	20.0	10.0	25.0 mL
2.0–100	10.0	5.00	25.0 mL
20–1000	5.00	1.00	25.0 mL
200–10000	1.00	0.50	25.0 mL

$$\frac{A \times 2500}{B \times C} = \text{Total mg/L Co}$$

A = mg/L reading from instrument

B = mL sample amount from table

C = mL analysis volume from table

Copper, Bicinchoninate (Methods 8026 and 8506)

Expected Cu conc. (mg/L)	Sample amount (mL)	Analysis volume (mL)	Dilute to
0.25–15	40.0	20.0	25.0 mL
1.0–60	20.0	10.0	25.0 mL
4.0–240	10.0	5.00	25.0 mL
40–2400	5.00	1.00	25.0 mL
400–24000	1.00	0.50	25.0 mL

$$\frac{A \times 2500}{B \times C} = \text{Total mg/L Cu}$$

A = mg/L reading from instrument

B = mL sample amount from table

C = mL analysis volume from table

Iron, 1,10 Phenanthroline (Method 8008)

Expected Fe conc. (mg/L)	Sample amount (mL)	Analysis volume (mL)	Dilute to
0.15–8.0	40.0	20.0	25.0 mL
0.6–35	20.0	10.0	25.0 mL
2.5–125	10.0	5.00	25.0 mL
25–1250	5.00	1.00	25.0 mL
250–12500	1.00	0.50	25.0 mL

$$\frac{A \times 2500}{B \times C} = \text{Total mg/L Fe}$$

A = mg/L reading from instrument

B = mL sample amount from table

C = mL analysis volume from table

Iron, FerroZine (Method 8147)

Expected Fe conc. (mg/L)	Sample amount (mL)	Analysis volume (mL)	Dilute to
0.07–4	40.0	20.0	25.0 mL
0.3–15	20.0	10.0	25.0 mL
1.1–65	10.0	5.00	25.0 mL
11–650	5.00	1.00	25.0 mL
110–6500	1.00	0.50	25.0 mL

$$\frac{A \times 2500}{B \times C} = \text{Total mg/L Fe}$$

A = mg/L reading from instrument

B = mL sample amount from table

C = mL analysis volume from table

Lead, Dithizone (Method 8033)

Expected Pb conc. (mg/L)	Sample amount (mL)	Analysis volume (mL)	Dilute to
0.1–5.0	40.0	20.0	250.0 mL
0.4–20	20.0	10.0	250.0 mL
1.5–80	10.0	5.00	250.0 mL
15–800	5.00	1.00	250.0 mL
150–8000	1.00	0.500	250.0 mL

$$\frac{A \times 25}{B \times C} = \text{Total mg/L Pb}$$

A = µg/L reading from instrument

B = mL sample amount from table

C = mL analysis volume from table

SECTION 1, continued

Manganese, PAN (Method 8149)

Expected Mn conc. (mg/L)	Sample amount (mL)	Analysis volume (mL)	Dilute to
0.05–2.1	40.0	20.0	25.0 mL
0.2–8.7	20.0	10.0	25.0 mL
0.8–35	10.0	5.00	25.0 mL
8.0–350	5.00	1.00	25.0 mL
80–3500	1.00	0.50	25.0 mL

$$\frac{A \times 2500}{B \times C} = \text{Total mg/L Mn}$$

A = mg/L reading from instrument

B = mL sample amount from table

C = mL analysis volume from table

Nickel, PAN (Method 8150)

Expected Ni conc. (mg/L)	Sample amount (mL)	Analysis volume (mL)	Dilute to
0.05–3	40.0	20.0	25.0 mL
0.2–12	20.0	10.0	25.0 mL
0.8–47	10.0	5.00	25.0 mL
8.0–470	5.00	1.00	25.0 mL
80–4700	1.00	0.50	25.0 mL

$$\frac{A \times 2500}{B \times C} = \text{Total mg/L Ni}$$

A = mg/L reading from instrument

B = mL sample amount from table

C = mL analysis volume from table

Nitrogen, TKN (Method 8075)

Expected TKN conc. (mg/L)	Sample amount (mL)	Analysis volume (mL)	Dilute to
0.5–28	40.0	10.0*	25.0 mL
2.0–112	20.0	5.00*	25.0 mL
11–560	10.0	2.00*	25.0 mL
45–2250	5.00	1.00*	25.0 mL
425–22500	1.00	0.50*	25.0 mL

* These are guidelines only. See the spectrophotometer procedure manual.

$$\frac{A \times 75}{B \times C} = \text{mg/L TKN}$$

A = mg/L reading from instrument

B = mL sample amount from table

C = mL analysis volume from table

Phosphorus, Ascorbic Acid (Method 8048)

Expected PO ₄ conc. (mg/L)	Sample amount (mL)	Analysis volume (mL)	Dilute to
0.12–6.0	40.0	20.0	25.0 mL
0.5–23	20.0	10.0	25.0 mL
2.0–90	10.0	5.00	25.0 mL
20–900	5.00	1.00	25.0 mL
200–9000	1.00	0.50	25.0 mL

$$\frac{A \times 2500}{B \times C} = \text{Total mg/L PO}_4$$

A = mg/L reading from instrument

B = mL sample amount from table

C = mL analysis volume from table

Potassium (Method 8049)

Expected K conc. (mg/L)	Sample amount (mL)	Analysis volume (mL)	Dilute to
4–20	40.0	20.0	25.0 mL
15–80	20.0	10.0	25.0 mL
60–300	10.0	5.00	25.0 mL
200–1000	5.0	2.0	25.0 mL
600–3000	5.00	1.00	25.0 mL
2000–10000	3.00	0.50	25.0 mL
6000–30000	1.00	0.50	25.0 mL

$$\frac{A \times 2500}{B \times C} = \text{Total mg/L K}$$

A = mg/L reading from instrument

B = mL sample amount from table

C = mL analysis volume from table

Silver (Method 8120)

Expected Ag conc. (mg/L)	Sample amount (mL)	Analysis volume (mL)	Dilute to
0.08–3.7	40.0	20.0	50.0 mL
0.3–15	20.0	10.0	50.0 mL
1.0–60	10.0	5.00	50.0 mL
12–600	5.00	1.00	50.0 mL
120–6000	1.00	0.50	50.0 mL

$$\frac{A \times 5000}{B \times C} = \text{Total mg/L Ag}$$

A = mg/L reading from instrument

B = mL sample amount from table

C = mL analysis volume from table

Zinc (Method 8009)

Expected Zn conc. (mg/L)	Sample amount (mL)	Analysis volume (mL)	Dilute to
0.2–12.5	40.0	20.0	50.0 mL
0.8–50	20.0	10.0	50.0 mL
3.0–200	10.0	5.00	50.0 mL
30–2000	5.00	1.00	50.0 mL
300–20000	1.00	0.500	50.0 mL

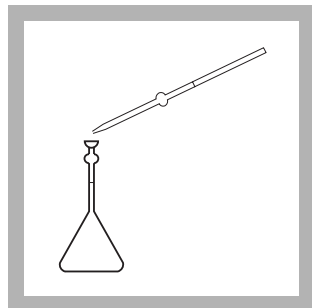
$$\frac{A \times 5000}{B \times C} = \text{Total mg/L Zn}$$

A = mg/L reading from instrument

B = mL sample amount from table

C = mL analysis volume from table

1.2 General Digesdahl Digestion



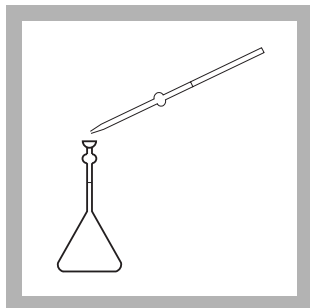
1. Transfer a pre-weighed or a pre-measured amount of sample into a 100-mL volumetric flask; see *Sample and Analysis Volumes* on page 46. The transferred amount should not contain more than 0.5 g of solids or organic liquids. The maximum volume for water samples is 40 mL. In samples with more than 1% solids present, use the formula below.

$$\frac{\text{WATER}}{\text{SAMPLE}} = \frac{40}{\text{VOLUME} \times \% \text{ solids}} \quad (\text{mL})$$

Note: If solids are 10% of total volume of sample, the maximum volume of liquid sample would be 5 mL.

Note: Several 50-mL sample aliquots of the sample may be digested in succession to concentrate a sample.

Note: If liquid is too viscous to measure, pre-weigh the sample into the digestion flask.

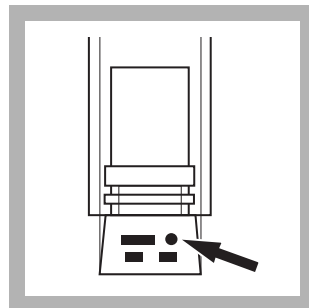


2. Add concentrated sulfuric acid according to the *Digestion Guidelines For Specific Sample Types* on page 59 to the volumetric flask and two or more silicon carbide (carborundum) boiling chips for liquid samples.

Note: Boiling chips can be pretreated by soaking in 1:1 nitric acid and rinsing thoroughly with deionized water. Treatment may be particularly important in low level work. Silicon carbide boiling chips are recommended.

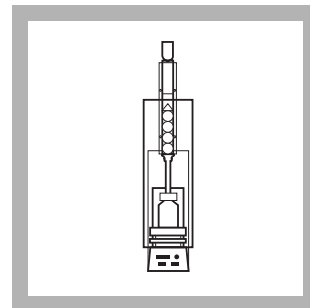
Note: Use only Hach digestion flasks. Volumetric flasks with concave bottom should not be used.

Note: Specific method manuals for a variety of sample types are available, free of charge from Hach Company. See Section 1.5 on page 60 for a complete listing.



3. Turn on the water to the aspirator. Make sure there is suction to the fractionating column. Turn the temperature dial to a heat setting of 440 °C (825 °F).

Note: Wait for the proper temperature to be reached before sample is placed on the heater.



4. Place the flask weight followed by the fractionating column with funnel on the flask. Place the flask on the heater. Heat until the boiling point of sulfuric acid is reached (refluxing sulfuric acid will be visible).

Note: White acid vapors usually will be present but their presence alone does not indicate that the boiling point of sulfuric acid has been reached.

Note: Liquid samples require total evaporation of water before vapors are visible.

Note: If sample starts to foam up into the neck of the flask, lower temperature to 335 °C (600 °F). Continue heating at lower temperature until all water is evaporated off. Then return to original digestion temperature.

Note: If foaming or bumping is not stopped by lowering temperature or volume, then liquid samples that will not clog the capillary funnel may be added to the flask via the capillary funnel, 10 mL at a time. Decrease amount added if foaming still persists.

Warning

A safety shield placed between the operator and the Digesdahl is required. Safety glasses are mandatory.

Caution

Experimentation with the Digesdahl Apparatus is not recommended. See the Safety Section in the Digesdahl Digestion Apparatus Manual.

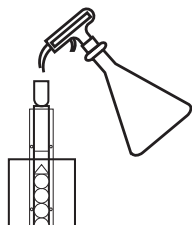
3 – 5 minutes



5. Heat 3–5 minutes. Do not boil sample to dryness.

Note: Discard sample if it evaporates to dryness and use larger amount of concentrated sulfuric acid for digestion in Step 2.

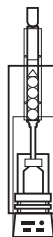
Note: Some organic samples may need more than five minutes for complex digestion. See the table Digestion Guidelines For Specific Sample Types on page 59.



6. Be sure you have added the correct amount of sulfuric acid. Add 10 mL of 50% hydrogen peroxide to the charred sample via the funnel on the fractionating head.

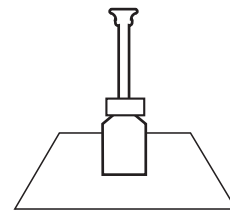
Note: If the digest does not turn colorless, add 5 mL increments of peroxide until the digest becomes clear.

Note: Visually confirm the presence of sulfuric acid in the flask before adding hydrogen peroxide.



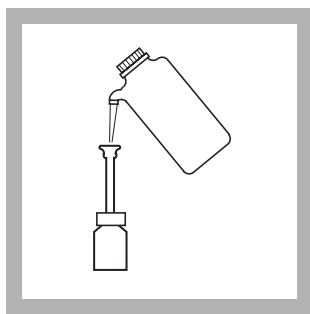
7. Boil off excess hydrogen peroxide by heating for one more minute after addition of hydrogen peroxide is complete. Do not heat to dryness.

Note: If the sample goes to dryness, turn off the Digesdahl and cool completely. Add water to flask before handling. Repeat digestion from the beginning.



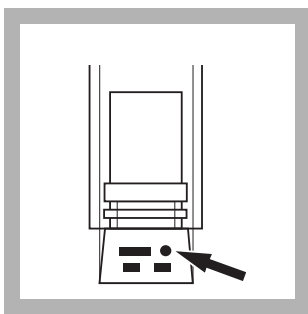
8. Take the flask off the heater and allow the flask contents to cool. Remove the fractionating column from the digestion flask.

Note: Use finger cots to remove the digestion flask. Place it on a cooling pad for at least one minute. Then remove the column.

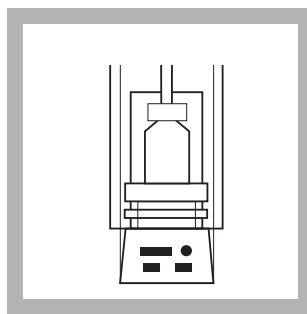


9. Dilute the digest to approximately 70 mL with deionized water. If analyzing for aluminum, nickel or iron, continue to Step 10. If not analyzing for aluminum, iron or nickel, dilute to the mark with deionized water. Skip steps 10–11 and go to Step 12.

Note: Add the deionized water slowly at first. If necessary, cool the flask for handling.

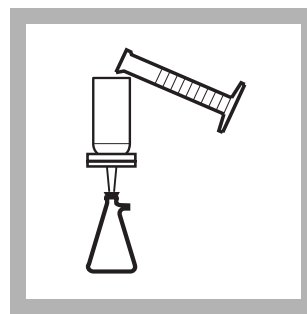


10. Turn the temperature dial to a heat setting of 204 °C (400 °F). Add 150 mL of water to a 400-mL beaker. Place the beaker on the heater.



11. Place the flask in the beaker and boil for 15 minutes. Cool to room temperature and dilute to the mark with deionized water. Invert several times to mix.

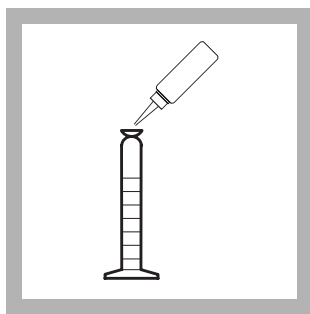
Note: When using the Digesdahl system without temperature control dials, reset to a lower setting that will cause the water to boil.



12. If the sample is turbid, filter or wait until the turbidity settles and the upper part of the sample is clear.

Filter as follows:

- a.** Place a filter paper into the filter holder with the wrinkled surface upward.
- b.** Place the filter holder assembly in the filtering flask and wet the filter with deionized water to make sure it stays on the holder.
- c.** While applying a vacuum to the filtering flask, pour the sample into the filter assembly.
- d.** After filtering, slowly release the vacuum from the filtering flask and transfer the sample to another container.



13. Adjust the pH of the aliquots taken for analysis using procedure under *pH Adjustment for Digested Samples* on page 56 following these steps. If using less than a 0.5 mL aliquot, pH adjustment is not necessary.

1.3 pH Adjustment for Digested Samples

1. Pipet the appropriate analysis volume into the appropriate graduated cylinder (see *Sample and Analysis Volumes* on page 46 before the digestion procedure).

Note: When analyzing small aliquots (< 0.5 mL), pH adjustment is not needed.

2. Dilute to about 20 mL with deionized water. No dilution is necessary for a 20-mL analysis volume.

All methods except TKN:

- a. Add one drop of 2,4-Dinitrophenol Indicator Solution.
- b. Add, dropwise, 8 N Potassium Hydroxide (KOH) Standard Solution, swirling between each addition, until the first flash of yellow appears (pH 3).

Note: Check for proper pH adjustment (pH 3) using pH paper. If pH is higher than 4, do not readjust with acid. Start over at Step 12 or 13 in the digestion procedure with a fresh aliquot.

Note: View the cylinder from the top against a white background. Compare the cylinder against a second cylinder filled to the same volume with deionized water.

Note: When analyzing for potassium, use 5 N sodium hydroxide to adjust the pH. When analyzing for potassium or silver, do not use a pH meter for pH adjustment because of potassium and silver contamination from the electrode.

- c. Add one drop of 1 N KOH, stopper the cylinder and invert several time to mix.
- d. Continue adding 1 N KOH in this manner until the first permanent yellow color appears (pH 3.5–4.0). Then go to Step e.

Note: Check for proper pH adjustment (pH 3) using pH paper. If pH is higher than 4, do not readjust with acid. Start over at Step 12 or 13 with a fresh aliquot.

SECTION 1, continued

TKN Method:

- a. Add one drop of TKN Indicator Solution.
- b. Add, dropwise, 8 N Potassium Hydroxide (KOH) Standard Solution dropwise, swirling between each addition, until the first flash of blue appears (pH 3).

Note: View the cylinder from the top against a white background. Compare the cylinder against a second cylinder filled to the same volume with distilled water.

- c. Add one drop of 1 N KOH, stopper the cylinder and invert several time to mix.
- d. Continue adding 1 N KOH in this manner until the first permanent blue color appears (pH 3.5–4.0). Then go to Step e.
- e. Add deionized water to the sample volume indicated in the Sample and Analysis Volumes table under “Dilute to”. Stopper and invert several times to mix.
- f. Using this solution as the sample, continue with the colorimetric method. Use the formula in the parameter table in Sample and Analysis Volumes to calculate the final concentration of analyte in the sample.

REQUIRED REAGENTS

Description	Quantity Required		Unit	Cat. No.
	Per Digestion			
Hydrogen Peroxide, 50%	10 mL	490 mL		21196-49
Potassium Hydroxide Standard Solution, 1.0 N	varies ...	50 mL* SCDB		23144-26
Potassium Hydroxide Standard Solution, 8 N	varies	500 mL		282-49
Sulfuric Acid, ACS (concentrated, specific gravity 1.84)	>3 mL	2.5 liters		979-09
Water, deionized	varies	4 liters		272-56

REQUIRED APPARATUS

Boiling Chips, silicon carbide	varies	500 g		20557-34
Dispenser with flask, pour-out, 10-mL	1	each		22200-38
Pipet, serological, 10-mL	1	each		532-38
Pipet Filler, safety bulb	1	each		14651-00
Safety Glasses	1	each		18421-00
Safety Shield, for Digesdahl	1	each		20974-00

Select one based on available voltage:

Digesdahl Apparatus, 115 VAC, 50/60 Hz	1	each		23130-20
Digesdahl Apparatus, 230 VAC, 50/60 Hz	1	each		23130-21

SECTION 1, continued

OPTIONAL REAGENTS

Description	Unit	Cat. No.
Hydrogen Peroxide, 30%, ACS.....	473 mL.....	144-11
Kjeldahl Reduction Reagent (for fluid fertilizers)	40 g.....	23653-04
2,4-Dinitrophenol Indicator Solution, pH 2.2–3.8	100 mL MDB.....	1348-32
Nitric Acid Solution, 1:1	500 mL.....	2540-49
Sodium Hydroxide, 5.0 N.....	59 mL* DB.....	2450-26
Sodium Hydroxide, 1.00 N	900 mL.....	1045-53
Total Kjeldahl Nitrogen Indicator Solution.....	50 mL SCDB.....	22519-26

OPTIONAL APPARATUS

Balance, Sartorius B310S, 110 VAC	each.....	24030-00
Balance, Sartorius B310S, 230 VAC	each.....	24030-02
Beaker, 400-mL	each.....	500-48
Beaker, Berzelius, 200-mL.....	12/pkg.....	22761-75
Bottle, Wash, 1-liter	each.....	620-16
Bulb, dropper, 2-mL.....	12/pkg.....	21189-00
Cylinder, graduated, 50-mL	each.....	508-41
Dispenser, 1–5 mL (for sulfuric acid, meat).....	each.....	23121-37
Dispenser, 10–50 mL (for sulfuric acid, meat)	each.....	23121-41
Filter Discs, glass, 47-mm.....	100/pkg.....	2530-00
Filter Holder, glass	each.....	2340-00
Flask, filtering, 500-mL	each.....	546-49
Flask, Digesdahl, flat-bottom, 100-mL, with stopper	each.....	23125-42
Fume Scrubber Apparatus, 115 VAC, 60 Hz	each.....	23266-00
Fume Scrubber Apparatus, 230 VAC, 50/60 Hz	each.....	23266-02
Oven, laboratory, 120 VAC, 50/60 Hz.....	each.....	14289-00
Paper, weighing, 76 x 76 mm	500/pkg.....	14738-00
pH Paper, pH 1.0–11.0	5 rolls/pkg.....	391-33
pH Meter, <i>sension</i> TM 1, portable	each.....	51700-00
Pipet, Pasteur, disposable, 229-mm.....	200/pkg.....	21234-01
Spatula, stainless, 10-cm	each.....	561-64
Spoon, measuring, 0.05-g.....	each.....	492-00
Stir Bar, Teflon, 1-inch.....	each.....	20953-51
Stir Plate, magnetic, Thermolyne, 120VAC, 50/60 Hz	each.....	23444-00
Stir Plate, magnetic, Thermolyne, 240VAC, 50/60 Hz	each.....	23444-02
Stopper, hollow, No. 5	6/pkg.....	14480-05
Syringe, 5cc, plastic	100/pkg.....	23433-33
Watch Glass, 65-mm	12/pkg.....	578-97

* Contact Hach for larger sizes.

1.4 Digestion Guidelines for Specific Sample Types

Digestion Guidelines For Specific Sample Types

Sample Type	Sample Weight	Amount of Acid	Preheat Time	Amount of Peroxide	Special Instructions
Plant Tissue	0.25 to 5 g	4 mL	4 minutes	10 mL	Use N-free paper to weigh sample
Meat & Poultry	0.5 g	4 mL	4 minutes	10 mL	None
Fluid Fertilizers	0.1 to 0.25 g	4 mL	4 minutes	10 mL	Add 0.4 g Kjeldahl Reduction Powder to flask before adding sulfuric acid. Place the flask in an 80 °C oven 15 minutes before digestion. Use N-free paper to weigh sample.
Feed & Forage	0.25 g	4 mL	4 minutes	10 mL	Use N-free paper to weigh sample.
Dairy	0.25 to 2.0 g	4 mL	4 minutes	10 mL	Use N-free paper to weigh dry samples (cheese).
Cereal	0.25 to 0.5 g	4 mL	4 minutes	10 mL	Use N-free paper to weigh sample.
Beverage	approx. 5 g (pipet into funnel)	4 mL	1 minute	10 mL	Preheat acid for 1 minute then add sample though funnel. Heat flask 30 seconds after sample is in flask.
Sludge	<2.5 g wet sludge <0.5 g dried sludge	4 mL	3 to 5 minutes	10 mL or increase in 5 mL increments	Heat the diluted digest for 15 minutes and filter
Water & Wastewater	not more than 0.5 g solids (mL = 50/C; C = % solids)	3 mL	until acid is refluxing	10 mL or increase in 5 mL increments	Water must evaporate before acid will reflux. Boiling chips required!
Bath Solutions	0.3 to 10 mL	4 mL	4 minutes	10 mL	Water must evaporate before acid will reflux. Boiling chips required!
Edible Oils	0.25 to 0.5 g	4 mL	4 minutes	5 mL immediately and 5 mL later	Weigh samples into flask and record exact weight.
Ion Exchange Resin	equivalent of 0.25 g dry resin	10–15 mL	12 minutes	20 mL	Digest will be clear with particles on bottom if metal oxides are not soluble in H ₂ SO ₄ . Add aqua regia or suitable solvent to dissolve particles. If particles are floating, start again using 15 mL H ₂ SO ₄ and longer char time.
Soil	0.25 to 1.0 g	6 mL	4 minutes	10 to 20 mL	None
Fuels & Lubricants	0.25 to 0.5 g	6 mL	4 minutes	20 mL	Heat the diluted digest for 15 minutes and filter. Temperature of heater may need to be lowered slightly if foaming or burning occur.

1.5 Application Specific Manuals

Procedures for the Digesdahl Digestion Apparatus are based on the type and form of the sample and are published in a series of procedure manuals dedicated to specific applications. They include step-by-step instructions for sample digestion and analysis of specific parameters. Specific setup and operating information is given in the *Digesdahl Digestion Apparatus Instruction Manual* included with each Digesdahl:

Literature Code	Title
8376	<i>Water Analysis Handbook</i>

To receive a free copy of these manuals, contact Customer Services (800-227-4224) and request by literature code number.

1.6 Distillation

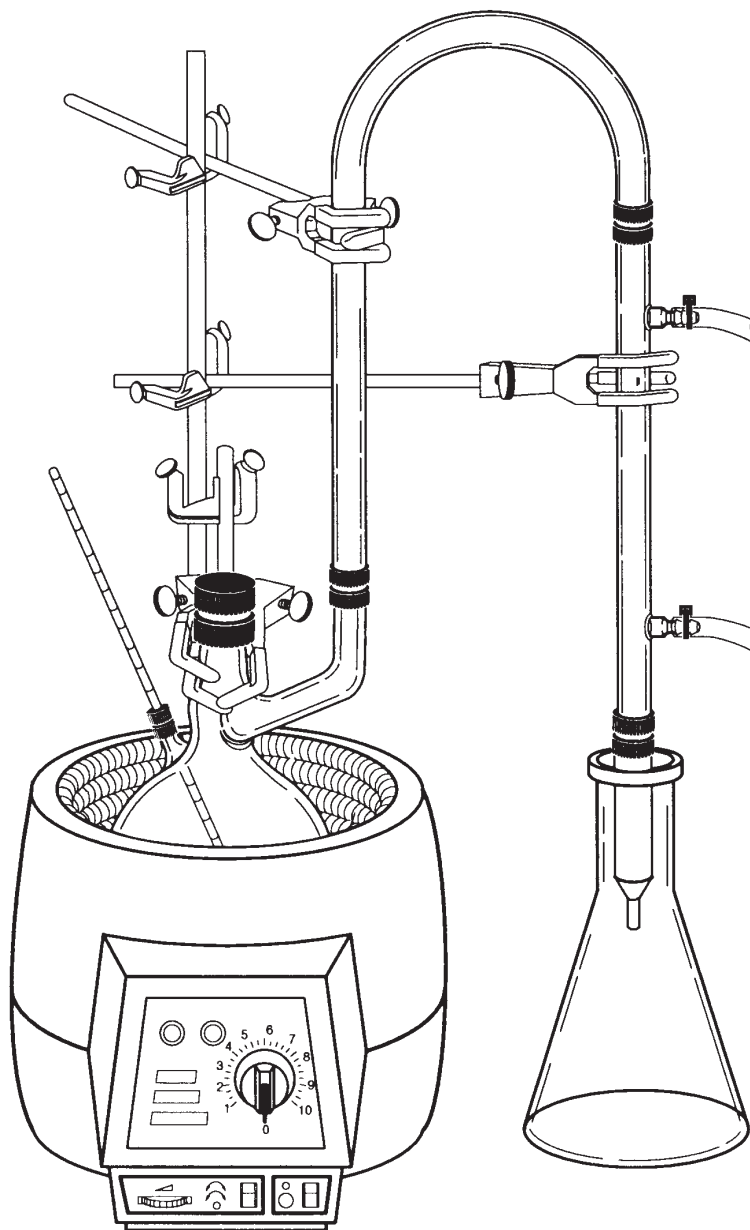
Distillation is an effective way of separating chemical components for analysis. The Hach Distillation Apparatus (see *Figure 1*) is adapted easily for many test needs and is suitable for water and wastewater samples. Sample distillations are easy and safe to perform.

Applications for the General Purpose Distillation Apparatus include:

- fluoride
- ammonia nitrogen
- selenium
- albuminoid nitrogen
- phenols
- volatile acids

Arsenic and cyanide require specialty glassware sets in addition to the General Purpose Set (the Arsenic Distillation Apparatus and the Cyanide Distillation Apparatus). All connecting glassware is manufactured with threaded connectors for ease and safety. The General Purpose Heater provide efficient heating and the Support Apparatus anchors the glassware.

Figure 1 **General Purpose Distillation Apparatus with Heater and Support Apparatus**



1.1 Waste Management

This section provides guidelines for laboratory waste management. It should assist you in complying with USEPA regulations governing waste management. It summarizes basic requirements, but it does not contain all USEPA regulations. It does not relieve people from complying with all regulations contained in the Code of Federal Regulations. Regulations change regularly and more state and local laws may apply to your waste. You are responsible for knowing and obeying these laws.

1.1.1 Waste Minimization

Waste minimization is the foundation of good waste management. Minimizing waste greatly reduces the disposal problems and expense. If possible, try to generate less waste rather than recycle or re-use it. For laboratories, ways to reduce waste include:

- Use the smallest sample size possible.
- Choose methods that use non-hazardous or “less” hazardous reagents when possible.
- Buy chemicals in small quantities which will be used before they expire. This eliminates disposal of out-dated materials.
- Clean glassware and laboratory apparatus with non-hazardous soaps when possible, rather than solvent or acids which may be hazardous.

1.1.2 Regulatory Overview

Federal waste disposal regulations were issued in accordance with the Resource Conservation and Recovery Act (RCRA). They are given in Title 40 Code of Federal Regulations (CFR) part 260. The Act controls all forms of solid waste disposal and encourages recycling and alternative energy sources. The major emphasis is controlling hazardous waste disposal. The regulations create a system to identify wastes and track waste generation, transport, and ultimate disposal. Each facility involved in managing hazardous waste must be registered with the USEPA. This includes the generator, transporters, and treatment, storage, and disposal facilities (TSDF).

Note: *If a laboratory generates acutely hazardous waste (as defined on 40 CFR 261) or accumulates over a certain amount of waste, the facility may be moved into a larger generator status. Check with your environmental compliance manager or state and local officials to determine which category your facility is in.*

Under federal regulations, there are three categories of generators with increasingly more strict regulation for larger quantity generators. The categories are based on the amount of hazardous waste generated in any given month. The categories are as follows:

- Conditionally Exempt Small Quantity Generator – less than 100 kg (220 lb.) per month
- Small Quantity Generator – between 100 kg (220 lb.) and 1,000 kg (2,200 lb.) per month
- Large Quantity Generator
- Greater than 1,000 kg (2,200 lb.) per month

1.1.3 Hazardous Waste Definition

For regulatory purposes, a “hazardous waste” is a material which is subject to special laws by the USEPA under 40 CFR 261. In addition, many states or local authorities regulate additional materials as hazardous waste. Be aware that many very toxic compounds are not regulated by this definition of hazardous waste. However, improper management or disposal of these compounds may lead to legal problems under other laws such as CERCLA (Superfund) or common law tortes.

The 40 CFR 261 defines a hazardous waste as a solid waste which is not excluded from regulation and meets any of the following criteria:

- It is a discarded commercial chemical product, off-specification species, container residue, or spill residue of materials specifically listed in 40 CFR 261.33;
- It is a waste from a specific source listed in 40 CFR 261.32;
- It is a waste from a non-specific source listed in 40 CFR 261.31; or
- It displays any of the following characteristics of hazardous waste defined in 40 CFR 261.20-24:
 - ignitability,
 - corrosivity,
 - reactivity, or
 - toxicity.

There are many exceptions to these regulations, and each generator should review the regulations and determine if they are excluded from the regulations.

1.1.4 Characteristic Hazardous Waste Codes

Hazardous wastes are managed by specific codes assigned in 40 CFR 261.20-261.33. In the DR/4000 Procedures Manual, these codes are given in the *Pollution Prevention and Waste Management* section of each procedure whenever the reagents used (as directed) will result in a characteristic hazardous waste. These codes are provided to help you identify hazardous waste. The generator is responsible for making the actual waste code determination.

Selected characteristic waste codes for chemicals which may be generated using Hach methods for water analysis are given in the following table. A complete list of waste codes is found in 40 CFR 261.24.

USEPA	Characteristic	CAS No.	Regulatory Level (mg/L)
D001	Ignitability	n/a	n/a
D002	Corrosivity	n/a	n/a
D003	Reactivity	n/a	n/a
D004	Arsenic	6440-38-2	5.0
D005	Barium	6440-39-3	100.0
D018	Benzene	71-43-2	0.5
D006	Cadmium	7440-43-9	1.0
D022	Chloroform	67-66-3	6.0
D007	Chromium	7440-47-3	5.0
D008	Lead	7439-92-1	5.0
D009	Mercury	7439-97-6	0.2
D010	Selenium	7782-49-2	1.0
D011	Silver	7440-22-4	5.0

1.1.5 Determining if Waste is Hazardous

Federal laws do not require you to test a material to decide if it is a hazardous waste. You may apply product knowledge to decide if a material is hazardous. Often, information on a material safety data sheet is enough to decide. If the product is specifically listed in the regulation, it is a hazardous waste.

You also need to decide if it has any characteristics of a hazardous waste. Physical information on the MSDS may help you decide. If the flash point is below 60 °F (15 °C) or is classified by DOT as an oxidizer, the material may be ignitable. If the pH of the material is ≤ 2 or ≥ 12.5 , the material may be corrosive. If the material is unstable, reacts violently with water, or may generate toxic gases, vapors, or fumes when mixed with water, it may be reactive.

Use the chemical composition data to decide if a material is toxic. This decision is based on the concentration of certain contaminants (heavy metals and a number of organic compounds). If the waste is a liquid, compare the concentration of the contaminants in the liquid to the concentrations listed in 40 CFR 261.24. If the waste is a solid, analyze the sample by the Toxicity Characteristic Leachability Procedure (TCLP) and compare the results to the concentration listed in the 40 CFR 261.24. Levels above the threshold amount listed in the table are considered hazardous.

See Section 1.2 *Material Safety Data Sheets* describing the MSDS, for help in finding information for making hazardous waste determinations.

1.1.6 Examples of Hazardous Waste

A number of chemicals used in and final solutions created from Hach procedures are hazardous wastes when they are disposed. In addition, substances in the sample matrix may be a hazardous waste. Sometimes, reagents which would be hazardous are neutralized or changed during the analytical procedure. In that case, the final solutions are not regulated. Finally, many reagents and final solutions may be non-regulated. The generator must either use their knowledge of the materials used or conduct analytical tests to determine if the final material is a hazardous waste.

Examples of tests using Hach reagents that generate hazardous waste include those containing mercury or mercury compounds such as COD tests or Nessler's reagent. Conversely, a test using non-regulated Hach reagents such as ManVer 2 Hardness Indicator Powder Pillows and EDTA Titration Cartridges do not produce a hazardous waste unless the sample contains a hazardous substance.

1.1.7 Disposing of Hazardous Waste

Hazardous waste must be managed and disposed of according to federal, state, and local regulations. The waste generator is responsible for making hazardous waste determinations. Analysts should check with the facility's environmental compliance people for specific instructions.

Hazardous wastes should be handled by treatment, storage, and disposal facilities (TSDF) that have USEPA permits. In some cases, the generator may treat the hazardous waste. In most cases, a permit from the USEPA is required to treat hazardous waste. Laboratories are not exempt from these regulations. If your facility is a "Conditionally Exempt Small Quantity Generator," special rules may apply. Check 40 CFR 261 to determine if have to comply with all the laws.

The most common allowed treatment is elementary neutralization. This refers to neutralizing wastes that are hazardous only because they are corrosive or are listed only for that reason. Neutralize acidic solutions by adding a base such as sodium hydroxide; neutralize basic solutions by adding an acid such as hydrochloric acid. Slowly add the neutralizing agent while stirring. Monitor the pH. When it is at or near 7, the material is neutralized and may be flushed down the drain. Many wastes generated from Hach procedures may be treated in this manner.

Other chemical or physical treatments such as cyanide destruction or evaporation may require a permit. Check with your environmental department or local regulators to determine which rules apply to your facility. See Section 1.1.8 for more information about cyanide-containing waste.

Laboratory chemicals may be mixed and disposed of with other hazardous wastes generated at your facility. They may also be accumulated in accordance with 40 CFR 262.34 satellite accumulation rules. After collection they may be disposed of in a "labpack." A number of environmental and hazardous waste companies offer labpacking services. They will inventory, sort, pack, and arrange proper disposal for hazardous waste. Find companies offering these services in the Yellow Pages under "Waste Disposal - Hazardous" or contact state and local regulators for assistance.

1.1.8 Managing Specific Wastes

Hach has several documents to assist customers in managing waste generated from our products. You can obtain the following documents by calling 1-800-227-4224 and requesting the literature codes given:

Literature Code	Title
1321	<i>Waste Reduction: A Primer</i>
9323	<i>COD/Mercury Waste Disposal Firms</i>
9325	<i>RCRA COD Waste Disposal Information Letter</i>
9326	<i>COD Heavy Metal Concentrations</i>

1.1.9 Special Considerations for Materials Containing Cyanide

Several procedures in this manual use reagents that contain cyanide compounds. These materials are regulated as reactive (D003) waste by the Federal RCRA. Waste disposal instructions provided with each procedure tell you how to collect these materials for proper disposal. It is imperative that these materials be handled safely to prevent the release of hydrogen cyanide gas (an extremely toxic material with the smell of bitter almonds). Most cyanide compounds are stable and can be safely stored for disposal in highly alkaline solutions (pH >11) such as 2 N sodium hydroxide. Never mix these wastes with other laboratory wastes that may contain lower pH materials such as acids or even water.

If a cyanide-containing compound is spilled, you must be careful not to be exposed to hydrogen cyanide gas. Take the following steps to destroy the cyanide compounds in an emergency:

- a. Use a fume hood, supplied air or self-contained breathing apparatus.
- b. While stirring, add the waste to a beaker containing a strong solution of sodium hydroxide and either calcium hypochlorite or sodium hypochlorite (household bleach).
- c. Add a lot of hydroxide and hypochlorite. Let the solution stand for 24 hours.
- d. Neutralize the solution and flush it down the drain with a large amount of water. If the solution contains other regulated materials such as chloroform or heavy metals, it may still need to be collected for hazardous waste disposal. Never flush hazardous wastes to the drain.

1.1.10 Resources

Many sources of information on proper waste management are available. The USEPA has a hot line number for questions about the Resource Conservation and Recovery Act (RCRA). The RCRA Hot Line number is 1-800-424-9346. You may also get a copy of the appropriate regulations. Federal hazardous waste regulations are found in 40 CFR 260- 99. Obtain this book from the U.S. Government Printing Office or a number of other vendors. Other documents which may be helpful to the laboratory hazardous waste manager include:

1. Task Force on Laboratory Waste Management. *Laboratory Waste Management, A Guidebook*; American Chemical Society, Department of Government Relations and Science Policy: Washington, DC 1994.
2. Task Force on Laboratory Waste Management. *Waste Management Manual for Laboratory Personnel*; American Chemical Society, Department of Government Relations and Science Policy: Washington, DC 1990.
3. Task Force on Laboratory Waste Management. *Less is Better*; 2nd ed.; American Chemical Society, Department of Government Relations and Science Policy: Washington, DC 1993.
4. Committee on Chemical Safety. *Safety in Academic Chemistry Laboratories*, 5th ed.; American Chemical Society: Washington, DC, 1990.
5. Armour, Margaret-Ann. *Hazardous Laboratory Chemicals Disposal Guide*; CRC Press: Boca Raton, FL, 1991.
6. *Environmental Health and Safety Manager's Handbook*; Government Institutes, Inc.: Rockville, MD, 1988.
7. Lunn, G.; Sansone, E.B. *Destruction of Hazardous Chemicals in the Laboratory*; John Wiley and Sons: New York, 1990.
8. National Research Council. *Prudent Practices for Disposal of Chemicals from Laboratories*; National Academy Press: Washington, DC, 1983.
9. National Research Council. *Prudent Practices for Handling Hazardous Chemicals in Laboratories*; National Academy Press: Washington, DC, 1981.
10. Environmental Protection Agency, Office of Solid Waste and Emergency Response. *The RCRA Orientation Manual*; U.S. Government Printing Office: Washington, DC, 1991.
11. Environmental Protection Agency, Office of Solid Waste and Emergency Response. *Understanding the Small Quantity Generator Hazardous Waste Rules: A Handbook for Small Business*; U.S. Government Printing Office: Washington, DC, 1986.

1.2 Material Safety Data Sheets

Material safety data sheets (MSDS) describe the hazards of chemical products. This section describes the information provided on a Hach MSDS and how to locate important information for safety and waste disposal. The information provided on the MSDS applies to the product as sold by Hach. The properties of any mixtures obtained by using this product will be different.

1.2.1 Obtaining an MSDS

Hach ships a MSDS to each customer with the first order of any chemical product. A new MSDS may be sent when the information on the data sheet is updated. Please review all new MSDSs for new information. If you need another copy of an MSDS, simply call 1-800-227-4227.

1.2.2 Sections of an MSDS

Each MSDS has ten sections. The sections and the information found in them are as follows.

Header Information

The Hach catalog number, MSDS date, change number, company address and telephone number, and emergency telephone numbers are listed at the top of the MSDS.

1. Product Identification

This section contains:

- Hach product name
- Chemical Abstract Services (CAS) number
- Chemical name
- Chemical formula, if appropriate
- Chemical family to which the material belongs

2. Ingredients

This section lists each component in the product. It contains the following information for each component:

- PCT: Percent by weight of this component
- CAS NO.: Chemical Abstract Services (CAS) registry number for this component
- SARA: Superfund Amendments and Reauthorization Act, better known as the “Community Right to Know Law.” Says if the component is listed in SARA 313. If the component is listed and you use more than the amount listed, you must report this to the USEPA every year.
- TLV: Threshold Limit Value. The maximum airborne concentration for an 8 hour exposure that is recommended by the American Conference of Governmental Industrial Hygienists (ACGIH).

- PEL: Permissible Exposure Limit. The maximum airborne concentration for an 8 hour exposure that is regulated by the Occupational Safety and Health Administration (OSHA).
- HAZARD: Physical and health hazards of the component are explained.

3. Physical Data

The physical properties of the product are given in this section. They include the physical state, color, odor, solubility, boiling point, melting point, specific gravity, pH, vapor density, evaporation rate, corrosivity, stability, and storage precautions.

4. Fire, Explosion Hazard And Reactivity Data

This section contains the flash point and flammable limits of the material. It also includes how to fight fires if the material catches on fire. Key terms in this section include:

- Flashpoint: The temperature at which a liquid will give off enough flammable vapor to ignite. Flammability and ignitability are usually defined by the flash point.
- Lower Flammable Limit (LFL or LEL): The lowest concentration that will produce a fire or flash when an ignition source is present.
- Upper Flammable Limit (UFL or UEL): The vapor concentration in air above which the concentration is too rich to burn.
- NFPA Codes: The National Fire Protection Association (NFPA) has a system to rate the degree of hazards presented by a chemical. These codes are usually placed in a colored diamond. The codes range from 0 for minimal hazard to 4 for extreme hazard. They are grouped into the following hazards: health (blue), flammability (red), reactivity (yellow), and special hazards (white).

5. Health Hazard Data

This section describes different ways the chemical can enter your body (ingestion, inhalation, skin contact). It also gives acute (immediate) and chronic (long-term) health effects. If the material causes cancer or genetic damage, it is identified in this section.

6. Precautionary Measures

This section contains special precautions for the material. These may include special storage instructions, handling instructions, conditions to avoid, and protective equipment required to use this material safely.

7. First Aid

First aid instructions for exposures to the chemical are given in this section. Be sure to read this section before inducing vomiting in a victim. Some chemicals are better treated by not inducing vomiting. Seek prompt medical attention for all chemical exposures.

8. Spill And Disposal Procedures

This section tells about safe work practices for cleaning up and disposing of spilled material. Please refer to the Waste Management section of this manual. Final determination of proper and legal disposal options is the responsibility of the waste generator. Be sure you know the federal, state, and local laws that apply to your facility.

9. Transportation Data

Domestic and International shipping information is provided in this section. It gives shipping name, hazard class, and ID number of the product.

10. References

This section lists the reference materials used to write the MSDS.

Following the *Reference* section, the product is listed as having SARA 313 chemicals or California Proposition 65 List Chemicals, if applicable. Also found here is any special information about the product.

1.3 Safety

Safety is the responsibility of each person performing analytical procedures. Because many of the procedures in this methods manual use potentially hazardous chemicals and equipment, it is important to prevent accidents by practicing good laboratory techniques. The following guidelines apply to water analysis. These guidelines do not cover every aspect of safety, but they are important for preventing injuries.

1.3.1 Material Safety Data Sheet

A material safety data sheet (MSDS) comes with the first shipment of all products. The MSDS provides environmental and safety information about the products. Always read the MSDS before using a new product. Section 1.2 gives more information on MSDS and explains how to read them.

1.3.2 Reading Labels Carefully

Read each reagent label carefully. Pay particular attention to the precautions given. Never remove or block the label on a reagent container while it contains reagent. Do not put a different reagent into a labeled container without changing the label. When preparing a reagent or standard solution, label the container clearly. If a label is hard to read, re-label promptly according to your facility's hazard communication program.

Warning labels also appear on some of the apparatus used with the test procedures. The protective shields with the COD Reactor and the Digesdahl Digestion Apparatus point out potential hazards. Be sure these shields are in place during use and observe the precautions on the label.

1.3.3 Protective Equipment

Use the right protective equipment for the chemicals and procedures. The MSDS contains this information. Protective equipment may include:

- Eye protection such as safety glasses or goggles to protect from flying objects or chemical splashes.

- Gloves to protect skin from toxic or corrosive materials, sharp objects, very hot or very cold materials, or broken glass. Use tongs or finger cots when transferring hot apparatus.
- Laboratory coats or splash aprons to protect skin and clothing from splashes.
- Footwear to protect feet from spills. Open toed shoes should not be worn in chemistry settings.
- Respirators may be needed to protect you from breathing toxic vapors if adequate ventilation, such as fume hoods, are not available.
- Use fume hoods as directed by the procedure or as recommended in the MSDS.
- For many procedures, adequate ventilation is enough. Be sure there is enough fresh air and air exhaust to protect against unnecessary exposure to chemicals.

1.3.4 First Aid Equipment and Supplies

Most first aid instructions for chemical splashes in eyes or on skin call for thorough flushing with water. Laboratories should have eyewash and shower stations. For field work, carry a portable eyewash unit. Laboratories should also have appropriate fire extinguishers and fume hoods.

1.3.5 General Safety Rules

Follow these rules to make work with toxic and hazardous chemicals safer:

1. **Never** pipet by mouth. Always use a mechanical pipet or pipet bulb to avoid ingesting chemicals.
2. Follow test procedures carefully and observe all precautionary measures. Read the entire procedure carefully before beginning.
3. Wipe up all spills promptly. Get proper training and have the right response equipment to clean up spills. See your safety director for more information.
4. **Do not** smoke, eat, or drink in an area where toxic or irritating chemicals are used.
5. Use reagents and equipment only as directed in the test procedure.
6. **Do not** use damaged labware and broken equipment.
7. Minimize all chemical exposures. **Do not** breathe vapors or let chemicals touch your skin. Wash your hands after using chemicals.
8. Keep work areas **neat** and **clean**.
9. **Do not** block exits or emergency equipment.

1.3.6 OSHA Chemical Hygiene Plan

The Occupational Safety and Health Administration (OSHA) enforces laws about the control exposure to hazardous chemicals in laboratories. These regulations are in Title 29 CFR 1910.1450. They apply to all employers who use hazardous chemicals. They require employers to develop and use a written Chemical Hygiene Plan and appoint a qualified person as the Chemical Hygiene Officer.



At Hach Company, customer service is an important part of every product we make.

With that in mind, we have compiled the following information for your convenience.

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Hach Company
P.O. Box 389
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U.S.A.

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Information Required

- Hach account number (if available)
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- Purchase order number
- Brief description or model number
- Billing address
- Shipping address
- Catalog number
- Quantity

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Hach Technical and Customer Service Department personnel are eager to answer questions about our products and their use. Specialists in analytical methods, they are happy to put their talents to work for you.
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HACH Company, c/o
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D-40549 Düsseldorf
Germany
Telephone: +49/[0]211.52.88.0
Fax: +49/[0]211.52.88.231

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Authorization must be obtained from Hach Company before sending any items for repair. Please contact the HACH Service Center serving your location.

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(800) 227-4224 (U.S.A. only)
Telephone: (515) 232-2533
FAX: (515) 232-1276

In Canada:

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Winnipeg, Manitoba
R3H 0X4
(800) 665-7635 (Canada only)
Telephone: (204) 632-5598
FAX: (204) 694-5134
E-mail: canada@hach.com

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Indian Subcontinent, Africa, Europe, or the Middle East:**

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Loveland, Colorado, 80539-0389
U.S.A.
Telephone: (970) 669-3050
FAX: (970) 669-2932
E-mail: intl@hach.com

Warranty

Hach Company warrants this product to the original purchaser against any defects that are due to faulty material or workmanship for a period of **one year from date of shipment**.

In the event that a defect is discovered during the warranty period, Hach Company agrees that, at its option, it will repair or replace the defective product or refund the purchase price, excluding original shipping and handling charges. Any product repaired or replaced under this warranty will be warranted only for the remainder of the original product warranty period.

This warranty does not apply to consumable products such as chemical reagents; or consumable components of a product, such as, but not limited to, lamps and tubing.

Contact Hach Company or your distributor to initiate warranty support. Products may not be returned without authorization from Hach Company.

Limitations

This warranty does not cover:

- damage caused by acts of God, natural disaster, labor unrest, acts of war (declared or undeclared), terrorism, civil strife or acts of any governmental jurisdiction
- damage caused by misuse, neglect, accident or improper application or installation
- damage caused by any repair or attempted repair not authorized by Hach Company
- any product not used in accordance with the instructions furnished by Hach Company
- freight charges to return merchandise to Hach Company
- freight charges on expedited or express shipment of warranted parts or product
- travel fees associated with on-site warranty repair

This warranty contains the sole express warranty made by Hach Company in connection with its products. All implied warranties, including without limitation, the warranties of merchantability and fitness for a particular purpose, are expressly disclaimed.

Some states within the United States do not allow the disclaimer of implied warranties and if this is true in your state the above limitation may not apply to you. This warranty gives you specific rights, and you may also have other rights that vary from state to state.

This warranty constitutes the final, complete, and exclusive statement of warranty terms and no person is authorized to make any other warranties or representations on behalf of Hach Company.

Limitation of Remedies

The remedies of repair, replacement or refund of purchase price as stated above are the exclusive remedies for the breach of this warranty. On the basis of strict liability or under any other legal theory, in no event shall Hach Company be liable for any incidental or consequential damages of any kind for breach of warranty or negligence.

SECTION 1 PROCEDURES





Scope and Application: For water

* This test is semi-quantitative. Results are expressed as greater or less than the threshold value used.

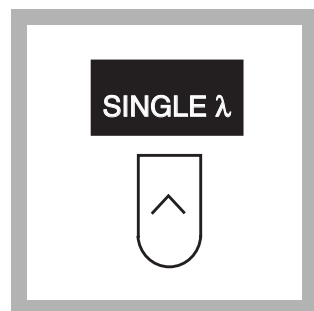
This method analyzes for Alachlor in water. Sample calibrators and reagents are added to cuvettes coated with Alachlor-specific antibodies. The color that develops is then measured and compared with the color measurements of the calibrators. The test requires about 30 minutes for complete analysis. As many as 20 cuvettes (18 samples and 2 calibrators) can be run simultaneously.

Tips and Techniques

- **Read the entire procedure before starting.** Identify and have ready all the necessary reagents, cuvettes, and other apparatus before beginning the analysis.
- **Timing is critical;** follow instructions carefully.
- **A consistent technique when mixing the cuvettes is critical to this test.** The best results come from using the cuvette rack and mixing as described in *Using the 1-cm MicroCuvette Rack*. Cuvettes can be mixed individually, but test results may not be as consistent.
- Handle the cuvettes carefully. Scratches on the inside or outside may cause erroneous results. Carefully clean the outside of the cuvettes with a clean absorbent cloth or tissue before placing them into the instrument. (Kimwipe® tissues are provided with the kit.)
- Antibody cuvettes and enzyme conjugate are made in matched lots. Do not mix reagent lots.
- To avoid damaging the Color Developing Solution, do not expose it to direct sunlight.
- The cuvette rack is designed to be inverted with the cuvettes in place. This is especially helpful when running many samples at once; the cuvettes can remain in the rack and be processed together until they are read in the Immunoassay Pocket Colorimeter.
- Twenty Antibody Cuvettes are provided with each reagent set. One Antibody Cuvette will be used for each calibrator and each sample. Cuvettes are not reusable.
- Ensure the 1cm MicroCell adapter is installed in the DR/4000.

Note: Hach Company recommends wearing protective nitrile gloves for this procedure.

Immunoassay



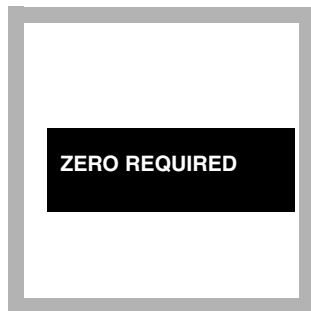
1. Press the soft key under **SINGLE λ**.

Press the soft key under **GO TO λ**.

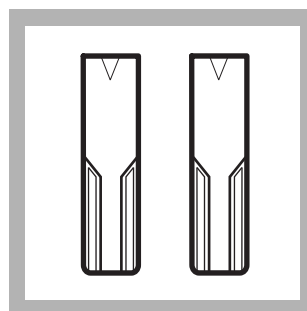
Select 450 nm by pressing the numeric keys **450**.

Press: **ENTER**

Note: The Flow Cell and Sipper Modules cannot be used with this procedure.

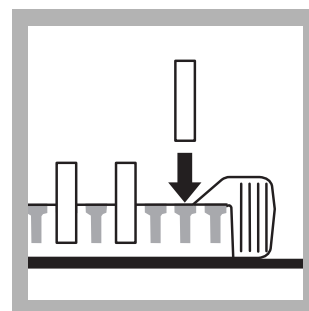


2. The display will show:
ZERO REQUIRED

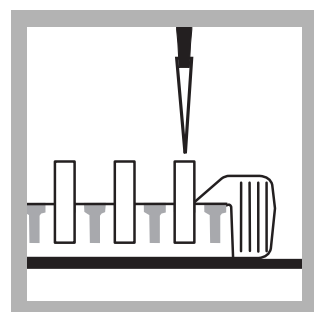


3. Label an Antibody Cuvette for each calibrator and each sample to be tested.

Note: As many as 20 cuvettes may be tested at one time and may comprise any combination of samples and calibrators.

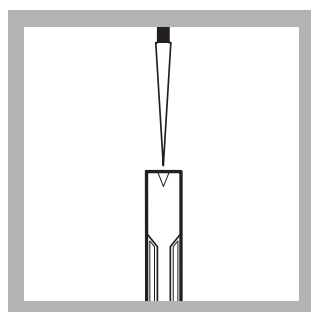


4. Place the cuvettes into the rack snugly.



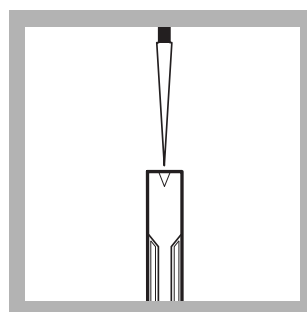
5. Pipet 0.5 mL of each calibrator into the appropriately labeled cuvette.

Note: Use a new pipette tip for each sample.

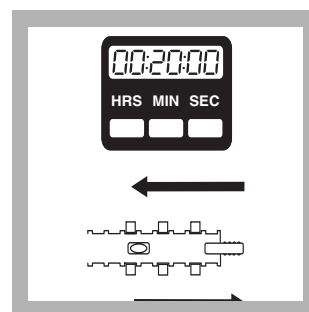


6. Pipet 0.5 mL of each sample to be tested into the appropriately labeled cuvette.

Note: Use a new pipette tip for each sample.



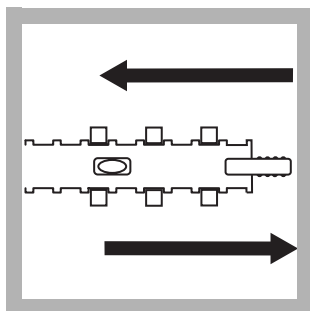
7. Immediately pipet 0.5 mL of Alachlor Enzyme Conjugate into each cuvette.



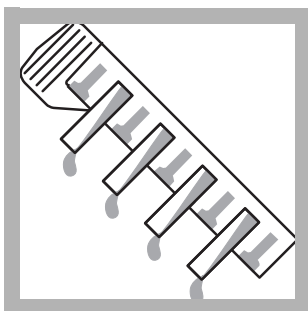
8. Key **2000**. Press the soft key under **START TIMER**.

A 20-minute reaction time will begin.

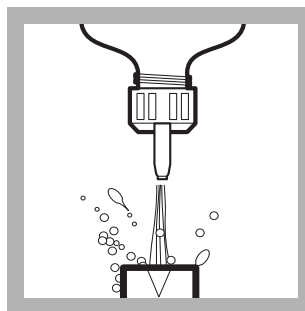
Immediately mix the contents of the cuvettes for 30 seconds using the technique described in *Using the 1-cm MicroCuvette Rack*.



9. After 10 minutes mix the contents of the rack for 30 seconds using the technique described in “Using the 1-cm MicroCuvette Rack” on page 5.



10. At the end of the 20-minute period, discard the contents of all the cuvettes into an appropriate waste container.

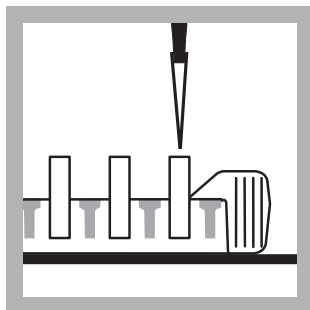


11. Wash each cuvette forcefully and thoroughly four times with deionized water. Empty the rinse water into the waste container.

Note: Ensure most of the water is drained from the cuvettes by turning the cuvettes upside down and tapping them lightly on a paper towel

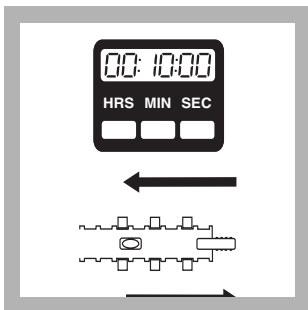
Color Development

Note: Timing is critical; follow instructions carefully.



12. With the cuvettes still held snugly in the rack, pipet 0.5 mL of Color Developing Solution into each Antibody Cuvette.

Note: Use a new pipette tip for each cuvette.



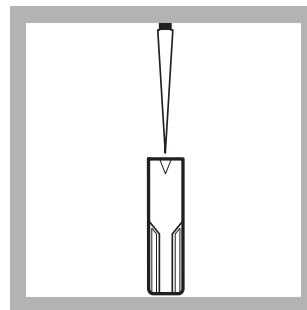
13. Key 1000. Press the soft key under **START TIMER**.

A reaction period will begin. Mix following the instructions in *Using the 1-cm MicroCuvette Rack*.



14. After 5 minutes, mix the contents of the rack a second time for a period of 30 seconds using the same technique.

Note: Solutions will turn blue in some or all of the cuvettes.



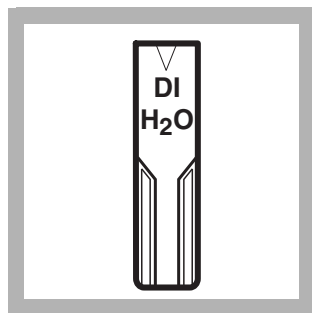
15. At the end of the 10-minute reaction period, pipette 0.5 mL of Stop Solution into each cuvette in the same order as the Color Developing Solution was added in step 12.

Slide the rack for 20 seconds using the technique described in *Using the 1-cm MicroCuvette Rack*.

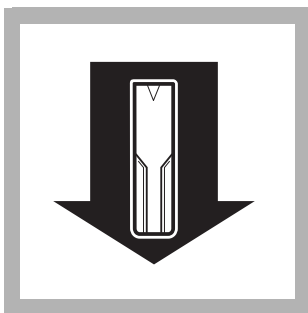
Note: Blue solutions will turn yellow with the addition of the Stop Solution.

Note: The same pipette tip can be used repeatedly for this step.

Measuring the Color

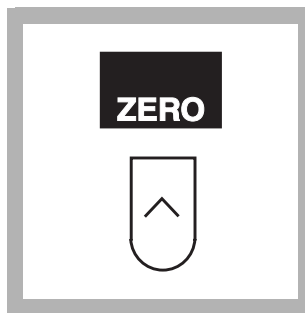


16. Label and fill a Zeroing Cuvette with deionized water. Wipe the outside of all the cuvettes with a tissue to remove water, smudges, and fingerprints.



17. Place the filled zeroing cuvette into the cell holder with the arrow pointing left.

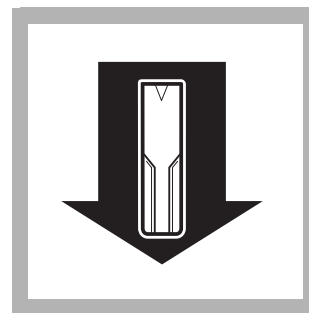
Orient the arrow in the same direction for all cuvettes.



18. Press the soft key under **ZERO**.

The display will show:

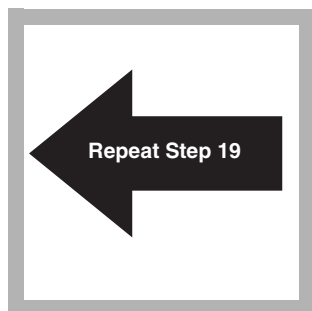
0.000 Abs



19. Place the first calibrator into the cell holder. Read the results.

The display will give an absorbance reading. Record the results for each calibrator and sample.

Note: See the *Instrument Manual* for more information on taking a reading.



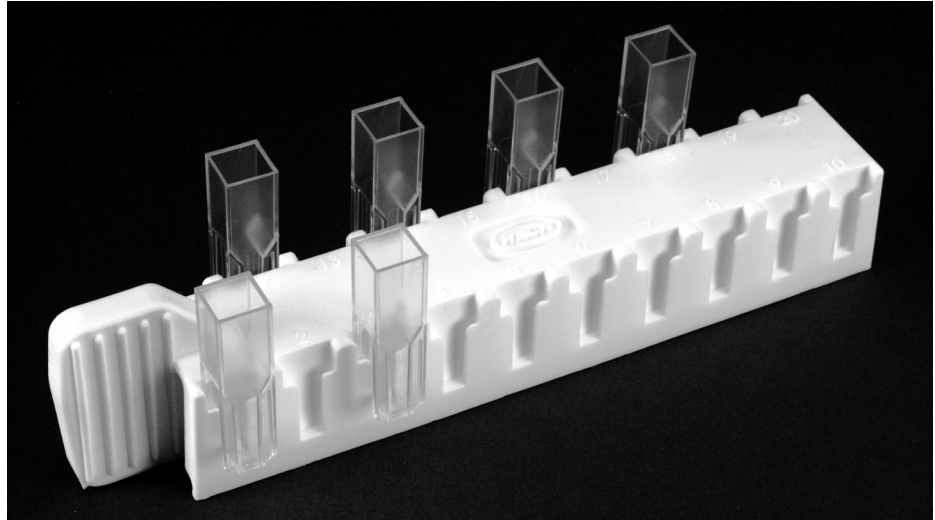
20. Repeat *step 19* for all remaining calibrators and samples.

See *Interpreting and Reporting Results* for help with interpretation of results.

Using the 1-cm MicroCuvette Rack

This rack (see *Figure 1*) has been designed specifically to aid in achieving precise and accurate results when using the immunoassay technique to analyze several samples at the same time.

Figure 1 **The 1-cm MicroCuvette Rack**



Loading the Rack — The cuvette rack is designed so that it may be inverted with the cuvettes in place. Identify each cuvette with a sample or calibrator number and place all the cuvettes in the rack before beginning the procedure. Fit the cuvettes snugly into the rack, but do not force them or they may be difficult to remove and their contents may spill. The cuvettes should remain in place when the rack is inverted and tapped lightly.

Mixing — Set the rack on a hard, flat surface that is at least twice the length of the rack. Hold the rack by one end and vigorously slide it back and forth along its long axis for 30 seconds. The rack should move through a distance equal to its own length in each direction.

Interpreting and Reporting Results

There is an inverse relationship between the concentration of Alachlor and the reading. In other words, the higher the reading, the lower the concentration of Alachlor.

If the sample reading is...	the sample Alachlor Concentration is...
...less than calibrator reading	...greater than the calibrator concentration
...greater than calibrator reading	...less than the calibrator concentration

Example

Readings:

0.1 ppb Alachlor Calibrator: **0.475 ABS**

0.5 ppb Alachlor Calibrator: **0.245 ABS**

Sample #1: **0.140 ABS**

Sample #2: **0.300 ABS**

Sample #3: **0.550 ABS**

Interpretation

Sample #1 — Sample reading is less than the readings for both calibrators. Therefore the sample concentration of Alachlor is greater than both 0.1 ppb and 0.5 ppb Alachlor.

Sample #2 — Sample reading is between the readings for the 0.1 ppb and 0.5 ppb Alachlor calibrators. Therefore the sample concentration of Alachlor is between 0.1 ppb and 0.5 ppb.

Sample #3 — Sample reading is greater than the readings for both calibrators. Therefore the sample concentration of Alachlor is less than both 0.5 ppb and 0.1 ppb.

Storing and Handling Reagents

- Wear protective gloves and eyewear.
- When storing reagent sets for extended periods of time, keep them out of direct sunlight. Store reagents at a temperature of 4 °C when not in use.
- Keep the foil pouch containing the Alachlor Antibody Cuvettes sealed when not in use.
- If Stop Solution comes in contact with eyes, wash thoroughly for 15 minutes with cold water and seek immediate medical help.

Sensitivity

The Alachlor immunoassay test cannot differentiate between the various acetanilide herbicides and metabolites, but it detects their presence to differing degrees. The following table shows the required concentration for selected chemicals.

Compound	Concentration required to give a positive response of 0.1 ppb Alachlor	Concentration required to give a positive response of 0.5 ppb Alachlor
Acetochlor	0.45 ppb	4 ppb
Butachlor	0.09 ppm	1 ppm
2 Chloro-2',6'-Diethylacetaniline	0.030 ppm	2 ppm
Metolachlor	0.085 ppm	2 ppm
2,6-Diethylaniline	0.3 ppm	9 ppm
Propachlor	0.72 ppm	12 ppm

The following compounds are not detectable at 10,000 ppb.

Alachlor	Carbofuran	Carbendazim
Aldicarb	2,4-D	
Diazotol	Chlorpyrifos	

Diluting Water Samples

Other levels of Alachlor can be tested by diluting the sample and comparing the results to the 0.1 ppb Calibrator. From the table choose the appropriate sample

volume, place it in a graduated mixing cylinder, and dilute it to 50 mL with deionized water.

mL Sample	Representative Concentration using 0.1 ppb Calibrator
0.5	10 ppb
1.0	5 ppb
2.5	2 ppb
5.0	1 ppb

Example:

Dilute 2.5 mL of sample to 50 mL with deionized water. Run the diluted sample in the procedure along with the 0.1 ppb calibrator. If the absorbance of the diluted sample is less than the 0.1 ppb calibrator, the concentration of the original sample is greater than 2 ppb.

Sample Collection and Storage

Collect samples in a clean glass bottle. Do not pre-rinse the bottle with the sample. If the sample cannot be analyzed immediately, store the sample at 4 °C. Samples may be kept for as long as 14 days. Warm the samples to room temperature before analysis.

Summary of Method

Hach immunoassay tests use antigen/antibody reactions to test for specific organic compounds in water and soil. Alachlor-specific antibodies, attached to the walls of plastic cuvettes, selectively bind and remove Alachlor from complex sample matrices. A prepared sample and a reagent containing enzyme-conjugate molecules (analyte molecules attached to molecules of an enzyme) are added to the Antibody Cuvettes. During incubation, enzyme-conjugate molecules and Alachlor compete for binding sites on the antibodies. Samples with higher levels of analyte will have more antibody sites occupied by Alachlor and fewer antibody sites occupied by the enzyme-conjugate molecules.

After incubation, the sample and unbound enzyme conjugate are washed from the cuvette and a color-development reagent is added. The enzyme in the conjugate catalyzes the development of color. Therefore, there is an inverse relationship between color intensity and the amount of Alachlor in the sample. The resulting color is then compared with a calibrator to determine whether the Alachlor concentration in the sample is greater or less than the threshold levels.

ALACHLOR in Water, continued

Required Reagents

Description	Unit	Cat. No.
Reagent Set, Alachlor*	20 cuvettes.....	28130-00

Required Apparatus

Caps, flip spout.....	2/pkg.....	25818-02
Cell Adapter, 1-cm MicroCell.....	each.....	48588-00
Marker, laboratory	each.....	20920-00
Rack, for 1-cm Micro Cuvettes	each.....	48799-00
Wipes, disposable.....	box.....	20970-00
TenSette®, Pipet, 0.1–1.0 mL.....	each.....	19700-01
Tips, for pipettor 19700-01	1000/pkg.....	21856-28

* Immunoassay components are manufactured for Hach Company by Beacon Analytical Systems, Inc.



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WORLD HEADQUARTERS
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Method 8012

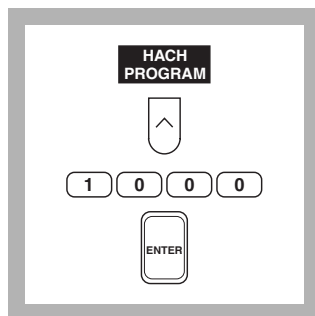
Aluminon Method*

Powder Pillows

(0 to 0.800 mg/L)

Scope and Application: For water and wastewater; digestion is required for determining total aluminum. See Section 2 for digestion procedure. The estimated detection limit for program number 1000 is 0.005 mg/L Al^{3+} .

* Adapted from *Standard Methods for the Examination of Water and Wastewater*.



1. Press the soft key under **HACH PROGRAM**.

Select the stored program number for aluminum (Al) by pressing **1000** with the numeric keys.

Press: **ENTER**

Note: If samples cannot be analyzed immediately, see *Sample Collection, Storage and Preservation* following these steps.

Note: Fluoride interferes at all levels. See *Interferences*.

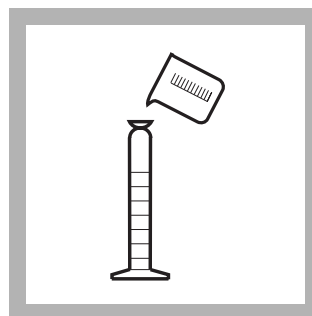
Note: The Flow Cell and Sipper Modules can be used if rinsed well with deionized water between the blank and prepared sample.



2. The display will show:

**HACH PROGRAM:
1000 Aluminum,
Aluminon**

The wavelength (λ), **522 nm**, is automatically selected.

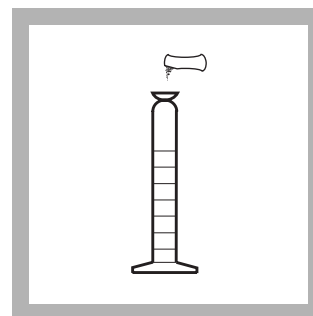


3. Fill a 50-mL graduated mixing cylinder to the 50-mL mark with sample.

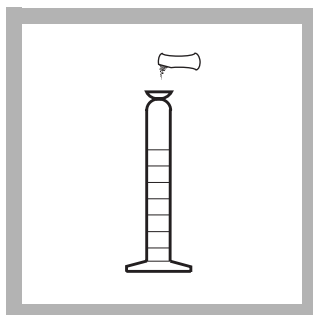
Note: Rinse cylinder with 1:1 hydrochloric acid and deionized water before use to avoid errors due to contaminants absorbed on the glass.

Note: The sample temperature must be between 20–25 °C (68–77 °F) for accurate results.

Note: For proof of accuracy, use a 0.4-mg/L aluminum standard solution (preparation given in the *Accuracy Check*) in place of the sample.



4. Add the contents of one Ascorbic Acid Powder Pillow. Stopper. Invert several times to dissolve powder.



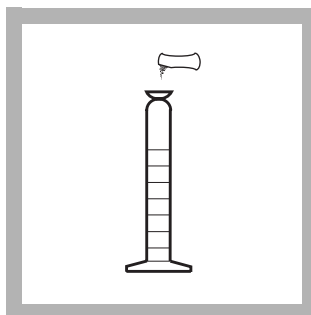
5. Add the contents of one AluVer 3 Aluminum Reagent Powder Pillow. Stopper. Press the soft key under **START TIMER**. Invert repeatedly for one minute to dissolve.

Note: An orange to orange-red color develops if aluminum is present.

Note: Inconsistent results will be obtained if any powder is undissolved.

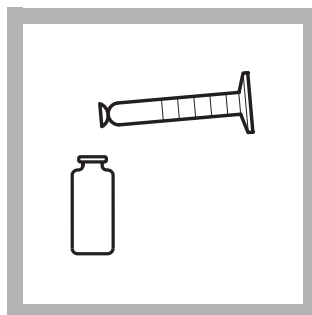


6. Pour 25 mL of mixture into a 25-mL sample cell (the prepared sample).

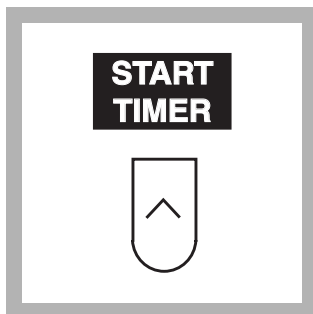


7. Add contents of one Bleaching 3 Reagent Powder Pillow to the remaining 25 mL in the mixing graduated cylinder. Stopper. Press the soft key under **START TIMER**. Vigorously shake for 30 seconds.

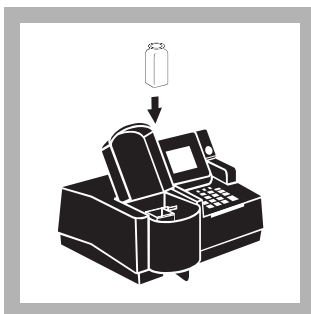
Note: This solution should turn a light to medium orange (but not colorless) upon bleaching.



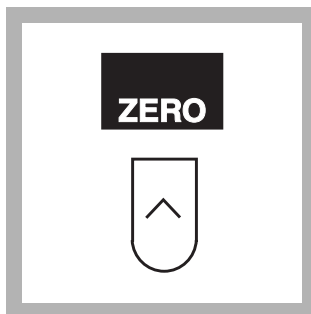
8. Pour the remaining 25 mL of mixture in the cylinder into a second 25-mL sample cell (the blank).



9. Press the soft key under **START TIMER**. A 15-minute reaction period will begin.



10. Within five minutes after the timer beeps, place the blank into the cell holder. Close the light shield.

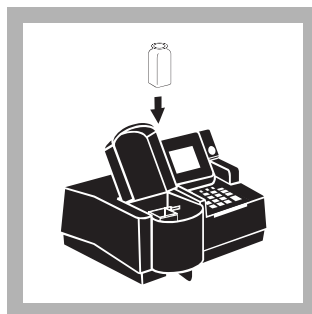


11. Press the soft key under **ZERO**.

The display will show:

0.000 mg/L Al³⁺

Note: For alternate concentration units, press the **OPTIONS** soft key. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



12. Immediately place the prepared sample into the cell holder. Close the lid. Results in mg/L aluminum (or chosen units) will be displayed.

Note: Clean the graduated cylinder and sample cells with soap and brush immediately following the test.

Note: Results may also be expressed as Al₂O₃. Press the soft key under **OPTIONS**, then under **FORM** to scroll through the available options. Press **ENTER** to return to the read screen.

Interferences

Table 1 Interfering Substances and Suggested Treatments

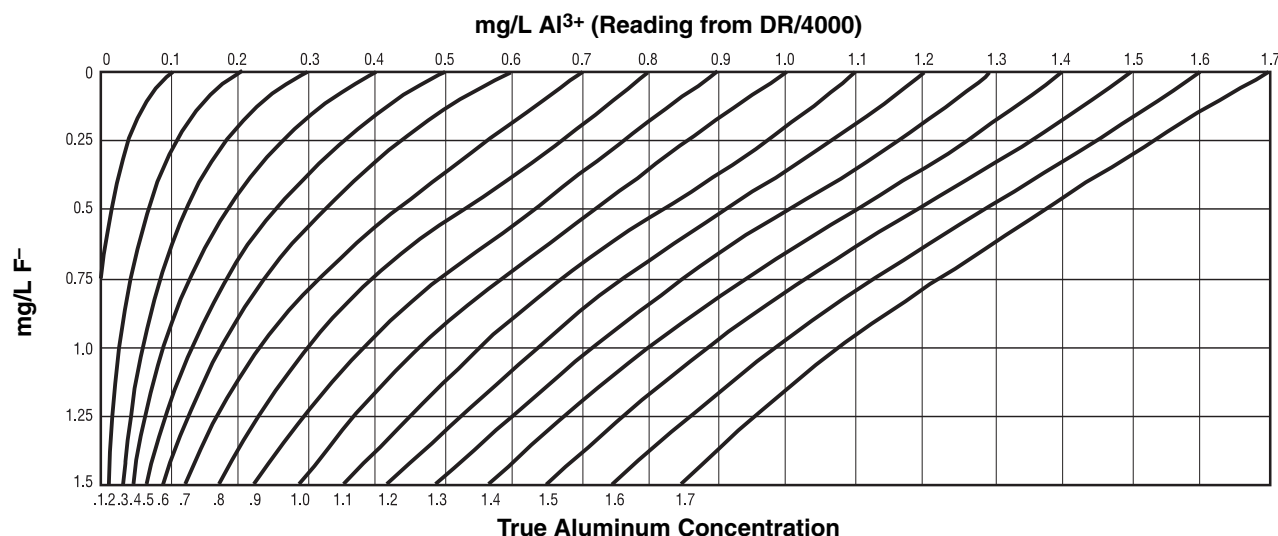
Interfering Substance	Interference Levels and Treatments
Acidity	Greater than 300 mg/L as CaCO_3 . Samples with greater than 300 mg/L acidity as CaCO_3 must be treated as follows: a) Add one drop of m-Nitrophenol Indicator Solution to the sample taken in Step 3. b) Add one drop of 5.0 N Sodium Hydroxide Standard Solution. Stopper the cylinder. Invert to mix. Repeat as often as necessary until the color changes from colorless to yellow. c) Add one drop of 5.25 N Sulfuric Acid Standard Solution to change the solution from yellow back to colorless. Continue with the test.
Alkalinity	1000 mg/L as CaCO_3 . Interferences from higher alkalinity concentrations can be eliminated by the following pretreatment: a) Add one drop of m-Nitrophenol Indicator Solution to the sample taken in Step 3. A yellow color indicates excessive alkalinity. b) Add one drop of 5.25 N Sulfuric Acid Standard Solution. Stopper the cylinder. Invert to mix. If the yellow color persists, repeat until the sample becomes colorless. Continue with the test.
Fluoride	Interferes at all levels. See graph below.
Iron	Greater than 20 mg/L
Phosphate	Greater than 50 mg/L
Polyphosphate	Polyphosphate interferes at all levels by causing negative errors and must not be present. Before running the test, polyphosphate must be converted to orthophosphate by acid hydrolysis as described under the phosphorus procedures.

Fluoride interferes at all levels by complexing with aluminum. The actual aluminum concentration can be determined using the Fluoride Interference Graph when the fluoride concentration is known.

To use the fluoride interference graph:

1. Select the vertical grid line along the top of the graph that represents the aluminum reading obtained in *step 12*.
2. Locate the point of the line where it intersects with the horizontal grid line that indicates how much fluoride is present in the sample.
3. Extrapolate the true aluminum concentration by following the curved lines on either side of the intersect point down to the true aluminum concentration.

Figure 1 Fluoride Interference Graph



For example, if the aluminum test result was 0.7 mg/L Al and the fluoride present in the sample was 1 mg/L F⁻, the point where the 0.7 grid line intersects with the 1 mg/L F⁻ grid line falls between the 1.2 and 1.3 mg/L Al curves. In this case, the true aluminum content would be 1.27 mg/L.

Sample Collection, Storage and Preservation

Collect samples in a clean glass or plastic containers. Preserve the sample by adjusting the pH to 2 or less with Nitric Acid (about 1.5 mL per liter). Preserved samples can be stored up to six months at room temperature. Before analysis, adjust the pH to 3.5–4.5 with 5.0 N Sodium Hydroxide. Correct the test result for volume additions.

Accuracy Check

Standard Additions Method

- Leave the unspiked sample in the sample compartment. Verify that the units displayed are in mg/L. Select standard additions mode by pressing the soft keys under **OPTIONS, (MORE)** and then **STD ADD**.
- Press **ENTER** to accept the default sample volume (mL), 50.
- Press **ENTER** to accept the default standard concentration (mg/L), 50.
- Press the soft key under **ENTRY DONE**.
- Snap the neck off a Aluminum Voluette® Ampule Standard, 50 mg/L Al.
- Use the TenSette Pipet to add 0.1 mL, 0.2 mL and 0.3 mL of standard, respectively to three 50-mL samples and mix each thoroughly.
- Analyze each standard addition sample as described above. Accept the standard additions reading by pressing the soft key under **READ** each time. Each addition should reflect approximately 100% recovery.
- After completing the sequence, the display will show the extrapolated concentration value and the “best-fit” line through the standard additions data points, accounting for matrix interferences.
- See Section 1.4.1 *Standard Additions* for more information.

Standard Solution Method

Prepare a 0.4-mg/L aluminum standard solution by pipetting 1.00 mL of Aluminum Standard Solution, 100 mg/L as Al^{3+} , into a 250-mL volumetric flask. Dilute to the mark with deionized water. Prepare this solution daily. Perform the aluminum procedure as described.

Or, using the TenSette Pipet, add 0.8 mL of solution from an Aluminum Voluette Ampule Standard Solution (50 mg/L as Al) into a 100-mL volumetric flask. Dilute to volume with deionized water.

Method Performance**Precision**

Standard: 0.40 mg/L Al^{3+}

Program	95% Confidence Limits
1000	0.398–0.402 mg/L Al^{3+}

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
1000	0.005 mg/L Al^{3+}

For more information on derivation and use of Hach's estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, Appendix B, see Section 1.5.1.

Sensitivity

Portion of Curve: Entire Range

Program	ΔAbs	$\Delta\text{Concentration}$
1000	0.010	0.0077 mg/L

See Section 1.5.3 *Sensitivity Explained* for more information.

Calibration Standard Preparation

To perform an aluminum calibration using the aluminon method, prepare a 10.0-mg/L Al stock solution by pipetting 10 mL of a 100-mg/L Aluminum Standard Solution into a 100-mL volumetric flask using Class A glassware. Dilute to the mark with deionized water and mix thoroughly.

Prepare calibration standards containing 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, and 0.8 mg/L Al as follows:

- a. Into eight different 100-mL Class A volumetric flasks, pipet 1, 2, 3, 4, 5, 6, 7 and 8 mL of the 10.0-mg/L Al stock solution using Class A glassware.
- b. Dilute to the mark with deionized water. Mix thoroughly.

- c. Using the aluminon method and the calibration procedure described in the *User-Entered Programs* section of the *DR/4000 Spectrophotometer Instrument Manual*, generate a calibration curve from the standards prepared above.

Summary of Method

Aluminon indicator combines with aluminum in the sample to form a red-orange color. The intensity of color is proportional to the aluminum concentration. Ascorbic acid is added to remove iron interference. The AluVer 3 Aluminum Reagent, packaged in powder form, shows exceptional stability and is applicable for fresh water samples.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to Section 3.

Pollution Prevention and Waste Management

For information on pollution prevention and waste management, refer to Section 3.

REQUIRED REAGENTS AND STANDARDS

	Cat. No.
Aluminum Reagent Set (100 Tests)	22420-00
Includes: (4) 14290-99, (1) 14577-99, (1) 14294-49	

Description	Quantity Required		Unit	Cat. No.
	per test			
AluVer 3 Aluminum Reagent Powder Pillows	1 pillow	100	/pkg	14290-99
Ascorbic Acid Powder Pillows	1 pillow	100	/pkg	14577-99
Bleaching 3 Reagent Powder Pillows	1 pillow	100	/pkg	14294-49

REQUIRED EQUIPMENT AND SUPPLIES

Cylinder, graduated mixing, 50 mL, with glass stopper	1	each	1896-41
DR/4000 1-inch Cell Adapter	1	each	48190-00

OPTIONAL REAGENTS AND STANDARDS

Aluminum Standard Solution, 100 mg/L	100 mL	14174-42
Aluminum Standard Solution, 10 mL Voluette Ampule, 50 mg/L as Al, 10 mL	16/pkg	14792-10
Hydrochloric Acid Solution, 6.0 N (1:1)	500 mL	884-49
m-Nitrophenol Indicator Solution, 10 g/L, pH 7.0–8.4	100 mL MDB	2476-32
Nitric Acid, ACS	500 mL	152-49
Nitric Acid Solution, 1:1	500 mL	2540-49
Sodium Hydroxide Standard Solution, 5.0 N	100 mL MDB	2450-32
Sodium Hydroxide Standard Solution, 5.0 N	59 mL DB	2450-26
Sulfuric Acid Standard Solution, 5.25 N	100 mL MDB	2449-32
Water, deionized	4 liters	272-56

OPTIONAL EQUIPMENT AND SUPPLIES

Description	Unit	Cat. No.
Brush, test tube	each.....	690-00
DR/4000 Carousel Module Kit	each.....	48070-02
DR/4000 Flow Cell Module Kit, 1-inch	each.....	48070-04
DR/4000 Flow Cell Module Kit, 1-cm	each.....	48070-05
DR/4000 Sipper Module Kit, 1-cm	each.....	48090-06
Flask, volumetric, 250-mL, with stopper	each.....	547-46
Flask, volumetric, Class A, 100-mL	each.....	14574-42
Flask, volumetric, Class A, 100-mL	6/pkg.....	14574-72
pH Indicator Paper, 1.0 to 11.0 pH.....	5 rolls/pkg.....	391-33
pH Meter, <i>sens^{ion}</i> TM 1, portable	each.....	51700-00
Pipet Filler, safety bulb.....	each.....	14651-00
Pipet, serological, 2-mL	each.....	532-36
Pipet, TenSette, 0.1 to 1.0 mL	each.....	19700-01
Pipet Tips, for 19700-01 TenSette Pipet	50/pkg.....	21856-96
Pipet, volumetric, Class A, 1.00-mL	each.....	14515-35
Pipet, volumetric, Class A, 2.00-mL	each.....	14515-36
Pipet, volumetric, Class A, 3.00-mL	each.....	14515-03
Pipet, volumetric, Class A, 4.00-mL	each.....	14515-04
Pipet, volumetric, Class A, 5.00-mL	each.....	14515-37
Pipet, volumetric, Class A, 6.00-mL	each.....	14515-06
Pipet, volumetric, Class A, 7.00-mL	each.....	14515-07
Pipet, volumetric, Class A, 8.00-mL	each.....	14515-08
Pipet, volumetric, Class A, 10.00-mL	each.....	14515-38
Thermometer, pocket, -10 to 110 °C	each.....	1877-01



FOR TECHNICAL ASSISTANCE, PRICE INFORMATION AND ORDERING:

In the U.S.A. – Call toll-free 800-227-4224

Outside the U.S.A. – Contact the HACH office or distributor serving you.

On the Worldwide Web – www.hach.com; E-mail – techhelp@hach.com

HACH COMPANY
WORLD HEADQUARTERS
Telephone: (970) 669-3050
FAX: (970) 669-2932



Method 8326

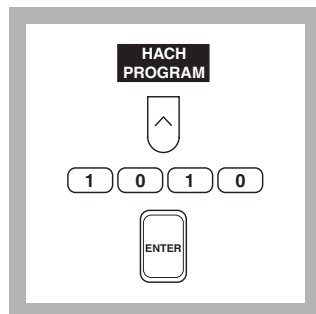
Eriochrome Cyanine R Method*

Powder Pillows

(0 to 0.250 mg/L Al³⁺)

Scope and Application: For water. The estimated detection limit for program number 1010 is 0.002 mg/L Al³⁺.

* Adapted from *Standard Methods for the Examination of Water and Wastewater*



- 1.** Press the soft key under **HACH PROGRAM**.

Select the stored program for aluminum (Al), ECR by pressing **1010** with the numeric keys.

Press: **ENTER**

Note: If samples cannot be analyzed immediately, see following these steps. Adjust the pH of preserved samples before analysis.

Note: The Flow Cell and Sipper Modules cannot be used for this procedure.

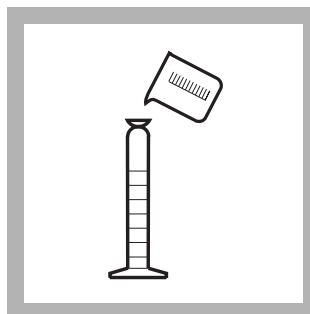


- 2.** The display will show:

**HACH PROGRAM:
1010 Aluminum, ECR**

The wavelength (λ), **535 nm**, is automatically selected.

Note: For best results, determine a reagent blank for each new lot of reagent as follows. Prepare a reagent blank by repeating steps 3 through 12, using deionized water as the sample. Zero the instrument on deionized water by pressing the soft key under **ZERO**. Insert the reagent blank and the blank value will be displayed. Correct for the reagent blank by pressing the soft keys under **OPTIONS, (MORE)**, and then **BLANK:OFF**. Enter the reagent blank value and press **ENTER**. Repeat for each new lot of reagent.

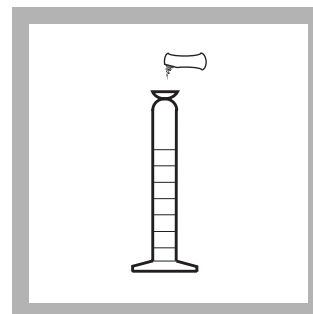


- 3.** Fill a 25-mL graduated mixing cylinder to the 20-mL mark with sample.

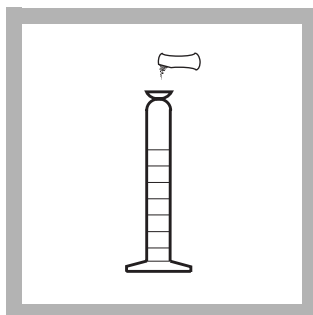
Note: Rinse cylinder with 1:1 hydrochloric acid and deionized water before use to avoid errors due to contaminants absorbed on the glass.

Note: The sample temperature must be between 20-25 °C (68-77 °F) for accurate results.

Note: For proof of accuracy, use a 0.1 mg/L aluminum standard solution (preparation given in the Accuracy Check section) in place of the sample.

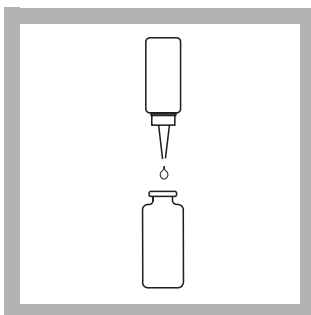


- 4.** Add the contents of one ECR Reagent Powder Pillow for 20-mL sample size. Stopper. Invert several times to dissolve powder. Then press the soft key under **START TIMER**. A 30-second reaction period will begin.



5. Add the contents of one Hexamethylenetetramine Buffer Reagent Powder Pillow for 20-mL sample size. Stopper. Invert repeatedly until the powder is dissolved.

Note: A red-orange color will develop if aluminum is present.



6. Put 1 drop of ECR Masking Reagent Solution into a clean sample cell. This will become the blank.

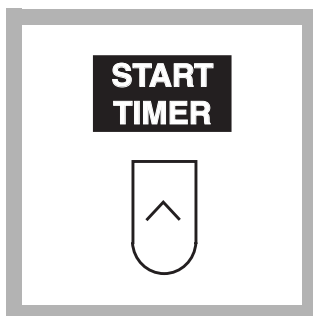


7. Pour 10 mL from the mixing cylinder into the sample cell with the ECR Masking Reagent (the blank). Swirl to mix.

Note: The solution will start to turn yellow.



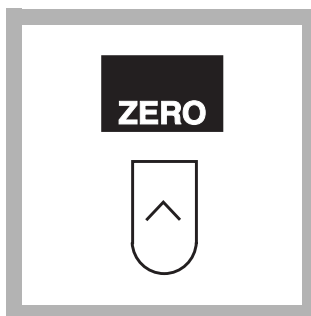
8. Fill a second sample cell to the 10-mL mark with the remaining solution in the cylinder (the prepared sample).



9. Press the soft key under **START TIMER**. A 5-minute reaction period will begin.



10. Within five minutes after the timer beeps, place the blank in the cell holder. Close the light shield.



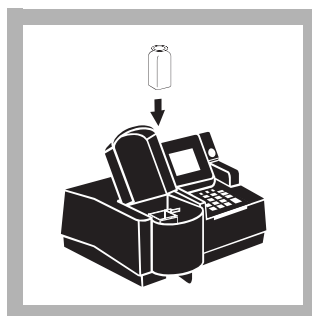
11. Press the softkey under **ZERO**.

The display will show:

0.000 mg/L Al³⁺

Note: If you are using a reagent blank correction, the display will show the correction.

Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the softkey under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



12. Immediately place the prepared sample into the cell holder. Close the light shield. Results in mg/L aluminum (or chosen units) will be displayed.

Note: If fluoride is present, it needs to be measured and the actual aluminum value determined from Table 2.

Note: The results can be expressed as mg/L aluminum oxide (Al₂O₃). Press the soft key under **OPTIONS**, then under **FORM**: to scroll through the available options. Press **ENTER** to return to the read screen.

Interferences

Table 1 Interfering Substances and Suggested Treatments

Interfering Substance	Interference Levels and Treatments
Acidity	Greater than 62 mg/L as CaCO_2
Alkalinity	Greater than 750 mg/L as CaCO_3
Ca^{2+}	Greater than 1000 mg/L as CaCO_3
Cl^-	Greater than 1000 mg/L
Cr^{6+}	0.2 mg/L (error is -5% of reading)
Cu^{2+}	2 mg/L (error is -5% of reading)
Fe^{2+}	Greater than 4 mg/L (error is positive and = mg/L Fe^{2+} x 0.0075)
Fe^{3+}	Greater than 4 mg/L (error is positive and = mg/L Fe^{3+} x 0.0075)
F^-	See Table 2.
Hexameta-phosphate	0.1 mg/L as PO_4^{3-} (error is -5% of reading)
Mg^{2+}	Greater than 1000 mg/L as CaCO_3
Mn^{2+}	Greater than 10 mg/L
NO_2^-	Greater than 5 mg/L
NO_3^-	Greater than 20 mg/L
pH	2.9–4.9 or 7.5–11.5. A sample pH between about 4.9 and 7.5 causes dissolved aluminum to partially convert to colloidal and insoluble forms. This method measures much of that hard-to-detect aluminum without any pH adjusting pretreatment as is necessary in some other methods.
PO_4^{3-} (ortho)	4 mg/L (error is -5% of reading)
Polyphosphate	See procedure below.
SO_4^{2-}	Greater than 1000 mg/L
Zn^{2+}	Greater than 10 mg/L

Polyphosphate interference can be reduced by converting polyphosphate to orthophosphate by the following steps:

- a. Rinse a 50-mL graduated mixing cylinder and a 125-mL erlenmeyer flask containing a magnetic stir bar with 6 N hydrochloric acid. Rinse again with deionized water. This will remove any aluminum present.

Note: Rinse two Erlenmeyer flasks if a reagent blank is used; see Step b below.

- b. Measure 50 mL deionized water into the 125-mL erlenmeyer flask using the graduated cylinder. This is the reagent blank. Because of the test sensitivity, this step must be done only when any of the reagents used in the following pretreatment are replaced even if the new reagent has a matching lot number. When the pretreated sample has been analyzed, correct for the aluminum concentration of the reagent blank by pressing the soft keys under **OPTIONS**, then **(MORE)**, then **BLANK:OFF**. Enter the reagent blank value using the numeric keys and press **ENTER**.
- c. Measure 50 mL sample into the 125-mL erlenmeyer flask using the graduated cylinder. Use a small amount of deionized water to rinse the cylinder contents into the flask.
- d. Add 4.0 mL of 5.25 N Sulfuric Acid Standard Solution.

- e. Use a combination hot plate/stirrer to boil and stir the sample for at least 30 minutes. Add deionized water as needed to maintain a sample volume of 20-40 mL. Do not boil dry.
- f. Cool the solution to near room temperature.
- g. Add 2 drops of Bromphenol Blue Indicator Solution.
- h. Add 1.5 mL of 12.0 N Potassium Hydroxide Standard Solution using the calibrated, plastic dropper provided. Swirl to mix. The solution color should be yellow or green but not purple. If the color is purple, begin with Step a again using an additional 1 mL Sulfuric Acid Standard Solution in Step d.
- i. While swirling the flask, add 1.0 N Potassium Hydroxide Solution, a drop at a time, until the solution turns a dirty green color.
- j. Pour the solution into the graduated cylinder. Rinse the flask contents into the graduated cylinder with deionized water to bring the total volume to 50 mL.
- k. Use this solution in Step 3 of the ECR method.

Fluoride interference can be corrected by using *Table 2*.

An Example:

If the fluoride concentration is known to be 1.00 mg/L F^- and the ECR method gives a DR/4000 reading of 0.060 mg/L aluminum, what is the true mg/L aluminum concentration?

Note: *Intermediate values can be found by interpolation. Do not use correction graphs or charts found in other publications.*

Answer: 0.183 mg/L

Sample Collection, Storage and Preservation

Collect samples in a clean glass or plastic containers. Preserve samples by adjusting the pH to 2 or less with concentrated nitric acid (about 1.5 mL per liter). Preserved samples can be stored up to six months at room temperature. Before analysis, adjust the pH to 2.9–4.9 with 12.0 N Potassium Hydroxide Standard Solution and/or 1 N Potassium Hydroxide Solution. Correct the test result for volume additions; see Section 1.2.2 *Correcting for Volume Additions*.

Accuracy Check

Standard Solution Method

Using Class A glassware, prepare a 0.100 mg/L aluminum standard solution by pipetting 1.00 mL of Aluminum Standard Solution, 100 mg/L as Al^{3+} , into a 1000-mL volumetric flask. Dilute to the mark with deionized water. Prepare this solution daily. Perform the aluminum procedure as described above.

Or, add 2.0 mL of solution from an Aluminum Voluette Ampule Standard Solution (50 mg/L as Al) into a 1000-mL volumetric flask. Dilute to volume with deionized water. Prepare this solution daily. Perform the aluminum procedure as described above.

To adjust the calibration curve using the reading obtained with the 0.100-mg/L standard solution, press the soft keys under **OPTIONS, MORE** then **STD:OFF**. Press

ENTER to accept the displayed concentration, the value of which depends on the selected units. If an alternate concentration is used, enter the actual concentration and press **ENTER** to return to the read screen. See Section 1.5.5 *Adjusting the Standard Curve* for more information.

**Table 2 True aluminum concentration (mg/L) vs.
DR/4000 reading (mg/L) and fluoride concentration (mg/L) for Eriochrome Cyanine R method**

DR/4000 Reading		Fluoride Concentration (mg/L)									
(mg/L)	0.00	0.20	0.40	0.60	0.80	1.00	1.20	1.40	1.60	1.80	2.00
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.010	0.010	0.019	0.030	0.040	0.052	0.068	0.081	0.094	0.105	0.117	0.131
0.020	0.020	0.032	0.046	0.061	0.077	0.099	0.117	0.137	0.152	0.173	0.193
0.030	0.030	0.045	0.061	0.077	0.098	0.124	0.146	0.166	0.188	0.214	0.243
0.040	0.040	0.058	0.076	0.093	0.120	0.147	0.174	0.192	0.222	True Aluminum Concentration (mg/L) Al	
0.050	0.050	0.068	0.087	0.109	0.135	0.165	0.188	0.217			
0.060	0.060	0.079	0.100	0.123	0.153	0.183	0.210	0.241			
0.070	0.070	0.090	0.113	0.137	0.168	0.201	0.230				
0.080	0.080	0.102	0.125	0.152	0.184	0.219					
0.090	0.090	0.113	0.138	0.166	0.200	0.237					
0.100	0.100	0.124	0.150	0.180	0.215						
0.120	0.120	0.146	0.176	0.209	0.246						
0.140	0.140	0.169	0.201	0.238							
0.160	0.160	0.191	0.226								
0.180	0.180	0.213									
0.200	0.200	0.235									
0.220	0.220										
0.240	0.240										

Method Performance

Precision

Standard: 0.100 mg/L Al³⁺

Program	95% Confidence Limits
1010	0.098-0.102 mg/L Al ³⁺

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
1010	0.002 mg/L Al ³⁺

For more information on derivation and use of Hach's estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, Appendix B, see Section 1.5.1.

Sensitivity

Program Number: 1010

Portion of Curve	Δ Abs	Δ Concentration
0.010 Abs	0.010	0.0023 mg/L
0.125 mg/L	0.010	0.0015 mg/L
0.225 mg/L	0.010	0.0015 mg/L

See Section 1.5.3 *Sensitivity Explained* for more information.

Calibration Standard Preparation

To perform an aluminum calibration using the ECR method, use a 10.0 mg/L Aluminum Standard Solution (Cat. No. 23058-42). Prepare calibration standards containing 0.01, 0.02, 0.04, 0.08, 0.12, 0.16, 0.200, 0.240 mg/L Al as follows:

- Into eight different 1000-mL Class A volumetric flasks, pipet 1.00, 2.00, 4.00, 8.00, 12.00, 16.00, 20.00 and 24.00 mL of the 10 mg/L Al stock solution using Class A glassware.
- Dilute to the mark with deionized water and mix thoroughly.
- Using the ECR method and the calibration procedure described in the *User-Entered Programs* section of the *DR/4000 Spectrophotometer Instrument Manual*, generate a calibration curve from the standards prepared above.

Summary of Method

Eriochrome Cyanine R combines with aluminum in a sample to produce an orange-red color. The intensity of color is proportional to the aluminum concentration.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to Section 1.

Pollution Prevention and Waste Management

For information on pollution prevention and waste management, refer to Section 1.

REQUIRED REAGENTS AND STANDARDS

	Cat. No.
Aluminum Reagent Set (100 Tests)	26037-00
Includes (1) 26038-49, (1) 26039-99, (1) 23801-23	

Description	Quantity Required		Cat. No.
	per test	Unit	
ECR Reagent Powder Pillows	1 pillow	100/pkg	26038-49
Hexamethylenetetramine Buffer Reagent Powder Pillows	1 pillow	100/pkg	26039-99
ECR Masking Reagent Solution	1 drops ...	25 mL SCDB	23801-23

REQUIRED EQUIPMENT AND SUPPLIES

Cylinder, 25 mL, graduated mixing, with glass stopper	1	each	1896-40
DR/4000 1-Inch Cell Adapter	1	each	48190-00

OPTIONAL REAGENTS AND STANDARDS

Description	Unit	Cat. No.
Aluminum Standard Solution, 10 mg/L	100 mL	23058-42
Aluminum Standard Solution, 100 mg/L	100 mL	14174-42
Aluminum Standard Solution, 10-mL Voluette Ampule, 50 mg/L as Al, 10 mL	16/pkg	14792-10
Aluminum Standard Solution, 2-mL Voluette Ampule, 25 mg/L as Al, 2 mL	20/pkg	25571-20
Bromphenol Blue Indicator Solution	100 mL MDB	14552-32
Hydrochloric Acid Solution, 6 N (1:1)	500 mL	884-49
Nitric Acid, ACS	500 mL	152-49
Nitric Acid Solution, 1:1	500 mL	2540-49
Potassium Hydroxide Solution, 1 N	50 mL SCDB	23144-26
Potassium Hydroxide Standard Solution, 12.0 N	100 mL	230-32
Potassium Hydroxide Standard Solution, 12.0 N	500 mL	230-49
Sulfuric Acid Standard Solution, 5.25 N	100 mL MDB	2449-32
Water, deionized	4 liters	272-56
Brush, test tube	each	690-00
DR/4000 Carousel Module Kit	each	48090-02
Flask, Erlenmeyer, 125-mL	each	505-43
Flask, volumetric, Class A, 100-mL	each	14574-42
Flask, volumetric, Class A, 1000-mL, with glass stopper	each	14574-53
Hot Plate/Stirrer, 120 V	each	23442-00
Hot Plate/Stirrer, 240 V	each	23442-02
Pad, cooling, 4 x 4 in.	each	18376-00
pH Paper, 1.0 to 11.0 pH	5 rolls/pkg	391-33
pH Meter, <i>sensio</i> TM 1, portable	each	51700-00
Pipet Filler, safety bulb	each	14651-00
Pipet, serological, 2-mL	each	532-36
Pipet, TenSette, 0.1 to 1.0 mL	each	19700-01
Pipet Tips, for 19700-01 TenSette Pipet	50/pkg	21856-96
Pipet, volumetric, Class A, 1.00-mL	each	14515-35
Pipet, volumetric, Class A, 2.00-mL	each	14515-36
Pipet, volumetric, Class A, 3.00-mL	each	14515-03
Pipet, volumetric, Class A, 4.00-mL	each	14515-04
Pipet, volumetric, Class A, 5.00-mL	each	14515-37
Pipet, volumetric, Class A, 6.00-mL	each	14515-06
Pipet, volumetric, Class A, 8.00-mL	each	14515-08
Pipet, volumetric, Class A, 20.00-mL	each	14515-20
Stir Bar, octagonal, 28.6 x 7.9 mm	each	20953-52
Thermometer, -10 to 110 °C	each	1877-01



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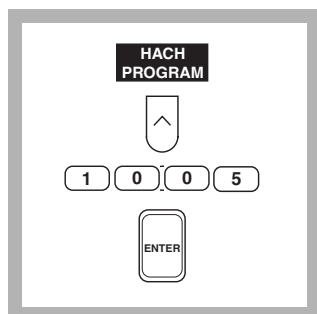


Chromazurol S Method

UniCell™ Vials

(0 to 0.50 mg/L Al³⁺)

Scope and Application: For drinking water, surface water, swimming pool water, and wastewater process control. The estimated detection limit for program number 1005 is 0.02 mg/L Al³⁺.



1. Press the soft key under **HACH PROGRAM**.

Select the stored program for UniCell Aluminum by pressing **1005** with the numeric keys.

Press: **ENTER**

Note: If samples cannot be analyzed immediately, see *Sample Collection, Storage and Preservation* following these steps. Adjust the pH of preserved samples before analysis.

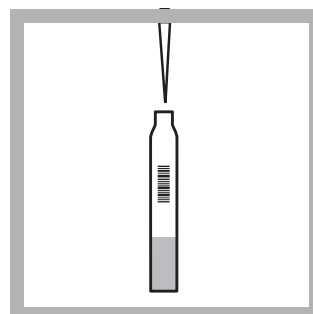


2. The display will show: **HACH PROGRAM: 1005 Aluminum, HCT 150**

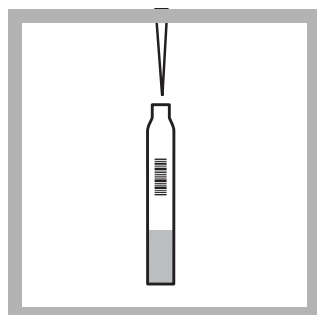
The wavelength (λ), **620 nm**, is automatically selected.



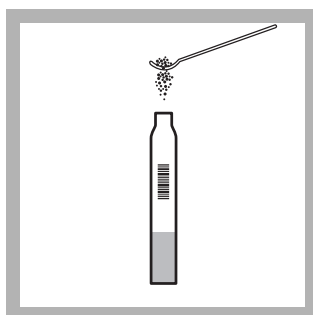
3. Insert the Test Tube Adapter into the sample cell module by sliding it under the thumb screw and into the alignment grooves. Fasten with the thumb screw.



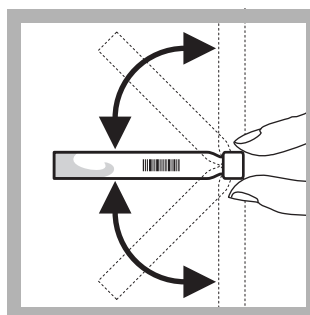
4. Pipet 2.0 mL of buffer solution A (HCT 150 A) into a sample vial.



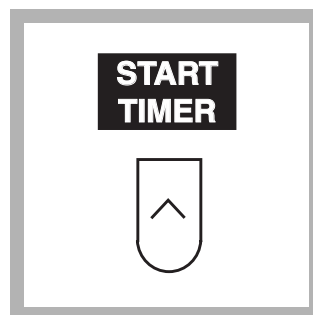
5. Pipet 3.0 mL sample into the sample vial.



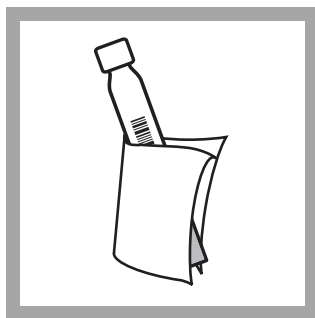
6. Use a dosing spoon to add one level spoonful of Masking Agent B (HCT 150 B) to the sample vial.



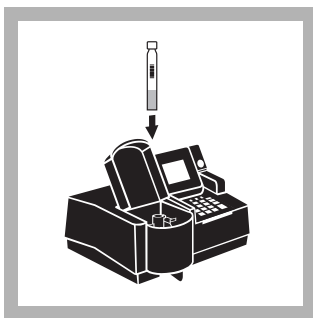
7. Cap the sample and invert repeatedly until the contents are completely dissolved.



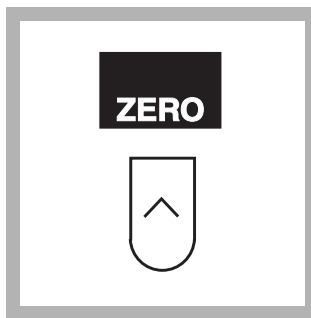
8. Press the soft key under **START TIMER**. A 25-minute reaction period will begin.



9. Wipe the zero vial (**white** cap) and the sample vial with a damp cloth followed by a dry one.



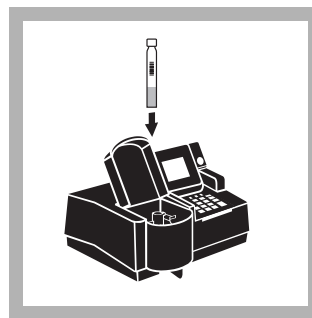
10. Place the zero vial into the cell holder. Close the light shield.



11. Press the soft key under **ZERO**.

The display will show:

0.00 mg/L Al³⁺



12. When the timer beeps, place the prepared sample into the cell holder. Close the light shield. Results in mg/L Al will be displayed.

Interference

The ions listed in the table have been individually checked up to the given concentrations. Cumulative effects and the influence of other ions have not been evaluated.

Ion	No interference above:
Mg ²⁺ , K ⁺ , Na ⁺ , NH ₄ ⁺ , Cl ⁻ , NO ₃ ⁻ , Ca ²⁺	500 mg/L
Ag ⁺ , Mn ²⁺	100 mg/L
Cd ²⁺ , Co ²⁺ , Ni ²⁺ , Sn ²⁺ , Pb ²⁺ , PO ₄ ³⁻	50 mg/L
Cu ²⁺ , Hg ²⁺	10 mg/L
Fe ²⁺ , Fe ³⁺ , Zn ²⁺ , Si ⁴⁺	5 mg/L
Cr ³⁺ , Cr ⁶⁺	0.5 mg/L
F ⁻	0.1 mg/L

Higher concentrations of heavy metals than those given, as well as fluoride, phosphate and relatively rare elements such as beryllium, thorium, titanium, zirconium, and vanadium interfere with the determination. Aluminum oxide hydrates and hydroxide are only partially determined.

Note: Sample pretreatment with the Metal Prep Set (HCT 200) will cause an interference.

Sample Collection, Storage and Preservation

Collect samples in acid-cleaned glass or plastic containers. No acid addition is necessary if analyzing the samples immediately. To preserve samples, adjust the pH to 2 or less with concentrated Nitric Acid (about 2 mL per liter). Preserved samples may be stored up to six months at room temperature.

Before analysis, adjust the pH to between 2.0 and 3.5 with 5.0 N Sodium Hydroxide Standard Solution. Do not exceed pH 3.5 or aluminum may precipitate or complex. The temperature of the water sample and the sample vial should be 18–22 °C (64–72 °F).

Accuracy Check

Standard Additions Method

- a. Select standard additions mode by pressing the soft keys under **OPTIONS, (MORE)** and then **STD ADD**.
- b. Press **ENTER** to accept the default sample volume (mL), 100.
- c. Press **ENTER** to accept the default standard concentration (mg/L), 250.
- d. Press the soft key under **ENTRY DONE**.
- e. Measure 250 mL of sample into three mixing cylinders.
- f. Using a pipette, add 0.10, 0.20, and 0.30 mL of 100-mg/L Aluminum Standard Solution to the three samples. Mix well.
- g. Analyze each standard addition sample as described above. Accept the standard additions reading by pressing the soft key under **READ** each time. The Al concentration should increase 0.04 mg/L for each 0.10 mL of standard added.
- h. After completing the sequence, the display will show the extrapolated concentration value and the “best-fit” line through the standard additions data points, accounting for matrix interferences.

See Section 1.4.1 *Standard Additions* for more information.

Standard Solution Method

Prepare a 0.30-mg/L Al standard solution by pipetting 0.30 mL of 100-mg/L Al Standard Solution into a 100-mL volumetric flask. Dilute to the mark with deionized water. Stopper and invert to mix. Prepare this solution daily. Analyze the sample as described above.

To adjust the calibration curve using the reading obtained with the 0.30-mg/L Al standard solution, press the soft keys under **OPTIONS, (MORE)** and then **STD:OFF**. Press **ENTER** to accept the default concentration, the value of which will depend on the selected units. If an alternate concentration is used, enter the actual concentration and press **ENTER** to return to the read screen. See Section 1.5.5 *Adjusting the Standard Curve* for more information.

Method Performance

Precision

Standard: 0.30 mg/L Al³⁺

Program	95% Confidence Limits
1005	0.25–0.35 mg/L Al ³⁺

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
1005	0.02 mg/L Al ³⁺

For more information on derivation and use of Hach's estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, appendix B, see Section 1.5.1.

Sensitivity

Program Number: 1005

Portion of Curve	Δ Abs	Δ Concentration
Entire Range	0.010	0.004 mg/L

See Section 1.5.3 *Sensitivity Explained* for more information.

Summary of Method

Chromazurol S forms a green complex with aluminum in a slightly acidic acetate buffer.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to Section 1.

REQUIRED REAGENTS AND STANDARDS

Description	Unit	Cat. No.
Aluminum - Al, UniCell™ HCT 150.....	23/pkg.....	HCT 150

OPTIONAL REAGENTS

Aluminum Standard Solution, 100-mg/L Al.....	100 mL.....	14174-42
Sodium Hydroxide Standard Solution, (5.0 N).....	1000 mL.....	2450-53
Nitric Acid, concentrated	500 mL.....	152-49

OPTIONAL EQUIPMENT AND SUPPLIES

Graduated cylinder, mixing 250-mL	each.....	20886-46
Flask, volumetric 100-mL	each.....	14574-42
Pipettor, (Jencons) 1–5 mL	each.....	27951-00
Replacement tips for 27951-00	pkg/100.....	27952-00
Pipettor, (Jencons) 100-1000 μ L	1 each.....	27949-00
Replacement tips for 27949-00	pkg/400.....	27950-00
pH Paper	pkg/100.....	26013-00



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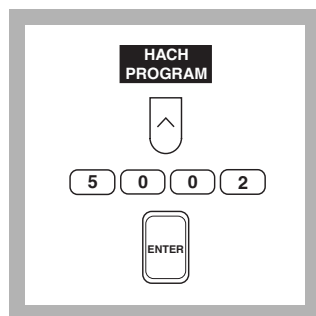


Salicylate Method

UniCell™ Vials

LR (0.00 to 1.50 mg/L NH_4^+)

Scope and Application: For surface water, wastewater, swimming pool water, and process control.
The estimated detection limit for program number 5002 is 0.04 mg/L NH_4^+-N .



- 1.** Press the soft key under **HACH PROGRAM**.

Select the stored program number for UniCell Ammonium by pressing **5002** with the numeric keys.

Press: **ENTER**

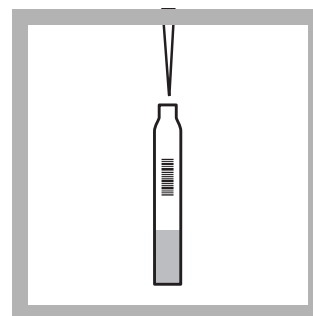
Note: If samples cannot be analyzed immediately, see Sample Collection, Storage, and Preservation following these steps. Adjust the pH of preserved samples before analysis.



- 2.** The display will show:
HACH PROGRAM: 5002
Ammonium, HCT 100
The wavelength (λ), **694 nm**, is automatically selected.

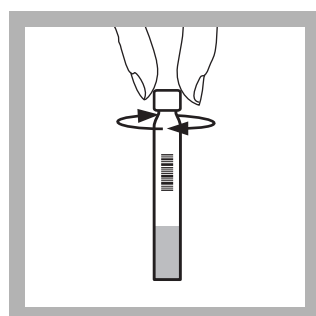


- 3.** Insert the Test Tube Adapter into the sample cell module by sliding it under the thumb screw and into the alignment grooves. Fasten with the thumb screw.



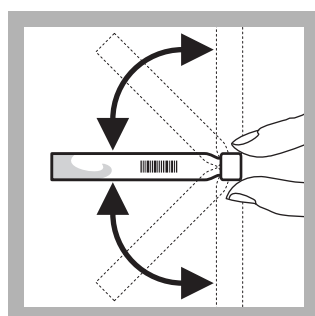
- 4.** Remove the cap from a sample vial. Add 5.0 mL of sample to the vial.

Note: For non-preserved samples with extreme pH, see the Sample Collection, Storage, and Preservation section.

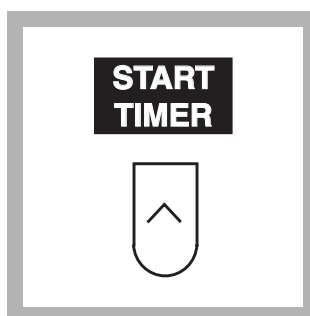


- 5.** Immediately screw a **light green** UniCap A (HCT 100-104 A) onto the sample vial.

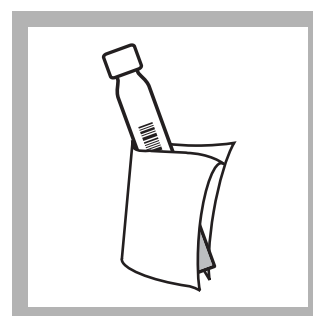
Note: Close the UniCap bottle **immediately** after use.



- 6.** Invert the sample vial repeatedly until the reagent in the cap is dissolved.



- 7.** Press the soft key under **START TIMER**. A 15-minute reaction period will begin.



- 8.** When the timer beeps, clean the outside of the zero (white cap) and sample vials with a towel, and place the zero vial into the cell holder. Close the light shield.

Note: Wiping with a damp cloth followed by a dry one removes fingerprints and other marks.



9. Press the soft key under **ZERO**.

The display will show:

0.00 mg/L NH₄⁺

Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



10. Place the prepared sample into the cell holder and close the light shield. The result in mg/L ammonium will be displayed.

Note: The results can also be expressed as ammonium nitrogen (NH₄⁺-N). Press the soft keys under **METHOD OPTIONS**, then **FORM**: to scroll through the available options. Press **ENTER** to return to the read screen.

Interferences

The ions listed in the following table have been individually tested up to the given concentrations. Cumulative effects and the influence of other ions have not been evaluated.

Ion	No interference up to:
Cl ⁻ , SO ₄ ²⁻	1000 mg/L
K ⁺ , Na ⁺ , Ca ²⁺	500 mg/L
CO ₃ ²⁻ , NO ₃ ⁻ , Fe ³⁺ , Cr ³⁺ , Cr ⁶⁺ , Zn ²⁺ , Cu ²⁺ , Co ²⁺ , Ni ²⁺ , Hg ²⁺	50 mg/L
Fe ²⁺	25 mg/L
Sn ²⁺	10 mg/L
Pb ²⁺	5 mg/L
Ag ⁺	2 mg/L

Primary amines and all reducing agents can cause high and low bias, respectively. Excess urea does not interfere.

Note: Excess ammonium can cause inaccurate readings. Verify results by performing an Accuracy Check.

Sample Collection, Storage, and Preservation

Collect samples in clean plastic or glass bottles. Analyze within 3 hours after collection for best results. For longer storage periods, add 1 mL of concentrated sulfuric acid per liter of sample and store at 4 °C.

Before testing the stored sample, warm to room temperature and adjust the pH to between 4–9 with 5.0 N Sodium Hydroxide Standard Solution.

Do not use mercury compounds as preservatives.

Correct for volume additions by dividing the total final volume (acid + base + sample) by the initial sample volume and multiplying the test result by this factor. See Section 1.2.2 *Correcting for Volume Additions* for more information.

Accuracy Check

Standard Additions Method

- a. Leave the unspiked sample in the sample compartment. Verify that the units displayed are in mg/L. Select standard additions mode by pressing the soft keys under **OPTIONS, (MORE)** and then **STD ADD**.
- b. Press **ENTER** to accept the default sample volume (mL), 100.00.
- c. Use the numeric keys to enter the standard concentration, 128.8 mg/L NH_4^+ .
Note: This is equivalent to 100-mg/L $\text{NH}_4^+\text{-N}$.
- d. Press the soft key under **ENTRY DONE**.
- e. Use a pipet to add 0.2, 0.4, and 0.6 mL of 100-mg/L $\text{NH}_4^+\text{-N}$ (128.8-mg/L NH_4^+) standard, respectively, to three 100-mL samples in 100-mL mixing cylinders. Mix each sample thoroughly.
- f. Analyze each standard addition sample as described above. Accept the standard additions reading by pressing the soft key under **READ** each time. Each addition should reflect approximately 100% recovery.
- g. After completing the sequence, the display will show the extrapolated concentration value and the “best-fit” line through the standard additions data points, accounting for matrix interferences.
- h. See Section 1.4.1 *Standard Additions* for more information.

Standard Solution Method

Prepare a 1 mg/L $\text{NH}_4\text{-N}$ standard solution by pipetting 1 mL of 100-mg/L $\text{NH}_4\text{-N}$ Standard Solution into a 100-mL volumetric flask. Dilute to the mark with deionized water. Stopper and invert to mix. Prepare this solution daily. Perform the ammonia procedure as described.

Method Performance

Precision

Standard: 1.00 mg/L $\text{NH}_4^+\text{-N}$

Program	95% Confidence Limits
5002	0.76–1.24 mg/L $\text{NH}_4^+\text{-N}$

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
5002	0.04 mg/L $\text{NH}_4^+\text{-N}$

AMMONIUM, continued

For more information on derivation and use of Hach's estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by 40 CFR part 136, appendix B, see Section 1.5.1.

Sensitivity

Program Number: 5002

Portion of Curve	Δ Abs	Δ Concentration
Entire Range	0.010	0.01 mg/L NH_4^+-N

See Section 1.5.3 *Sensitivity Explained* for more information.

Summary of Method

Ammonium ions react at pH 12.6 with hypochlorite ions and salicylate ions in the presence of sodium nitroferricyanide to form indophenol blue.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to Section 1.

UniCap –(HCT 100-104 A) contains sodium dichloroisocyanurate and sodium nitroferricyanide.

Pollution Prevention and Waste Management

The ammonia salicylate reagent contains sodium nitroferricyanide. Cyanide solutions are regulated as hazardous wastes by the Federal RCRA. Collect cyanide solutions for disposal as reactive (D001) waste. Be sure cyanide solutions are stored in a caustic solution with pH >11 to prevent release of hydrogen cyanide gas. See Section 1 for further information on proper disposal of these materials.

REQUIRED REAGENTS AND STANDARDS

	Unit	Cat. No.
Ammonium NH_4-N UniCell™ HCT 100.....	23/pkg.....	HCT 100

OPTIONAL REAGENTS AND STANDARDS

Ammonium as N Standard Solution, 100-mg/L as NH_3-N	500 mL.....	24065-49
Sodium Hydroxide Standard Solution, 5.0 N.....	50 mL SCDB.....	2450-26
Sulfuric Acid, ACS.....	500 mL.....	979-49

OPTIONAL EQUIPMENT AND SUPPLIES

Cylinder, graduated, 100-mL	each.....	20886-42
Flask, volumetric, 1000-mL	each.....	14574-53
Pipettor, Jencon, 1–5 mL1.....	each.....	27951-00
Replacement tips for 27951-00	100/pkg.....	27952-00



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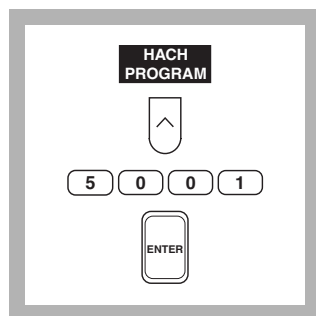


Salicylate Method

UniCell™ Vials

HR (0.0 to 45.0 mg/L NH_4^+)

Scope and Application: For surface water, wastewater, swimming pool water, and process control.
The estimated detection limit for program number 5001 is 1.2 mg/L NH_4^+-N .

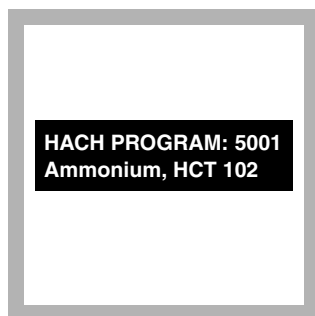


- 1.** Press the soft key under **HACH PROGRAM**.

Select the stored program number for UniCell Ammonium by pressing **5001** with the numeric keys.

Press: **ENTER**

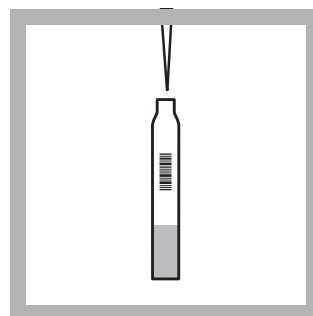
Note: If samples cannot be analyzed immediately, see Sample Collection, Storage, and Preservation following these steps. Adjust the pH of preserved samples before analysis.



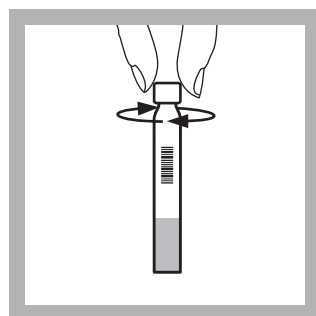
- 2.** The display will show:
HACH PROGRAM:
5001
Ammonium, HCT 102
The wavelength (λ), **694 nm**, is automatically selected.



- 3.** Insert the Test Tube Adapter into the sample cell module by sliding it under the thumb screw and into the alignment grooves. Fasten with the thumb screw.

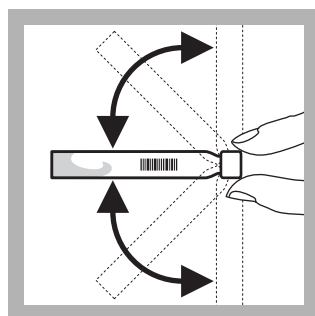


- 4.** Remove the cap from a sample vial. Add 0.2 mL of sample to the vial.
Note: For non-preserved samples with extreme pH, see the Sample Collection, Storage, and Preservation section.

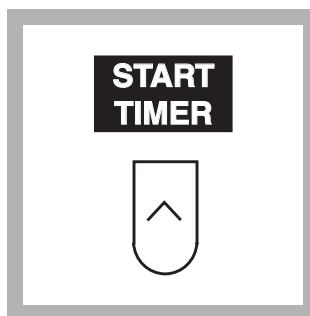


- 5.** Immediately screw a light green UniCap A (HCT 100-104 A) onto the sample vial.

Note: Close the UniCap bottle **immediately** after use.



- 6.** Invert the sample vial repeatedly until the reagent in the cap is dissolved.



- 7.** Press the soft key under **START TIMER**. A 15-minute reaction period will begin.



- 8.** When the timer beeps, clean the outside of the zero (white cap) and sample vials with a towel, and place the zero vial into the cell holder. Close the light shield.

Note: Wiping with a damp cloth followed by a dry one removes fingerprints and other marks.



9. Press the soft key under **ZERO**.

The display will show:

0.0 mg/L NH₄⁺

Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



10. Place the prepared sample into the cell holder and close the light shield. The result in mg/L ammonium will be displayed.

Note: The results can also be expressed as ammonium nitrogen (NH₄⁺-N). Press the soft keys under **METHOD OPTIONS**, then **FORM**: to scroll through the available options. Press **ENTER** to return to the read screen.

Interferences

The ions listed in the following table have been individually tested up to the given concentrations. Cumulative effects and the influence of other ions have not been evaluated.

Ion	No interference up to:
Cl ⁻ , SO ₄ ²⁻	1000 mg/L
K ⁺ , Na ⁺ , Ca ²⁺	500 mg/L
CO ₃ ²⁻ , NO ₃ ⁻ , Fe ³⁺ , Cr ³⁺ , Cr ⁶⁺ , Zn ²⁺ , Cu ²⁺ , Co ²⁺ , Ni ²⁺ , Hg ²⁺	50 mg/L
Fe ²⁺	25 mg/L
Sn ²⁺	10 mg/L
Pb ²⁺	5 mg/L
Ag ⁺	2 mg/L

Primary amines and all reducing agents can cause high and low bias, respectively. Excess urea does not interfere.

Note: Excess ammonium can cause inaccurate readings. Verify results by performing an Accuracy Check.

Sample Collection, Storage, and Preservation

Analyze samples within 3 hours after collection for best results. Store in a cool place in clean plastic or glass bottles. Preserve samples for longer periods by adding 1 mL of concentrated sulfuric acid per liter and store at 4 °C.

Warm samples to room temperature and adjust the pH to 4–9 with 5.0 N Sodium Hydroxide before analysis. Correct the test result for volume additions by dividing the total final volume (acid + base + sample) by the initial sample volume and multiplying the result by this factor.

Accuracy Check

Standard Additions Method

- a. Leave the unspiked sample in the sample compartment. Verify that the units displayed are in mg/L. Select standard additions mode by pressing the soft keys under **OPTIONS, (MORE)** and then **STD ADD**.
- b. Press **ENTER** to accept the default sample volume (mL), 100.
- c. Use the numeric keys to enter the standard concentration, 1287.8 mg/L NH_4^+ .
Note: This is equivalent to 1000 mg/L NH_4^+-N .
- d. Press the soft key under **ENTRY DONE**.
- e. Use the pipet to add 0.2mL, 0.4 mL, and 0.6 mL of 1000-mg/L NH_4^+-N (1287.8 mg/L NH_4^+) standard, respectively, to three 100-mL samples in 100-mL mixing cylinders. Mix each thoroughly.
- f. Analyze each standard addition sample as described above. Accept the standard additions reading by pressing the soft key under **READ** each time. Each addition should reflect approximately 100% recovery.
- g. After completing the sequence, the display will show the extrapolated concentration value and the “best-fit” line through the standard additions data points, accounting for matrix interferences.
- h. See Section 1.4.1 *Standard Additions* for more information.

Standard Solution Method

Prepare a 25 mg/L NH_4^+-N standard solution by pipetting 25 mL of 1000-mg/L NH_4^+-N into a 1000-mL volumetric flask. Dilute to the mark with deionized water. Stopper and invert to mix. Prepare this solution daily. Perform the ammonia procedure as described.

Method Performance

Precision

Standard: 25 mg/L NH_4^+-N

Program	95% Confidence Limits
5001	21.4–28.6 mg/L NH_4^+-N

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
5001	1.2 mg/L NH_4^+-N

For more information on derivation and use of Hach’s estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by 40 CFR part 136, appendix B, see Section 1.5.1.

AMMONIUM, continued

Sensitivity

Program Number: 5001

Portion of Curve	Δ Abs	Δ Concentration
Entire Range	0.010	0.1 mg/L NH_4^+-N

See Section 1.5.3 *Sensitivity Explained* for more information.

Summary of Method

Ammonium ions react with hypochlorite ions and salicylate ions in the presence of sodium nitroferricyanide to form indophenol blue.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to Section 1.

UniCap –(HCT 100-104 A) contains sodium dichloroisocyanurate and sodium nitroferricyanide.

Pollution Prevention and Waste Management

The ammonia salicylate reagent contains sodium nitroferricyanide. Cyanide solutions are regulated as hazardous wastes by the Federal RCRA. Collect cyanide solutions for disposal as reactive (D001) waste. Be sure cyanide solutions are stored in a caustic solution with pH >11 to prevent release of hydrogen cyanide gas. See Section 1 for further information in proper disposal of these materials.

REQUIRED REAGENTS AND STANDARDS

Description	Unit	Cat. No.
Ammonium NH_4^+-N UniCell™ HCT 102.....	23/pkg	HCT 102

OPTIONAL REAGENTS AND STANDARDS

Description	Quantity Required per test	Unit	Cat. No.
Ammonium as N, standard solution, 1000-mg/L as NH_3-N	1	L	23541-53
Sodium Hydroxide Standard Solution, 5.0 N.....	50 mL	SCDB	2450-26

OPTIONAL EQUIPMENT AND SUPPLIES

Cylinder, graduated, 100-mL	3	each	20886-42
Flask, volumetric, 1000-mL	1	each	14574-53
Pipettor, Jencon, 100–1000 μL	1	each	27949-00
Replacement tips for 27949-00	400	/pkg	27950-00
Pipettor, Jencon, 1–5 mL.....	1	each	27951-00
Replacement tips for 27951-00	100	/pkg	27952-00



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HACH COMPANY
WORLD HEADQUARTERS
Telephone: (970) 669-3050
FAX: (970) 669-2932



✓ Method 8013

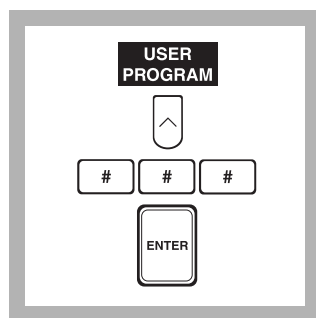
Silver Diethyldithiocarbamate Method*

(0 to 0.200 mg/L)

Scope and Application: For water, wastewater and seawater; distillation is required; USEPA accepted for reporting for drinking and wastewater analysis (digestion required).** See Section 2 for digestion procedure.

* Adapted from Standard Methods for the Examination of Water and Wastewater

** Procedure is equivalent to USEPA method 206.4 for wastewater and Standard Method 3500-As for drinking water analyses.



1. This procedure requires a user-entered calibration for each new lot of arsenic absorber solution. See *Calibration Standard Preparation* section. Press the soft key under **USER PROGRAM**. Select the stored program for arsenic (As) using the numeric keys.

Press: **ENTER**

Note: If samples cannot be analyzed immediately, see *Sample Collection, Storage and Preservation* following these steps.

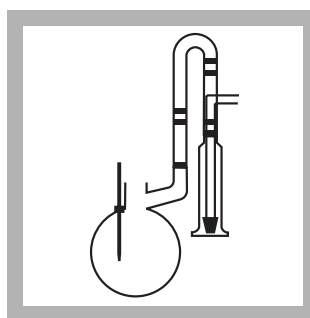
Note: The Flow Cell and Sipper Modules cannot be used with this procedure.



2. The display will show:
HACH PROGRAM: ###
Arsenic As

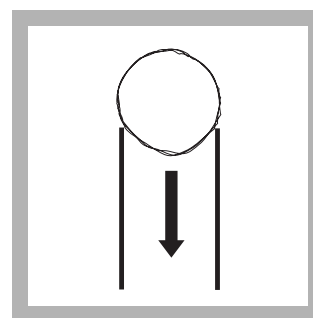
The wavelength (λ), **520 nm**, is automatically selected.

Note: ### refers to the number assigned during calibration.

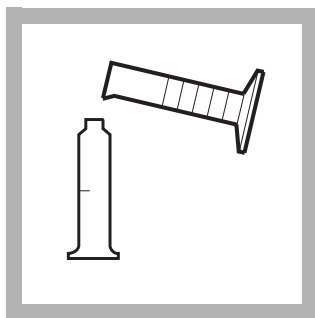


3. Prepare the Hach Distillation Apparatus for arsenic recovery. Place it under a fume hood to vent toxic fumes.

Note: See the Hach Distillation Manual for assembly instructions.

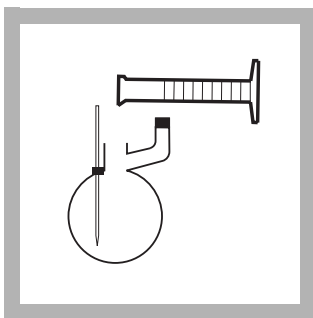


4. Dampen a cotton ball with 10% Lead Acetate Solution. Place it in the gas scrubber. Be certain the cotton seals against the glass.

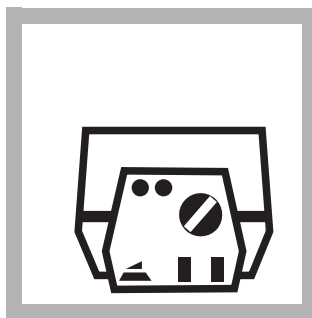


5. Measure 25 mL of prepared arsenic absorber solution into the cylinder/gas bubbler assembly with a graduated cylinder. Attach it to the distillation apparatus.

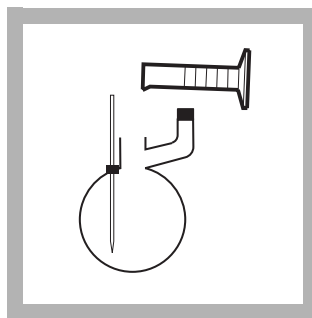
Note: Prepare the arsenic absorber solution as directed under Reagent Preparation below.



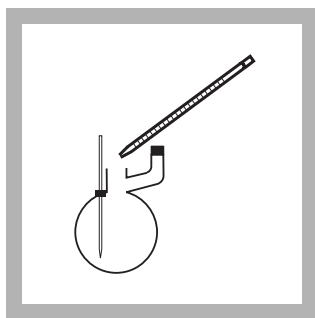
6. Measure 250 mL of sample into the distillation flask using a graduated cylinder.



7. Turn on the power switch. Set the stir control to 5. Set the heat control to 0.

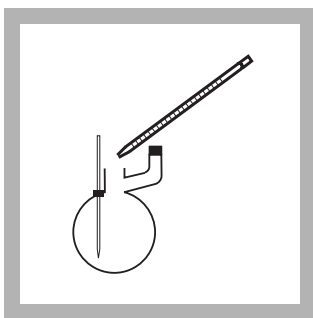


8. Add 25 mL of Hydrochloric Acid, ACS, to the distillation flask using a graduated cylinder.



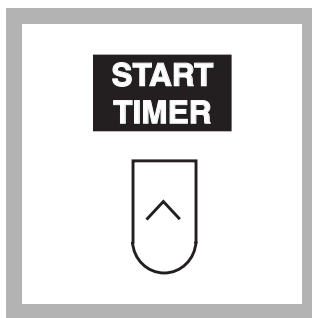
9. Add 1 mL of Stannous Chloride Solution to the flask.

Note: Use a serological pipet to measure the solution.

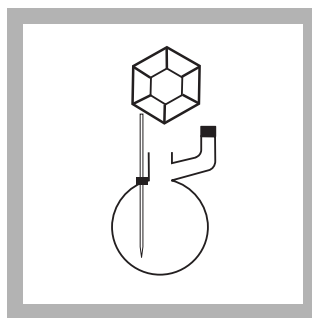


10. Add 3 mL of Potassium Iodide Solution to the flask. Cap.

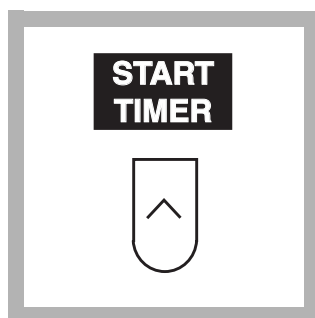
Note: Use a serological pipet to measure the solution.



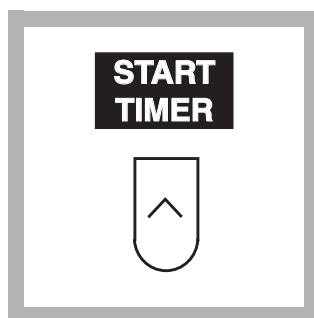
11. Press the soft key under **START TIMER**. A 15-minute reaction period will begin.



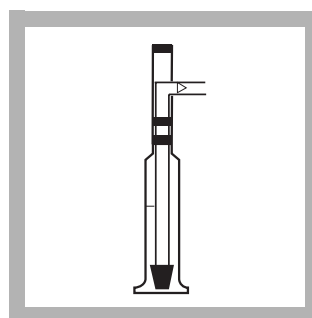
12. When the timer beeps, add 6.0 g of 20-mesh zinc to the flask. Cap immediately.



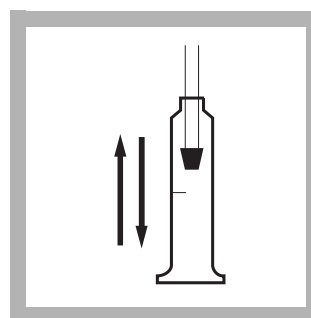
13. Set the heat control to 3.
Press the soft key under **START TIMER**.
A second 15-minute reaction period will begin.



14. When the timer beeps, set the heat control to 1.
Press the soft key under **START TIMER**.
A third 15-minute reaction period will begin.



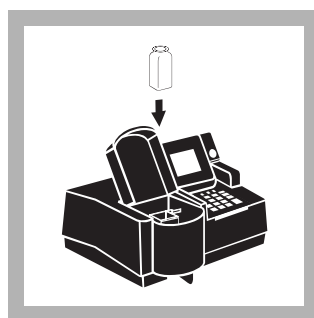
15. When the timer beeps, turn off the heater. Remove the cylinder/gas bubbler assembly as a unit.



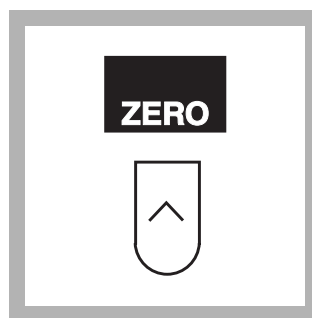
16. Rinse the gas bubbler by moving it up and down in the arsenic absorber solution.



17. Fill a dry sample cell with unreacted arsenic absorber solution (the blank). Stopper. Place it into the cell holder.



18. Place the blank into the cell holder. Close the light shield.

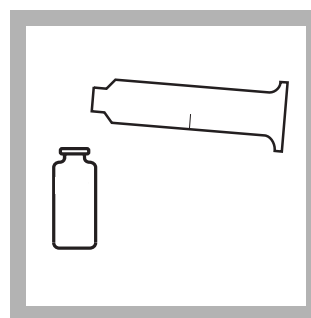


19. Press the soft key under **ZERO**.

The display will show:

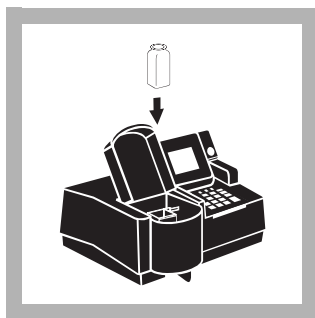
0.000 mg/L As

Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen. Some units may not be applicable for this calibration even though they appear as options.



20. Pour the reacted arsenic absorber sample into a sample cell. Stopper.

Note: If the solution volume is less than 25 mL, add pyridine to bring the volume to exactly 25 mL. Swirl to mix.



21. Place the reacted sample into the cell holder. Close the light shield. Results in mg/L arsenic (or chosen units) will be displayed.

Note: See *Pollution Prevention and Waste Management* following these steps about proper disposal of arsenic solutions.

Interferences

Table 1 Interfering Substances and Suggested Treatments

Interfering Substance	Interference Levels and Treatments
Antimony Salts	May interfere with color development

Sample Collection, Storage and Preservation

Collect samples in acid washed glass or plastic bottles. Adjust the pH to 2 or less with sulfuric acid (about 2 mL per liter). Preserved samples may be stored up to six months at room temperature. Correct the test result for volume additions; see Section 1.2.2 *Correcting for Volume Additions*.

Reagent Preparation

Prepare the arsenic absorber solution as follows:

1. Weigh 1.00 g of silver diethyldithiocarbamate on an analytical balance.
2. Transfer the powder to a 200-mL volumetric flask. Dilute to volume with pyridine. You must use pyridine only in a fume hood and wear chemical resistant gloves. Read the MSDS before using pyridine.
3. Mix well to dissolve. Store the reagent, tightly sealed, in an amber bottle. The reagent is stable for one month if stored in this manner. Larger volumes of reagent can be prepared if the reagent is used within one month.

Accuracy Check

Standard Additions Method

- a. Leave the unspiked sample in the sample compartment. Verify that the units displayed are in mg/L. Select standard additions mode by pressing the soft keys under **OPTIONS, (MORE)** and then **STD ADD**.
- b. Press **ENTER** to accept the default sample volume (mL), 250.
- c. Press **ENTER** to accept the default standard concentration (mg/L), 100.
- d. Press the soft key under **ENTRY DONE**.
- e. Prepare a 100-mg/L arsenic working standard by pipetting 10.0 mL of Arsenic Standard Solution, 1000-mg/L As (Cat. No. 14571-42) into a 100-mL volumetric flask. Dilute to volume with deionized water.
- f. Use the TenSette Pipet to add 0.1 mL, 0.2 mL and 0.3 mL of standard, respectively to three 250-mL samples and mix each thoroughly.
- g. Analyze each standard addition sample as described above. Accept the standard additions reading by pressing the soft key under **READ** each time. Each addition should reflect approximately 100% recovery.
- h. After completing the sequence, the display will show the extrapolated concentration value and the “best-fit” line through the standard additions data points, accounting for matrix interferences.
- i. See Section 1.4.1 *Standard Additions* for more information.

Standard Solution Method

To check accuracy, use a 0.100-mg/L arsenic standard solution prepared as follows:

- a. Pipet 1 mL of a 1000-mg/L Arsenic Standard Solution into a 100-mL Class A volumetric flask. Dilute to the mark with deionized water and mix thoroughly.
- b. Pipet 1 mL of the solution prepared in step a into a 100-mL class A volumetric flask. Dilute to the mark with deionized water and mix thoroughly. This is a 0.100-mg/L arsenic standard solution.

Calibration Standard Preparation

Standard Preparation

Perform a new calibration for each lot of arsenic absorber solution. Prepare a 10.0-mg/L arsenic working standard by pipetting 10.0 mL of Arsenic Standard Solution, 1000-mg/L As into a 100-mL volumetric flask. Dilute to volume with deionized water.

Into three different 500-mL volumetric flasks, pipet 1.0, 2.0, and 10.0 mL of the 10.0-mg/L As stock solution using Class A glassware. Dilute to the mark with deionized water and mix thoroughly. These standards have concentrations of 0.02, 0.04, and 0.20 mg/L As.

User Programming

- a. Start from the “MAIN MENU.” Press the soft key under **USER PROGRAM**.

- b. If you have not performed an arsenic calibration before, press the soft key under **CREATE**. Key any available program number you wish to use for arsenic testing. Press **ENTER**. Press the soft key under **COPY PROGRAM**. Select program number **1050** and press **ENTER**. If you already have a working arsenic program, press the soft key under **EDIT**, select the program number and press **ENTER**.
- c. Press the **DOWN ARROW** key until you highlight the parameter **Calib. table**. Press the soft key under **EDIT TABLE**. Press the **UP ARROW** key to highlight the very first concentration in the list. Press the soft key under **EDIT ABS**. Press **CE** and then **ENTER** to erase the first, inaccurate, absorbance value. Repeat to erase all the absorbance values. Press the soft key under **ENTRY DONE**.

Note: A user calibration **MUST** be performed to obtain accurate results. The original absorbance values are generic and are included only as an example.

- d. Note which mg/L value is highlighted. Perform steps 3 through 21 of the arsenic Silver Diethyldithiocarbamate Method on deionized water (this is the reagent blank). Place the sample cell into the cell holder. Press the soft key under **ZERO**. Then press the soft key under **READ**. Repeat steps 3 through 21 of the procedure on the other arsenic standards. Place the prepared samples in the cell holder and press the soft key under **READ** to accept the absorbance value.
- e. In the **Curve fit**: display, press the soft key under **NEXT FORMULA** until **C = a + bA** is displayed. Press the soft key under **FORCE 0**: once so that **ON** is selected. Press **EXIT** until **Store Changes?** is displayed. Press the soft key under **YES**. The program is ready for use.

Note: Some variations of the calibration procedure are possible. See the DR/4000 Instrument Manual for details.

Summary of Method

Arsenic is reduced to arsine gas by a mixture of zinc, stannous chloride, potassium iodide and hydrochloric acid in a specially equipped distillation apparatus. The arsine is passed through a scrubber containing cotton saturated with lead acetate and then into an absorber tube containing silver diethyldithiocarbamate in pyridine. The arsenic reacts to form a red complex which is read colorimetrically. This procedure requires a manual calibration.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to Section 1.

Pollution Prevention and Waste Management

The arsenic absorber in this test is a silver solution in pyridine. Both silver (D011) and pyridine (D038) are regulated by the Federal RCRA as hazardous waste. In addition, the cotton ball soaked in lead acetate (D008) solution is a hazardous waste. These materials should not be poured down the drain. See Section 3 for more information on proper disposal of these materials.

REQUIRED REAGENTS AND STANDARDS

Description	Quantity Required per test	Unit	Cat. No.
Arsenic Standard Solution, 1000-mg/L As	varies.....	100 mL.....	14571-42
Hydrochloric Acid, ACS	25 mL	500 mL.....	134-49
Lead Acetate Solution, 10%	1 mL	100 mL.....	14580-42
Potassium Iodide Solution, 20%	3 mL	100 mL.....	14568-42
Pyridine, ACS.....	50 mL	500 mL.....	14469-49
Silver Diethyldithiocarbamate.....	1 g	25 g	14476-24
Stannous Chloride Solution.....	1 mL	100 mL.....	14569-42
Zinc, 20-mesh, ACS	6 g	454 g	795-01

REQUIRED EQUIPMENT AND SUPPLIES

Balance, analytical, 110/220 VAC.....	1	each.....	26103-00
Balls, cotton.....	1	100/pkg.....	2572-01
Boat, weighing	2	500/pkg.....	21790-00
Bottle, amber, 237-mL, glass	1	6/pkg.....	7144-41
Cap, polypropylene, for amber bottle.....	1	6/pkg.....	21667-06
Cylinder, graduated, 25-mL	2	each.....	508-40
Cylinder, graduated, 250-mL	1	each.....	508-46
Distillation Apparatus, Arsenic Accessories.....	1	set.....	22654-00
Distillation Apparatus, General Purpose Accessories.....	1	set.....	22653-00
DR/4000 1-Inch Cell Adapter	1	each.....	48190-00
Flask, volumetric, 1000-mL, Class A, with glass stopper.....	1	each.....	14574-53
Flask, volumetric, 200-mL, Class A	1	each.....	14574-45
Flask, volumetric, 500-mL, Class A	6	each.....	14574-49
Pipet Filler, safety bulb.....	1	each.....	14651-00
Pipet, serological, 5-mL	2	each.....	532-37
Pipet, volumetric, Class A, 1.00-mL	2	each.....	14515-35
Pipet, volumetric, Class A, 2.00-mL	1	each.....	14515-36
Pipet, volumetric, Class A, 4.00-mL	1	each.....	14515-04
Pipet, volumetric, Class A, 6.00-mL	1	each.....	14515-06
Pipet, volumetric, Class A, 8.00-mL	1	each.....	14515-08
Pipet, volumetric, Class A, 10.00-mL	1	each.....	14515-38
Stopper, hollow, poly., No. 1	2	6/pkg.....	14480-00

Select one based on available voltage:

Distillation Apparatus Heater, 115 VAC, 60 Hz	each.....	22744-00
Distillation Apparatus Heater, 230 VAC, 50 Hz	each.....	22744-02

OPTIONAL REAGENTS AND STANDARDS

Description	Unit	Cat. No.
Hydrochloric Acid, ACS	2.5 liters.....	134-06
Nitric Acid, ACS	500 mL.....	152-49
Nitric Acid Solution, 1:1	500 mL.....	2540-49
Pyridine, ACS.....	4 liters.....	14469-17
Water, deionized	4 liters.....	272-56

OPTIONAL EQUIPMENT AND SUPPLIES

Description	Quantity Required per test	Unit	Cat. No.
DR/4000 Carousel Module Kit		each.....	48090-02
Flask, volumetric, Class A, 100-mL		each.....	14574-42
Gloves, chemical resistant:			
Size 7-7½.....		pair.....	24101-02
Size 8-8½.....		pair.....	24101-03
Size 9-9½.....		pair.....	24101-04
Size 10.....		pair.....	24101-05
Size 11.....		pair.....	24101-06
pH Meter, <i>sens^{ion}</i> TM 1, portable.....		each.....	51700-00
pH Indicator Paper, 1.0 to 11.0 pH.....	5 rolls/pkg		391-33
Pipet, serological, 2-mL		each.....	532-36
Pipet, TenSette, 0.1 to 1.0 mL		each.....	19700-01
Pipet Tips, for TenSette Pipet 19700-01	50/pkg		21856-96



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HACH COMPANY

WORLD HEADQUARTERS

Telephone: (970) 669-3050

FAX: (970) 669-2932



Scope and Application: For water

* This test is semi-quantitative. Results are expressed as greater or less than the threshold value used.

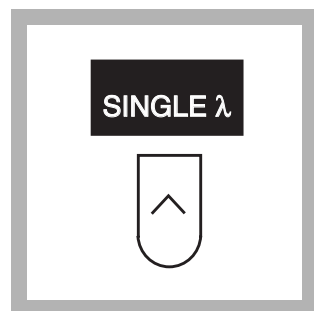
This method analyzes for Atrazine in water. Sample calibrators and reagents are added to cuvettes coated with Atrazine-specific antibodies. The color that develops is then measured and compared with the color measurements of the calibrators. The test requires about 30 minutes for complete analysis. As many as 20 cuvettes (18 samples and 2 calibrators) can be run simultaneously.

Tips and Techniques

- **Read the entire procedure before starting.** Identify and have ready all the necessary reagents, cuvettes, and other apparatus before beginning the analysis.
- **Timing is critical;** follow instructions carefully.
- **A consistent technique when mixing the cuvettes is critical to this test.** The best results come from using the cuvette rack and mixing as described in *Using the 1-cm MicroCuvette Rack*. Cuvettes can be mixed individually, but test results may not be as consistent.
- Handle the cuvettes carefully. Scratches on the inside or outside may cause erroneous results. Carefully clean the outside of the cuvettes with a clean absorbent cloth or tissue before placing them into the instrument. (Kimwipe® tissues are provided with the kit.)
- Antibody cuvettes and enzyme conjugate are made in matched lots. Do not mix reagent lots.
- To avoid damaging the Color Developing Solution, do not expose it to direct sunlight.
- The cuvette rack is designed to be inverted with the cuvettes in place. This is especially helpful when running many samples at once; the cuvettes can remain in the rack and be processed together until they are read in the Immunoassay Pocket Colorimeter.
- Twenty Antibody Cuvettes are provided with each reagent set. One Antibody Cuvette will be used for each calibrator and each sample. Cuvettes are not reusable.
- Ensure the 1cm MicroCell adapter is installed in the DR/4000.

Note: Hach Company recommends wearing protective nitrile gloves for this procedure.

Immunoassay



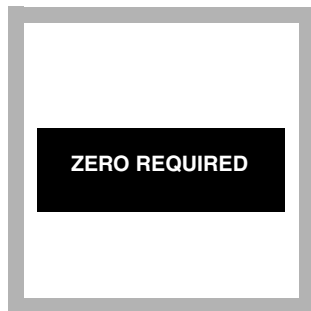
1. Press the soft key under **SINGLE λ**.

Press the soft key under **GO TO λ**.

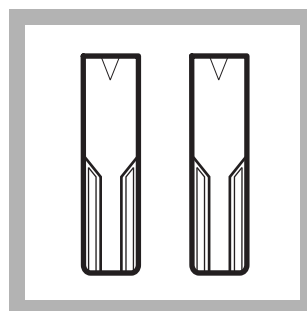
Select 450 nm by pressing the numeric keys **450**.

Press: **ENTER**

Note: The Flow Cell and Sipper Modules cannot be used with this procedure.

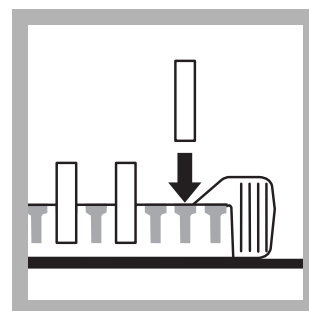


2. The display will show:
ZERO REQUIRED

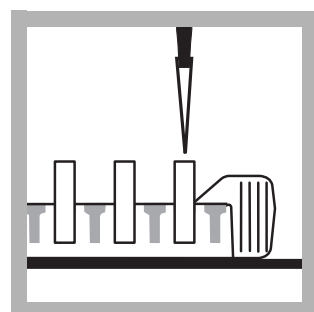


3. Label an Antibody Cuvette for each calibrator and each sample to be tested.

Note: As many as 20 cuvettes may be tested at one time and may comprise any combination of samples and calibrators.

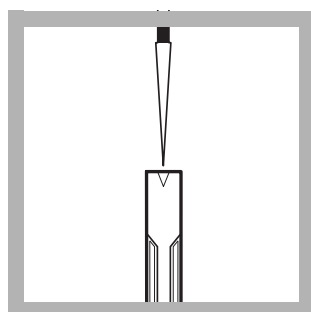


4. Place the cuvettes into the rack snugly.



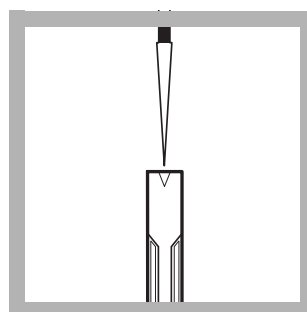
5. Pipet 0.5 mL of each calibrator into the appropriately labeled cuvette.

Note: Use a new pipette tip for each sample.

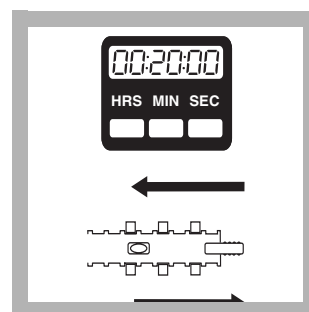


6. Pipet 0.5 mL of each sample to be tested into the appropriately labeled cuvette.

Note: Use a new pipette tip for each sample.



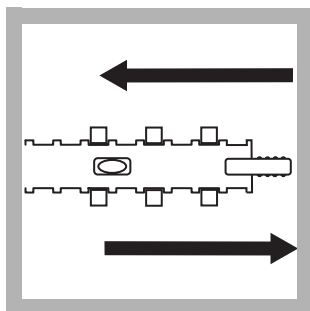
7. Immediately pipet 0.5 mL of Atrazine Enzyme Conjugate into each cuvette.



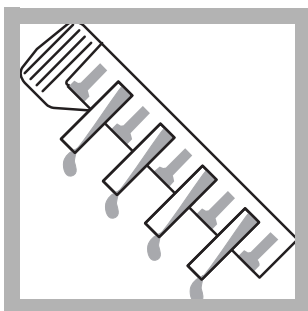
8. Key **2000**. Press the soft key under **START TIMER**.

A 20-minute reaction time will begin.

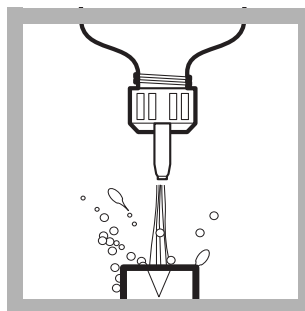
Immediately mix the contents of the cuvettes for 30 seconds using the technique described in *Using the 1-cm MicroCuvette Rack*.



9. After 10 minutes mix the contents of the rack for 30 seconds using the technique described in “Using the 1-cm MicroCuvette Rack” on page 5.



10. At the end of the 20-minute period, discard the contents of all the cuvettes into an appropriate waste container.

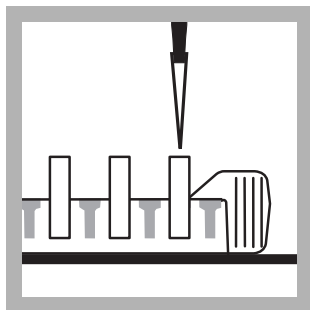


11. Wash each cuvette forcefully and thoroughly four times with deionized water. Empty the rinse water into the waste container.

Note: Ensure most of the water is drained from the cuvettes by turning the cuvettes upside down and tapping them lightly on a paper towel

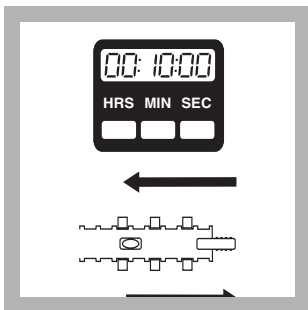
Color Development

Note: Timing is critical; follow instructions carefully.



12. With the cuvettes still held snugly in the rack, pipet 0.5 mL of Color Developing Solution into each Antibody Cuvette.

Note: Use a new pipette tip for each cuvette.



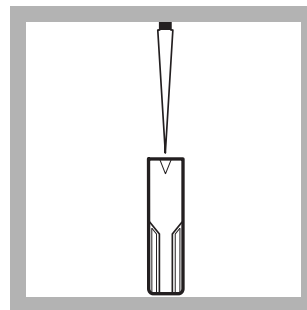
13. Key 1000. Press the soft key under **START TIMER**.

A reaction period will begin. Mix following the instructions in *Using the 1-cm MicroCuvette Rack*.



14. After 5 minutes, mix the contents of the rack a second time for a period of 30 seconds using the same technique.

Note: Solutions will turn blue in some or all of the cuvettes.



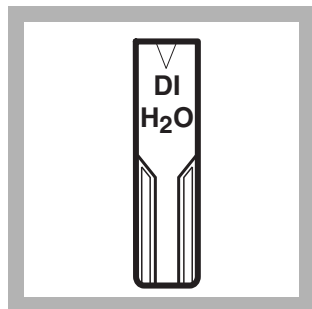
15. At the end of the 10-minute reaction period, pipette 0.5 mL of Stop Solution into each cuvette in the same order as the Color Developing Solution was added in step 12.

Slide the rack for 20 seconds using the technique described in *Using the 1-cm MicroCuvette Rack*.

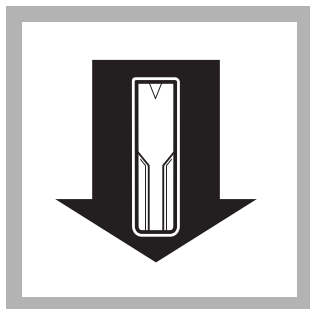
Note: Blue solutions will turn yellow with the addition of the Stop Solution.

Note: The same pipette tip can be used repeatedly for this step.

Measuring the Color

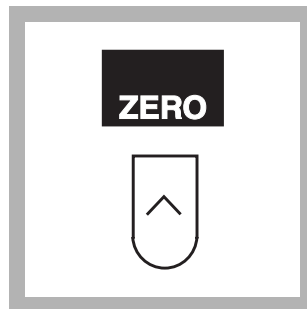


16. Label and fill a Zeroing Cuvette with deionized water. Wipe the outside of all the cuvettes with a tissue to remove water, smudges, and fingerprints.



17. Place the filled zeroing cuvette into the cell holder with the arrow pointing left.

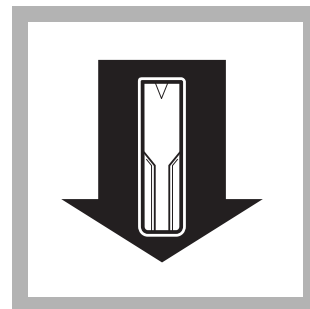
Orient the arrow in the same direction for all cuvettes.



18. Press the soft key under **ZERO**.

The display will show:

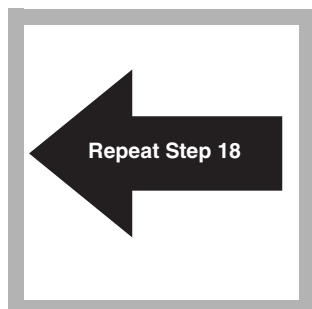
0.000 Abs



19. Place the first calibrator into the cell holder. Read the results.

The display will give an absorbance reading. Record the results for each calibrator and sample.

Note: See the *Instrument Manual* for more information on taking a reading.



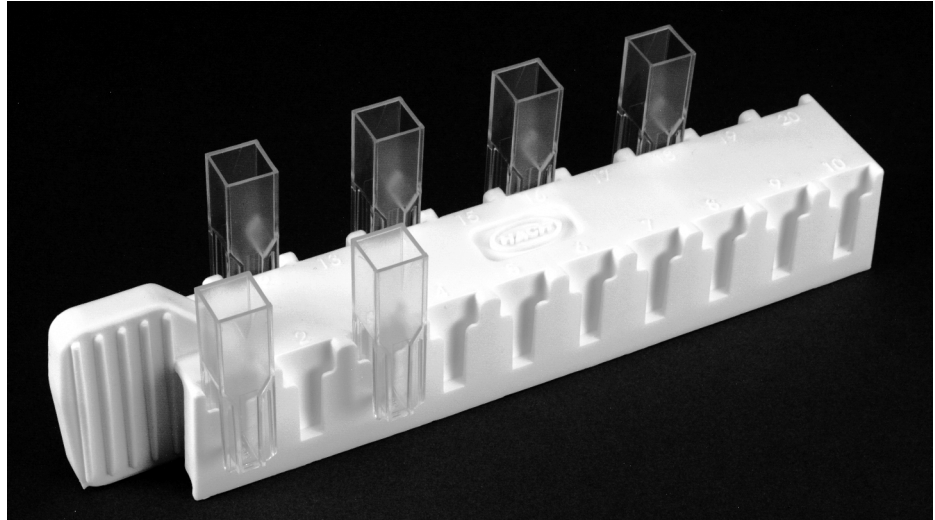
20. Repeat *step 19* for all remaining calibrators and samples.

See *Interpreting and Reporting Results* for help with interpretation of results.

Using the 1-cm MicroCuvette Rack

This rack (see *Figure 1*) has been designed specifically to aid in achieving precise and accurate results when using the immunoassay technique to analyze several samples at the same time.

Figure 1 The 1-cm MicroCuvette Rack



Loading the Rack — The cuvette rack is designed so that it may be inverted with the cuvettes in place. Identify each cuvette with a sample or calibrator number and place all the cuvettes in the rack before beginning the procedure. Fit the cuvettes snugly into the rack, but do not force them or they may be difficult to remove and their contents may spill. The cuvettes should remain in place when the rack is inverted and tapped lightly.

Mixing — Set the rack on a hard, flat surface that is at least twice the length of the rack. Hold the rack by one end and vigorously slide it back and forth along its long axis for 30 seconds. The rack should move through a distance equal to its own length in each direction.

Interpreting and Reporting Results

There is an inverse relationship between the concentration of Atrazine and the reading. In other words, the higher the reading, the lower the concentration of Atrazine.

If the sample reading is...	the sample Atrazine Concentration is...
...less than calibrator reading	...greater than the calibrator concentration
...greater than calibrator reading	...less than the calibrator concentration

Example

Readings:

0.5 ppm Atrazine Calibrator: **0.475 ABS**

2.0 ppm Atrazine Calibrator: **0.245 ABS**

Sample #1: **0.140 ABS**

Sample #2: **0.300 ABS**

Sample #3: **0.550 ABS**

Interpretation

Sample #1 — Sample reading is less than the readings for both calibrators. Therefore the sample concentration of Atrazine is greater than both 0.5 ppb and 2.0 ppb Atrazine.

Sample #2 — Sample reading is between the readings for the 0.5 ppb and 2.0 ppb Atrazine calibrators. Therefore the sample concentration of Atrazine is between 0.5 ppb and 2.0 ppb.

Sample #3 — Sample reading is greater than the readings for both calibrators. Therefore the sample concentration of Atrazine is less than both 2.0 ppb and 0.5 ppb.

Storing and Handling Reagents

- Wear protective gloves and eyewear.
- When storing reagent sets for extended periods of time, keep them out of direct sunlight. Store reagents at a temperature of 4 °C when not in use.
- Keep the foil pouch containing the Atrazine Antibody Cuvettes sealed when not in use.
- If Stop Solution comes in contact with eyes, wash thoroughly for 15 minutes with cold water and seek immediate medical help.

Sensitivity

The Atrazine immunoassay cannot differentiate between the various triazines and metabolites, but it detects their presence in differing degrees.

Table 1 Required Concentrations for Selected Chemicals

Compound	Concentration to give a positive result at 0.5 ppb Atrazine	Concentration to give a positive result at 2.0 ppb Atrazine
Acetochlor	74 ppm	398 ppm
Butachlor	84 ppb	550 ppb
2 Chloro-2'6'-Diethylacetaniline	8 ppm	60 ppm
2,6-Diethylaniline	61 ppm	313 ppm
Propachlor	60 ppb	295 ppb

The following compounds are not detectable at 10,000 ppb.

Atrazine	Carbofuran	Carbendazim
Aldicarb	2,4-D	
Diazotol	Chlorpyrifos	

Sample Collection and Storage

Collect samples in a clean glass bottle. Do not pre-rinse the bottle with the sample. If the sample cannot be analyzed immediately, store the sample at 4 °C. Samples may be kept for as long as 14 days. Warm the samples to room temperature before analysis.

Summary of Method

Hach immunoassay tests use antigen/antibody reactions to test for specific organic compounds in water and soil. Atrazine-specific antibodies, attached to the walls of plastic cuvettes, selectively bind and remove Atrazine from complex sample matrices. A prepared sample and a reagent containing enzyme-conjugate molecules (analyte molecules attached to molecules of an enzyme) are added to the Antibody Cuvettes. During incubation, enzyme-conjugate molecules and Atrazine compete for binding sites on the antibodies. Samples with higher levels of analyte will have more antibody sites occupied by Atrazine and fewer antibody sites occupied by the enzyme-conjugate molecules.

After incubation, the sample and unbound enzyme conjugate are washed from the cuvette and a color-development reagent is added. The enzyme in the conjugate catalyzes the development of color. Therefore, there is an inverse relationship between color intensity and the amount of Atrazine in the sample. The resulting color is then compared with a calibrator to determine whether the Atrazine concentration in the sample is greater or less than the threshold levels.

Required Reagents

Description	Unit	Cat. No.
Reagent Set, Atrazine*	20 cuvettes.....	27627-00

Required Apparatus

Caps, flip spout.....	2/pkg.....	25818-02
Cell Adapter, 1-cm MicroCell.....	each.....	48588-00
Marker, laboratory	each.....	20920-00
Rack, for 1-cm Micro Cuvettes	each.....	48799-00
Wipes, disposable.....	box.....	20970-00
TenSette®, Pipet, 0.1–1.0 mL.....	each.....	19700-01
Tips, for pipettor 19700-01	1000/pkg.....	21856-28

* Immunoassay components are manufactured for Hach Company by Beacon Analytical Systems, Inc.



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HACH COMPANY

WORLD HEADQUARTERS

Telephone: (970) 669-3050

FAX: (970) 669-2932



Method 8014

Turbidimetric Method*

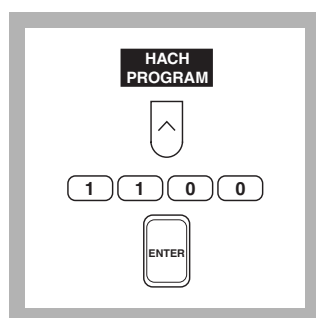
Powder Pillows or AccuVac® Ampuls

(0 to 100 mg/L)

Scope and Application: For water, wastewater, oil-field water and seawater.

* Adapted from Snell and Snell, *Colorimetric Methods of Analysis*, Vol. II, 769 (1959).

Using Powder Pillows



- 1.** Press the soft key under **HACH PROGRAM**.

Select the stored program for barium (Ba) by pressing **1100** with the numeric keys.

Press: **ENTER**

Note: If samples cannot be analyzed immediately, see *Sample Collection, Storage and Preservation* following these steps. Adjust the pH of preserved samples before analysis.

Note: The Pour-Thru and Sipper Cell Modules cannot be used with this procedure.

Note: For the best results, perform a new calibration for each new lot of reagent. See *Calibration Standard Preparation* following these steps.



- 2.** The display will show:
**HACH PROGRAM:
1100 Barium**

The wavelength (λ), **450 nm**, is automatically selected.

Note: You must determine a reagent blank for each new lot of reagent as follows. Prepare a reagent blank by repeating Steps 3 through 5, using deionized water as the sample. Zero the instrument on deionized water by pressing the soft key under **ZERO**. Insert the reagent blank and the blank value will be displayed. Correct for the reagent blank by pressing the soft keys under **OPTIONS, (MORE)** and then **BLANK:OFF**. Enter the reagent blank value and press **ENTER**. Repeat for each new lot of reagent.

Note: You must perform a standard curve adjustment for each new lot of reagent. See *Standard Curve Adjustment* following these steps.



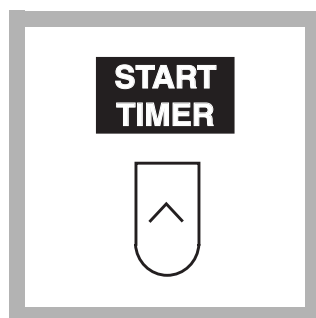
- 3.** Fill a sample cell with 25 mL of sample.

Note: Filter highly colored or turbid water samples using labware listed under **OPTIONAL EQUIPMENT AND SUPPLIES**. Large amounts of color or turbidity will interfere and cause high readings. Use the filtered sample in steps 3 and 6.



- 4.** Add the contents of one BariVer 4 Barium Reagent Powder Pillow to the cell (the prepared sample). Swirl to mix.

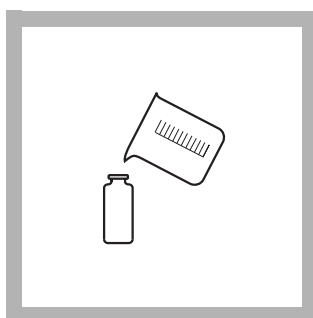
Note: A white turbidity will develop if barium is present.



5. Press the soft key under **START TIMER**.

A 5-minute reaction period will begin.

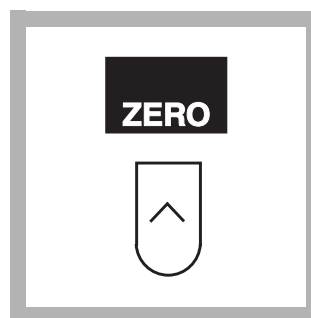
Note: The sample should not be disturbed during the 5-minute turbidity development period. If the BariVer 4 Barium Reagent does not dissolve readily in the sample, use a 25-mL graduated mixing cylinder. Mix the reagent with the sample in the graduate before pouring it into the sample cell.



6. Fill another sample cell (the blank) with 25-mL of sample.



7. When the timer beeps, place the blank into the cell holder. Close the light shield.



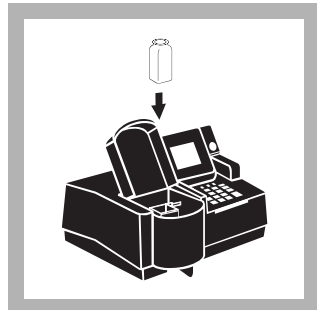
8. Press the soft key under **ZERO**.

The display will show:

0.0 mg/L Ba

Note: If you are using a reagent blank correction, the display will show the correction.

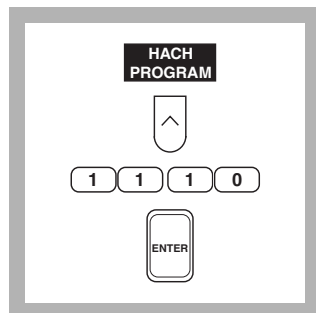
Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



9. Within 10 minutes after the timer beeps, place the prepared sample into the cell holder. Close the lid. Results in mg/L barium (or chosen units) will be displayed.

Note: Immediately after each test the sample cell should be cleaned with soap, water, and a brush to prevent a film of barium sulfate from developing on the inside of the sample cell.

Using AccuVac Ampuls



1. Press the soft key under **HACH PROGRAM**.

Select the stored program for barium (Ba) AccuVac Ampul method by pressing **1110** with the numeric keys.

Press: **ENTER**

Note: If samples cannot be analyzed immediately, see *Sample Collection, Storage and Preservation* following these steps. Adjust the pH of preserved samples before analysis.

Note: For the best results, perform a new calibration for each new lot of reagent. See *Calibration Standard Preparation* following these steps.

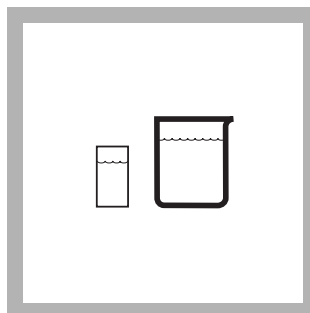


2. The display will show:
HACH PROGRAM:
1110 Barium, AV

The wavelength (λ), **450 nm**, is automatically selected.

Note: You must determine a reagent blank for each new lot of reagent as follows. Prepare a reagent blank by repeating steps 5 through 7, using deionized water as the sample. Zero the instrument on deionized water by pressing the soft key under **ZERO**. Insert the reagent blank and the blank value will be displayed. Correct for the reagent blank by pressing the soft keys under **OPTIONS, (MORE)**, and then **BLANK:OFF**. Enter the reagent blank value and press **ENTER**. Repeat for each new lot of reagent.

Note: You must perform a standard curve adjustment for each new lot of reagent. See *Standard Curve Adjustment* following these steps.

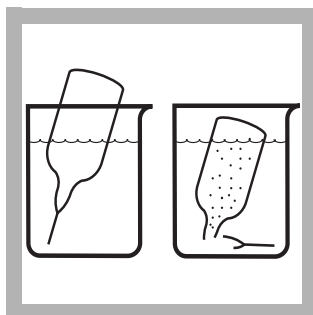


3. Fill a zeroing vial with at least 10 mL of sample (the blank). Collect at least 40 mL of sample in a 50-mL beaker.

Note: Filter highly colored or turbid samples using labware listed under **OPTIONAL EQUIPMENT AND SUPPLIES**.

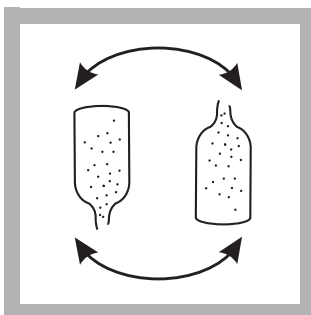


4. Insert and align the AccuVac Ampul Adapter in the Sample Cell Module. Fasten it with the threaded screw.



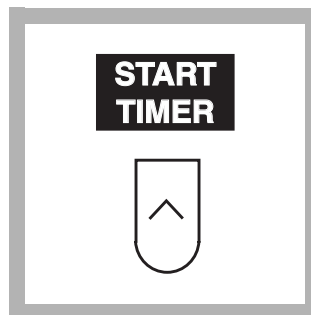
5. Fill a Barium AccuVac Ampul with sample (the prepared sample).

Note: Keep the tip immersed while the ampul fills completely.



6. Quickly invert the ampul several times to mix, then wipe off any liquid or fingerprints.

Note: A white turbidity will develop if barium is present.



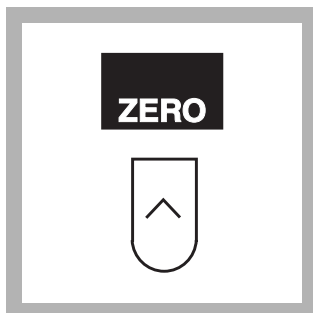
7. Press the soft key under **START TIMER**.

A 5-minute reaction period will begin.

Note: The sample should not be disturbed during the 5-minute turbidity development period.



8. When the timer beeps, place the blank into the cell holder. Close the light shield.



9. Press the soft key under **ZERO**.

The display will show:

0.0 mg/L Ba

Note: If you are using a reagent blank correction, the display will show the correction.

Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



10. Place the prepared sample into the cell holder. Close the light shield.

Note: Take reading within 5 minutes after beeper sounds.

Interferences

The following may interfere when present in concentrations exceeding those listed below.

Table 1 Interfering Substances and Suggested Treatments

Interfering Substance	Interference Levels and Treatments
Calcium	10,000 mg/L as CaCO ₃
Magnesium	100,000 mg/L as CaCO ₃
Silica	500 mg/L
Sodium Chloride	130,000 mg/L as NaCl
Strontium	Interferes at any level. If present, the total concentration between barium and strontium may be expressed as a PS (Precipitated by Sulfate). While this does not distinguish between barium and strontium, it gives an accurate indication of scaling tendency.
Highly buffered samples or extreme sample pH	May exceed the buffering capacity of the reagents and require sample pretreatment (see Section 1.3.1 <i>pH Interference</i>).

Sample Collection, Storage and Preservation

Collect samples in an acid cleaned glass or plastic container. Adjust the pH to 2 or less with nitric acid (about 2 mL per liter). Preserved samples can be stored up to six months at room temperature. Before analysis, adjust the pH to 5 with 5.0 N sodium hydroxide. Correct the test result for volume additions (see section 1.2.2 *Correcting for Volume Additions*).

Standard Curve Adjustment

Using Class A glassware, prepare a 90.0-mg/L barium standard solution by pipetting 9.00 mL of Barium Standard Solution, 1000 mg/L, into a 100-mL volumetric flask and diluting to the mark with deionized water. Prepare this solution daily. Perform the barium procedure as described above.

To adjust the calibration curve using the reading obtained with the 90.0-mg/L standard solution, press the soft keys under **OPTIONS, MORE** then **STD: OFF**. Press **ENTER** to accept the displayed concentration, the value of which depends on the selected units. If an alternate concentration is used, enter the actual concentration and press **ENTER** to return to the read screen. See section 1.5.5 *Adjusting the Standard Curve* for more information.

Accuracy Check

Standard Additions Method

- Leave the unspiked sample in the sample compartment. Verify that the units displayed are in mg/L. Select standard additions mode by pressing the soft keys under **OPTIONS, (MORE)** and then **STD ADD**.
- Press **ENTER** to accept the default sample volume (mL), 25.
- Press **ENTER** to accept the default standard concentration (mg/L), 1000.
- Press the soft key under **ENTRY DONE**.
- Open a Barium Standard Solution, 1000 mg/L Ba.
- Use the TenSette Pipet to add 0.1 mL, 0.2 mL and 0.3 mL of standard, respectively, to three 25-mL samples and mix each thoroughly (for AccuVac Ampuls, use 50-mL beakers).

- g. Analyze each standard addition sample as described above. Accept the standard additions reading by pressing the soft key under **READ** each time. Each addition should reflect approximately 100% recovery.
- h. After completing the sequence, the display will show the extrapolated concentration value and the “best-fit” line through the standard additions data points, accounting for matrix interferences.
- i. See section 1.4.1 *Standard Additions* for more information.

Method Performance

Precision and Estimated Detection Limits are not available due to the large variation in reagent lots.

Sensitivity

This information is only an estimate. The sensitivity will vary with the reagent lot.

Program Number: 1100

Portion of Curve	Δ Abs	Δ Concentration
0.010 Abs	0.010	0.5 mg/L
50 mg/L	0.010	1.01 mg/L
90 mg/L	0.010	1.3 mg/L

Program Number 1110

Portion of Curve	Δ Abs	Δ Concentration
0.010 Abs	0.010	0.6 mg/L
50 mg/L	0.010	0.9 mg/L
90 mg/L	0.010	1.1 mg/L

See section 1.5.3 *Sensitivity Explained* for more information.

Calibration Standard Preparation

Prepare calibration standard containing 10, 20, 30, 50, 80, 90, and 100 mg/L Ba as follows:

- a. Into seven different 100-mL Class A volumetric flasks, pipet 1, 2, 3, 5, 8, 9 and 10 mL of the 1000-mg/L Barium Standard Solution (Cat. No. 14611-42) using Class A glassware.
- b. Dilute to the mark with deionized water. Mix thoroughly.
- c. Using the turbidimetric method and the calibration procedure described in the *User-Entered Programs* section of the *DR/4000 Spectrophotometer Instrument Manual*, generate a calibration curve from the standards prepared above.

Summary of Method

The BariVer 4 Barium Reagent Powder combines with barium to form a barium sulfate precipitate, which is held in suspension by a protective colloid. The amount of turbidity present caused by the fine white dispersion of particles is directly proportional to the amount of barium present.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to Section 1.

Pollution Prevention and Waste Management

For information on pollution prevention and waste management, refer to Section 3.

REQUIRED REAGENTS AND APPARATUS (Using Powder Pillows)

Description	Quantity Required		Unit	Cat. No.
	per test			
BariVer 4 Barium Reagent Powder Pillows	1 pillow	100/pkg		12064-99
Pipet, volumetric, Class A, 9.00 mL	1	each		14515-09
Pipet Filler, safety bulb	1	each		14651-00

REQUIRED REAGENTS (Using AccuVac Ampuls)

BariVer 4 Barium Reagent AccuVac Ampuls	1 ampul	25/pkg		25130-25
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REQUIRED EQUIPMENT AND SUPPLIES

DR/4000 AccuVac Ampul Adapter	1	each		48187-00
Beaker, 50-mL	1	each		500-41
Pipet, volumetric, Class A, 9.00-mL	1	each		14515-09
Pipet Filler, safety bulb	1	each		14651-00
Sample Cell, 10-ml with cap (zeroing vial)	1	each		21228-00

OPTIONAL REAGENTS AND STANDARDS

Barium Standard Solution, 50 mg/L Ba	500 mL			1951-49
Barium Standard Solution, 1000 mg/L Ba	100 mL			14611-42
Barium Standard Solution, 10-mL Voluette Ampule, 5000-mg/L	16/pkg			14251-10
Nitric Acid, ACS	500 mL			152-49
Nitric Acid Solution, 1:1	500 mL			2540-49
Sodium Hydroxide Standard Solution, 5.0 N	1 liters			2450-53
Water, deionized	4 liters			272-56

OPTIONAL EQUIPMENT AND SUPPLIES

Description	Unit	Cat. No.
Brush, test tube	each	690-00
DR/4000 Carousel Module	each	48070-02
Filter Paper, folded, 12.5-cm	100/pkg	1894-57
Funnel, poly, 65-mm	each	1083-67
pH Paper, pH 1.0 to 11.0	5 rolls/pkg	391-33
pH Meter, <i>sension</i> TM 1, portable	each	51700-00
Pipet, serological, 2-mL	each	532-36
Pipet, TenSette, 0.1 to 1.0 mL	each	19700-01
Pipet Tips, for 19700-01 TenSette Pipet	50/pkg	21856-96
Pipet, volumetric, Class A, 1.00-mL	each	14515-35
Pipet, volumetric, Class A, 2.00-mL	each	14515-36
Pipet, volumetric, Class A, 3.00-mL	each	14515-03
Pipet, volumetric, Class A, 5.00-mL	each	14515-37
Pipet, volumetric, Class A, 8.00-mL	each	14515-08
Pipet, volumetric, Class A, 10.00-mL	each	14515-38



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Method 8079

UV Photolysis Method*

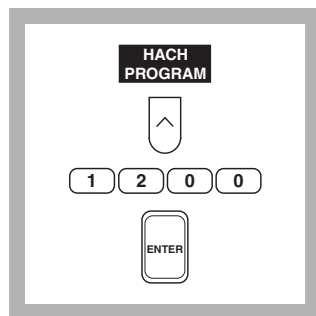
Powder Pillows

(0 to 16.0 mg/L)

Scope and Application: For cooling or boiler water. The estimated detection limit for program numbers 1200 and 3600 are 0.3 and 0.4 mg/L, respectively.

* Adapted from Harp, D., *Proceedings 45th International Water Conference*, 299 (October 22-24, 1984)

Using Powder Pillows



1. Press the soft key under **HACH PROGRAM**.

Select the stored program for benzotriazole by pressing **1200** with the numeric keys.

1 2 0 0

For tolyltriazole, press **3600** with the numeric keys.

3 6 0 0

Press: **ENTER**

Note: If samples cannot be analyzed immediately, see *Sample Collection, Storage and Preservation* following these steps.

Note: The Flow Cell and Sipper Modules can be used with this procedure.



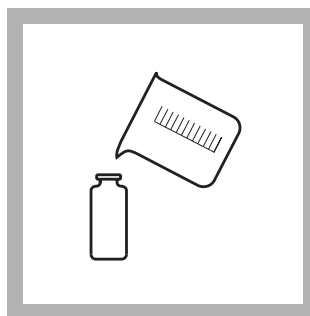
2. The display will show:

**HACH PROGRAM:
1200 Benzotriazole**

or

**HACH PROGRAM:
3600 Tolyltriazole**

The wavelength (λ), **425 nm**, is automatically selected.



3. Fill a sample cell with 25 mL of sample.

Note: For proof of accuracy, use a 5.0 mg/L benzotriazole standard solution (preparation given in the Accuracy Check section) in place of the sample.

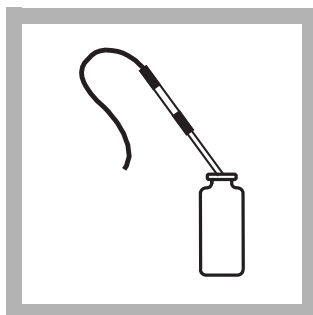
Note: Sample temperature should be 20–25 °C (68–78 °F).

Note: If sample contains nitrite or borax (sodium borate), adjust the pH to 4–6 with 1 N sulfuric acid.



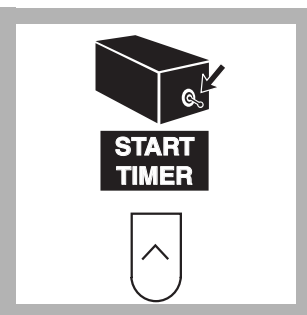
4. Add the contents of one Triazole Reagent Powder Pillow. Swirl to dissolve completely.

Note: If the sample contains more than 500 mg/L hardness (as CaCO₃), add 10 drops of Rochelle Salt Solution.



5. Insert the ultraviolet lamp into the sample cell.

Note: UV safety goggles should be worn while the lamp is on.

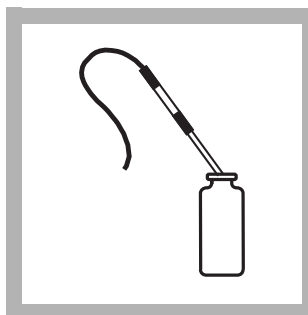


6. Turn the UV lamp ON.

Press the soft key under **START TIMER**.

A 5-minute reaction period will start.

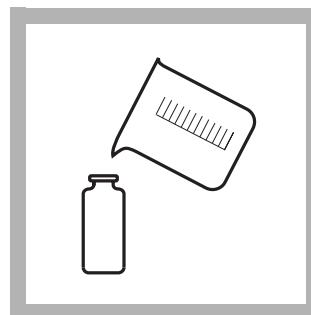
Note: A yellow color will form if triazole is present.



7. When the timer beeps, turn the lamp off. Remove the lamp from the cell (the prepared sample). Swirl the cell to mix thoroughly.

Note: Low results will occur if photolysis (lamp ON) takes place for more or less than five minutes.

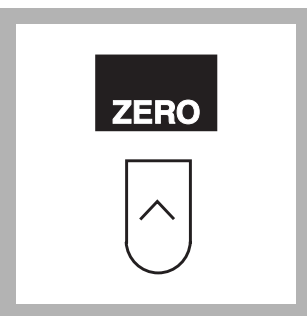
Note: Avoid fingerprints on the quartz surface of the lamp. Rinse the lamp and wipe with a soft, clean tissue between tests.



8. Fill another sample cell with 25 mL of sample (the blank).



9. Place the blank into the cell holder. Close the light shield.



10. Press the soft key under **ZERO**.

The display will show:

0.0 mg/L Benzo

or

0.0 mg/L Toly

Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



11. Place the prepared sample into the cell holder. Close the lid. Results in mg/L benzotriazole or tolyltriazole (or chosen units) will be displayed.

Interferences

Table 1 Interfering Substances and Suggested Treatments

Interfering Substance	Interference Levels and Treatments
Acrylates (as methyl acrylate)	Greater than 50 mg/L
Alum	Greater than 400 mg/L
Borate (as sodium tetraborate)	Greater than 4000 mg/L
Chlorine (as Cl ₂)	Greater than 20 mg/L
Chromium (as chromate)	Greater than 12 mg/L
Copper	Greater than 10 mg/L
Hardness	Greater than 500 mg/L as CaCO ₃
Iron	Greater than 20 mg/L
Lignosulfonates	Greater than 40 mg/L
Magnesium	Greater than 300 mg/L as CaCO ₃
Molybdenum (as molybdate)	Greater than 200 mg/L
Nitrite	Greater than 4000 mg/L
Phosphonates (AMP or HEDP)	Greater than 100 mg/L
Sulfate	Greater than 200 mg/L
Zinc	Greater than 80 mg/L
Strong oxidizing or reducing agents	Interfere at all levels

Sample Collection, Storage and Preservation

The most reliable results are obtained when samples are analyzed as soon as possible after collection.

Accuracy Check

Standard Additions Method

- a. Leave the unspiked sample in the sample compartment. Verify that the units displayed are in mg/L. Select standard additions mode by pressing the soft keys under **OPTIONS, (MORE)** and then **STD ADD**.
- b. Press **ENTER** to accept the default sample volume (mL), 25.
- c. Press **ENTER** to accept the default standard concentration (mg/L benzotriazole), 500.
- d. Press the soft key under **ENTRY DONE**.
- e. Open a 500-mg/L Benzotriazole Standard Solution.
- f. Use the TenSette Pipet to add 0.1 mL, 0.2 mL and 0.3 mL of standard, respectively to three 25-mL samples and mix each thoroughly.
- g. Analyze each standard addition sample as described above. Accept the standard additions reading by pressing the soft key under **READ** each time. Each addition should reflect approximately 100% recovery.
- h. After completing the sequence, the display will show the extrapolated concentration value and the “best-fit” line through the standard additions data points, accounting for matrix interferences.
- i. See section 1.4.1 *Standard Additions* for more information.

BENZOTRIAZOLE or TOLYLTRIAZOLE, continued

UV Lamp Check

To verify the ultraviolet lamp (normal life equals 5000 hours) is working properly, perform the following test:

- a. Prepare a 5.0 mg/L benzotriazole standard solution by pipetting 10.0 mL of Benzotriazole Standard Solution, 500-mg/L benzotriazole, into a 1-liter volumetric flask. Dilute to volume.
- b. Analyze according to the above procedure. If the result is significantly below 5.0 mg/L, replace the lamp.

Method Performance

Precision

Standard: 8.0 mg/L benzotriazole or tolyltriazole

Program	95% Confidence Limits
1200	7.9–8.1 mg/L
3600	7.8–8.2 mg/L

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
1200	0.3 mg/L benzotriazole
3600	0.4 mg/L tolyltriazole

For more information on derivation and use of Hach's estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, Appendix B, see Section 1.5.1.

Sensitivity

Program Number: 1200

Portion of Curve	Δ Abs	Δ Concentration
Entire Range	0.010	0.17 mg/L

Program Number 3600

Portion of Curve	Δ Abs	Δ Concentration
Entire Range	0.010	0.20 mg/L

See Section 1.5.3 for more information.

Calibration Standard Preparation

To perform a benzotriazole calibration using the UV photolysis method, prepare a 100 mg/L benzotriazole stock solution by pipetting 20 mL of a 500-mg/L Benzotriazole Standard Solution into a 100-mL volumetric flask using Class A glassware. Dilute to the mark with deionized water and mix thoroughly.

BENZOTRIAZOLE or TOLYLTRIAZOLE, continued

Prepare calibration standard containing 2.0, 4.0, 6.0, 8.0, 10.0, 12.0, 14.0, and 18.0 mg/L benzotriazole as follows:

- a. Into eight different 100-mL Class A volumetric flasks, pipet 2.00, 4.00, 6.00, 8.00, 10.00, 12.00, 14.00, and 18.00 mL of the 100-mg/L stock solution using Class A glassware.
- b. Dilute to the mark with deionized water and mix thoroughly.
- c. Using the UV photolysis method and the calibration procedure described in the *User-Entered Programs* section in the *DR/4000 Spectrophotometer Instrument Manual*, generate a calibration curve from the standards prepared above.

Summary of Method

Benzotriazole or tolyltriazole, used in many applications as corrosion inhibitors for copper and copper alloys, are determined by a proprietary catalytic ultraviolet (UV) photolysis procedure requiring less than 10 minutes to perform.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to Section 1.

Pollution Prevention and Waste Management

For information on pollution prevention and waste management, refer to Section 1.

REQUIRED REAGENTS AND STANDARDS

Description	Quantity Required		Unit	Cat. No.
	per test			
Triazole Reagent Powder Pillows.....	1 pillow	100/pkg		21412-99

REQUIRED EQUIPMENT AND SUPPLIES

UV Safety Goggles.....	1	each	21134-00
DR/4000 1-Inch Cell Adapter	1	each	48190-00

Select one based on available voltage:

Lamp Kit, UV, with power supply, 115 VAC, 60 Hz	each	20828-00
Lamp Kit, UV, with power supply, 230 VAC, 50 Hz	each	20828-02

OPTIONAL REAGENTS

Benzotriazole Standard Solution, 500-mg/L.....	100 mL	21413-42
Rochelle Salt Solution	29 mL* DB	1725-33
Sulfuric Acid Standard Solution, 1.000 N.....	100 mL MDB	1270-32

* Contact Hach for larger sizes.

BENZOTRIAZOLE or TOLYLTRIAZOLE, continued

OPTIONAL EQUIPMENT AND SUPPLIES

Description	Quantity Required per test	Unit	Cat. No.
DR/4000 Carousel Module Kit		each.....	48070-02
DR/4000 Flow Cell Module Kit, 1-inch.....		each.....	48070-04
DR/4000 Sipper Module Kit, 1-inch.....		each.....	48090-03
Flask, volumetric, Class A, 1000-mL, with stopper.....		each.....	14574-53
Lamp, UV (lamp only)		each.....	20823-00
pH Paper, pH 1.0 to 11.0	5 rolls/pkg.....		391-33
Pipet Filler, safety bulb.....		each.....	14651-00
Pipet, volumetric, Class A, 10.0-mL		each.....	14515-38
Cord Adapter, single to dual UV lamp.....		each.....	19485-00
Stopwatch, 60-second.....		each.....	14645-00
Timer, 3-channel, 1 second to ninety-nine hours		each.....	23480-00



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FAX: (970) 669-2932



Method 10061

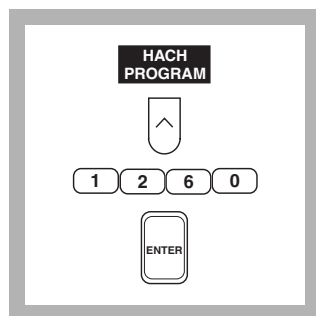
Azomethine-H Method*

Powder Pillows

LR (0 to 1.50 mg/L as B)

Scope and Application: For testing low levels of boron (boric acid or borates) in drinking water, cooling water, industrial process waters or wastewaters. The estimated detection limit for program number 1260 is 0.02 mg/L as B.

* Adapted from ISO Method 9390



1. Press the soft key under **HACH PROGRAM**.

Select the stored program number for boron (B), low range by pressing **1260** with the numeric keys.

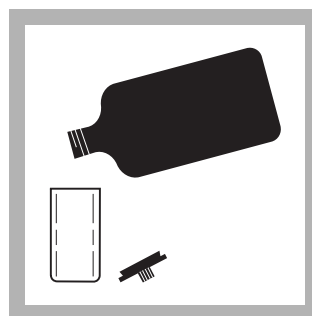
Press: **ENTER**

Note: The Flow Cell and Sipper Modules can be used with this procedure.



2. The display will show:
**HACH PROGRAM:
1260 Boron, LR.**

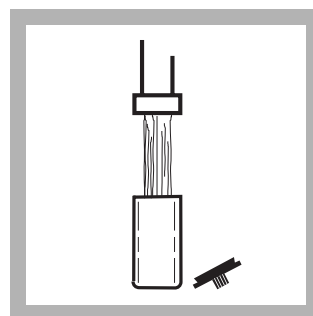
The wavelength (λ), **410 nm**, is automatically selected.



3. Fill a clean plastic sample cell to the 25-ml mark with Ultra-Pure Water. Label this cell as the “blank.”

Note: For most accurate work, perform a blank analysis with each sample analysis.

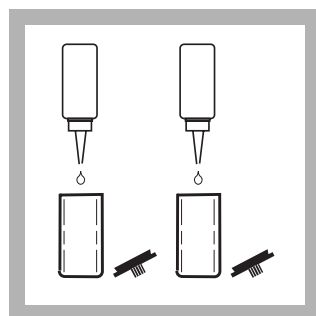
Note: For most accurate work, the sample cell pair should be matched. See the Cell Matching Procedure following these steps.



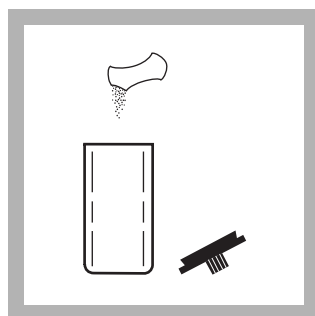
4. Fill a second clean plastic sample cell to the 25-mL mark with the water to be tested.

Note: If the sample is highly colored, turbid, or contains interferences, see the Interferences section for sample pre-treatment.

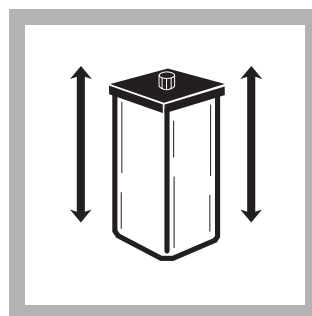
Note: Sample temperature should be 22–24 °C (72-75 °F) or most accurate results. If outside this range, measure and record the sample temperature.



5. Add 10 drops of EDTA Solution, 1 M, to each cell. Cap and invert each cell twice to mix.

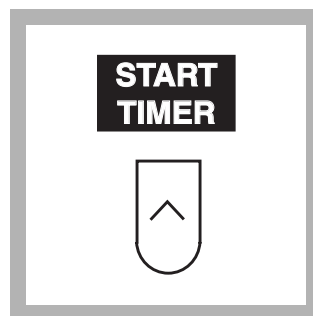


6. Open one pillow BoroTrace #2 Reagent and add the contents to the cell containing sample.



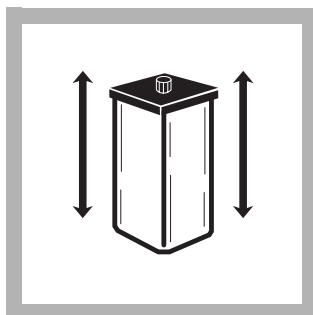
7. Cap and begin to shake to dissolve the powder.

Note: Proceed immediately with steps 8 and 9.

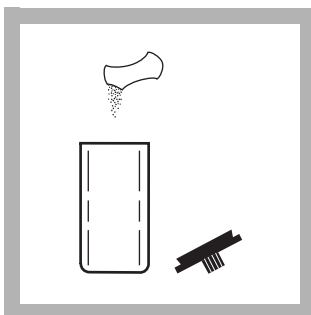


8. Press the soft key under **START TIMER**.

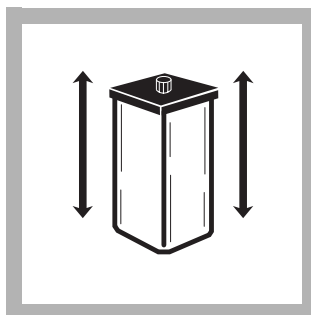
A 10-minute reaction period will begin.



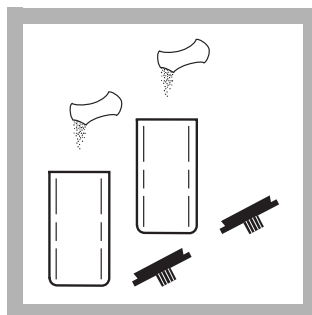
9. Continue shaking vigorously for 30 seconds. Let the cell sit capped for the duration of the timed reaction.



10. During the timed reaction, add the contents of a second BoroTrace #2 Reagent pillow to the cell containing the blank.



11. Cap the cell containing the blank and shake vigorously until the powder is dissolved.



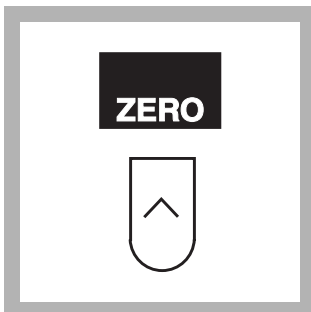
12. After the timer beeps, add the contents of one BoroTrace #3 Reagent to each cell. Cap and shake to dissolve.

Note: The addition of BoroTrace #3 Reagent “stops” the reaction.



13. Place the “blank” cell into the cell holder. Close the light shield.

Note: Clean the cell walls with a soft tissue or cloth to remove fingerprints before placing in the cell holder.



14. Press the soft key under **ZERO**.

The display will show:

0.00 mg/L B

Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



15. Place the prepared sample into the cell holder. Close the light shield. Results in mg/L boron (or chosen units) will be displayed.

Note: Correct the result for sample temperature. If outside 22–24 °C (72–75 °F), see the Sample Temperature Compensation section.

Sample Collection, Preservation and Storage

Collect samples in clean polyethylene bottles. Do not use borate-based detergents or soaps to clean sample containers or labware used for this method. After use, rinse all plastic containers with copious amounts of deionized water, allow to air dry, and keep covered.

Cell Matching Procedure

1. Rinse and fill two cells with deionized water.
2. Wipe sides with soft cloth or tissue.
3. Set the instrument to zero absorbance at 410 nm with one of the cells.
4. Read the absorbance of the other cell.
5. Cells which read within 0.002 absorbance are matched.

Interferences

The following have been tested for interference and found *not* to interfere up to the indicated levels (in mg/L):

Interfering Substance		Interference Levels
Aluminum (³⁺)		10
Benzotriazole		20
Biocides:		
	Carbamate-type	120
	Isothiazolin-type	120
	Quat-type	90
	Thiocyanate-type	60
Calcium		1000 (as CaCO ₃)
Chloride		2500
Copper (²⁺)		20
Magnesium		1000 (as CaCO ₃)
Manganese (⁷⁺)		5
Molybdate (Mo ⁶⁺)		60
Phosphonates, AMP		20
Phosphonates, HEDP		20
Polyacrylates		20 (as Acumer 1000, 1100)
Polymaleic Acid		40 (as Belcene 200)
Silica		120
Sulfate		1800
Sulfite		40
Tolyltriazole		20
Zinc (²⁺)		10

Table 1 Interfering Substances and Suggested Treatments

Interfering Substance, Interference Level, (positive or negative interference)	Recommended Treatment
Alkalinity >500 mg/L (+ or -)	<ol style="list-style-type: none"> 1. Adjust sample pH to between 5–7 using 1.0 N Sulfuric Acid Solution. 2. Continue with Step 5 of the analytic procedure.
Color (+)	<ol style="list-style-type: none"> 1. Zero the instrument (0.00 mg/L B) using Ultra-Pure Water. 2. Measure and record the apparent concentration, in mg/L B, due to the samples color. 3. Subtract the apparent concentration from the result in Step 15 of the test procedure.
Halogens (Bromine or Chlorine) all levels (+)	<p>Halogen disinfectants in the sample can produce a red-color after the addition of BoroTrace #2 Reagent. To eliminate this interference:</p> <ol style="list-style-type: none"> 1. Add 1 pillow Dechlorinating Reagent to 25-mL each of Ultra-Pure Water and sample. 2. Cap and shake to dissolve. 3. Continue with Step 5 of the test procedure.
Iron (Fe^{3+} or Fe^{2+}), above 8 mg/L (+)	<p>High levels of iron in the sample can produce a red-color after the addition of BoroTrace #2 Reagent. To compensate, increase the amount of EDTA from 10 drops to 15 drops to be added to each cell (Step 5). Alternatively, dilute the sample with Ultra-Pure Water and continue with Step 5 of the test procedure. Correct the results (Step 15) using the appropriate dilution factor.</p>
Nitrites, all levels (+)	<ol style="list-style-type: none"> 1. Add 0.1 gram scoop Sulfamic Acid to 25-mL each Ultra-Pure Water and sample in plastic cells. 2. Cap and shake to dissolve. 3. Uncap and wait 5 minutes. 4. Add 5N Sodium Hydroxide Reagent solution to each to adjust pH 5–8 using pH paper. 5. Continue with Step 5 of the test procedure.
Turbidity (+)	<p>Filter the sample through a 3 μm membrane prior to testing. Do not use a glass fiber filter.</p>

Sample Temperature Compensation

The reaction chemistry is very dependent on the sample temperature. Hach calibrations are performed at 23 °C (73 °F). If the sample temperature is outside the range of 22–24 °C (72–75 °F), multiply the results, in mg/L, by the appropriate multiplier:

Sample Temp.		Multiplier
°C	(°F)	
5	(41)	0.70
7	(45)	0.73
10	(50)	0.78
12	(54)	0.81
14	(57)	0.84
16	(61)	0.87
18	(64)	0.91

Sample Temp.		Multiplier
°C	(°F)	
20	(68)	0.94
25	(77)	1.04
26	(79)	1.06
27	(81)	1.08
28	(82)	1.10
29	(84)	1.12
30	(86)	1.15

Method Performance

Precision

Program 1260:

Boron conc., at mg/L	99% Confidence Limits
0.15	± 0.01 mg/L B
0.75	± 0.03 mg/L B
1.25	± 0.03 mg/L B

Estimated Detection Limit

Program	EDL
1260	0.02 mg/L

Sensitivity

Program 1260:

Portion of Curve:	ΔAbs	ΔConcentration
0.25 mg/L B	0.010	0.012 mg/L
0.75 mg/L B	0.010	0.013 mg/L
1.25 mg/L B	0.010	0.013 mg/L

Accuracy Check

Standard Additions Method

- Leave the unspiked sample in the sample compartment. Prepare a 50.0 mg/L boron standard by pipetting 5.0 mL of a 1000-mg/L Boron Standard Solution into a 100-mL plastic volumetric flask. Dilute with deionized water, stopper and invert to mix.
- Verify that the units displayed are in mg/L. Select standard additions mode by pressing the soft keys under **OPTIONS, (MORE)** and then **STD ADD.**
- Press **ENTER** to accept the default sample volume (mL), 25.
- Press **ENTER** to accept the default standard concentration (mg/L), 50.
- Press the soft key under **ENTRY DONE.**
- Use the TenSette Pipet to add 0.1, 0.2, and 0.3 mL of the 50.0-mg/L boron standard to three 25-mL water samples. Mix thoroughly.
- Analyze each standard addition as described above. Accept the standard additions reading by pressing the soft key under **READ** each time. Each addition should reflect approximately 100% recovery.
- After completing the sequence, the display will show the extrapolated concentration value and the “best-fit” line through the standard additions points, accounting for matrix interferences.

See Section 1.4.1 *Standard Additions* for more information.

Standard Solution Method

Prepare a 1.0-mg/L B standard as follows: Using plastic pipet, transfer 4.0 mL of Boron Standard Solution, 250-mg/L as B, into a 1000-mL plastic volumetric flask. Dilute to volume with deionized water, stopper and invert to mix.

To adjust the calibration curve using the reading obtained with the 1.0 mg/L standard solution, press the soft keys under **OPTIONS, MORE** then **STD:OFF**. Press **ENTER** to accept the displayed concentration, the value of which depends on the selected units. If an alternate concentration is used, entered the actual concentration and press **ENTER** to return to the read screen. See Section 1.5.5 *Adjusting the Standard Curve* for more information.

Calibration Standard Preparation

To perform a boron calibration using the azomethine-H, prepare a 25 mg/L boron stock solution by pipetting 10.0 mL of a 250-mg/L Boron Standard Solution (Cat. No. 14249-10) into a 100-mL plastic volumetric flask. Dilute to the mark with deionized water and mix thoroughly. Prepare calibration standards containing 0.25, 0.50, 0.75, 1.00, 1.25, and 1.50 mg/L B as follows:

- a. Into six different 100-mL plastic volumetric flasks, pipet 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0 mL of the 25-mg/L Boron stock solution.
- b. Dilute to the mark with deionized water and mix thoroughly.
- c. Using the azomethine-H method and the calibration procedure described in the *User-Entered Programs* section in the *DR/4000 Spectrophotometer Instrument Manual*, generate a calibration curve from the standards prepared above.

Summary of Method

Azomethine-H, a Schiff base, is formed by the condensation of an aminonaphthol with an aldehyde by the catalytic action of boron.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to *Section 1*.

Pollution Prevention and Waste Management

For information on pollution prevention and waste management, refer to *Section 1*.

REQUIRED REAGENTS AND STANDARDS

Description	Cat. No.
BoroTrace Reagent Set.....	26669-00
Includes: (1) 26666-69, (1) 26667-99, (1) 22419-26, (1) 25946-49	

Description	Quantity Required		Unit	Cat. No.
	Per Test			
EDTA Solution, 1 M	20 drops	.. 50 mL	SCDB.....	22419-26
BoroTrace #2 Reagent Powder Pillows.....	2		100/pkg.....	26666-69
BoroTrace #3 Reagent Powder Pillows.....	2		100/pkg.....	26667-99
Water, Ultra-Pure, Aldehyde-Free.....	25 mL		500 mL.....	25946-49

REQUIRED EQUIPMENT AND SUPPLIES

Cell, sample, 1-inch, polystyrene.....	2		2/pkg.....	24102-22
Clippers, for opening powder pillows	1		each.....	968-00
DR4000 1-Inch Cell Adapter	1		each.....	48190-00

OPTIONAL REAGENTS AND STANDARDS

Description	Unit	Cat. No.
Boron Standard Solution, 10-mL Voluette Ampule, 250-mg/L B,	16/pkg.....	14249-10
Boron Standard Solution, 1000-mg/L as B	100 mL.....	1914-42
Dechlorinating Reagent Powder Pillows.....	100/pkg.....	14363-69
Sodium Hydroxide Standard Solution, 5.0 N.....	59 mL DB.....	2450-26
Sulfamic Acid Standard Solution.....	113 g.....	2344-14
Sulfuric Acid Standard Solution, 1.000 N.....	100 mL MDB.....	1270-32

OPTIONAL EQUIPMENT AND SUPPLIES

Description	Unit	Cat. No.
Cell, sample, 1-inch, polystyrene.....	12/pkg.....	24102-12
DR/4000 Flow Cell Module Kit, 1-inch.....	each.....	48070-04
DR/4000 Flow Cell Module Kit, 1-cm.....	each.....	48070-05
DR/4000 Sipper Module Kit, 1-cm.....	each.....	48090-06
Filter Holder Assembly	each.....	2468-00
Filters, membrane, 3-micron	25/pkg.....	25940-25
Flask, volumetric, polypropylene, 100-mL	each.....	14060-42
Flask, volumetric, polypropylene, 1000-mL	each.....	14060-53
pH Paper, pH 1.0-11.0	each.....	391-33
Pipet, Mohr-type, polypropylene, 5-mL.....	each.....	2106-37
Pipet, TenSette, 0.1 to 1.0 mL	each.....	19700-01
Pipet Tips, for 19700-01 TenSette Pipet	each.....	21856-96
Scoop, 0.1-g.....	each.....	511-00
Thermometer, pocket, -10 to 110 °C	each.....	1877-01



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Method 8015

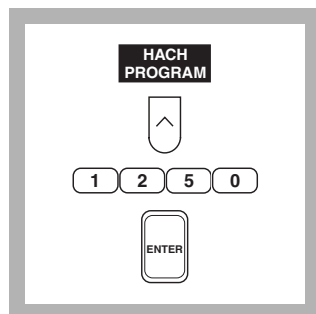
Carmine Method*

Powder Pillows

(0 to 14.0 mg/L)

Scope and Application: For water and wastewater. The estimated detection limit for program number 1250 is 0.4 mg/L B.

* Adapted from *Standard Methods for the Examination of Water and Wastewater*.



- 1.** Press the soft key under **HACH PROGRAM**.

Select the stored program number for Boron (B) by pressing **1250** with the numeric keys.

Press: **ENTER**

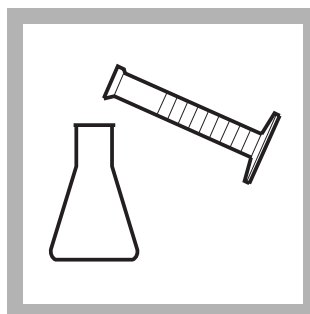
Note: If samples cannot be analyzed immediately, see *Sample Collection, Preservation and Storage* following these steps.

Note: The Flow Cell and Sipper Modules cannot be used with this procedure.



- 2.** The display will show: **HACH PROGRAM: 1250 Boron**

The wavelength (λ), **605 nm**, is automatically selected.



- 3.** Measure 75 mL of concentrated sulfuric acid, ACS, using a 100-mL graduated cylinder, into a 250-mL plastic erlenmeyer flask.

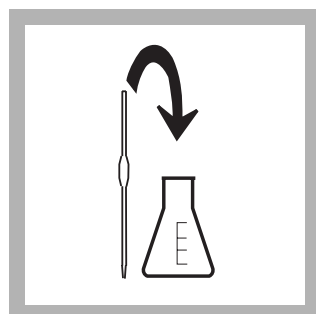
Note: All labware must be completely dry. Excess water will cause low results.

Note: For proof of accuracy, use a 4.0 mg/L boron standard solution in place of the sample (see *Accuracy Check*).



- 4.** Add the contents of one BoroVer 3 Reagent Powder Pillow to the flask. Swirl to mix. Allow 5 minutes for the powder to dissolve completely.

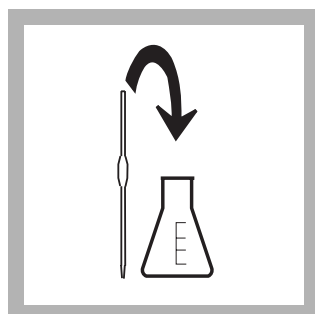
Note: Use with adequate ventilation; see *Reagent Preparation* below.



- 5.** Accurately pipet 2.0 mL of deionized water into a 125-mL plastic erlenmeyer flask (the blank).

Warning!

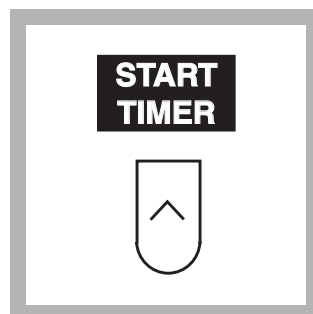
Do not use a stoppered or capped vessel to complete Steps 6 and 7.



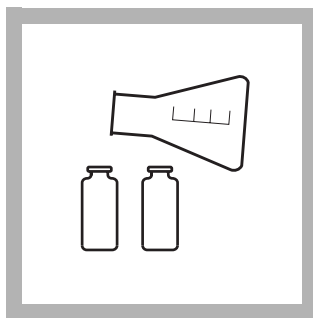
- 6.** Accurately pipet 2.0 mL of sample into another 125-mL plastic erlenmeyer flask (the prepared sample).



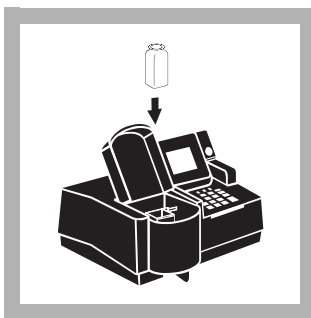
- 7.** Add 35 mL of the BoroVer 3/Sulfuric Acid Reagent Solution to each erlenmeyer flask using a 50-mL graduated cylinder. Swirl to mix completely.



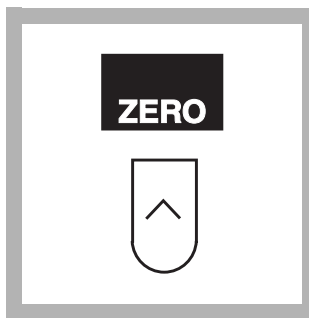
- 8.** Press the soft key under **START TIMER**. A 25-minute reaction period will begin.



9. When the timer beeps, pour at least 10 mL from each flask into separate 1-inch sample cells.



10. Place the blank into the cell holder. Close the light shield.

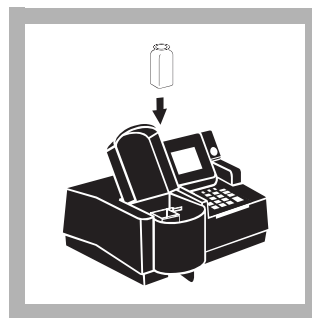


11. Press the soft key under **ZERO**.

The display will show:

0.00 mg/L B

***Note:** For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.*



12. Place the prepared sample into the cell holder. Close the light shield. Results in mg/L boron (or chosen units) will be displayed.

Sample Collection, Preservation and Storage

Collect samples in clean polyethylene or polypropylene bottles.

Reagent Preparation

To prepare additional BoroVer 3/Sulfuric Acid Solution, mix one BoroVer 3 Reagent Powder Pillow per 75 mL of concentrated sulfuric acid, adding the powder pillows individually while stirring. Preparation of this solution generates gaseous HCl when the indicator pillow is added to the sulfuric acid. Use of a fume hood or other well-ventilated lab area is strongly advised. This solution is stable up to 48 hours if stored in plastic containers. Prepare the reagent solution in a polypropylene flask.

Accuracy Check

Standard Additions Method

- Leave the unspiked sample in the sample compartment. Verify that the units displayed are in mg/L. Select standard additions mode by pressing the soft keys under **OPTIONS, (MORE)** and then **STD ADD**.
- Press **ENTER** to accept the default sample volume (mL), 25.
- Press **ENTER** to accept the default standard concentration (mg/L), 250.
- Press the soft key under **ENTRY DONE**.
- Snap the neck off a Boron Voluette Ampule Standard, 250-mg/L B.
- Use the TenSette Pipet to add 0.1 mL, 0.2 mL and 0.3 mL of standard, respectively to three 25-mL samples and mix each thoroughly.

- g. Analyze each standard addition sample as described above. Accept the standard additions reading by pressing the soft key under **READ** each time. Each addition should reflect approximately 100% recovery.
- h. After completing the sequence, the display will show the extrapolated concentration value and the “best-fit” line through the standard additions data points, accounting for matrix interferences.
- i. See Section 1.4.1 *Standard Additions* for more information.

Standard Solution Method

Check the accuracy of the test using Boron Standard Solution, 4 mg/L as B, listed under *OPTIONAL REAGENTS AND STANDARDS*.

Or, prepare this solution by pipetting 4.00 mL of the Boron Voluette Ampule Standard, 250-mg/L B, into a 250-mL volumetric flask. Dilute to volume with deionized water. Swirl to mix.

To adjust the calibration curve using the reading obtained with the 4.0-mg/L standard solution, press the soft keys under **OPTIONS, MORE** then **STD: OFF**. Press **ENTER** to accept the displayed concentration, the value of which depends on the selected units. If an alternate concentration is used, enter the actual concentration and press **ENTER** to return to the read screen. See Section 1.5.5 *Adjusting the Standard Curve* for more information.

Method Performance

Precision

Standard: 4.0 mg/L B

Program	95% Confidence Limits
1250	3.9–4.1 mg/L B

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
1250	0.4 mg/L B

For more information on derivation and use of Hach’s estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, Appendix B, see Section 1.5.1.

Sensitivity

Program Number: 1250

Portion of Curve	ΔAbs	ΔConcentration
0.010 Abs	0.010	0.15 mg/L
7 mg/L	0.010	0.16 mg/L
12.6 mg/L	0.010	0.17 mg/L

See Section 1.5.3 *Sensitivity Explained* for more information.

Calibration Standard Preparation

To perform a boron calibration using the carmine method, prepare a 100.0-mg/L boron stock solution by pipetting 10.00 mL of 1000-mg/L Boron Standard Solution (Cat. No. 1914-42) into a 100-mL volumetric flask using Class A glassware. Dilute to the mark with deionized water and mix thoroughly. Prepare calibration standards containing 2.0, 4.0, 6.0, 8.0, 10.0, 12.0, and 14.0 mg/L B as follows:

- a. Into seven different 100-mL Class A volumetric flasks, pipet 2.00, 4.00, 6.00, 8.00, 10.00, 12.00 and 14.00 mL of the 100.0-mg/L B stock solution using Class A glassware.
- b. Dilute to the mark with deionized water and mix thoroughly.
- c. Using the carmine method and the calibration procedure described in the *User-Entered Programs* section in the *DR/4000 Spectrophotometer Instrument Manual*, generate a calibration curve from the standards prepared above.

Summary of Method

Boron is determined by its reaction with carminic acid in the presence of sulfuric acid to produce a reddish to bluish color. The amount of color is directly proportional to the boron concentration.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to *Section 1*.

Pollution Prevention and Waste Management

The BoroVer 3/Sulfuric Acid Solution is highly acidic. Neutralize to pH 6–9 and flush down the drain for disposal. For more information on waste management, see *Section 1*.

REQUIRED REAGENTS AND STANDARDS

Description	Quantity Required		Unit Cat. No.
	Per Test		
BoroVer 3 Boron Reagent Powder Pillows	1 pillow	100/pkg	14170-99
Sulfuric Acid, ACS, concentrated	75 mL	2.5 liters	979-09
Water, deionized	2.0 mL	4 liters	272-56

REQUIRED EQUIPMENT AND SUPPLIES

Cylinder, graduated, 50-mL	1	each	508-41
Cylinder, graduated, 100-mL, plastic	1	each	1081-42
DR/4000 1-Inch Cell Adapter	1	each	48190-00
Flask, Erlenmeyer, 125-mL, plastic	2	each	1082-43
Flask, Erlenmeyer, 250-mL, plastic	1	each	1082-46
Pipet, volumetric, 2.0-mL	2	each	14515-36

OPTIONAL REAGENTS AND STANDARDS

Boron Standard Solution, 4-mg/L B	500 mL	1963-49
Boron Standard Solution, 10-mL Voluette Ampule, 250-mg/L B	16/pkg	14249-10
Boron Standard Solution, 1000-mg/L B	100 mL	1914-42

OPTIONAL EQUIPMENT AND SUPPLIES

Cylinder, graduated, 500-mL	each	20885-49
DR/4000 Carousel Module Kit	each	48090-02
Flask, erlenmeyer, 1000-mL	each	505-53
Pipet, TenSette, 0.1 to 1.0 mL	each	19700-01
Pipet Tips, for 19700-01 TenSette Pipet	50/pkg	21856-96
Pipet, volumetric, 4.00-mL	each	14515-04
Pipet Filler, safety bulb	each	14651-00
Sample cell, with 25-mL mark, 1-inch	2/pkg	13537-02



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WORLD HEADQUARTERS
Telephone: (970) 669-3050
FAX: (970) 669-2932



Method 8016

DPD Method*

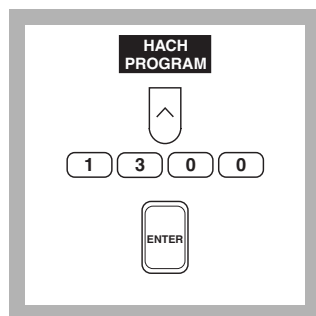
Powder Pillows or AccuVac® Ampuls

(0 to 4.50 mg/L)

Scope and Application: For testing bromine residuals (including hypobromite, hypobromous acid and bromamines) used as disinfectants in process waters, treated water, estuary and seawater. The estimated detection limits for program numbers 1300 and 1310 are 0.03 and 0.02 mg/L Br₂, respectively.

* Adapted from *Standard Methods for the Examination of Water and Wastewater*

Using Powder Pillows



1. Press the soft key under **HACH PROGRAM**.

Select the stored program for bromine (Br₂) by pressing **1300** with the numeric keys.

Press: **ENTER**

Note: Samples must be analyzed immediately and cannot be preserved for later analysis.

Note: The Flow Cell and Sipper Modules may be used with this procedure if rinsed with deionized water between sample readings. Use a 25 mL sample and reagents with the Flow Cell Module.



2. The display will show: **HACH PROGRAM: 1300 Bromine**

The wavelength (λ), **530 nm**, is automatically selected.

Note: For best results, determine a reagent blank for each new lot of reagent as follows. Prepare a reagent blank by repeating Steps 3 through 9, using deionized water as the sample. Zero the instrument on deionized water by pressing the soft key under **ZERO**. Insert the reagent blank and the blank value will be displayed. Correct for the reagent blank by pressing the soft keys under **OPTIONS, (MORE)**, and **BLANK:OFF**. Enter the reagent blank value and press **ENTER**. Repeat for each new lot of reagent.



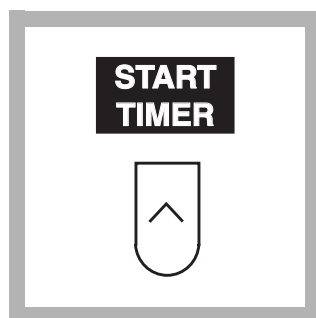
3. Fill a sample cell with 10 mL of sample.

Note: For samples with extreme pH, see the *Interferences* section.



4. Add the contents of one DPD Total Chlorine Powder Pillow to the sample cell (the prepared sample). Swirl to mix.

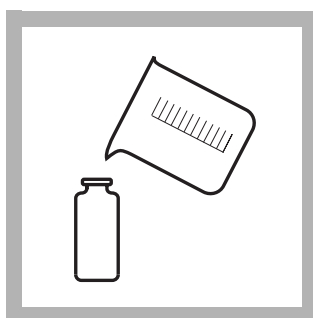
Note: A pink color will develop if bromine is present.



5. Press the soft key under **START TIMER**.

A 3-minute reaction period will begin.

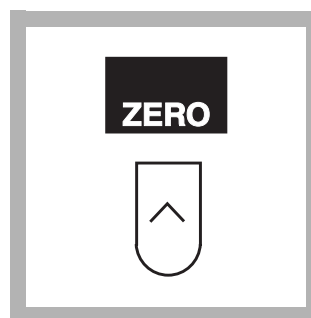
Note: Perform Steps 6–8 during this 3-minute period.



6. Fill a second sample cell (the blank) with 10 mL of sample.



7. Place the blank into the cell holder. Close the light shield.



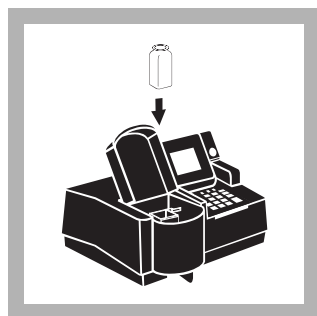
8. Press the soft key under **ZERO**.

The display will show:

0.000 mg/L Br₂

Note: If you are using a reagent blank correction, the display will show the correction.

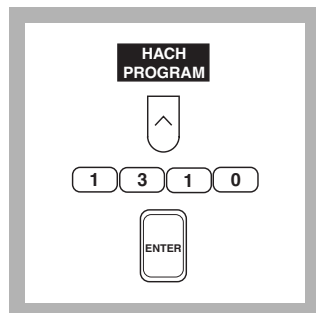
Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



9. Within 3 minutes after the timer beeps, place the prepared sample into the cell holder. Close the light shield. Results in mg/L bromine (or chosen units) will be displayed.

Note: If the sample temporarily turns yellow after reagent addition, or the display shows **OVER!**, dilute a fresh sample and repeat the test. A slight loss of bromine may occur because of the dilution. Multiply the result by the appropriate dilution factor; see Section 1.2.6 Sample Dilution Techniques.

Using AccuVac Ampuls



1. Press the soft key under **HACH PROGRAM**.

Select the stored program for AccuVac bromine by pressing **1310** with the numeric keys.

Press: **ENTER**

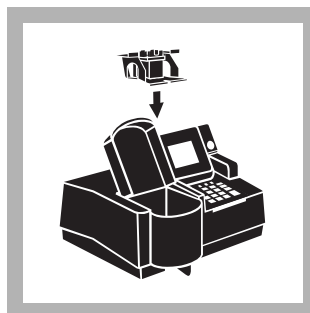
Note: Samples must be analyzed immediately and cannot be preserved for later analysis.



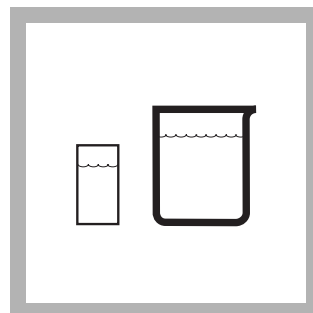
2. The display will show:
HACH PROGRAM:
1310 Bromine, AV

The wavelength (λ), **530 nm**, is automatically selected.

Note: For best results, determine a reagent blank for each new lot of reagent as follows. Prepare a reagent blank by repeating steps 3 through 10, using deionized water as the sample. Zero the instrument on deionized water by pressing the soft key under **ZERO**. Insert the reagent blank and the blank value will be displayed. Correct for the reagent blank by pressing the soft keys under **OPTIONS**, (**MORE**), and then **BLANK:OFF**. Enter the reagent blank value and press **ENTER**. Repeat for each new lot of reagent.

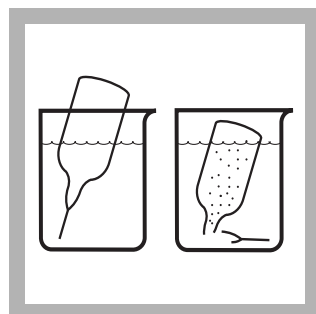


3. Insert the AccuVac adapter into the sample cell module by sliding it under the thumb screw and into the alignment grooves. Fasten with the thumb screw.



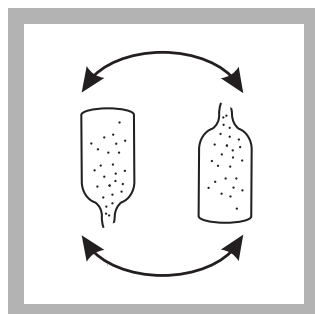
4. Fill a zeroing vial (the blank) with at least 10 mL of sample. Cap. Collect at least 40 mL of sample in a 50-mL beaker.

Note: For samples with extreme pH, see *Interferences* section.



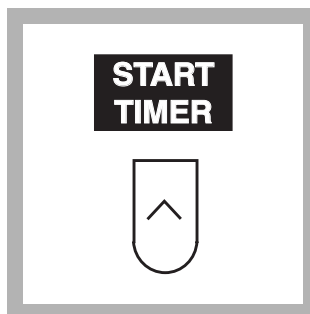
5. Fill a DPD Total Chlorine Reagent AccuVac Ampul with sample.

Note: Keep the tip immersed while the ampul fills completely.



6. Quickly invert the ampuls several times to mix. Wipe off any liquid or fingerprints.

Note: A pink color will form if bromine is present.

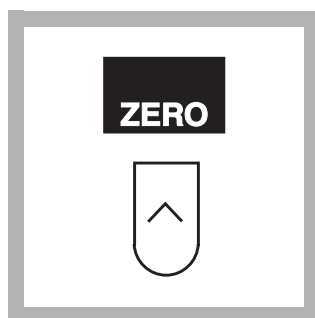


7. Press the soft key under **START TIMER**. A 3-minute reaction period will begin.

Note: Perform steps 8–9 during the 3-minute period.



8. Place the blank into the cell holder. Close the light shield.



9. Press the soft key under **ZERO**.

The display will show:

0.00 mg/L Br₂

Note: if you are using a reagent blank correction, the display will show the correction.

Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



10. Within 3 minutes after the timer beeps, place the AccuVac Ampul into the cell holder. Close the light shield. Results in mg/L bromine (or chosen units) will be displayed.

Note: If the sample temporarily turns yellow after reagent addition, or the display shows **OVER!**, dilute a fresh sample and repeat the test. A slight loss of bromine may occur because of the dilution. Multiply the result by the appropriate dilution factor; see Section 1.2.6 Sample Dilution Techniques.

Interferences

Interfering Substance	Interference Levels and Treatments
Acidity	Greater than 150 mg/L CaCO_3 . May not develop full color or color may fade instantly. Neutralize to pH 6–7 with 1 N sodium hydroxide. Determine amount to be added on separate sample aliquot, then add the same amount to the sample being tested. Correct for volume addition (see Section 1.2.2 <i>Correcting for Volume Additions</i>).
Alkalinity	Greater than 250 mg/L CaCO_3 . May not develop full color or color may fade instantly. Neutralize to pH 6–7 with 1 N sulfuric acid. Determine amount to be added on separate sample aliquot, then add the same amount to the sample being tested. Correct for volume addition (see Section 1.2.2 <i>Correcting for Volume Additions</i>).
Chlorine	Interferes at all levels
Chlorine Dioxide	Interferes at all levels
Chloramines, organic	May interfere
Hardness	No effect at less than 1,000 mg/L as CaCO_3
Iodine	Interferes at all levels
Manganese, Oxidized (Mn^{4+} , Mn^{7+}) or Chromium, Oxidized (Cr^{6+})	<ol style="list-style-type: none"> 1. Adjust sample pH to 6–7. 2. Add 3 drops potassium iodide (30 g/L) to a 25-mL sample. 3. Mix and wait minute. 4. Add 3 drops sodium arsenite (5 g/L) and mix. 5. Analyze 10 mL of the treated sample as described in the procedure. 6. Subtract the result from this test from the original analysis to obtain the correct bromine concentration.
Monochloramine	Interferes at all levels
Ozone	Interferes at all levels
Peroxides	May interfere
Extreme sample pH	Adjust to pH 6–7. See Section 1.3.1 <i>pH Interference</i> .
Highly Buffered Samples	Adjust to pH 6–7. See Section 1.3.1 <i>pH Interference</i> .

Sample Collection, Storage and Preservation

Collect samples in clean, dry glass containers. If sampling from a tap, allow the water to flow at least 5 minutes to ensure a representative sample. Avoid excessive agitation and exposure to sunlight when sampling. Allow several volumes of water to overflow the container and cap the container so there is not headspace above the sample. If sampling with a DR cell, rinse the cell several times with the sample, then carefully fill to the 10-mL mark. Proceed with the analysis immediately.

Accuracy Check

Standard Additions Method

- Leave the unspiked sample in the sample cell compartment. Verify that the units displayed are in mg/L. Select standard additions mode by pressing the soft keys under **OPTIONS, (MORE)** and then **STD ADD**.
- Press **ENTER** to accept the default sample volume (mL), 10.

- c. Multiply the average chlorine concentration shown on the certificate enclosed with the LR Voluettes by 2.25 to obtain the equivalent concentration of bromine. When prompted for the standard concentration, use the numeric keys to enter the calculated bromine value. Press **ENTER**.
- d. Press the soft key under **ENTRY DONE**.
- e. Snap the neck off a LR Chlorine Voluette Ampule Standard, 20–30 mg/L Cl₂.
- f. Use the TenSette Pipet to add 0.1 mL, 0.2 mL and 0.3 mL of standard, respectively to three 10-mL samples and mix each thoroughly.
- g. Analyze each standard addition sample as described above. Accept the standard additions reading by pressing the soft key under **READ** each time. Each addition should reflect approximately 100% recovery.
- h. After completing the sequence, the display will show the extrapolated concentration value and the “best-fit” line through the standard additions data points, accounting for matrix interferences.
- i. See Section 1.4.1 *Standard Additions* for more information.

Standard Solution Method (using AccuVac Ampuls)

- a. Leave the unspiked sample in the sample compartment. Verify that the units displayed are in mg/L. Select standard additions mode by pressing the soft keys under **OPTIONS, (MORE)** and then **STD ADD**.
- b. Press **ENTER** to accept the default sample volume (mL), 25.
- c. Multiply the average chlorine concentration shown on the certificate enclosed with the LR Voluettes by 2.25 to obtain the equivalent concentration of bromine. When prompted for the standard concentration, use the numeric keys to enter the calculated bromine value. Press **ENTER**.
- d. Press the soft key under **ENTRY DONE**.
- e. Snap the neck off a LR Chlorine Voluette Ampule Standard, 20–30 mg/L Cl₂.
- f. Use graduated cylinder to measure 25 mL of sample into each of three 50-mL beakers. Use a TenSette Pipet to add 0.2, 0.4 and 0.6 mL of standard, respectively, to each of the 25-mL samples. Swirl gently to mix.
- g. Fill a DPD Total Chlorine AccuVac completely from each beaker and analyze each standard addition sample as described above. Accept the standard additions readings by pressing the soft key under **READ** each time. Each addition should reflect approximately 100% recovery.
- h. After completing the sequence, the display will show the extrapolated concentration value and the “best-fit” line through the standard additions data points, accounting for matrix interferences.
- i. See *Standard Additions* for more information.

Method Performance

Precision

Standard: 2.25 mg/L Br₂

Program	95% Confidence Limits
1300	2.23–2.27 mg/L Br ₂
1310	2.23–2.27 mg/L Br ₂

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
1300	0.03 mg/L Br ₂
1310	0.02 mg/L Br ₂

For more information on derivation and use of Hach's estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, Appendix B, see Section 1.5.1.

Sensitivity

Portion of Curve: Entire Range

Program	ΔAbs	ΔConcentration
1300	0.010	0.042 mg/L
1310	0.10	0.042 mg/L

See Section 1.5.3 *Sensitivity Explained* for more information.

Summary of Method

Bromine residuals reacts with DPD (N,N-diethyl-p-phenylenediamine) to form a pink color which is proportional to the total bromine concentration.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to Section 1.

Pollution Prevention and Waste Management

Samples treated with sodium arsenite for manganese or chromium interferences will be hazardous wastes as regulated by the Federal RCRA for arsenic (D004). See Section 1 for more information on proper disposal of these materials.

BROMINE, continued

REQUIRED REAGENTS AND STANDARDS (Using Powder Pillows)

Description	Quantity Required per test	Unit	Cat. No.
DPD Total Chlorine Reagent Powder Pillows.....	1 pillow	100/pkg.....	21056-69

REQUIRED REAGENTS AND STANDARDS (Using AccuVac Ampuls)

DPD Total Chlorine Reagent AccuVac Ampuls.....	1 ampul.....	25/pkg.....	25030-25
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REQUIRED EQUIPMENT AND SUPPLIES (Using Powder Pillows)

DR/4000 1-Inch Cell Adapter	1	each.....	48190-00
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REQUIRED EQUIPMENT AND SUPPLIES (Using AccuVac Ampuls)

DR/4000 AccuVac Ampul Adapter.....	1	each.....	48187-00
Beaker, 50-mL.....	1	each.....	500-41
Sample Cell, 10-mL with cap (zeroing vial).....	1	each.....	21228-00

OPTIONAL REAGENTS AND STANDARDS

Chlorine Standard Solution, 2-mL Voluette Ampule, 20–30 mg/L	20/pkg.....	26300-20
Potassium Iodide Solution, 30-g/L.....	100 mL* MDB.....	343-32
Sodium Arsenite Solution, 5-g/L.....	100 mL* MDB.....	1047-32
Sodium Hydroxide Standard Solution, 1.00 N	100 mL* MDB.....	1045-32
Sulfuric Acid Standard Solution, 1.00 N.....	100 mL* MDB.....	1270-32
Water, deionized	4 liters.....	272-56

OPTIONAL APPARATUS

AccuVac Snapper	each.....	24052-00
Ampule Breaker, PourRite	each.....	24846-00
Cylinder, graduated, 25-mL, poly	each.....	1081-40
DR/4000 Carousel Module Kit	each.....	48070-02
DR/4000 Flow Cell Module Kit, 1-inch.....	each.....	48070-04
DR/4000 Sipper Module Kit, 1-inch.....	each.....	48090-03
pH Meter, <i>sension</i> TM 1, portable.....	each.....	51700-00
Pipet, TenSette, 0.1 to 1.0 mL	each.....	19700-01
Pipet Tips, for 19700-01 TenSette Pipet	50/pkg.....	21856-96
Sample Cells, 1-inch, polystyrene.....	12/pkg.....	24102-1

* Contact Hach for larger sizes



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Method 8017

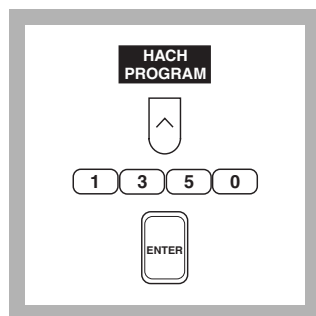
Dithizone Method*

Powder Pillows

(0 to 80.0 µg/L)

Scope and Application: For water and wastewater; digestion is required to determine total cadmium. See Section 2 for digestion procedure. The estimated detection limit for program number 1350 is 1.3 µg/L Cd.

* Adapted from *Standard Methods for the Examination of Water and Wastewater*



1. Press the soft key under **HACH PROGRAM**.

Select the stored program number for cadmium (Cd) by pressing **1350** with the numeric keys.

Press: **ENTER**

Note: If samples cannot be analyzed immediately, see *Sample Collection, Storage and Preservation* following these steps. Adjust the pH of preserved samples before analysis.

Note: The Flow Cell and Sipper Modules cannot be used with this procedure.

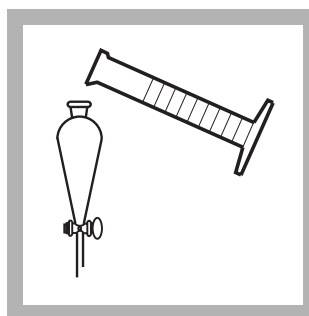


2. The display will show: **HACH PROGRAM: 1350 Cadmium**

The wavelength (λ), **515 nm**, is automatically selected.

Note: For proof of accuracy, use a 40 µg/L cadmium standard solution (preparation given in the *Accuracy Check*) in place of the sample.

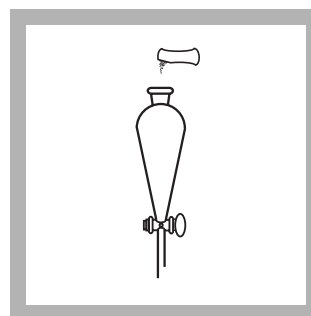
Note: For best results, determine a reagent blank for each new lot of reagent as follows. Prepare a reagent blank by repeating steps 3 through 14, using deionized water as the sample. Zero the instrument on chloroform by pressing the soft key under **ZERO**. Insert the reagent blank and the blank value will be displayed. Correct for the reagent blank by pressing the soft keys under **OPTIONS**, **(MORE)**, and then **BLANK:OFF**. Enter the reagent blank value and press **ENTER**. Repeat for each new lot of reagent.



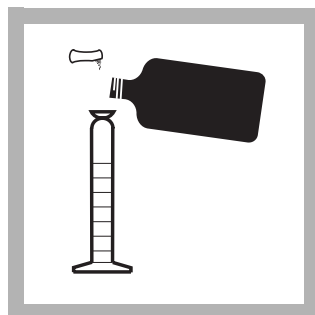
3. Fill a 250-mL graduated cylinder to the 250-mL mark with sample. Pour the sample into a 500-mL separatory funnel.

Note: Clean all glassware with 6 N Hydrochloric Acid Solution and rinse with deionized water.

Note: Cloudy and turbid samples may require filtering before running the test. Report results as µg/L soluble cadmium. Use glass membrane type filter to avoid loss of cadmium by adsorption onto the filter paper.



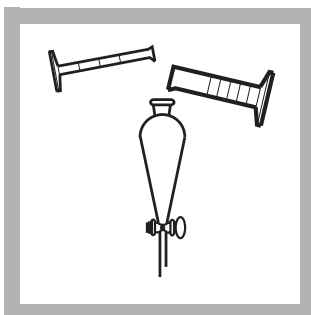
4. Add the contents of one Buffer Powder Pillow for heavy metals, citrate type. Stopper the funnel and shake to dissolve.



5. Add 30 mL of chloroform to a 50-mL mixing graduated cylinder. Add the contents of one DithiVer Metals Reagent Powder Pillow. Stopper the cylinder. Invert several times to mix (this is the DithiVer solution).

Note: Use adequate ventilation for the extraction, preferably a fume hood.

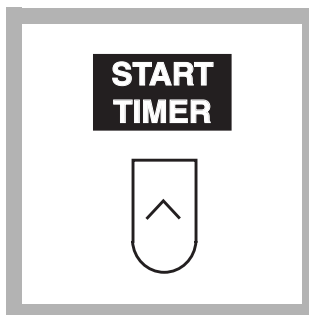
Note: The DithiVer powder will not completely dissolve in the chloroform. For further notes see DithiVer Solution Preparation and Storage.



6. Add 20 mL of 50% Sodium Hydroxide Solution. Add a 0.1-g scoop of potassium cyanide to the funnel. Stopper. Shake vigorously for 15 seconds.

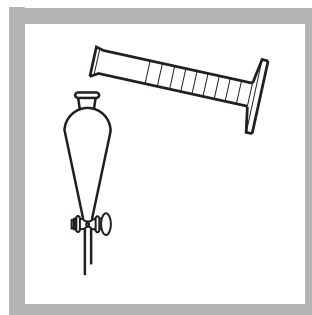
Warning: Cyanide is a deadly poison. Use a fume hood. Maintain cyanide solutions at pH 11 or greater to prevent formation of cyanide gas.

Note: Spilled reagent will affect test accuracy and is hazardous to skin and other materials. Please read the MSDS before testing.



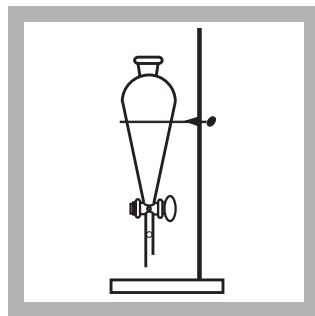
7. Remove the stopper. Press the soft key under **START TIMER**.

A 1-minute reaction period will begin.



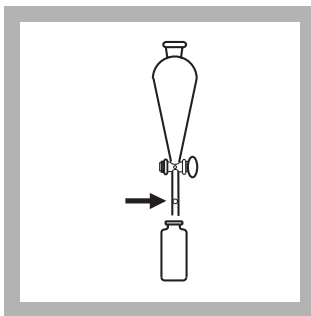
8. Add 30 mL of the DithiVer solution to the 500-mL separatory funnel. Stopper, invert, and open stopcock to vent. Close the stopcock and shake funnel once or twice; vent again.

Press the soft key under **START TIMER**. Close the stopcock and shake the funnel vigorously during the 1 minute time period.



9. Press the soft key under **START TIMER**, and allow the funnel to stand undisturbed until the timer beeps.

Note: The bottom (chloroform) layer will be orange to pink if cadmium is present.

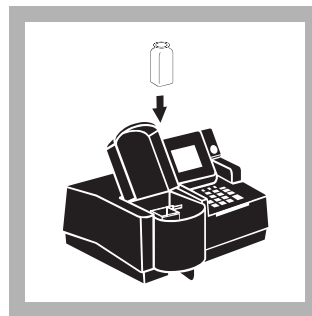


10. Insert a cotton plug the size of a pea into the delivery tube of the funnel and slowly drain the bottom (chloroform) layer into a dry 25-mL sample cell (the prepared sample). Stopper.

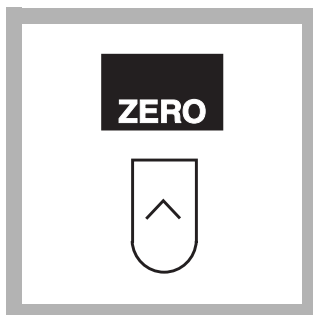
Note: The cadmium-dithizone complex is stable for more than one hour if the sample cell is kept tightly capped and out of direct sunlight.



11. Fill a dry 25-mL sample cell with chloroform (the blank). Stopper.



12. Place the blank into the cell holder. Close the light shield.



13. Press the soft key under **ZERO**.

The display will show:

0.0 µg/L Cd

Note: If you are using a reagent blank correction, the display will show the correction.

Note: For other concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return the read screen.



14. Place the prepared sample into the cell holder. Close the light shield. Results in µg/L cadmium (or chosen units) will be displayed.

Note: See Pollution Prevention and Waste Management following these steps for proper disposal of chloroform solutions.

Interferences

The following do not interfere:

Aluminum	Cobalt	Manganese
Antimony	Iron	Nickel
Arsenic	Lead	Tin
Calcium	Magnesium	Zinc
Chromium		

Table 1 Interfering Substances and Suggested Treatments

Interfering Substance	Interference Levels and Treatments
Highly buffered samples or Extreme sample pH	May exceed the buffering capacity of the reagents and require sample pretreatment; see Section 1.3.1 pH Interference.
Bismuth	Greater than 80 mg/L. See treatment below.
Copper	Greater than 2 mg/L. See treatment below.
Mercury	All levels. See treatment below.
Silver	Greater than 2 mg/L. See treatment below.

Eliminate interference from the metals in the table by the following treatment, beginning after Step 5.

- a. Measure about 5-mL of the DithiVer solution into the separatory funnel. Stopper the funnel, invert and open the stopcock to vent. Close the stopcock and shake the solution vigorously for 15 seconds. Allow the funnel to stand undisturbed until the layers separate (about 30 seconds). A yellow, red, or bronze color in the bottom (chloroform) layer confirms the presence of interfering metals. Draw off and collect the bottom (chloroform) layer for proper disposal.
- b. Repeat extraction with fresh 5 mL portions of the DithiVer solution (discarding the bottom layer each time) until the bottom layer shows a pure dark green color for three successive extracts. Extractions can be repeated several times without appreciably affecting the amount of cadmium in the sample.
- c. Extract the solution with several 2- or 3-mL portions of pure chloroform to remove any remaining DithiVer, collecting the bottom layer each time for proper disposal.
- d. Continue with Step 6 of the procedure.
- e. In Step 8, substitute 28.5 mL of DithiVer solution for the 30 mL.
- f. Continue with Step 9 of the procedure.

Sample Collection, Storage and Preservation

Collect samples in an acid-washed glass or plastic containers. Adjust the pH to 2 or less with nitric acid (about 2 mL per liter). Store preserved samples up to six months at room temperature. Adjust the pH to 2.5 with 5.0 N sodium hydroxide before analysis. Correct the test result for volume additions; see Section 1.2.2 *Correcting for Volume Additions*.

DithiVer Solution Preparation and Storage

Store DithiVer Powder Pillows away from light and heat. A convenient way to prepare this solution is to add the contents of 16 DithiVer Metals Reagent Powder Pillows to a 500-mL bottle of chloroform and invert several times until well mixed (carrier powder may not dissolve completely). Store dithizone solution in an amber glass bottle. This solution is stable for 24 hours.

Accuracy Check

Standard Additions Method

- a. Leave the unspiked sample in the sample compartment. Verify that the units displayed are in $\mu\text{g/L}$. Select standard additions mode by pressing the soft keys under **OPTIONS, (MORE)** and then **STD ADD**.
- b. Press **ENTER** to accept the default sample volume (mL), 250.
- c. Press **ENTER** to accept the default standard concentration ($\mu\text{g/L}$), 25,000.
- d. Press the soft key under **ENTRY DONE**.
- e. Snap the neck off a Cadmium Voluette Ampule Standard, 25-mg/L Cd (25,000- $\mu\text{g/L}$ Cd).

- f. Use the TenSette Pipet to add 0.1 mL, 0.2 mL and 0.3 mL of standard, respectively to three 250-mL samples and mix each thoroughly.
- g. Analyze each standard addition sample as described above. Accept the standard additions reading by pressing the soft key under **READ** each time. Each addition should reflect approximately 100% recovery.
- h. After completing the sequence, the display will show the extrapolated concentration value and the “best-fit” line through the standard additions data points, accounting for matrix interferences.
- i. See Section 1.4.1 *Standard Additions* for more information.

Standard Solution Method

Prepare a 5.0-mg/L cadmium standard solution by pipetting 5.00 mL of Cadmium Standard Solution, 100-mg/L Cd, into a 100-mL volumetric flask. Dilute to the mark with deionized water. Prepare this solution daily. Pipet 2.00 mL of the 5.0-mg/L Cadmium Standard Solution into 248 mL of deionized water in a 500-mL separatory funnel. This is a 40-μg/L cadmium solution. Perform the cadmium test on this solution beginning with Step 4 of the procedure.

Method Performance

Precision

Standard: 40.0 μg/L Cd

Program	95% Confidence Limits
1350	39.3–40.7 μg/L Cd

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
1350	1.3 μg/L Cd

For more information on derivation and use of Hach’s estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, Appendix B, see Section 1.5.1.

Sensitivity

Program Number: 1350

Portion of Curve:	ΔAbs	ΔConcentration
Entire Range	0.010	0.73 μg/L

See Section 1.5.3 *Sensitivity Explained* for more information.

Calibration Standard Preparation

To perform a cadmium calibration using the dithizone method, prepare a 1,000-μg/L cadmium stock solution by pipetting 10.00 mL of a 100-mg/L Cadmium Standard Solution (Cat. No. 14024-42) into a 1000-mL volumetric flask using Class A glassware. Dilute to the mark with deionized water and mix thoroughly.

Prepare calibration standard containing 12.00, 36.00, 64.00 and 80.00 µg/L Cd as follows:

- a. Into four different 250-mL volumetric flasks, pipet 3.00, 9.00, 16.00 and 20.00 mL of the 1,000-µg/L Cd stock solution using Class A glassware.
- b. Dilute to the mark with deionized water and mix thoroughly.
- c. Using the dithizone method and the calibration procedure described in the *User-Entered Programs* section of the *DR/4000 Spectrophotometer Instrument Manual*, generate a calibration curve from the standards prepared above.

Summary of Method

The dithizone method is designed for the determination of cadmium in water and wastewater. The DithiVer Metals Reagent is a stable powder form of dithizone. Cadmium ions in basic solution react with dithizone to form a pink to red cadmium-dithizonate complex, which is extracted with chloroform.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to Section 3.

Pollution Prevention and Waste Management

Both chloroform (D022) and cyanide (D003) solutions are regulated as hazardous wastes by the Federal RCRA. Do not pour these solutions down the drain. Chloroform solutions and the cotton plug used in the delivery tube of the separatory funnel should be collected for disposal with laboratory solvent waste. Collect the cyanide solution as a reactive waste. Be sure that cyanide solutions are stored in a caustic solution with a pH >11 to prevent potential release of hydrogen cyanide gas. See Section 3 for more information on proper disposal of these materials.

REQUIRED REAGENTS AND STANDARDS

	Cat. No.
Cadmium Reagent Set (100 Tests)	22422-00
Includes: (1) 14202-99, (1) 14458-17, (1) 12616-99, (1) 767-14, (4) 2180-49, (1) 2572-01	

Description	Quantity Required		Cat. No.
	per test	Unit	
Buffer Powder Pillows, citrate	1 pillow	100/pkg	14202-99
Chloroform, ACS	30 mL	4 L	14458-17
DithiVer Metals Reagent Powder Pillows	1 pillow	100/pkg	12616-99
Potassium Cyanide	0.1 g	125 g	767-14
Sodium Hydroxide Solution, 50%	20 mL	500 mL	2180-49
Cotton Balls, absorbent	1	100/pkg	2572-01

REQUIRED EQUIPMENT AND SUPPLIES

Clippers, for opening powder pillows	1	each	968-00
Cylinder, graduated, 25-mL	1	each	508-40
Cylinder, graduated, 250-mL	1	each	508-46
Cylinder, graduated, mixing, 50-mL	1	each	1896-41
Sample Cells, matched pair, 1-inch, glass, with stopper	2	pair	26126-02
DR/4000 1-Inch Cell Adapter	1	each	48190-00
Funnel, separatory, 500-mL	1	each	520-49
Spoon, measuring, 0.1-g	1	each	511-00
Support Ring, 4"	1	each	580-01
Support Ring Stand, 5" x 8" base	1	each	563-00

OPTIONAL REAGENTS AND STANDARDS

Cadmium Standard Solution, 100-mg/L Cd	100 mL	14024-42
Cadmium Standard Solution, 10-mL Voluette Ampule, 25-mg/L Cd	16/pkg	14261-10
Hydrochloric Acid Solution, 6.0 N	500 mL	884-49
Sodium Hydroxide Standard Solution, 5.0 N	100 mL MDB	2450-32
Sodium Hydroxide Standard Solution, 5.0 N	59 mL SCDB	2450-26
Water, deionized	4 liters	272-56
Chloroform, ACS	500 mL	14458-49

OPTIONAL EQUIPMENT AND SUPPLIES

Description	Unit	Cat. No.
Ampule Breaker Kit	each.....	21968-00
Cylinder, graduated, 5-mL	each.....	508-37
DR/4000 Carousel Module Kit	each.....	48090-02
Filter Discs, glass, 47 mm	100/pkg.....	2530-00
Filter Holder, glass, for 47-mm filter	each.....	2340-00
Flask, Erlenmeyer, 500-mL	each.....	505-49
Flask, filtering, 500-mL	each.....	546-49
Flask, volumetric, Class A, 100-mL	each.....	14574-42
Flask, volumetric, Class A, 250-mL	each.....	14574-46
Flask, volumetric, Class A, 1000-mL, with glass stopper.....	each.....	14574-53
Hot Plate, 3½-in. diameter, 120 VAC, 50/60 Hz	each.....	12067-01
Hot Plate, 3½-in. diameter, 240 VAC, 50/60 Hz, variable control	each.....	12067-02
pH Paper, pH 1.0 to 11.0	5 rolls/pkg.....	391-33
pH Meter, <i>sens^{ion}</i> TM 1, portable	each.....	51700-00
Pipet, serological, 2-mL	each.....	532-36
Pipet, TenSette, 0.1 to 1.0 mL	each.....	19700-01
Pipet Tips, for 19700-01 TenSette Pipet	50/pkg.....	21856-96
Pipet, volumetric, 2.00-mL, Class A	each.....	14515-36
Pipet, volumetric, 3.00-mL, Class A	each.....	14515-03
Pipet, volumetric, 6.00-mL, Class A	each.....	14515-06
Pipet, volumetric, 8.00-mL, Class A	each.....	14515-08
Pipet, volumetric, 9.00-mL, Class A	each.....	14515-09
Pipet, volumetric, 10.00-mL, Class A	each.....	14515-38
Pipet, volumetric, 20.00-mL, Class A	each.....	14515-20
Pipet Filler, safety bulb.....	each.....	14651-00
Tongs, crucible, 9-inch	each.....	569-00



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FAX: (970) 669-2932

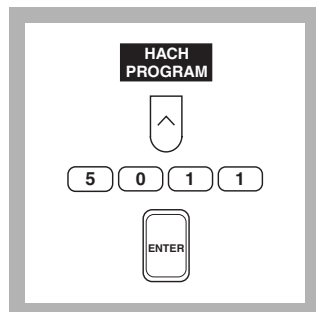


Cadion Method

UniCell™ Vials

(0 to 0.30 mg/L Free Cd)

Scope and Application: For wastewater process control. The estimated detection limit for program number 5011 is 0.02 mg/L Cd.



1. Press the soft key under **HACH PROGRAM**.

Select the stored program number for UniCell cadmium by pressing **5011** with the numeric keys.

Press: **ENTER**

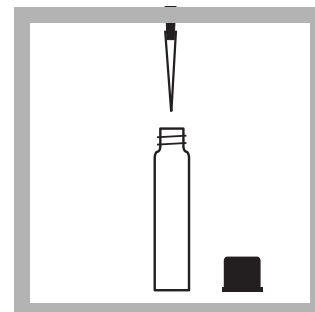


2. The display will show:
**HACH PROGRAM: 5011
Cadmium, HCT 152**

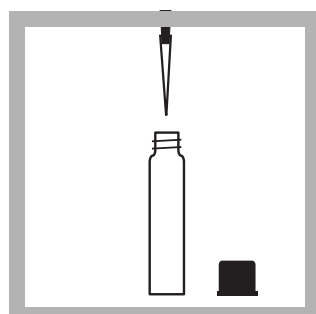
The wavelength (λ), **552 nm**, is automatically selected.



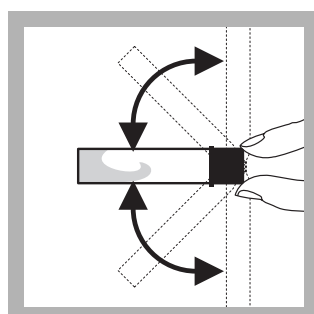
3. Insert the Test Tube Adapter into the sample cell module by sliding it under the thumb screw and into the alignment grooves. Fasten with the thumb screw.



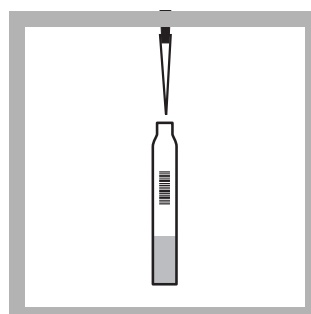
4. Pipet 10.0 mL of sample into the reaction tube (red cap).



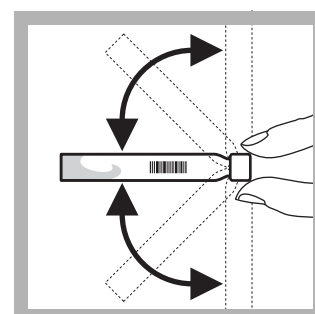
5. Pipet 1.0 mL of Complexing Agent A (HCT 154 A) to the reaction tube.



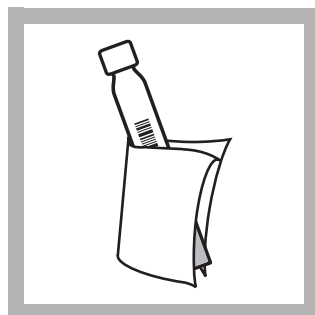
6. Close the reaction tube and invert several times to mix.



7. Pipet 0.5 mL of Stabilizer Solution B (HCT 154 B) into a sample vial (light red cap).



8. Close the sample vial and invert several times to mix.

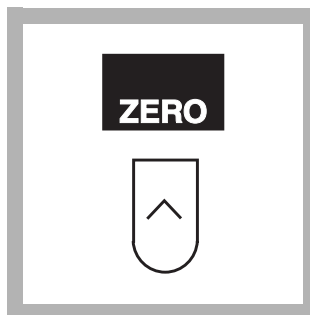


9. Clean the outside of the vial with a towel.

Note: Wiping with a damp towel, followed by a dry one, removes fingerprints and other marks.



10. Place the pretreated sample vial into the cell holder. Close the light shield.

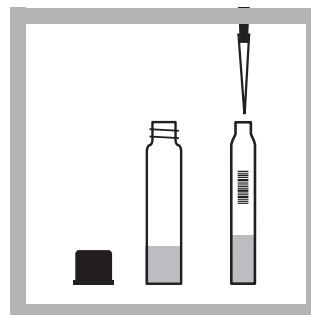


11. Press the soft key under **ZERO**.

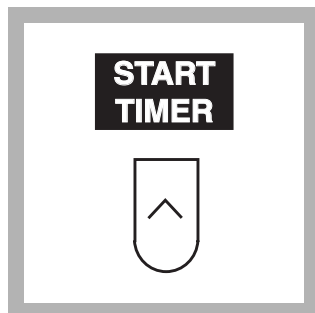
The display will show:

0.00 mg/L Free Cd

Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



12. Pipet 5.0 mL of sample from the reaction tube into the same sample vial.



13. Press the soft key under **START TIMER**.

A 30-second reaction period will begin.



14. When the timer beeps, place the sample vial into the cell holder. Close the light shield. The results in mg/L free Cd (or chosen units) will be displayed.

Interference

The ions listed in the table have been individually checked up to the given concentrations. Cumulative effects and the influence of other ions have not been evaluated.

Ion	No interference above:
Ca ²⁺ , Mg ²⁺	50 mg/L
Ag ⁺ , Au ⁺ , Cr ⁶⁺ , Zn ²⁺ , Cu ²⁺ , Fe ²⁺ , Pb ²⁺ , Co ²⁺ , Ni ²⁺	25 mg/L
Mn ²⁺	2 mg/L

Total cadmium, including undissolved cadmium and complexed cadmium, can only be determined after digesting with the Metal Prep Set, HCT 200.

Note: The total cadmium measuring range is 0.02 – 0.36 mg/L.

Sample Collection, Storage and Preservation

Collect samples in acid-cleaned glass or plastic containers. No acid addition is necessary if analyzing the samples immediately. To preserve samples, adjust the pH to 2 or less with concentrated nitric acid (about 2 mL per liter). Preserved samples may be stored up to six months at room temperature.

Before analysis, adjust the pH to between 3 and 6 with 5.0 N Sodium Hydroxide Standard Solution. Water samples which are free from complexing agents and organic compounds can be analyzed directly. Other water samples have to be digested with the Metal Prep Set in order to bring undissolved or complexed cadmium compounds into solution.

Accuracy Check

Standard Additions Method

- a. Select standard additions mode by pressing the soft keys under **OPTIONS, (MORE)** and then **STD ADD**.
- b. Press **ENTER** to accept the default sample volume (mL), 250.
- c. Press **ENTER** to accept the default standard concentration (mg/L), 100.
- d. Press the soft key under **ENTRY DONE**.
- e. Use a pipet to add 0.1 mL, 0.2 mL and 0.3 mL of standard, respectively, to three 250-mL samples. Mix each thoroughly.
- f. Analyze each standard addition sample as described above. Accept the standard additions reading by pressing the soft key under **READ** each time. Each addition should reflect approximately 100% recovery.
- g. After completing the sequence, the display will show the extrapolated concentration value and the “best-fit” line through the standard additions data points, accounting for matrix interferences.
- h. See Section 1.4.1 *Standard Additions* for more information.

Standard Solution Method

Prepare a 0.20-mg/L Cd standard solution by pipetting 0.20 mL of 100-mg/L Cd Standard into a 100-mL volumetric flask. Dilute to the mark with deionized water. Stopper and invert to mix. Prepare this solution daily. Perform the Cadmium procedure as described.

To adjust the calibration curve using the reading obtained with the 0.20-mg/L standard solution, press the soft keys under **OPTIONS, (MORE)** then **STD: OFF**. Press **ENTER** to accept the displayed concentration, the value of which depends on the selected units. If an alternate concentration is used, enter the actual concentration and press **ENTER** to return to the read screen. See Section 1.5.5 *Adjusting the Standard Curve* for more information.

Method Performance

Precision

Standard: 0.20 mg/L free Cd

Program	95% Confidence Limits
5011	0.13–0.27 mg/L free Cd

For more information on determining precision data and method detection limits, refer to *Section 1.5*.

Estimated Detection Limit

Program	EDL
5011	0.02 mg/L free Cd

For more information on derivation and use of Hach's estimated detection limit, see *Section 1.5.2*. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, appendix B, see *Section 1.5.1*.

Sensitivity

Program Number: 5011

Δ Abs	Δ Concentration
0.010	0.01 mg/L

See *Section 1.5.3 Sensitivity Explained* for more information.

Summary of Method

Cadmium ions react with cadion to form a red complex, the destruction of which results in a loss of color intensity that is directly related to the amount of cadmium in the sample.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used.

REQUIRED REAGENTS

Description	Unit	Cat. No.
Cadmium - Cd UniCell™ HCT 154	24/pkg	HCT 154

OPTIONAL REAGENTS

Cadmium Standard Solution, 100-mg/L as Cd	100 mL	14024-42
Metal Prep Set	50 digestions	HCT 200

OPTIONAL APPARATUS

Graduated cylinder, mixing, 250-mL	each	20886-46
Flask, volumetric, 100-mL	each	14574-42
Pipettor, (Jencons) 1–5 mL	each	27951-00
Replacement tips for 27951-00	100/pkg	27952-00
Pipettor, (Jencons) 100- to 1000- μ L	1 each	27949-00
Replacement tips for 27949-00	400/pkg	27950-00
pH Paper	100/pkg	26013-00



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DR/4000 PROCEDURE

CHLORAMINE, Mono, Low Range

Method 10171

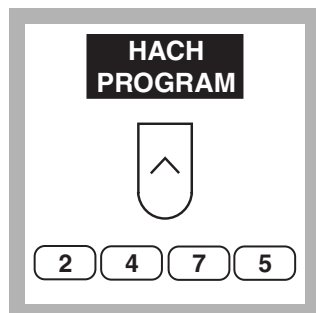
Indophenol method*

LR (0–4.50 mg/L Cl₂)

Scope and Application: Chloraminated drinking water and chlorinated wastewater.

* Patent pending

Note: For best results, determine a reagent blank for each lot of reagent using deionized water in place of the sample. Correct for the reagent blank by pressing the soft keys under **OPTIONS**, (**MORE**), and then **BLANK:OFF**. Enter the reagent blank value and press **ENTER**. Repeat for each lot of reagent.



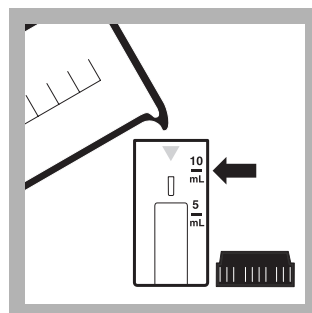
1. Press the soft key under **HACH PROGRAM**. Select the stored program number for Monochloramine, LR, by pressing **2475** using the numeric keys.



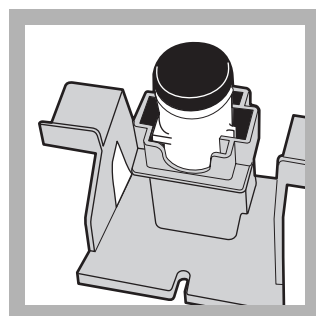
2. Press: **ENTER**.
The display will show:
HACH PROGRAM 2475 Monochlor F, LR
The wavelength (λ), **655 nm**, is automatically selected.



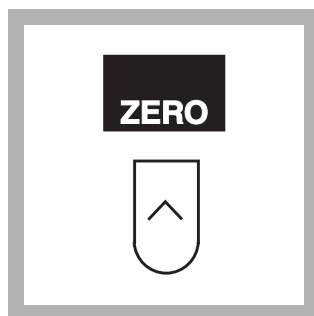
3. Insert the AccuVac[®] adapter into the sample cell module by sliding it under the thumbscrew and into the alignment grooves. Fasten with the thumbscrew.



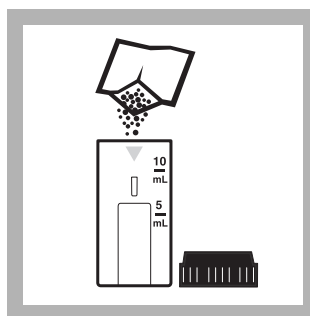
4. Fill the 10-mL/1-cm cell to the 10-mL line with sample.



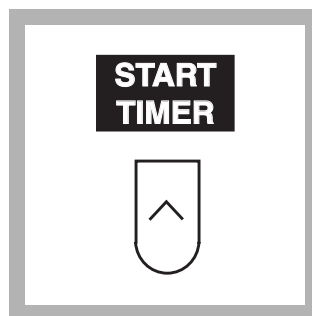
5. Place the cell into the cell holder so that the locking ridge on the cell is oriented to the left. Press the top rim of the cell on the right side to lock it in place. Close the light shield.



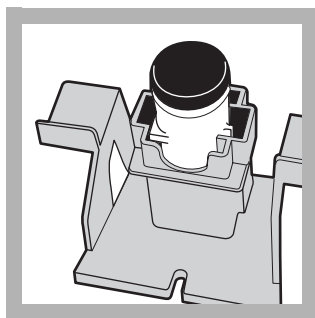
6. Press the soft key under **ZERO**.
The display will show:
mg/L Cl₂



7. Remove the cell from the cell holder and add the contents of one pillow Monochlor-F to the sample. Cap and shake the cell about 20 seconds to dissolve.



8. Press the soft key under **START TIMER**. A 5-minute reaction period will begin.
Note: Samples colder than 18 °C will require additional time. See Table 3.



9. After the color has developed fully, place the cell into the cell holder so the locking ridge on the cell is oriented to the left. Press the top rim of the cell on the right side to lock it in place.



10. Close the light shield. The result in mg/L monochloramine (as Cl_2) will be displayed.

Note: Results may be expressed as NH_2Cl or N. Press the soft keys under **OPTIONS** and then **FORM:** to scroll through the available options. Press **ENTER** to return to the read screen.

Sampling and Storage

Analyze samples for monochloramine immediately after collection. Rinse the sample container several times with sample, letting the container overflow each time. If sampling from a tap, let the water flow for at least 5 minutes before sampling. Then cap the container so that there is no head space (air) above the sample.

Method Performance

Precision

In a single laboratory, using a monochloramine standard of 2.10-mg/L Cl_2 and representative lots of reagent, a single operator obtained a standard deviation of ± 0.06 mg/L Cl_2 .

Estimated Detection Limit

The estimated detection limit (EDL) for Method 10172 is 0.09 mg/L Cl_2 . For more information on the EDL, see Section 1 of the *DR/4000 Procedures Manual*.

Accuracy Check

1. Prepare the following monochloramine standard fresh before use.
2. Add the contents of one Buffer Powder Pillow, pH 8.3 to about 50-mL of organic-free water in a clean 100-mL Class A volumetric flask. Swirl to dissolve the powder.
3. Using a Class A volumetric pipet, transfer 2.00 mL of Nitrogen, Ammonia Standard Solution, 100-mg/L as $\text{NH}_3\text{-N}$, into the flask.
4. Dilute to volume with organic-free water, cap and mix thoroughly. This is a 2.00-mg/L buffered ammonia standard.

5. Pipet 50.00 mL of the buffered ammonia standard into a clean 100-mL beaker. Add a stir bar.
6. Obtain a recent lot of Chlorine Solution Ampules, 50–70 mg/L, and note the actual free chlorine concentration for this lot.
7. Calculate the amount of Chlorine Solution to be added to the ammonia standard using the following equation:
$$\text{mL chlorine solution required} = \frac{455}{\text{free chlorine concentration}}$$
8. Open an ampule and, using a glass Mohr pipet, add the calculated amount of Chlorine Solution slowly to the ammonia standard, while mixing at medium speed on a stir-plate.
9. Allow the monochloramine solution to mix for 1 minute after all Chlorine Solution is added.
10. Quantitatively transfer the monochloramine solution to a clean 100-mL Class A volumetric flask. Dilute to the mark with organic-free water, cap, and mix thoroughly. This is a nominal 4.5-mg/L (as Cl₂) monochloramine standard.

Use this standard within 1 hour of preparation.

Interferences

The following have been tested for interference and found *not* to interfere up to the indicated levels:

Table 1 Non-interfering Substances

Substance	Maximum Level Tested
Alanine	1 mg/L N
Aluminum	10 mg/L
Bromide	100 mg/L Br ⁻
Bromine	15 mg/L Br ₂
Calcium	1000 mg/L as CaCO ₃
Chloride	18,000 mg/L
Chlorine Dioxide	5 mg/L ClO ₂
Chromium (III)	5 mg/L
Copper	10 mg/L
Cyanide	10 mg/L CN ⁻
Dichloramine	10 mg/L as Cl ₂
Fluoride	5 mg/L
Free Chlorine	10 mg/L Cl ₂
Glycine	1 mg/L N
Iron (II)	10 mg/L
Iron (III)	10 mg/L
Lead	10 mg/L
Nitrate	100 mg/L N
Nitrite	50 mg/L N
Phosphate	100 mg/L PO ₄

CHLORAMINE, Mono, Low Range, continued

Table 1 Non-interfering Substances (Continued)

Substance	Maximum Level Tested
Silica	100 mg/L SiO ₂
Sulfates	2600 mg/L
Sulfite	50 mg/L SO ₃ ²⁻
Tyrosine	1 mg/L N
Urea	10 mg/L N
Zinc	5 mg/L

Table 2 Interfering Substances

Interfering Substance and its effect		Interference Level	Recommended Treatment
Magnesium	+	Above 400 mg/L CaCO ₃	Add 5 drops Rochelle Salt Solution prior to testing. OR: use the high range (HR) test.
Manganese (+7)	–	Above 3 mg/L	Use the HR test; it will tolerate up to 10 mg/L.
Ozone	–	Above 1 mg/L	Usually doesn't coexist with monochloramine.
Sulfide	+	Turns a "rust" color if present.	Usually doesn't coexist with monochloramine.
Thiocyanate	–	Above 0.5 mg/L	Use the HR test; it will tolerate up to 2 mg/L.

Table 3 Minimum color development time as it relates to temperature*

Sample Temperature		Minutes
° C	° F	
5	40	10
7	42	9
9	48	8
10	50	8
12	54	7
14	58	7
16	61	6
18	68	4
20	73	3
23	75	2.5
25	77	2
>25	>77	2

* Exact timing is not critical. Once the color has developed fully, it will remain stable for a period of hours.

Summary of Method

In the presence of a cyanoferrate catalyst, monochloramine (NH_2Cl) in the sample reacts with a substituted phenol to form an intermediate monoimine compound. The intermediate couples with excess substituted phenol to form a green-colored indophenol, which is proportional to the amount of monochloramine present in the sample.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the Material Safety Data Sheet (MSDS) for information specific to the reagent used.

REQUIRED REAGENTS

Description	Quantity Required		Cat. No.
	Per Test	Unit	
Monochlor F Reagent Pillows	1	50/pkg	28022-46

REQUIRED APPARATUS

Sample Cell, 10-mL/1-cm	1	2/pkg	48643-02
Clippers	1	each	23694-00

OPTIONAL REAGENTS

Rochelle Salt Solution	29-mL DB	1725-33
Organic-Free Water	500-mL	26415-49
Buffer Powder Pillows	25/pkg	898-68
Nitrogen, Ammonia Standard Solution, 100-mg/L as $\text{NH}_3\text{-N}$	500-mL	24065-49
Chlorine Solution Voluette Ampule	16/pkg	14268-10

OPTIONAL APPARATUS

Beaker, 100-mL	each	500-42
Flask, Volumetric, Class A, 100-mL	each	14574-42
Pipet, Mohr, Glass, 10-mL	each	20934-38
Pipet, Volumetric, Class A, 2.00-mL	each	14515-36
Pipet, Volumetric, Class A, 50.00-mL	each	14515-41
Stir Bar, Octagonal	each	20953-52
Stirrer, Magnetic	each	23436-00



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FAX: (970) 669-2932



Method 10172

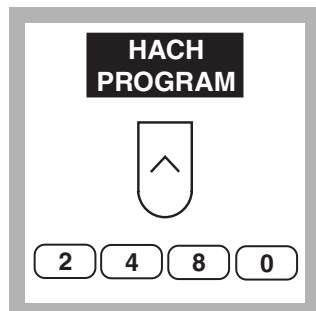
Indophenol method*

HR (0–10.0 mg/L Cl₂)

Scope and Application: Chlorinated wastewater.

* Patent pending

Note: For best results, determine a reagent blank for each lot of reagent using deionized water in place of the sample. Correct for the reagent blank by pressing the soft keys under **OPTIONS**, (**MORE**), and then **BLANK:OFF**. Enter the reagent blank value and press **ENTER**. Repeat for each lot of reagent.



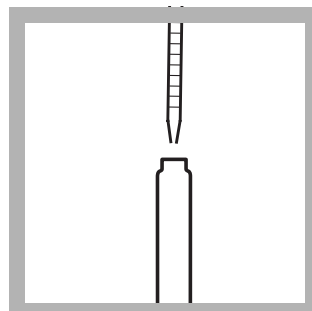
1. Press the soft key under **HACH PROGRAM**. Select the stored program number for Monochloramine, HR, by pressing **2480** using the numeric keys.



2. Press: **ENTER**.
The display will show:
**HACH PROGRAM 2480
Monochlor F, HR**
The wavelength (λ), 655 nm, is automatically selected.



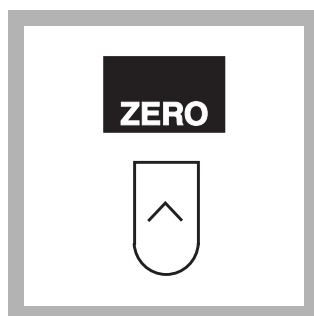
3. Insert the test tube adapter into the sample cell module by sliding it under the thumbscrew and into the alignment grooves. Fasten with the thumbscrew.



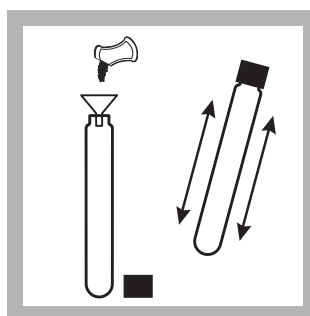
4. Remove the cap from one HR Monochloramine Diluent vial. Use a glass pipet to add 2.0 mL sample to the vial. Re-cap and invert several times to mix.



5. Wipe the outside of the vial clean. Place the vial into the test tube adapter with the Hach logo facing forward. Close the light shield.

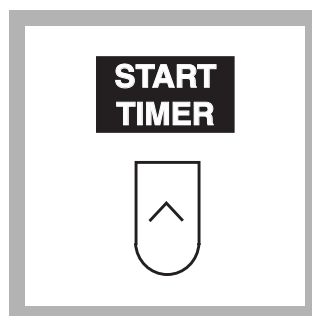


6. Press the soft key under **ZERO**.
The display will show:
mg/L Cl₂



7. Remove the vial from the adapter and add the contents of one Monochlor-F pillow to the sample. Cap and shake the cell about 20 seconds to dissolve.

Note: Use the micro-funnel as an aid in adding reagent powder to the vial.



8. Press the soft key under **START TIMER**. A 5-minute reaction period will begin.



9. After the timer beeps, place the vial into the test tube adapter with the Hach logo facing forward. Close the light shield. The result will be displayed in mg/L monochloramine (as Cl_2).

Note: Results may be expressed as NH_2Cl or N . Press the soft keys under **OPTIONS** and then **FORM:** to scroll through the available options. Press **ENTER** to return to the read screen.

Sampling and Storage

Analyze samples for monochloramine immediately after collection. Rinse the sample container several times with sample, letting the container overflow each time. If sampling from a tap, let the water flow for at least 5 minutes. Then cap the container so that there is no head space (air) above the sample.

Method Performance

Precision

In a single laboratory, using a monochloramine standard of 4.3 mg/L Cl_2 and representative lots of reagent, a single operator obtained a standard deviation of ± 0.1 mg/L Cl_2 .

Estimated Detection Limit

The estimated detection limit (EDL) for Method 10172 is 0.1 mg/L Cl_2 . For more information on the EDL, see See Section 1 of the *DR/4000 Procedures Manual*.

Accuracy Check

1. Prepare the following monochloramine standard fresh before use.
2. Add the contents of one Buffer Powder Pillow, pH 8.3 to about 50-mL of organic-free water in a clean 100-mL Class A volumetric flask. Swirl to dissolve the powder.
3. Using a Class A volumetric pipet, transfer 2.00 mL of Nitrogen, Ammonia Standard Solution, 100 mg/L as $\text{NH}_3\text{-N}$ into the flask.
4. Dilute to volume with organic-free water, cap and mix thoroughly. This is a 2.00 mg/L buffered ammonia standard.
5. Pipet 50.00 mL of the buffered ammonia standard into a clean 100-mL beaker. Add a stir bar.
6. Obtain a recent lot of Chlorine Solution Ampules, 50–70 mg/L, and note the actual free chlorine concentration for this lot.
7. Calculate the amount of Chlorine Solution to be added to the ammonia standard using the following equation:

$$\text{mL chlorine solution required} = \frac{455}{\text{free chlorine concentration}}$$
8. Open an ampule and, using a glass Mohr pipet, add the calculated amount of Chlorine Solution slowly to the ammonia standard, while mixing at medium speed on a stir-plate.
9. Allow the monochloramine solution to mix for 1 minute after all Chlorine Solution is added.
10. Quantitatively transfer the monochloramine solution to a clean 100-mL Class A volumetric flask. Dilute to the mark with organic-free water, cap, and mix thoroughly. This is a nominal 4.5 mg/L (as Cl_2) monochloramine standard.

Use this standard within 1 hour of preparation.

Interferences

The following have been tested for interference and found *not* to interfere up to the indicated levels:

Table 1 Non-interfering Substances

Substance	Maximum Level Tested
Alanine	1 mg/L as N
Aluminum	10 mg/L
Bromide	100 mg/L Br^-
Bromine	15 mg/L Br_2
Calcium	1000 mg/L as CaCO_3
Chloride	18,000 mg/L
Chlorine Dioxide	5 mg/L ClO_2
Chromium (III)	5 mg/L
Copper	10 mg/L

Table 1 Non-interfering Substances (Continued)

Substance	Maximum Level Tested
Cyanide	10 mg/L CN ⁻
Dichloramine	10 mg/L as Cl ₂
Fluoride	5 mg/L
Free Chlorine	10 mg/L Cl ₂
Glycine	1 mg/L as N
Iron (II)	10 mg/L
Iron (III)	10 mg/L
Magnesium	1000 mg/L as CaCO ₃
Manganese (VII)	10 mg/L
Lead	10 mg/L
Nitrate	100 mg/L as N
Nitrite	50 mg/L N
Phosphate	100 mg/L PO ₄
Silica	100 mg/L SiO ₂
Silver	10 mg/L
Sulfate	2600 mg/L
Sulfite	50 mg/L SO ₃ ²⁻
Tyrosine	1 mg/L as N
Urea	10 mg/L as N
Zinc	5 mg/L

Table 2 Interfering Substances

Interfering Substance and its effect		Interference Level	Recommended Treatment
Ozone	–	Above 1 mg/L	Usually doesn't coexist with monochloramine
Sulfide	+	Turns a "rust" color if present.	Usually doesn't coexist with monochloramine
Thiocyanate	–	Above 2 mg/L	

Summary of Method

The sample is first diluted in a Test 'N Tube™. In the presence of a cyanoferrate catalyst, monochloramine (NH₂Cl) in the sample reacts with a substituted phenol to form an intermediate monoimine compound. The intermediate couples with excess substituted phenol to form a green-colored indophenol, which is proportional to the amount of monochloramine present in the sample.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the Material Safety Data Sheet (MSDS) for information specific to the reagent used.

REQUIRED REAGENTS

HR Monochloramine Test 'N Tubes, 50 tests

Includes: (50) HR Monochloramine Diluent Vials*, (1) 25843-35, (1) 28022-4628051-45

Description	Quantity Required		Unit	Cat. No.
	Per Test			
Funnel, micro	1		each.....	25843-35
Monochlor F Reagent Pillows.....	1		50/pkg.....	28022-46

REQUIRED APPARATUS

Adapter, DR/4000 Test Tube.....	1		each.....	48189-00
Pipet, Mohr, glass, 2.00-mL	1		each.....	20936-36
Test Tube Rack	1		each.....	18641-00

OPTIONAL REAGENTS

Organic-Free Water	500-mL.....	26415-49
Buffer Powder Pillows	25/pkg.....	898-68
Nitrogen, Ammonia Standard Solution, 100 mg/L as NH ₃ -N	500-mL.....	24065-49
Chlorine Solution Voluette Ampule	16/pkg.....	14268-10

OPTIONAL APPARATUS

Beaker, 100-mL	each.....	500-42
Clippers, for medium powder pillows	each.....	968-00
Flask, Volumetric, Class A, 100-mL	each.....	14574-42
Pipet, Mohr, Glass, 10-mL	each.....	20934-38
Pipet, Volumetric, Class A, 2.00 mL.....	each.....	14515-36
Pipet, Volumetric, Class A, 50.00 mL.....	each.....	14515-41
Stir Bar, Octagonal	each.....	20953-52
Stirrer, Magnetic.....	each.....	23436-00
Clippers (shears).....	each.....	23694-00

* This item cannot be purchased separately.



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Method 8113

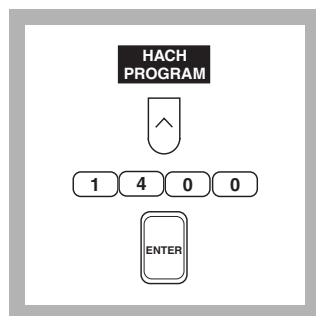
Mercuric Thiocyanate Method*

(0 to 25.00 mg/L Cl⁻)

Scope and Application: For water and wastewater.

The estimated detection limit for program number 1400 is 0.24 mg/L Cl⁻.

* Adapted from Zall, et. al., *Analytical Chemistry*, 28 (11) 1665 (1956)



1. Press the soft key under **HACH PROGRAM**.

Select the stored program number for chloride (Cl⁻) by pressing **1400** with the numeric keys.

Press: **ENTER**

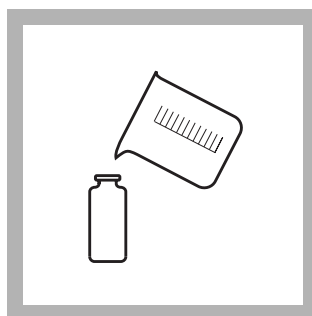
Note: If samples cannot be analyzed immediately, see *Sample Collection, Storage and Preservation* following these steps.

Note: The Flow Cell and Sipper Modules cannot be used with this procedure.



2. The display will show:
HACH PROGRAM: 1400 Chloride

The wavelength (λ), **455 nm**, is automatically selected.



3. Fill a sample cell with 25 mL of sample (the prepared sample).

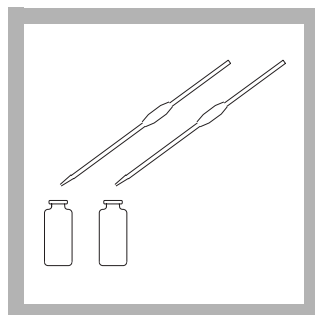
Note: Filter turbid samples through moderately rapid filter paper before analysis.

Note: For proof of accuracy, use a 10.0 mg/L chloride standard solution (see *Accuracy Check*) in place of the sample.

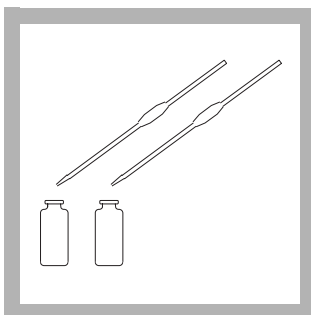
Note: For best results, determine a reagent blank for each new lot of reagent as follows. Prepare a reagent blank by repeating steps 3 through 10, using deionized water as the sample. Zero the instrument on deionized water by pressing the soft key under **ZERO**. Insert the reagent blank and the blank value will be displayed. Correct for the reagent blank by pressing the soft keys under **OPTIONS, (MORE)**, and then **BLANK:OFF**. Enter the reagent blank value and press **ENTER**. Repeat for each new lot of reagent.



4. Fill another sample cell with 25 mL of deionized water (the blank).

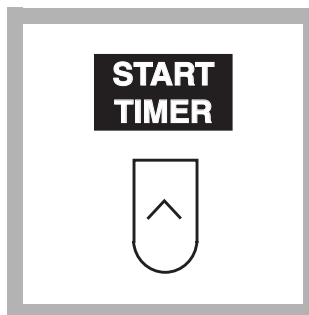


5. Pipet 2.0 mL of Mercuric Thiocyanate Solution into each sample cell. Swirl to mix.



6. Pipet 1.0 mL of Ferric Ion Solution into each sample cell. Swirl to mix.

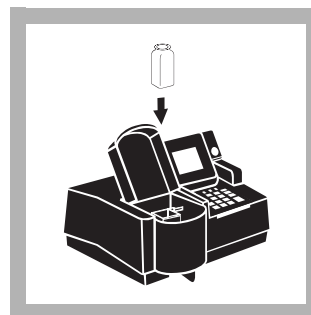
Note: An orange color will develop if chloride is present.



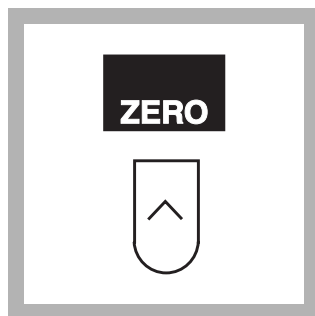
7. Press the soft key under **START TIMER**.

A 2-minute reaction period will begin.

Note: Read the sample within 5 minutes after the timer beeps.



8. Place the blank into the cell holder. Close the light shield.



9. Press the soft key under **ZERO**.

The display will show:

0.00 mg/L Cl⁻

Note: If you are using a reagent blank correction, the display will show the correction.

Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



10. Place the prepared sample into the cell holder. Close the light shield. Results in mg/L chloride (or chosen units) will be displayed.

Interferences

Table 1 Interfering Substances and Suggested Treatments

Interfering Substance	Interference Levels and Treatments
Extreme pH	<p>Should be about 2</p> <p>If the sample is strongly acidic or alkaline, adjust a portion of sample before testing to a pH of about 7. Use either 5.0 N Sodium Hydroxide Standard Solution or a 1:5 dilution of perchloric acid. Use pH paper, as most pH electrodes will contaminate the sample with chloride.</p>

Sample Collection, Storage and Preservation

Collect samples in glass or plastic containers. Samples can be stored for at least 28 days at room temperature.

Accuracy Check

Standard Additions Method

- a. Leave the unspiked sample in the sample compartment. Verify that the units displayed are in mg/L. Select standard additions mode by pressing the soft keys under **OPTIONS, (MORE)** and then **STD ADD**.
- b. Press **ENTER** to accept the default sample volume (mL), 25.
- c. Press **ENTER** to accept the default standard concentration (mg/L), 1000.
- d. Press the soft key under **ENTRY DONE**.
- e. Use the TenSette Pipet to add 0.1 mL, 0.2 mL and 0.3 mL of 1000-mg/L Chloride Standard Solution, respectively, to three 25-mL samples and mix each thoroughly.
- f. Analyze each standard addition sample as described above. Accept the standard additions reading by pressing the soft key under **READ** each time. Each addition should reflect approximately 100% recovery.
- g. After completing the sequence, the display will show the extrapolated concentration value and the “best-fit” line through the standard additions data points, accounting for matrix interferences.
- h. See Section 1.4.1 *Standard Additions* for more information.

Standard Solution Method

Prepare a 10.00-mg/L chloride standard solution by pipetting 5.00 mL of Chloride Standard Solution, 1000-mg/L, into a 500-mL volumetric flask and diluting to the mark with deionized water. Use Class A glassware. Perform the chloride procedure as described above.

To adjust the calibration curve using the reading obtained with the 10.00-mg/L standard solution, press the soft keys under **OPTIONS, (MORE)** then **STD: OFF**. Press **ENTER** to accept the displayed concentration, the value of which depends on the selected units. If an alternate concentration is used, enter the actual concentration and press **ENTER** to return to the read screen. See Section 1.5.5 *Adjusting the Standard Curve* for more information.

Method Performance

Precision

Standard: 10.00 mg/L Cl⁻

Program	95% Confidence Limits
1400	9.84–10.16 mg/L Cl ⁻

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
1400	0.24 mg/L Cl ⁻

For more information on derivation and use of Hach's estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, Appendix B, see Section 1.5.1.

Sensitivity

Program Number: 1400

Portion of Curve:	ΔAbs	ΔConcentration
0.010 Abs	0.010	0.122 mg/L
12.50 mg/L	0.010	0.338 mg/L
22.50 mg/L	0.010	0.544 mg/L

See Section 1.5.3 *Sensitivity Explained* for more information.

Calibration Standard Preparation

To perform a chloride calibration using the mercuric thiocyanate method, prepare a 100.0-mg/L chloride stock solution by pipetting 50.00 mL of 1000-mg/L Chloride Standard Solution into a 500-mL volumetric flask using Class A glassware. Dilute to the mark with deionized water and mix thoroughly.

Prepare calibration standards containing 3.00, 6.00, 9.00, 12.00, 15.00, 18.00, and 20.00 mg/L Cl⁻ as follows:

- Into seven different 100-mL volumetric flasks, pipet 3.00, 6.00, 9.00, 12.00, 15.00, 18.00, and 20.00 mL of the 100.0-mg/L Cl⁻ stock solution using Class A glassware.
- Dilute to the mark with deionized water and mix thoroughly.
- Using the mercuric thiocyanate method and the calibration procedure described in the *User-Entered Programs* section in the *DR/4000 Spectrophotometer Instrument Manual*, generate a calibration curve from the standards prepared above.

Summary of Method

Chloride in the sample reacts with mercuric thiocyanate to form mercuric chloride and liberate thiocyanate ion. Thiocyanate ions react with the ferric ions to form an orange ferric thiocyanate complex. The amount of this complex is proportional to the chloride sample concentration. Chloride at these levels also can be determined directly using the Chloride Ion Selective Electrode (Cat. No. 50255-00).

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to *Section 1*.

Pollution Prevention and Waste Management

Both the sample and the blank will contain mercury (D009) at a concentration regulated as a hazardous waste by the Federal RCRA. Do not pour these solutions down the drain. See *Section 3* for more information on proper disposal of these materials.

CHLORIDE, continued

REQUIRED REAGENTS AND STANDARDS

	Cat. No.
Chloride Reagent Set (50 Tests*).....	23198-00
Includes: (1) 22122-42, (1) 22121-29	

Description	Quantity Required		Unit	Cat. No.
	per test			
Ferric Ion Solution	2 mL	100	mL	22122-42
Mercuric Thiocyanate Solution	4 mL	200	mL	22121-29
Water, deionized	25 mL	4	liters	272-56

REQUIRED EQUIPMENT AND SUPPLIES

DR/4000 1-Inch Cell Adapter	1	each.....	48190-00
Pipet, volumetric, Class A, 1.00-mL	1	each.....	14515-35
Pipet, volumetric, Class A, 2.00-mL	1	each.....	14515-36
Pipet Filler, safety bulb.....	1	each.....	14651-00
<i>or</i>			
Pipet, TenSette, 0.1 to 1.0 mL	1	each.....	19700-01
Pipet Tips, for 19700-01 TenSette Pipet	1	50/pkg.....	21856-96

OPTIONAL REAGENTS AND STANDARDS

Chloride Standard Solution, 1000-mg/L Cl ⁻	500 mL	183-49
Chloride Standard Solution, 2-mL Voluette Ampule, 12,500-mg/L Cl ⁻	20/pkg	14250-20
Perchloric Acid, ACS, 70%	680 g	757-65
Sodium Hydroxide Standard Solution, 5.0 N	50 mL DB	2450-26

OPTIONAL EQUIPMENT AND SUPPLIES

Combination Chloride Electrode	each	50255-00
DR/4000 Carousel Module Kit	each	48070-02
Filter Paper, folded, 12.5-cm	100/pkg	692-57
Flask, Erlenmeyer, 125-mL	each	505-43
Flask, volumetric, 100-mL, Class A	each	14574-42
Flask, volumetric, 100-mL, Class A	6/pkg	14574-72
Flask, volumetric, 500-mL, Class A, with glass stopper	each	14574-49
Funnel, analytical, filtering, polypropylene, 75-mm	each	1083-68
pH Paper, 1.0 to 11.0 pH	5 rolls/pkg	391-33

OPTIONAL EQUIPMENT AND SUPPLIES

Pipet, volumetric, Class A, 3.00-mL	each	14515-03
Pipet, volumetric, Class A, 5.00-mL	each	14515-37
Pipet, volumetric, Class A, 6.00-mL	each	14515-06
Pipet, volumetric, Class A, 9.00-mL	each	14515-09
Pipet, volumetric, Class A, 15.00-mL	each	14515-39
Pipet, volumetric, Class A, 20.00-mL	each	14515-20
Pipet, volumetric, Class A, 50.00-mL	each	14515-41

* 50 tests equals 25 samples and 25 blanks.



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Outside the U.S.A. – Contact the HACH office or distributor serving you.
On the Worldwide Web – www.hach.com; E-mail – techhelp@hach.com

HACH COMPANY
WORLD HEADQUARTERS
Telephone: (970) 669-3050
FAX: (970) 669-2932



✓ Method 8021

DPD Method*

Powder Pillows or AccuVac® Ampuls

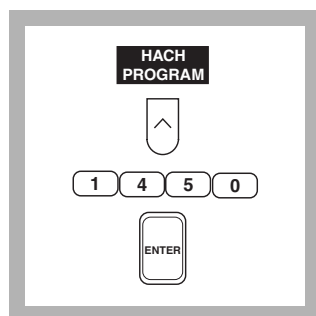
(0 to 2.00 mg/L)

Scope and Application: For testing free chlorine (hypochlorous acid and hypochlorite ion) in water, treated waters, estuary and seawater. USEPA accepted for reporting for drinking water analyses**. The estimated detection limit for program numbers 1450 and 1460 is 0.01 mg/L Cl₂.

* Adapted from *Standard Methods for the Examination of Water and Wastewater*

** Procedure is equivalent to USEPA method 330.5 and Standard Method 4500-Cl G for drinking water.

Using Powder Pillows



1. Press the soft key under **HACH PROGRAM**.

Select the stored program number for free chlorine (Cl₂) by pressing **1450** with the numeric keys.

Press: **ENTER**

Note: Samples must be analyzed immediately and cannot be preserved for later analysis. See Sample Collection, Storage and Preservation following these steps.

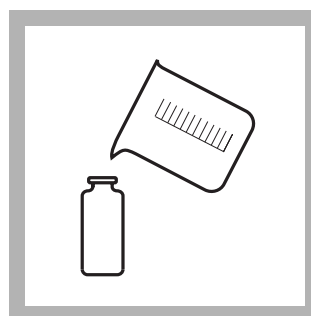
Note: The Flow Cell and Sipper Modules can be used with this procedure if rinsed between samples. Use a 25-mL sample and reagents with the Flow Cell Module.



2. The display will show:
HACH PROGRAM: 1450 Chlorine, F&T

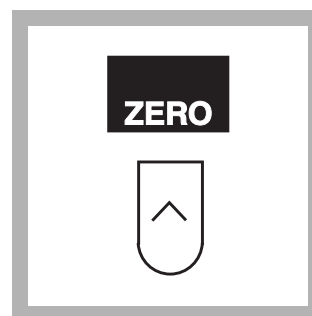
The wavelength (λ), **530 nm**, is automatically selected.

Note: For best results, determine a reagent blank for each new lot of reagent as follows. Prepare a reagent blank by repeating Steps 3 through 7, using deionized water as the sample. Zero the instrument on deionized water by pressing the soft key under **ZERO**. Insert the reagent blank and the blank value will be displayed. Correct for the reagent blank by pressing the soft keys under **OPTIONS, (MORE)**, and then **BLANK:OFF**. Enter the reagent blank value and press **ENTER**. Repeat for each new lot of reagent.



3. Fill a sample cell with 10 mL of sample (the blank). Place it into the cell holder and close the light shield.

Note: For sample with extreme pH, see Interferences section.



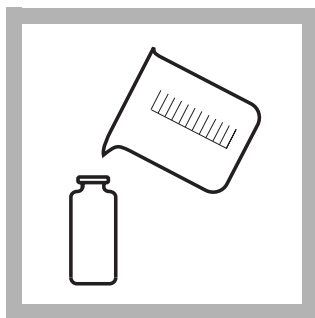
4. Press the soft key under **ZERO**.

The display will show:

0.00 mg/L Cl₂

Note: If you are using a reagent blank correction, the display will show the correction.

Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.

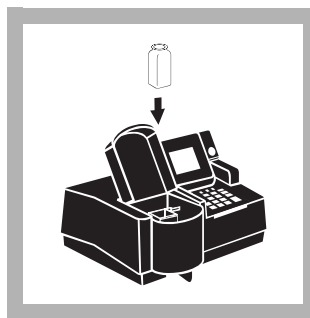


5. Fill another cell with 10 mL of sample.



6. Add the contents of one DPD Free Chlorine Powder Pillow to the sample cell (the prepared sample). Swirl the sample cell for 20 seconds to mix. Proceed to Step 7 immediately.

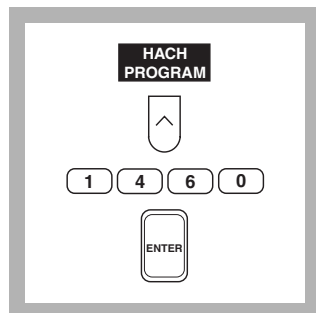
Note: A pink color will develop if free chlorine is present.



7. Place the prepared sample into the cell holder. Close the light shield. Read results in mg/L chlorine (or chosen units) within 1 minute of reagent addition.

Note: If the chlorine concentration in the sample exceeds the upper limit of the test, the color may fade or the display may show **OVER!** Dilute the sample with high quality water that is chlorine demand-free, and repeat the test. Some loss of chlorine may occur due to the dilution. Multiply the result by the appropriate dilution factor; See Section 1.2.6 Sample Dilution Techniques.

Using AccuVac Ampuls



1. Press the soft key under **HACH PROGRAM**.

Select the stored program number for free chlorine (Cl_2) by pressing **1460** with the numeric keys.

Press: **ENTER**

Note: Samples must be analyzed immediately and cannot be preserved for later analysis. See Sample Collection, Storage and Preservation following these steps.



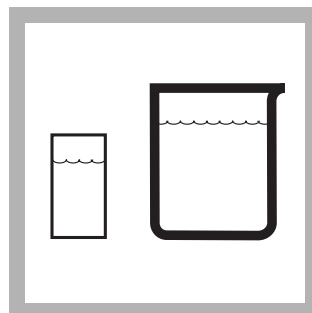
2. The display will show:
HACH PROGRAM: 1460 Chlorine, F&T AV

The wavelength (λ), **530 nm**, is automatically selected.

Note: For best results, determine a reagent blank. See Step 2 in the powder pillow procedure for instructions.



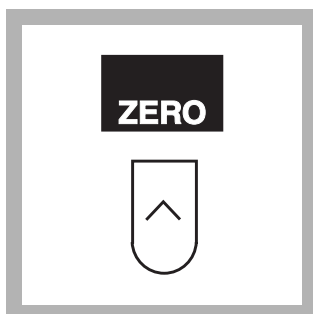
3. Insert the AccuVac Ampul Adapter into the sample cell module by sliding it under the thumb screw and into the alignment grooves. Fasten with the thumb screw.



4. Fill a zeroing vial (the blank) with at least 10 mL of sample. Collect at least 40 mL of sample in a 50-mL beaker.



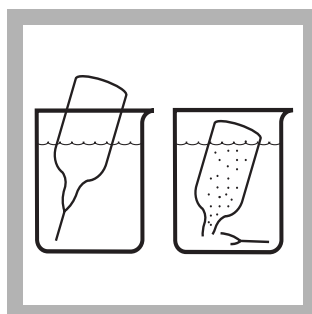
5. Place the blank into the cell holder. Close the light shield.



6. Press the soft key under **ZERO**.
The display will show:
0.00 mg/L Cl_2

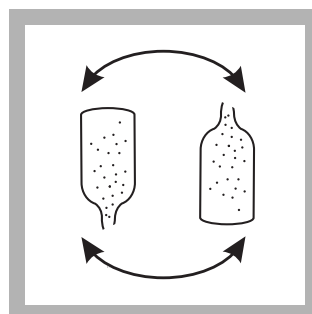
Note: If you are using a reagent blank correction, the display will show the correction.

Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



7. Fill a DPD Free Chlorine Reagent AccuVac Ampul with sample.

Note: Keep the tip immersed while the ampul fills completely



8. Quickly invert the ampul several times to mix. Wipe off any liquid or fingerprints.

Note: A pink color will form if free chlorine is present.



9. Immediately (within one minute of sample addition) place the AccuVac Ampul into the cell holder. Close the light shield. Results in mg/L chlorine (or chosen units) will be displayed.

Note: *If the chlorine concentration in the sample exceeds the upper limit of the test, the color may fade or the display may show **OVER!** Dilute the sample with high quality water that is chlorine demand-free, and repeat the test. Some loss of chlorine may occur due to the dilution. Multiply the result by the appropriate dilution factor; see Section 1.2.6 Sample Dilution Techniques.*

Interferences

Interfering Substance	Interference Levels and Treatments
Acidity	Greater than 150 mg/L CaCO_3 . May not develop full color or color may fade instantly. Neutralize to pH 6–7 with 1 N sodium hydroxide. Determine amount to be added on separate sample aliquot, then add the same amount to the sample being tested. Correct for volume addition (see Section 1.2.2 <i>Correcting for Volume Additions</i>).
Alkalinity	Greater than 250 mg/L CaCO_3 . May not develop full color or color may fade instantly. Neutralize to pH 6–7 with 1 N sulfuric acid. Determine amount to be added on separate sample aliquot, then add the same amount to the sample being tested. Correct for volume addition (see Section 1.2.2 <i>Correcting for Volume Additions</i>).
Bromine, Br_2	Interferes at all levels
Chlorine Dioxide, ClO_2	Interferes at all levels
Chloramines, organic	May interfere
Hardness	No effect at less than 1,000 mg/L as CaCO_3
Iodine, I_2	Interferes at all levels
Manganese, Oxidized (Mn^{4+} , Mn^{7+}) or Chromium, Oxidized (Cr^{6+})	<ol style="list-style-type: none"> 1. Adjust sample pH to 6–7. 2. Add 3 drops potassium iodide (30-g/L) to a 25-mL sample. 3. Mix and wait one minute. 4. Add 3 drops sodium arsenite (5-g/L) and mix. 5. Analyze 10 mL of the treated sample as described in the procedure. 6. Subtract the result from this test from the original analysis to obtain the correct chlorine concentration.
Monochloramine	Causes a gradual drift to higher readings. When read within 1 minute after reagent addition, 3 mg/L monochloramine causes less than a 0.1 mg/L increase in the reading.
Ozone	Interferes at all levels
Peroxides	May interfere
Extreme sample pH	Adjust to pH 6-7. See Section 1.3.1 <i>pH Interference</i> .
Highly Buffered Samples	Adjust to pH 6-7. See Section 1.3.1 <i>pH Interference</i> .

Sample Collection, Storage and Preservation

Analyze samples for chlorine immediately after collection. Free chlorine is a strong oxidizing agent and it is unstable in natural waters. It reacts rapidly with various inorganic compounds and more slowly oxidizes organic compounds. Many factors, including reactant concentrations, sunlight, pH, temperature and salinity influence decomposition of free chlorine in water.

Avoid plastic containers since these may have a large chlorine demand. Pretreat glass sample containers to remove any chlorine demand by soaking in a dilute bleach solution (1 mL commercial bleach to 1 liter of deionized water) for at least 1 hour. Rinse thoroughly with deionized or distilled water. If sample containers are rinsed thoroughly with deionized or distilled water after use, only occasional pre-treatment is necessary.

Do not use the same sample cells for free and total chlorine. If trace iodide from the total chlorine reagent is carried over into the free chlorine determination, monochloramine will interfere. It is best to use separate, dedicated sample cells for free and total chlorine determinations.

A common error in testing for chlorine is obtaining a representative sample. If sampling from a tap, let the water flow for at least 5 minutes to ensure a

representative sample. Let the container overflow with the sample several times, then cap the sample containers so there is no headspace (air) above the sample. If sampling with a sample cell, rinse the cell several times with the sample, then carefully fill to the 10-mL mark. Perform the chlorine analysis immediately.

Accuracy Check

Standard Additions Method (using powder pillows)

- a. Leave the unspiked sample in the sample compartment. Verify that the units displayed are in mg/L. Select standard additions mode by pressing the soft keys under **OPTIONS, (MORE)** and then **STD ADD**.
- b. Press **ENTER** to accept the default sample volume (mL), 10.
- c. Locate the average chlorine concentration shown on the certificate enclosed with the LR Voluettes. When prompted for the standard concentration, use the numeric keys to enter the certificate value. Press **ENTER**.
- d. Press the soft key under **ENTRY DONE**.
- e. Snap the neck off a LR Chlorine Voluette Ampule Standard, 20-30 mg/L Cl₂.
- f. Use the TenSette Pipet to add 0.1 mL, 0.2 mL and 0.3 mL of standard, respectively to three 10-mL samples and mix each thoroughly.
- g. Analyze each standard addition sample as described above. Accept the standard additions readings by pressing the soft key under **READ** each time. Each addition should reflect approximately 100% recovery.
- h. After completing the sequence, the display will show the extrapolated concentration value and the “best-fit” line through the standard additions data points, accounting for matrix interferences.
- i. See Section 1.4.1 *Standard Additions* for more information.

Standard Additions Method (using AccuVac Ampuls)

- a. Leave the unspiked sample in the sample compartment. Verify that the units displayed are in mg/L. Select standard additions mode by pressing the soft keys under **OPTIONS, (MORE)** and then **STD ADD**.
- b. Press **ENTER** to accept the default sample volume (mL), 25.
- c. Locate the average chlorine concentration shown on the certificate enclosed with the LR Voluettes. When prompted for the standard concentration, use the numeric keys to enter the certificate value. Press **ENTER**.
- d. Press the soft key under **ENTRY DONE**.
- e. Snap the neck off a LR Chlorine Voluette Ampule Standard, 20-30 mg/L Cl₂.
- f. Use graduated cylinder to measure 25 mL of sample into each of three 50-mL beakers. Use a TenSette Pipet to add 0.2, 0.4 and 0.6 mL of standard, respectively, to each of the 25-mL samples. Swirl gently to mix.
- g. Fill a DPD Free Chlorine AccuVac completely from each beaker and analyze each standard addition sample as described above. Accept the

standard additions readings by pressing the soft key under **READ** each time. Each addition should reflect approximately 100% recovery.

- h. After completing the sequence, the display will show the extrapolated concentration value and the “best-fit” line through the standard additions data points, accounting for matrix interferences.
- i. See Section 1.4.1 *Standard Additions* for more information.

Method Performance

Precision

Standard: 1.00 mg/L Cl₂

Program	95% Confidence Limits
1450	0.99–1.01 mg/L Cl ₂
1460	0.99–1.01 mg/L Cl ₂

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
1450	0.01 mg/L Cl ₂
1460	0.01 mg/L Cl ₂

For more information on derivation and use of Hach’s estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, Appendix B, see Section 1.5.1.

Sensitivity

Program Number: 1450

Portion of Curve	ΔAbs	ΔConcentration
Entire Range	0.010	0.019 mg/L

Program Number: 1460

Portion of Curve	ΔAbs	ΔConcentration
Entire Range	0.010	0.020 mg/L

See Section 1.5.3 *Sensitivity Explained* for more information.

Summary of Method

Chlorine in the sample as hypochlorous acid or hypochlorite ion (free chlorine or free available chlorine) immediately reacts with DPD (N,N-diethyl-p-phenylenediamine) indicator to form a pink color which is proportional to the chlorine concentration.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to Section 1.

Pollution Prevention and Waste Management

Samples treated with sodium arsenite for interferences will be hazardous waste as regulated by Federal RCRA for arsenic (D004). See Section 1 for further information on proper disposal of these materials.

REQUIRED REAGENTS AND STANDARDS (Using Powder Pillows)

Description	Quantity Required per test	Unit	Cat. No.
DPD Free Chlorine Reagent Powder Pillows, 10-mL.....	1 pillow	100/pkg	21055-69

REQUIRED REAGENTS AND STANDARDS (Using AccuVac Ampuls)

DPD Free Chlorine Reagent AccuVac Ampuls.....	1 ampul	25/pkg	25020-25
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REQUIRED EQUIPMENT AND SUPPLIES (Using Powder Pillows)

DR/4000 1-Inch Cell Adapter	1	each.....	48190-00
Stopper, rubber, No. 2	1	12/pkg.....	2118-02

REQUIRED EQUIPMENT AND SUPPLIES (Using AccuVac Ampuls)

Beaker, 50-mL.....	1	each.....	500-41
DR/4000 AccuVac Ampul Adapter.....	1	each.....	48187-00
Sample Cell, 10-mL with cap (zeroing vial).....	1	each.....	21228-00

OPTIONAL REAGENTS AND STANDARDS

Chlorine Standard Solution, 2-mL Voluette Ampule, 20–30 mg/L	20/pkg	26300-20
Potassium Iodide Solution, 30-g/L.....	100 mL * MDB.....	343-32
Sodium Arsenite Solution, 5-g/L	100 mL * MDB.....	1047-32
Sodium Hydroxide Standard Solution, 1.00 N.....	100 mL * MDB.....	1045-32
Sulfuric Acid Standard Solution, 1.000 N.....	100 mL * MDB.....	1270-32
SwifTest DPD Free Chlorine Reagent, with dispenser	250 tests.....	28023-00
SwifTest Replacement Vial	250 tests.....	21055-60
Water, deionized	4 liters.....	272-56

OPTIONAL EQUIPMENT AND SUPPLIES

AccuVac Snapper	each.....	24052-00
Ampule Breaker Kit	each.....	21968-00
Cylinder, graduated, 25-mL, poly	each.....	1081-40
DR/4000 Carousel Module Kit	each.....	48070-02
DR/4000 Flow Cell Module Kit, 1-inch.....	each.....	48070-04
DR/4000 Flow Cell Module Kit, 1-cm.....	each.....	48070-05
DR/4000 Sipper Module Kit, 1-inch	each.....	48090-03
Graph Paper, linear, 10 x 10	100/pkg.....	22313-00
pH Meter, <i>sensio</i> TM 1, portable	each.....	51700-00
Pipet, TenSette, 0.1 to 1.0 mL	each.....	19700-01
Pipet Tips, for 19700-01 TenSette Pipet	50/pkg.....	21856-96

* Contact Hach for larger sizes.



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Method 10102

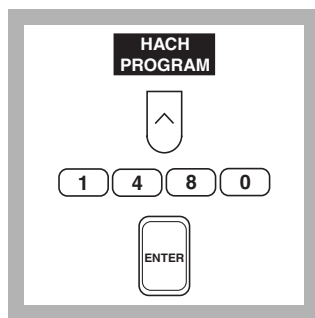
DPD Method*

Test 'N Tube™ Vials

(0 to 5.00 mg/L)

Scope and Application: For testing higher levels of free chlorine (hypochlorous acid and hypochlorite ion) in drinking water, cooling water, and industrial process waters. The estimated detection limit for program number 1480 is 0.04 mg/L Cl_2 .

* Adapted from *Standard Methods for the Examination of Water and Wastewater*



1. Press the soft key under **HACH PROGRAM**.

Select the stored program number for Test 'N Tube free chlorine (Cl_2) by pressing **1480** with the numeric keys.

Press: **ENTER**

Note: Samples must be analyzed immediately and cannot be preserved for later analysis.

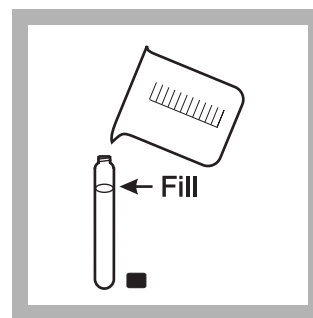


2. The display will show:
HACH PROGRAM: 1480
Chlorine, TNT

The wavelength (λ), **530 nm**, is automatically selected.



3. Insert the COD adapter into the sample module by sliding it under the thumbscrew and into the alignment grooves. Fasten with the thumbscrew.



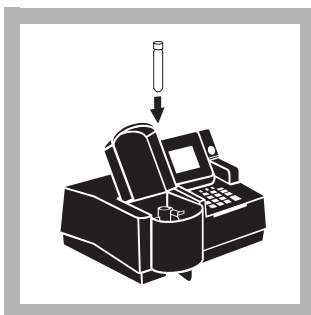
4. Fill an empty Test 'N Tube (TNT) vial with sample (the blank).

Note: Fill to the top of the label.

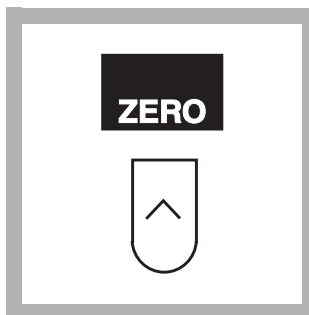


5. Clean the outside of the sample blank vial with a towel.

Note: Wiping with a damp towel followed by a dry one will remove fingerprints and other marks.



6. Place the blank into the adapter with the Hach logo facing the front of the instrument. Close the light shield.

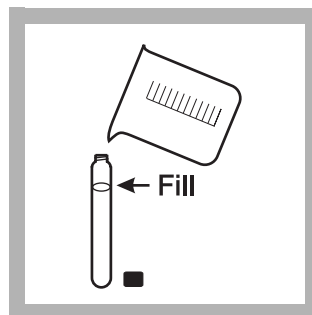


7. Press the soft key under **ZERO**.

The display will show:

0.00 mg/L Cl₂

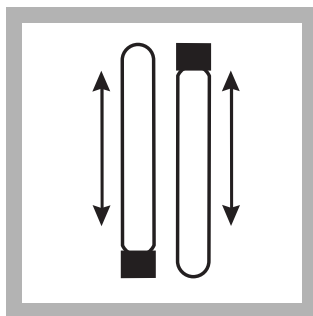
Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



8. Remove the cap from a Free Chlorine DPD-TNT tube. Add 10 mL of sample.

Note: Fill to the top of the label.

Note: A pink color will develop if free chlorine is present.

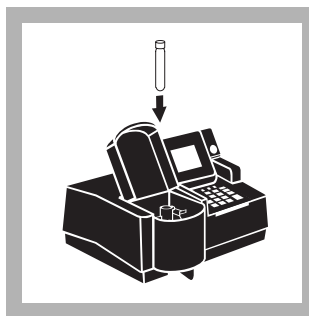


9. Cap and invert at least 10 times to dissolve the powder (the prepared sample).

Note: Use slow, deliberate inversion for complete recovery. Ten inversions should take at least 30 seconds. One inversion equals turning the vial upside down, then returning it to an upright position.



10. Within 30 seconds after mixing, wipe the vial containing the prepared sample.



11. Place the sample in the adapter with the Hach logo facing the front of the instrument. Close the light shield.

Results in mg/L chlorine (or chosen units) will be displayed.

Interferences

Interfering Substance	Interference Levels and Treatments																									
Acidity	Greater than 150 mg/L CaCO ₃ . May not develop full color or color may fade instantly. Neutralize to pH 6–7 with 1 N sodium hydroxide. Determine amount to be added on separate sample aliquot, then add the same amount to the sample being tested. Correct for volume addition (see <i>Correcting for Volume Additions</i> in the <i>DR/4000 Procedures Manual</i>).																									
Alkalinity	Greater than 250 mg/L CaCO ₃ . May not develop full color or color may fade instantly. Neutralize to pH 6–7 with 1 N sulfuric acid. Determine amount to be added on separate sample aliquot, then add the same amount to the sample being tested. Correct for volume addition (see <i>Correcting for Volume Additions</i> in the <i>DR/4000 Procedures Manual</i>).																									
Bromine, Br ₂	Interferes at all levels																									
Chlorine Dioxide, ClO ₂	Interferes at all levels																									
Chloramines, organic	May interfere																									
Hardness	No effect at less than 1,000 mg/L as CaCO ₃																									
Iodine, I ₂	Interferes at all levels																									
Manganese, oxidized (Mn ⁴⁺ , Mn ⁷⁺) or Chromium, oxidized (Cr ⁶⁺)	<ol style="list-style-type: none">1. Adjust sample pH to 6–7.2. Add 3 drops potassium iodide (30-g/L) to a 25-mL sample.3. Mix and wait 1 minute.4. Add 3 drops sodium arsenite (5-g/L) and mix.5. Analyze 10 mL of the treated sample as described in the procedure.6. Subtract the result from this test from the original analysis to obtain the correct chlorine concentration in the sample.																									
Monochloramine	<p>For conventional free chlorine disinfection (beyond the breakpoint), typical monochloramine concentrations are very low. If monochloramine is present in the sample, its interference in the free chlorine test depends on the sample temperature, relative amount of monochloramine to free chlorine, and the time required to do the analysis. Typical interference levels of monochloramine in the free chlorine test are listed below (as mg/L Cl₂).</p> <table><tr><th>NH₂Cl</th><th colspan="4">Sample Temp. °C (°F)</th></tr><tr><th>(as Cl₂)</th><th>5 (40)</th><th>10 (50)</th><th>20 (68)</th><th>30 (83)</th></tr><tr><td>1.2 mg/L</td><td>+0.15</td><td>+0.19</td><td>+0.30</td><td>+0.29</td></tr><tr><td>2.5 mg/L</td><td>0.35</td><td>0.38</td><td>0.55</td><td>0.61</td></tr><tr><td>3.5 mg/L</td><td>0.38</td><td>0.56</td><td>0.69</td><td>0.73</td></tr></table>	NH ₂ Cl	Sample Temp. °C (°F)				(as Cl ₂)	5 (40)	10 (50)	20 (68)	30 (83)	1.2 mg/L	+0.15	+0.19	+0.30	+0.29	2.5 mg/L	0.35	0.38	0.55	0.61	3.5 mg/L	0.38	0.56	0.69	0.73
NH ₂ Cl	Sample Temp. °C (°F)																									
(as Cl ₂)	5 (40)	10 (50)	20 (68)	30 (83)																						
1.2 mg/L	+0.15	+0.19	+0.30	+0.29																						
2.5 mg/L	0.35	0.38	0.55	0.61																						
3.5 mg/L	0.38	0.56	0.69	0.73																						
Ozone, O ₃	Interferes at all levels																									
Peroxides	May interfere																									
Extreme sample pH	Adjust to pH 6-7. See <i>pH Interferences</i> in the <i>DR/4000 Procedures Manual</i> .																									
Highly Buffered Samples	Adjust to pH 6-7. See <i>pH Interferences</i> in the <i>DR/4000 Procedures Manual</i> .																									

Sample Collection, Storage and Preservation

Analyze samples for chlorine immediately after collection. Free chlorine is a strong oxidizing agent and it is unstable in natural waters. It reacts rapidly with various inorganic compounds and more slowly oxidizes organic compounds. Many factors, including reactant concentrations, sunlight, pH, temperature and salinity influence decomposition of free chlorine in water.

Avoid plastic containers since these may have a large chlorine demand. Pretreat glass sample containers to remove any chlorine demand by soaking in a dilute bleach solution (1 mL commercial bleach to 1 liter of deionized water) for at least 1 hour. Rinse thoroughly with deionized or distilled water. If sample containers are rinsed thoroughly with deionized or distilled water after use, only occasional pre-treatment is necessary.

Do not use the same sample cells for free and total chlorine. If trace iodide from the total chlorine reagent is carried over into the free chlorine determination, monochloramine will interfere. It is best to use separate, dedicated sample containers for free and total chlorine determinations.

A common error in testing for chlorine is obtaining an unrepresentative sample. If sampling from a tap, let the water flow for at least 5 minutes to ensure a representative sample. Let the container overflow with the sample several times, then cap the sample containers so there is no headspace (air) above the sample. Perform the chlorine analysis immediately.

Accuracy Check

Standard Additions Method

- a. Leave the unspiked sample in the cell compartment. Verify that the units displayed are in mg/L. Select standard additions mode by pressing the soft keys under **OPTIONS, (MORE)** and then **STD ADD**.
- b. Press **ENTER** to accept the default sample volume (mL), 10.
- c. Locate the average chlorine concentration shown on the certificate enclosed with the HR Chlorine PourRite® Ampules. When prompted for the standard concentration, use the numeric keys to enter the certificate value. Press **ENTER**.
- d. Press the soft key under **ENTRY DONE**.
- e. Snap the neck off a HR Chlorine PourRite Ampule Standard, 50-75 mg/L Cl₂.
- f. Use the TenSette® Pipet to add 0.1 mL to a 10-mL sample. Mix thoroughly.
- g. Analyze the standard addition sample as described above. Accept the standard addition readings by pressing the soft key under **READ** each time. Each addition should reflect approximately 100% recovery.
- h. See *Standard Additions* in the *DR/4000 Procedures Manual* for more information.

Method Performance

Precision

at mg/L Cl ₂	95% Confidence Limits
0.10	±0.01 mg/L
2.50	±0.01 mg/L
3.40	±0.01 mg/L

For more information on determining precision data and method detection limits, refer to *Section 1.5 of the DR/4000 Procedures Manual*.

Estimated Detection Limit

The estimated detection limit for program 1480 is 0.04 mg/L Cl₂. For more information on derivation and use of Hach's estimated detection limit, see *Section 1.5.2 of the DR/4000 Procedures Manual*. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, Appendix B, see *Section 1.5.1 of the DR/4000 Procedures Manual*.

Sensitivity

Portion of Curve	ΔAbs	ΔConcentration
0.10	0.010	0.032 mg/L
2.50	0.010	0.035 mg/L
3.40	0.010	0.036 mg/L

See *Section 1.5.3 Sensitivity Explained* in the *DR/4000 Procedures Manual* for more information.

Summary of Method

Chlorine in the sample as hypochlorous acid or hypochlorite ion (free chlorine or free available chlorine) immediately reacts with DPD (N,N-diethyl-p-phenylenediamine) indicator to form a pink color which is proportional to the chlorine concentration.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to *Section 1 of the DR/4000 Procedures Manual*.

Pollution Prevention and Waste Management

Samples treated with sodium arsenite for interferences will be hazardous waste as regulated by Federal RCRA for arsenic (D004). See *Section 1 of the DR/4000 Procedures Manual* for further information on proper disposal of these materials.

CHLORINE, Free, continued

REQUIRED REAGENTS AND STANDARDS

Description	Quantity Required per test	Unit	Cat. No.
Test 'N Tube DPD Free Chlorine Reagent.....	1 vial.....	25/pkg.....	21055-45
Test 'N Tube Vials.....	1 vial.....	25/pkg.....	25831-25

REQUIRED EQUIPMENT AND SUPPLIES

COD/TNT Vial Adapter, DR/4000	1	each.....	48189-00
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OPTIONAL REAGENTS AND STANDARDS

Chlorine Standard Solution, 2-mL PourRite Ampule, 50-75mg/L	20/pkg.....	14268-20
Potassium Iodide Solution, 30-g/L.....	100 mL * MDB.....	343-32
Sodium Arsenite Solution, 5-g/L	100 mL * MDB.....	1047-32
Sodium Hydroxide Standard Solution, 1.00 N.....	100 mL * MDB.....	1045-32
Sulfuric Acid Standard Solution, 1.000 N.....	100 mL * MDB.....	1270-32
Water, deionized	4 liters	272-56

OPTIONAL EQUIPMENT AND SUPPLIES

Ampule Breaker Kit	each.....	21968-00
Beaker, 50-mL.....	each.....	500-41
pH Indicator Paper, 1 to 11 pH.....	5 rolls/pkg.....	391-33
Pipet, TenSette, 0.1 to 1.0 mL	each.....	19700-01
Pipet Tips, for 19700-01 TenSette Pipet	50/pkg.....	21856-96
Test Tube Rack	each.....	18641-00

* Contact Hach for larger sizes.



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WORLD HEADQUARTERS
Telephone: (970) 669-3050
FAX: (970) 669-2932



Method 10069

DPD method*

UHR (0.1–10.0 mg/L as Cl₂)

Scope and Application: For testing higher levels of free chlorine (hypochlorous acid and hypochlorite ion) in drinking water, cooling water, and industrial process waters.

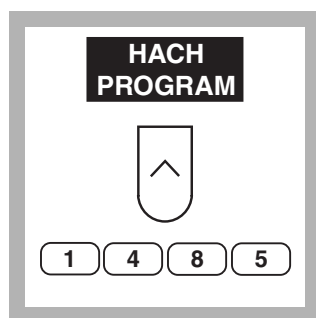
* Adapted from *Standard Methods for the Examination of Water and Wastewater*.

Tips and Techniques

- Analyze samples immediately. Do not preserve for later analysis.
- If chlorine is present, a pink color will develop after adding DPD Free Chlorine Reagent
- If the chlorine concentration is typically less than 2 mg/L, use method 8021, program number 1450.

Powder Pillow

Method 10069



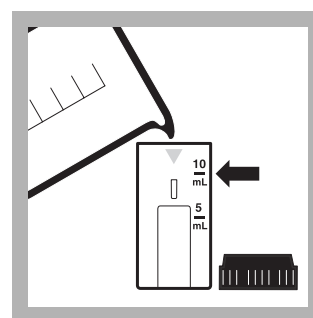
1. Press the soft key under **HACH PROGRAM**. Select the stored program number for Chlorine, HR by pressing **1485**. Use the numeric keys.



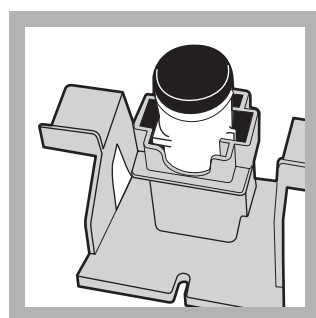
2. Press: **ENTER**.
The display will show:
HACH PROGRAM 1485 Chlorine, UHR
The wavelength (λ), **530 nm**, is automatically selected.



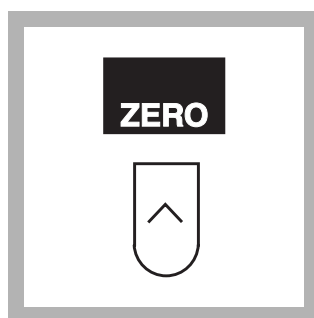
3. Insert the AccuVac[®] adapter into the sample cell module by sliding it under the thumbscrew and into the alignment grooves. Fasten with the thumbscrew.



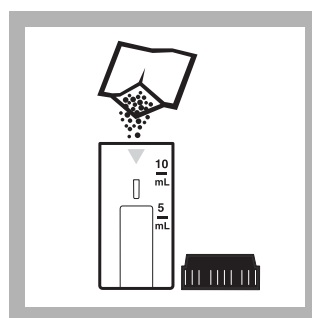
4. Fill the 10-mL/1-cm cell to the 5-mL line with sample.



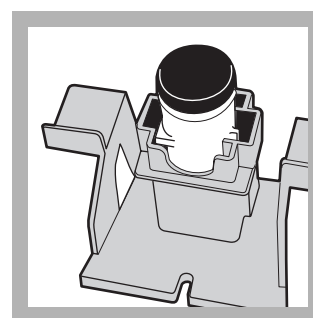
5. Place the cell into the cell holder so that the locking ridge on the cell is oriented to the left. Press the top rim of the cell on the right side to lock it in place. Close the light shield.



6. Press the soft key under **ZERO**.
The display will show:
0.0 mg/L Cl₂



7. Remove the cell from the cell holder and add the contents of one pillow of DPD Free Chlorine to the sample. Proceed immediately to step 8. Cap and shake the cell about 20 seconds to dissolve.



8. Place the cell into the cell holder so the locking ridge on the cell is oriented to the left. Press the top rim of the cell on the right side to lock it in place.



9. Close the light shield. Within 1 minute after reagent addition, read the results in mg/L chlorine (as Cl_2).

Sampling and Storage

Analyze samples for chlorine immediately after collection. free and combined chlorine are strong oxidizing agents and react rapidly with various compounds. Many factors such as sunlight, pH, temperature, and sample composition will influence decomposition of free chlorine in water.

Avoid plastic containers, which may have a large chlorine demand. Pretreat glass containers to remove chlorine demand by soaking in a dilute bleach solution (1 mL commercial bleach to 1 L of deionized water) for at least 1 hour. Rinse thoroughly with deionized or distilled water. If sample containers are rinsed thoroughly with deionized or distilled water after use, only occasional pretreatment is necessary.

Do not use the same sample cells for free and total chlorine. If trace iodide from the total chlorine reagent is carried over to the free chlorine test, monochloramine could interfere. It is best to use separate, dedicated sample cells for free and total chlorine determinations.

A common error in testing for chlorine is obtaining a representative sample. If sampling from a tap, let the water flow for at least 5 minutes to ensure a representative sample. Let the container overflow with the sample several times, then cap the sample container so there is no air above the sample. If sampling with a sample cell, rinse the cell several times with the sample, then carefully fill to the 5-mL mark. Proceed with the chlorine test immediately.

Method Performance

Precision

Standard: 5.6 mg/L Cl_2

Program	95% Confidence Limits of Distribution
1485	5.5–5.7 mg/L Cl_2

Sensitivity

Portion of Curve	Δ Abs	Δ Concentration
Entire range	0.010	0.1 mg/L Cl ₂

Accuracy Check

Standard Additions Method (Sample Spike)

1. Leave the unspiked sample in the sample compartment. Verify that the units displayed are in mg/L. Select standard additions mode by pressing the soft keys under **OPTIONS, (MORE)** and then **STD ADD**.
2. Press **ENTER** to accept the default sample volume (mL), 10.
3. Locate the average chlorine concentration shown on the certificate enclosed with the LR Voluette® Ampules. When prompted for the standard concentration, use the numeric keys to enter the certificate value. Press **ENTER**.
4. Press the soft key under **ENTRY DONE**.
5. Snap the neck off of a Chlorine Voluette® Ampule Standard, 50–75 mg/L Cl₂.
6. Use the Tensette® Pipet to add 0.1 mL, 0.2 mL, and 0.3 mL of standard, respectively, to three 10-mL samples. Mix each thoroughly.
7. Analyze each standard addition sample as described above. Accept the standard additions readings by pressing the soft key under **READ** each time. Each additions should reflect approximately 100% recovery.
8. After completing the sequence, the display will show the extrapolated concentration value and the “best-fit” line through the standard additions data points, accounting for matrix interferences.
9. See Section 1.4.1 *Standard Additions* for more information.

Interferences

The following have been tested for interference and found to interfere at the indicated levels:

Table 1 Interfering Substances

Substance	Level	Correction
Acidity	Greater than 150 mg/L CaCO ₃ May not develop full color or color may fade instantly.	1 Neutralize to pH 6–7 with 1 N Sodium Hydroxide. 2 Determine the amount to be added on separate sample aliquot, then add the same amount to the sample being tested. 3 Correct for volume additions.
Alkalinity	Greater than 250 mg/L CaCO ₃ May not develop full color or color may fade instantly.	1 Neutralize to pH 6–7 with 1 N Sulfuric Acid. 2 Determine the amount to be added on separate sample aliquot, then add the same amount to the sample being tested. 3 Correct for volume additions.

Table 1 Interfering Substances (Continued)

Substance	Level	Correction
Bromine, Br ₂	Interferes at all levels	
Chlorine dioxide, ClO ₂	Interferes at all levels	
Chloramines, organic	May interfere	
Chromium, oxidized (Cr ⁶⁺)		1 Adjust sample pH to 6–7. 2 Add 2 drops Potassium Iodide (30 g/L) to a 5-mL sample. 3 Mix and wait 1 minute. 4 Add 2 drops Sodium Arsenite* (5 g/L) and mix. 5 Analyze the treated sample as described in the procedure. Subtract the result from this test from the original analysis to obtain the correct chlorine concentration.
Iodine, I ₂	Interferes at all levels	
Manganese, oxidized (Mn ⁴⁺ , Mn ⁷⁺)		1 Adjust sample pH to 6–7. 2 Add 2 drops Potassium Iodide (30 g/L) to a 5-mL sample. 3 Mix and wait 1 minute. 4 Add 2 drops Sodium Arsenite* (5 g/L) and mix. 5 Analyze the treated sample as described in the procedure. 6 Subtract the result from this test from the original analysis to obtain the correct chlorine concentration.
Ozone	Interferes at all levels	
Peroxides	May interfere	
pH (extreme sample pH or highly buffered samples)		Adjust to pH 6–7 using acid (1.000 N Sulfuric Acid) or base (1.00 N Sodium Hydroxide).

* Samples treated with sodium arsenite for manganese or chromium interference will be hazardous wastes as regulated by the Federal RCRA for arsenic (D004).

Summary of Method

The range of analysis using the DPD method for free chlorine can be extended by adding more indicator in proportion to sample volume. Thus, a larger fill powder pillow of DPD Free Chlorine Reagent is added to a 5-mL sample portion.

Chlorine in the sample as hypochlorous acid or hypochlorite ion (free chlorine or free available chlorine) immediately reacts with DPD (N, N-diethyl-p-phenylenediamine) indicator to form a magenta color which is proportional to the chlorine concentration.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the Material Safety Data Sheet (MSDS) for information specific to the reagent used.

REQUIRED REAGENTS

Description	Quantity Required		Unit	Cat. No.
	Per Test			
DPD Free Chlorine Reagent Powder Pillows.....	1		100/pkg.....	14070-99

REQUIRED APPARATUS

Sample Cell, 10-mL/1-cm, with cap	1		2/pkg.....	48643-02
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OPTIONAL REAGENTS

Sodium Hydroxide, 1 N	100 mL MDB.....	1045-32
Sulfuric Acid, 1 N	100 mL MDB.....	1270-32
Potassium Iodide, 30 g/L.....	100 mL MDB.....	343-32
Sodium Arsenite, 5 g/L	100 mL.....	1047-32

OPTIONAL APPARATUS

Cylinder, graduated mixing.....	each.....	1896-40
TenSette Pipet, 0.1–1.0 mL	each.....	19700-01
Replacement Tips for 19700-01	50/pkg.....	21856-96

REQUIRED STANDARDS

Chlorine Standard Solution, PourRite™ Ampules, 50–75 mg/L.....	20/pkg.....	14268-20
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✓ Method 8370

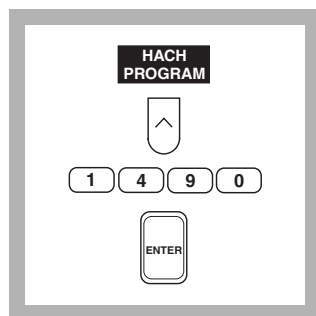
DPD Method*

ULR (0 to 500 µg/L as Cl₂)

Scope and Application: For testing trace levels of chlorine and chloramines in clean waters relatively free of color and turbidity. USEPA accepted for reporting for drinking water analysis.

The estimated detection limit for program number 1490 is 3 µg/L Cl₂.

* U.S. Patent 5,362,650



1. Press the soft key under **HACH PROGRAM**.

Select the stored program number for ultra low range (ULR) chlorine by pressing **1490** with the numeric keys.

Press: **ENTER**

Note: Samples should be analyzed immediately after collection, as chlorine is not stable in aqueous solution.

Note: See Treating Analysis Labware section for more information on cleaning labware. The Flow-Thru and Sipper Cells must be cleaned and treated for chlorine demand.

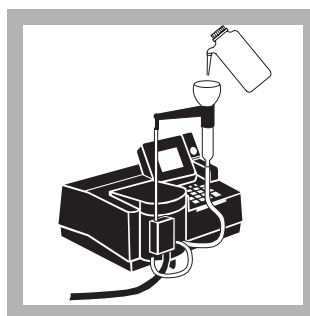
Note: The Single Cell Module cannot be used in this procedure. See the Summary of Method section.



2. The display will show:
**HACH PROGRAM: 1490
Chlorine, Tot. ULR**

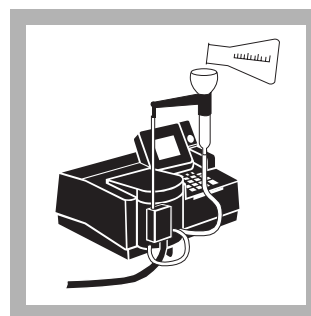
The wavelength (λ), **515 nm**, is automatically selected.

Note: A reagent blank value for a combined lot of indicator/buffer reagent solutions should be determined at least once a day. If sample color or turbidity fluctuates frequently during the day, determine a reagent blank for each sample. See Determining the Reagent Blank Value after this procedure.



3. Install the 1-inch Flow-Thru or Sipper Cell Module in the instrument. Flush it with at least 50 mL of deionized water.

Note: See the DR/4000 Optional Modules Instrument Manual for operation of the Flow-Thru or Sipper Cell modules.



4. Pour at least 50 mL of sample into the Flow-Thru or Sipper Cell.

**START
TIMER**



5. After the flow stops, press the soft key under **START TIMER**.

A 3-minute reaction period will begin.

Note: Wait 3 minutes before zeroing the sample to allow any turbidity or solids in the sample to settle. This ensures a stable reading.

ZERO



6. When the timer beeps, press the soft key under **ZERO**.

The display will show:

0 µg/L Cl₂

Note: If you are using a reagent blank correction, the display will show the correction.

Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



7. Break open 1 ampule of ULR Chlorine Buffer Solution.



8. Using a TenSette Pipet and clean tip, transfer 1.0 mL of buffer from the ampule to a clean, treated 50-mL graduated mixing cylinder.

Note: See Treating Analysis Labware following these steps for cleaning glassware.

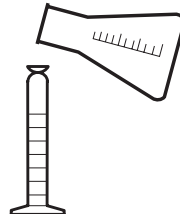
Note: The ampules contain more than 1.0 mL of solution for ease of reagent transfer. Discard any excess reagent in the ampule.



9. Break open 1 ampule of DPD Indicator Solution for Ultra Low-Range Chlorine.



10. Using a TenSette Pipet and clean tip, transfer 1.0 mL of indicator from the ampule to the graduated mixing cylinder. Swirl to mix the reagents. Proceed with Step 11 within 1 minute.



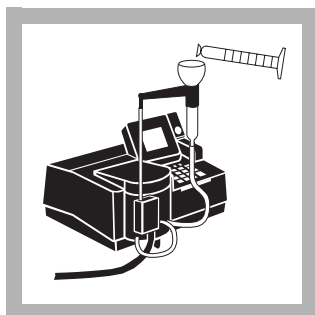
11. Avoiding extra agitation, carefully fill the cylinder to the 50-mL mark with sample. Stopper the cylinder. Gently invert it twice to mix (the prepared sample).

**START
TIMER**

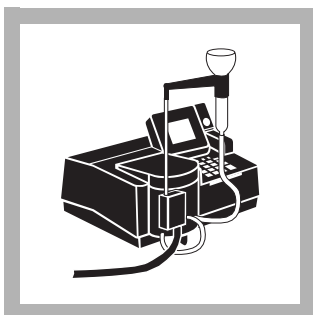


12. Press the soft key under **START TIMER**. A 3-minute reaction time will begin.

Note: Measure the reacted sample 3–4 minutes after mixing the sample and reagents. If less than 3 minutes elapses, reaction with chloramines may be incomplete. A reading after 4 minutes may result in higher reagent blank values.



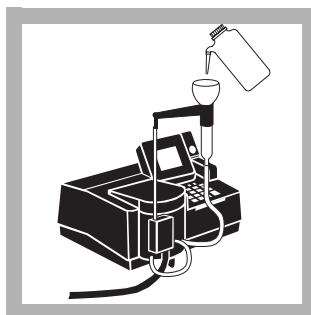
13. Introduce the contents of the graduated mixing cylinder into the Flow-Thru or Sipper Cell.



14. When the timer beeps, the result in $\mu\text{g/L}$ (or chosen units) chlorine will be displayed.

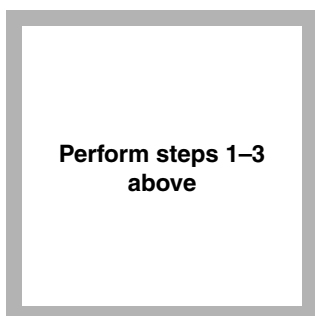
Note: If a de-chlorinating agent (e.g. sulfite or sulfur dioxide) is present, the sample result, corrected for the reagent blank, will read "0" or a slightly negative value.

Note: If a reagent blank value is entered, a correction is not necessary. If not, correct the result for the blank value (see Determining the Reagent Blank Value following these steps).

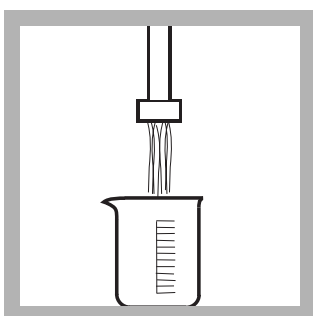


15. Flush the Flow-Thru or Sipper Cell with at least 50 mL of deionized water immediately after use.

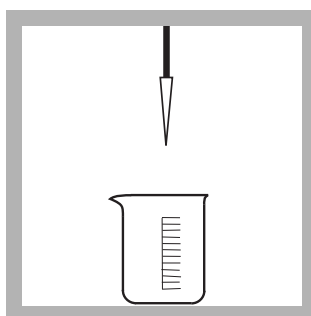
Determining the Reagent Blank Value



1. Set up the DR/4000 Spectrophotometer as described in steps 1–3 of the procedure.

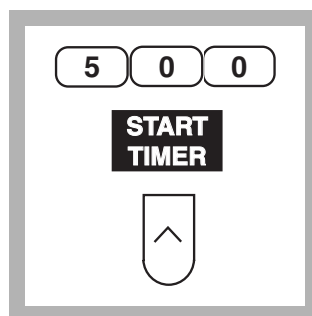


2. Collect about 100 mL of deionized or tap water in a clean 250-mL beaker.



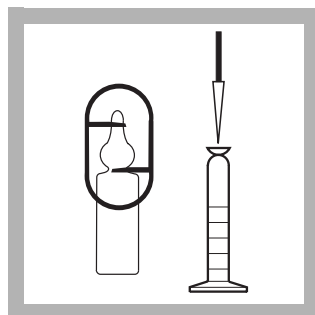
3. Using a TenSette Pipet, add 1.0 mL of Blanking Reagent to the beaker. Swirl several times to mix.

Note: The Blanking Reagent removes chlorine and chloramines from the water.

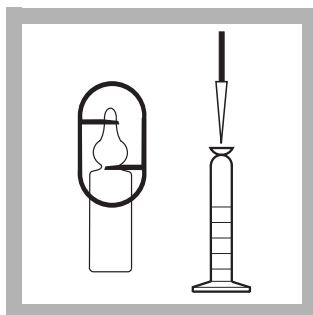


4. Press **500** followed by **START TIMER**.

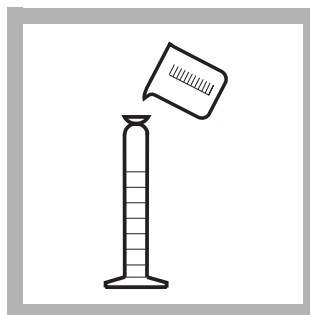
A 5-minute dechlorination period will begin.



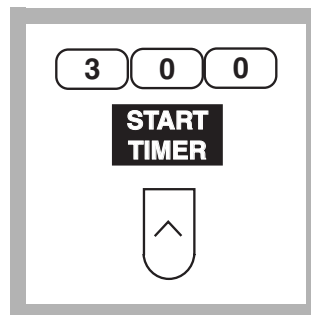
5. After the timer beeps, break open 1 ampule of ULR Chlorine Buffer Solution. Using a TenSette Pipet and clean tip, transfer 1.0 mL of buffer from the ampule to a clean 50-mL mixing graduated cylinder.



6. Break open 1 ampule of DPD Indicator Solution for Ultra Low-Range Chlorine. Using a TenSette Pipet and clean tip, transfer 1.0 mL of indicator from the ampule to the cylinder. Swirl to mix the reagents. Proceed with Step 7 within 1 minute.

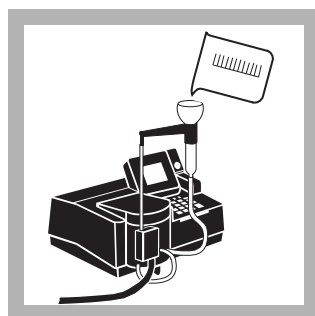


7. Fill the cylinder to the 50-mL mark with the dechlorinated water from Step 3. Cap and invert twice to mix. Save the remaining water for Step 9.

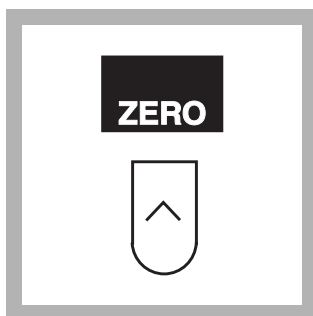


8. Press **300** followed by the soft key under **START TIMER**.

A 3-minute reaction period will begin.



9. During the reaction period flush the Flow-Thru or Sipper Cell with the remainder of the original dechlorinated water from Step 7.

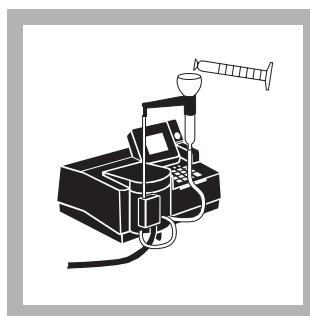


10. When the flow stops, press the soft key under **ZERO**.

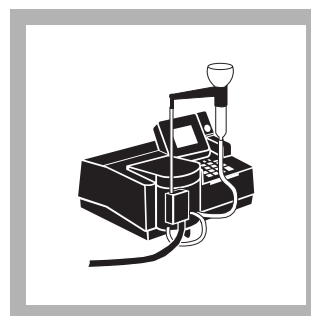
The display will show:

0 $\mu\text{g/L Cl}_2$

Note: Make sure the Blank is set to OFF under **OPTIONS, (MORE)**.



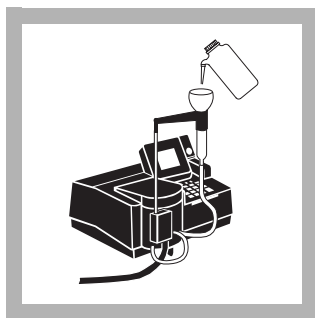
11. When the timer beeps, introduce the contents of the cylinder into the Flow-Thru Cell or Sipper Cell.



12. After the flow stops, the reagent blank value will be displayed in $\mu\text{g/L}$ (or chosen units) chlorine.

Note: Store the reagent blank value by pressing the soft keys under **OPTIONS, (MORE)**, and then **BLANK: (OFF)**. Enter the reagent blank value and press **ENTER**. Repeat daily or for each new combined lot of reagent.

Note: The reagent blank value is normally less than $5 \mu\text{g/L}$. If the value is greater than $5 \mu\text{g/L}$, an interfering substance may be present in the blanking water or the DPD Indicator may be degrading. If there is doubt about the reagents, repeat the reagent blank determination using chlorine demand-free water for the sample. Blanks up to $10 \mu\text{g/L}$ may be entered.



13. Flush the Flow-Thru or Sipper Cell with at least 50 mL of deionized water immediately after use.

Interferences

Table 1 Interfering Substances and Suggested Treatments

Interfering Substance	Interference Levels and Treatments												
Bromine, Br ₂	Interferes at all levels												
Chlorine Dioxide, ClO ₂	Interferes at all levels												
Chloramines, organic	May interfere												
Copper, Cu ²⁺	Greater than 1000 µg/L												
Iodine, I ₂	Interferes at all levels												
Iron (Fe ³⁺)	Greater than 1000 µg/L												
Manganese, Oxidized (Mn ⁴⁺ , Mn ⁷⁺) or Chromium, Oxidized (Cr ⁶⁺)	<ol style="list-style-type: none"> 1. Adjust sample pH to 6–7. 2. Add 6 drops potassium iodide (30-g/L) to a 50-mL sample. 3. Mix and wait 3 minutes. 4. Add 6 drops sodium arsenite (5-g/L) and mix. 5. Analyze the treated sample as described in the procedure. 6. Subtract the result from this test from the original analysis to obtain the correct chlorine concentration. 												
Nitrite, NO ₂ ⁻ (uncommon in clean waters)	<table border="1"> <thead> <tr> <th>mg/L nitrite</th><th>Apparent µg/L chlorine</th></tr> </thead> <tbody> <tr> <td>2.0 mg/L</td><td>3 µg/L</td></tr> <tr> <td>5.0 mg/L</td><td>5 µg/L</td></tr> <tr> <td>10.0 mg/L</td><td>7 µg/L</td></tr> <tr> <td>15.0 mg/L</td><td>16 µg/L</td></tr> <tr> <td>20.0 mg/L</td><td>18 µg/L</td></tr> </tbody> </table>	mg/L nitrite	Apparent µg/L chlorine	2.0 mg/L	3 µg/L	5.0 mg/L	5 µg/L	10.0 mg/L	7 µg/L	15.0 mg/L	16 µg/L	20.0 mg/L	18 µg/L
mg/L nitrite	Apparent µg/L chlorine												
2.0 mg/L	3 µg/L												
5.0 mg/L	5 µg/L												
10.0 mg/L	7 µg/L												
15.0 mg/L	16 µg/L												
20.0 mg/L	18 µg/L												
Ozone, O ₃	Interferes at all levels												
Peroxides	May interfere												
Extreme sample pH	Adjust to pH 6–7. See Section 1.3.1 <i>pH Interference</i> .												
Highly Buffered Samples	Adjust to pH 6–7. See Section 1.3.1 <i>pH Interference</i> .												

Sample Collection, Storage and Preservation

Analyze samples for chlorine immediately after collection. Many factors, including reactant concentrations, sunlight, pH, temperature and salinity influence decomposition of chlorine in water.

Avoid plastic containers since these may have a large chlorine demand. Pretreat glass sample containers to remove any chlorine demand by soaking in a dilute bleach solution (0.5 mL commercial bleach to 1 liter of deionized water) for at least 1 hour. Rinse thoroughly with deionized or distilled water. If sample containers are rinsed thoroughly with deionized or distilled water after use, only occasional pre-treatment is necessary.

Do not use the same sample cells for free and total chlorine. If trace iodide from the total chlorine reagent is carried over into the free chlorine determination, monochloramine will interfere. It is best to use separate, dedicated sample cells for free and total chlorine determinations.

A common error in testing for chlorine is obtaining a representative sample. If sampling from a tap, let the water flow for at least 5 minutes to ensure a representative sample. Let the container overflow with the sample several times, then cap the sample containers so there is no headspace (air) above the sample. Perform the chlorine analysis immediately.

Treating Analysis Labware

Glassware used in this test must be chlorine demand-free. Treat all glassware with a dilute solution of chlorine bleach prepared by adding 0.5 mL of commercial bleach to 1 liter of water. Soak glassware in this solution at least one hour. After soaking, rinse the glassware with copious amounts of deionized water and allow to dry before use.

Treat the Flow-Thru or Sipper Cell similarly with dilute bleach and let stand for several minutes and then rinse several times with deionized water.

Cleaning the Flow-Thru Cell

The Flow-Thru or Sipper Cell may accumulate a buildup of colored reaction products, especially if the reacted solutions are allowed to remain in the cell for long periods after measurement. Remove the buildup by rinsing the cell with 5.25 N sulfuric acid followed by several rinsings with deionized water.

Accuracy Check

Standard Additions Method

- a. Leave the unspiked sample in the sample compartment. Verify that the units displayed are in $\mu\text{g/L}$. Select standard additions mode by pressing the soft keys under **OPTIONS, (MORE)** and then **STD ADD**.
- b. Press **ENTER** to accept the default sample volume (mL), 50.
- c. Locate the average chlorine concentration shown on the certificate enclosed with the LR Voluettes. Multiply the mg/L Voluette concentration by 1000 to convert to $\mu\text{g/L}$. When prompted for the standard concentration, use the numeric keys to enter the $\mu\text{g/L}$ value. Press **ENTER**.

- d. Press the soft key under **ENTRY DONE**.
- e. Snap the neck off a LR Chlorine Voluette Ampule Standard, 20–30 mg/L Cl₂.
- f. Use the TenSette Pipet to add 0.1 mL, 0.2 mL and 0.3 mL of standard, respectively to three 50-mL samples and mix each thoroughly.
- g. Analyze each standard addition sample as described above. Accept the standard additions readings by pressing the soft key under **READ** each time. Each addition should reflect approximately 100% recovery.
- h. After completing the sequence, the display will show the extrapolated concentration value and the “best-fit” line through the standard additions data points, accounting for matrix interferences.
- i. See Section 1.4.1 *Standard Additions* for more information.

Method Performance

Precision

Standard: 250 µg/L Cl₂

Program	95% Confidence Limits
1490	248–252 µg/L Cl ₂

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
1490	3 µg/L Cl ₂

For more information on derivation and use of Hach’s estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, Appendix B, see Section 1.5.1.

Sensitivity

Program Number: 1490

Portion of Curve	ΔAbs	ΔConcentration
Entire Range	0.010	15.8 µg/L

See Section 1.5.3 *Sensitivity Explained* for more information.

Summary of Method

This method is designed for clean water, low in color and turbidity. The main applications include monitoring for trace chlorine break-through of activated carbon beds and feedwater to reverse osmosis membranes or ion-exchange resins.

Several modifications to the normal DPD chlorine method are necessary to measure trace levels of chlorine. The 1-inch Flow-Thru or Sipper Cell must be used in the spectrophotometer. Liquid reagents are also required. The reproducible optics of the Flow-Thru or Sipper Cell gives more stable readings than is possible with movable sample cells, resulting in more stable measurements.

CHLORINE, Total, continued

The reagents are packaged in ampules and sealed under argon gas to ensure stability. Use of liquid reagents eliminates any slight turbidity that might be caused by using powdered reagents. Due to the possible oxidation of the reagents (which could give a positive chlorine reading in the blank), a reagent blank must be determined at least once a day for each lot of reagent used. This reagent blank value is subtracted from the sample result and the corrected value is the actual chlorine concentration.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to Section 1.

Pollution Prevention and Waste Management

Samples treated with sodium arsenite for manganese or chromium interferences will be hazardous wastes as regulated by the Federal RCRA for arsenic (D004). See Section 1 for more information on proper disposal of these materials.

REQUIRED REAGENTS AND STANDARDS

Description	Quantity Required Per Test	Unit	Cat. No.
ULR Chlorine Reagent Set (about 20 tests)			25630-00
Includes: (1) 24930-23, (1) 24931-20, (1) 24932-20			
ULR Chlorine Buffer Solution, 1.5 mL ampules	1 mL	20/pkg	24931-20
DPD Indicator Solution for ULR Chlorine, 1.5 mL ampules	1 mL	20/pkg	24932-20
Blanking Reagent for ULR Chlorine	1 mL	29 mL	24930-23

REQUIRED EQUIPMENT AND SUPPLIES

Beaker, 250-mL	1	each	500-46
Cylinder, mixing, graduated, 50-mL	1	each	1896-41
DR/4000 Flow Cell Module Kit, 1-inch	1	each	48070-04
DR/4000 Sipper Module Kit, 1-inch	1	each	48090-03
Pipet, TenSette, 0.1 to 1.0 mL	1	each	19700-01
Pipet Tips, for 19700-01 TenSette Pipet	2	50/pkg	21856-96

OPTIONAL REAGENTS AND STANDARDS

Chlorine Standard Solution, 2-mL Voluette Ampules, 20–30 mg/L	20/pkg	26300-20
Sodium Hydroxide Solution, 50%	500 mL	2180-49
Sulfuric Acid Standard Solution, 5.25 N	1 liter	2449-53

OPTIONAL EQUIPMENT AND SUPPLIES

Beaker, 150-mL	each	500-44
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In the U.S.A. – Call toll-free 800-227-4224
Outside the U.S.A. – Contact the HACH office or distributor serving you.
On the Worldwide Web – www.hach.com; E-mail – techhelp@hach.com

HACH COMPANY
WORLD HEADQUARTERS
Telephone: (970) 669-3050
FAX: (970) 669-2932



DR/4000 PROCEDURE

CHLORINE, Total

✓ Method 10014

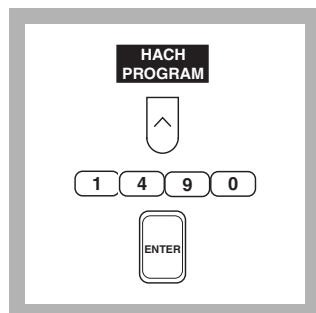
DPD Method* **

ULR (0 to 500 µg/L as Cl₂)

Scope and Application: For testing trace levels of chlorine and chloramines in treated domestic and industrial wastewater. USEPA accepted for reporting wastewater analyses. The estimated detection limit for program number 1490 is 3 µg/L Cl₂.

* Adapted from *Standard Methods for the Examination of Water and Wastewater*

** U.S. Patent number 5,362,650 covers the procedure. U.S. Patent 5,549,816 covers the OriFlo filter.



1. Press the soft key under **HACH PROGRAM**.

Select the stored program number for ultra low range (ULR) chlorine by pressing **1490** with the numeric keys.

Press: **ENTER**

Note: Samples should be analyzed immediately after collection, as chlorine is not stable in aqueous solution. See *Sample Collection, Storage and Preservation* following these steps.

Note: See *Treating Analysis Labware* section for more information on cleaning labware. The Flow-Thru and Sipper Cells must be cleaned and treated for chlorine demand.

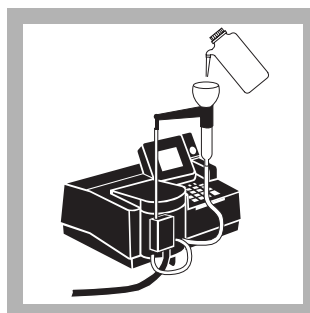
Note: The Single Cell Module cannot be used in this procedure.



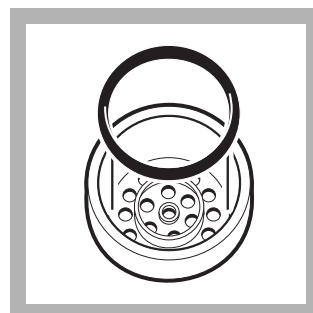
2. The display will show: **HACH PROGRAM: 1490 Chlorine, Tot. ULR**

The wavelength (λ), **515 nm**, is automatically selected.

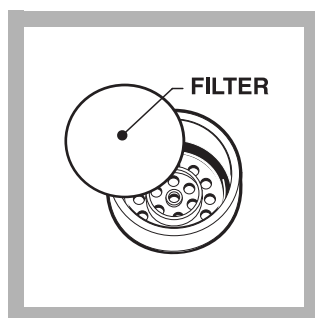
Note: A reagent blank value for a combined lot of Indicator/Buffer reagent solutions should be determined at least once a day. Determine the reagent blank as described in *Determining the Reagent Blank Value after this procedure*.



3. Install the 1-inch Flow-Thru or Sipper Cell Module in the instrument. Flush it with at least 50 mL of deionized water.

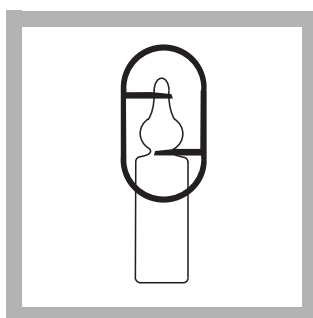


4. Unscrew the cap from the OriFlo plunger assembly. Be sure the O-ring is properly seated in the cap.

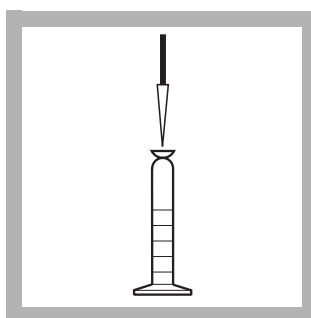


5. Install a new 3-micron filter into the cap well. Wet the filter with a few drops of deionized water. Re-assemble and hand-tighten the cap onto the plunger.

Note: Use a new filter for each test. Using an unspecified filter may give low analysis results or inability to filter the required volume.



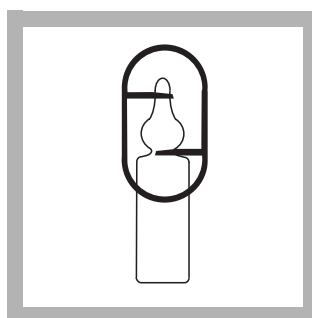
6. Break open 1 ampule of ULR Chlorine Buffer Solution.



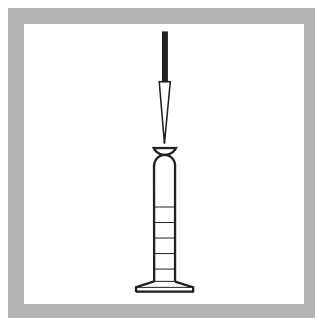
7. Using a TenSette Pipet and clean tip, transfer 1.0 mL of buffer from the ampule to a clean, treated 50-mL graduated mixing cylinder.

Note: See Treating Analysis Labware following these steps for cleaning glassware.

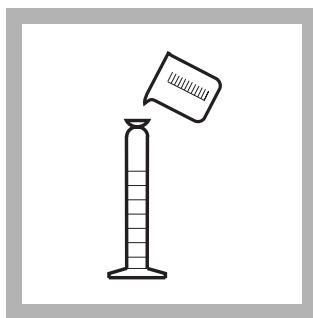
Note: The ampules contain more than 1.0 mL of solution for ease of reagent transfer. Discard any excess reagent in the ampule.



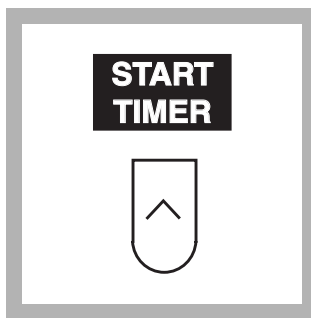
8. Break open 1 ampule of DPD Indicator Solution for Ultra Low-Range Chlorine.



9. Using a TenSette Pipet and clean tip, transfer 1.0 mL of indicator from the ampule to the graduated mixing cylinder. Swirl to mix the reagents. Proceed with Step 10 within 1 minute.



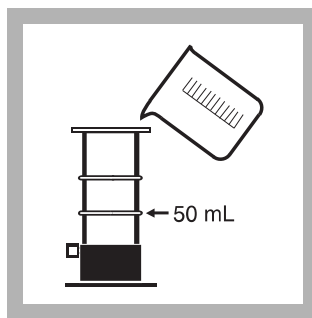
10. Avoiding extra agitation, carefully fill the cylinder to the 50-mL mark with sample. Stopper. Gently invert it twice to mix (the prepared sample).



11. Press the soft key under **START TIMER**. A 3-minute reaction period will begin.

Note: Measure the reacted sample 3–6 minutes after mixing the sample and reagents. If less than 3 minutes elapses, reaction with chloramines may be incomplete. A reading after 6 minutes may result in higher reagent blank values.

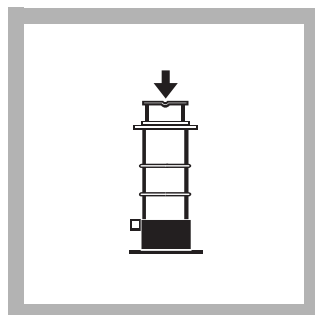
Note: Perform Steps 12-17 during the 3-minute reaction period.



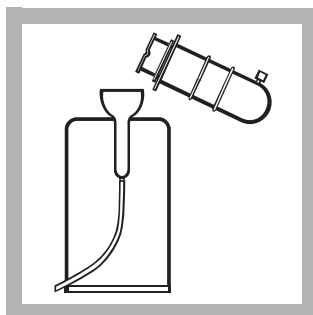
12. During the 3-minute reaction period, push the valve button on the OriFlo barrel assembly to the “closed” position. Place the barrel assembly into its stand. Pour approximately 50-mL of the original sample into the barrel.

Note: Perform steps 11-16 within the 3-minute reaction period.

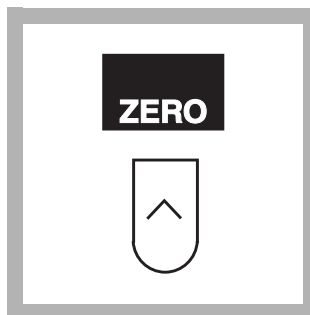
Note: The lower ring on the barrel assembly represents about a 50-mL volume.



13. Insert the plunger into the barrel and slowly push the plunger down with even pressure, until the plunger is fully seated.



14. Introduce the filtered sample in the beaker into the Flow-Thru or Sipper Cell.



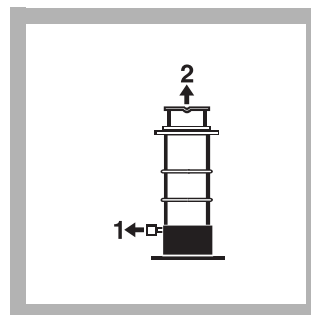
15. After the flow stops, press the soft key under **ZERO**.

The display will show:

0 µg/L Cl₂

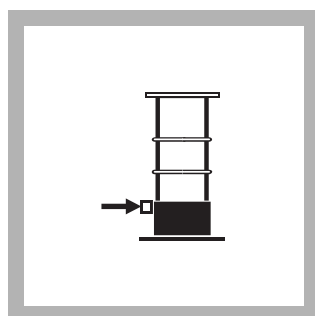
Note: If a reagent blank value is entered, the display will show a negative number.

Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.

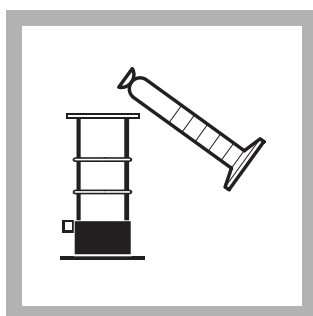


16. Pull the barrel's valve button out to the "open" position. Pull the plunger up to separate it completely from the barrel assembly. Discard the remaining unfiltered sample.

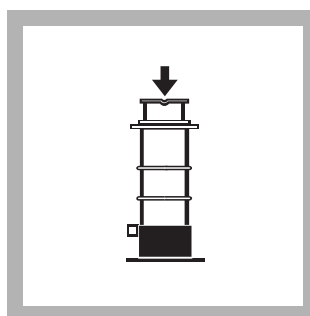
Note: For very turbid samples, a new membrane may need to be installed. Alternatively, use a second Quick Filter unit with a new membrane filter installed.



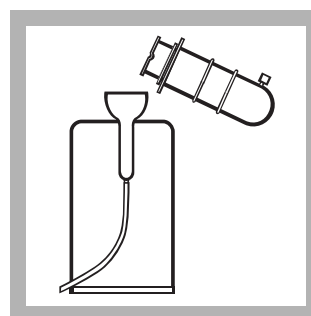
17. Push the barrel's valve button to the "closed" position. Place the barrel assembly into its stand.



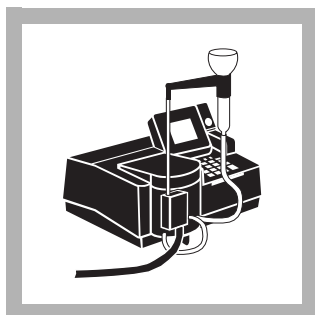
18. When the timer beeps, pour the contents of the mixing graduated cylinder into the barrel.



19. Insert the plunger into the barrel and slowly push the plunger down with even pressure, until the plunger is fully seated.

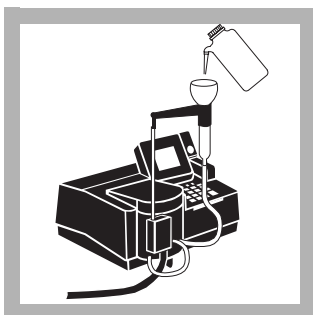


20. Introduce the filtered reacted sample from the beaker into the Flow-Thru or Sipper Cell.

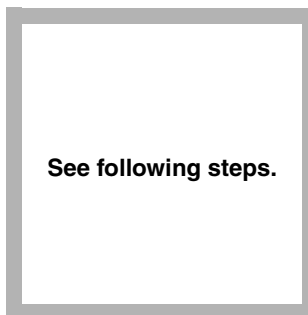


21. After the flow stops and the reading stabilizes, results in $\mu\text{g/L}$ (or chosen units) chlorine will be displayed.

Note: *If a dechlorinating agent (e.g., sulfite or sulfur dioxide) is present, the sample result, corrected for the reagent blank, will read "0" or a slightly negative value.*



22. Flush the Flow-Thru or Sipper Cell with at least 50 mL of deionized water immediately after use.



See following steps.

23. Determine a reagent blank using the procedure following these steps.

Determining the Reagent Blank Value

Perform steps 1–3 of procedure.



5 0 0

START
TIMER



1. Set up the DR/4000 Spectrophotometer as described in steps 1–3 of the procedure.

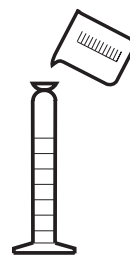
2. Collect about 100 mL deionized or tap water in a clean 250-mL beaker.

3. Using a TenSette Pipet, add 1.0 mL of Blanking Reagent to the beaker. Swirl several times to mix.

Note: The Blanking Reagent removes chlorine and chloramines from the water.

4. Press **500** followed by the soft key under **START TIMER**.

A 5-minute dechlorination period will begin.



3 0 0

START
TIMER



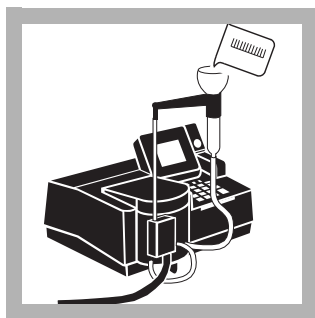
5. After the timer beeps, break open 1 ampule of DPD Indicator Solution for Ultra Low-Range Chlorine. Using a TenSette Pipet and clean tip, transfer 1.0 mL of buffer from the ampule to a clean 50-mL mixing graduated cylinder.

6. Break open 1 ampule of DPD Indicator Solution for Ultra Low-Range Chlorine. Using a TenSette Pipet and clean tip, transfer 1.0 mL of indicator from the ampule to the cylinder. Swirl to mix the reagents. Proceed with Step 7 within 1 minute.

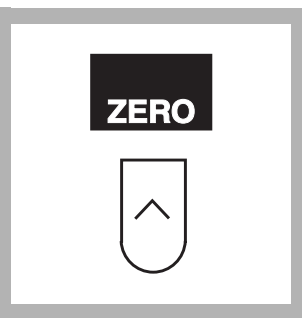
7. Fill the cylinder to the 50-mL mark with the dechlorinated water from Step 3. Cap and invert twice to mix. Save the remaining water for Step 9.

8. Press **300** followed by the soft key under **START TIMER**.

A 3-minute reaction period will begin.



9. During the reaction period flush the Flow-Thru or Sipper Cell with the remainder of the original dechlorinated water from Step 7.

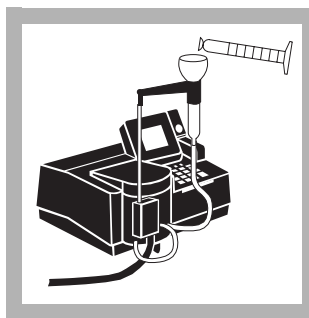


10. When the flow stops, press the soft key under **ZERO**.

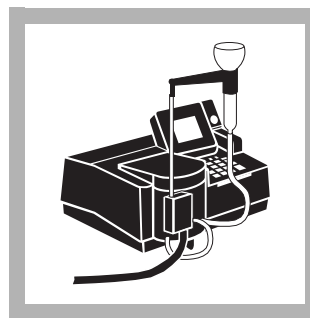
The display will show:

0 $\mu\text{g/L Cl}_2$

Note: Make sure the Blank is set to **OFF** under **OPTIONS: (MORE)**, soft key.



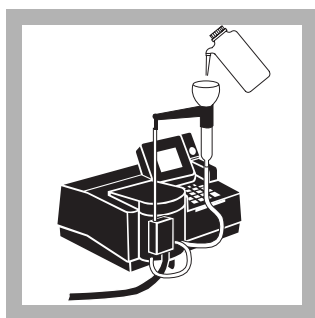
11. When the timer beeps, introduce the contents of the cylinder into the Flow-Thru Cell or Sipper Cell.



12. After the flow stops, the reagent blank value will be displayed in $\mu\text{g/L}$ (or chosen units) chlorine.

Note: Store the reagent blank value by pressing the soft keys under **OPTIONS, (MORE)**, and then **BLANK (OFF)**. Enter the reagent blank value and press **ENTER**. Repeat for each new combined lot of reagent.

Note: The reagent blank value is normally less than 5 $\mu\text{g/L}$. If the value is greater than 5 $\mu\text{g/L}$, an interfering substance may be present in the blanking water or the DPD Indicator may be degrading. If there is doubt about the reagents, repeat the reagent blank determination using chlorine-demand-free water for the sample. Blanks up to 5 $\mu\text{g/L}$ may be used.



13. Flush the Flow-Thru or Sipper Cell with at least 50 mL deionized water immediately after use.

Interferences

Table 1 Interfering Substances and Suggested Treatments

Interfering Substance	Interference Levels and Treatments												
Bromine, Br ₂	Interferes at all levels												
Chlorine Dioxide	Interferes at all levels												
Chloramines, organic	May interfere												
Hardness	No effect at less than 1,000 mg/L as CaCO ₃												
Iodine, I ₂	Interferes at all levels												
Manganese, Oxidized (Mn ⁴⁺ , Mn ⁷⁺) or Chromium, Oxidized (Cr ⁶⁺)	<ol style="list-style-type: none"> 1. Adjust sample pH to 6–7 2. Add 6 drops potassium iodide (30-g/L) to a 50-mL sample. 3. Mix and wait one minute. 4. Add 6 drops sodium arsenite (5-g/L) and mix. 5. Analyze the treated sample as described in the procedure. 6. Subtract the result from this test from the original analysis to obtain the correct chlorine concentration. 												
Nitrite, NO ₂ ⁻	<p>Causes a positive interference which varies with the nitrite concentration:</p> <table border="1"> <thead> <tr> <th>mg/L nitrite</th><th>Apparent µg/L chlorine</th></tr> </thead> <tbody> <tr> <td>2.0 mg/L</td><td>3 µg/L</td></tr> <tr> <td>5.0 mg/L</td><td>5 µg/L</td></tr> <tr> <td>10.0 mg/L</td><td>7 µg/L</td></tr> <tr> <td>15.0 mg/L</td><td>16 µg/L</td></tr> <tr> <td>20.0 mg/L</td><td>18 µg/L</td></tr> </tbody> </table>	mg/L nitrite	Apparent µg/L chlorine	2.0 mg/L	3 µg/L	5.0 mg/L	5 µg/L	10.0 mg/L	7 µg/L	15.0 mg/L	16 µg/L	20.0 mg/L	18 µg/L
mg/L nitrite	Apparent µg/L chlorine												
2.0 mg/L	3 µg/L												
5.0 mg/L	5 µg/L												
10.0 mg/L	7 µg/L												
15.0 mg/L	16 µg/L												
20.0 mg/L	18 µg/L												
Ozone, O ₃	Interferes at all levels												
Peroxides	May interfere												
Extreme sample pH	Adjust to pH 6–7. See Section 1.3.1 <i>pH Interference</i> .												
Highly Buffered Samples	Adjust to pH 6–7. See Section 1.3.1 <i>pH Interference</i> .												

Sample Collection, Storage and Preservation

Analyze samples for chlorine immediately after collection. Free chlorine is a strong oxidizing agent and it is unstable in natural waters. It reacts rapidly with various inorganic compounds and more slowly oxidizes organic compounds. Many factors, including reactant concentrations, sunlight, pH, temperature and salinity influence decomposition of free chlorine in water.

Avoid plastic containers since these may have a large chlorine demand. Pretreat glass sample containers to remove any chlorine demand by soaking in a dilute bleach solution (1 mL commercial bleach to 1 liter of deionized water) for at least 1 hour. Rinse thoroughly with deionized or distilled water. If sample containers are rinsed thoroughly with deionized or distilled water after use, only occasional pre-treatment is necessary.

Do not use the same sample cells for free and total chlorine. If trace iodide from the total chlorine reagent is carried over into the free chlorine determination, monochloramine will interfere. It is best to use separate, dedicated sample cells for free and total chlorine determinations.

A common error in testing for chlorine is obtaining a representative sample. If sampling from a tap, let the water flow for at least 5 minutes to ensure a representative sample. Let the container overflow with the sample several times, then cap the sample containers so there is no headspace (air) above the sample. Perform the chlorine analysis immediately.

Treating Analysis Labware

Glassware used in this test must be chlorine demand-free. Treat all glassware with a dilute solution of chlorine bleach prepared by adding 0.5 mL of commercial bleach to 1 liter of water. Soak glassware in this solution at least one hour. After soaking, rinse the glassware with copious amounts of deionized water and allow to dry before use.

Treat the Flow-Thru or Sipper Cell similarly with dilute bleach and let stand for several minutes and then rinse several times with deionized water.

Cleaning the Flow-Thru and Sipper Cells

The Flow-Thru or Sipper Cell may accumulate a buildup of colored reaction products, especially if the reacted solutions are allowed to remain in the cell for long periods after measurement. Remove the buildup by rinsing the cell with 5.25 N sulfuric acid followed by several rinsings with deionized water.

Accuracy Check

Standard Additions Method

Note: The Standard Additions technique is not applicable for samples that contain excess reducing agents such as sulfur dioxide, sulfites or bisulfites.

- a. Leave the unspiked sample in the sample cell compartment. Verify that the units displayed are in $\mu\text{g/L}$. Select standard additions mode by pressing the soft keys under **OPTIONS, (MORE)** and then **STD ADD**.
- b. Press **ENTER** to accept the default sample volume (mL), 50.
- c. Locate the average chlorine concentration shown on the certificate enclosed with the LR Voluettes. Multiply the mg/L Voluette concentration by 1000 to convert to $\mu\text{g/L Cl}_2$. When prompted for the standard concentration, use the numeric keys to enter the $\mu\text{g/L}$ value. Press **ENTER**.
- d. Press the soft key under **ENTRY DONE**.
- e. Snap the neck off a LR Chlorine Voluette Ampule Standard, 20–30 mg/L.
- f. Use the TenSette Pipet to add 0.1 mL, 0.2 mL and 0.3 mL of standard, respectively to three 50-mL samples in 150-mL beakers. Swirl gently to mix.
- g. Analyze each standard addition sample as described above. Accept the standard additions readings by pressing the soft key under **READ** each time. Each addition should reflect approximately 100% recovery.
- h. After completing the sequence, the display will show the extrapolated concentration value and the “best-fit” line through the standard additions data points, accounting for matrix interferences.
- i. See Section 1.4.1 *Standard Additions* for more information.

Method Performance

Precision

Standard: 250 µg/L Cl₂

Program	95% Confidence Limits
1490	248–252 µg/L Cl ₂

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
1490	3 µg/L Cl ₂

For more information on derivation and use of Hach's estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, Appendix B, see Section 1.5.1.

Sensitivity

Program Number: 1490

Portion of Curve	ΔAbs	ΔConcentration
Entire Range	0.010	15.8 µg/L

See Section 1.5.3 *Sensitivity Explained* for more information.

Summary of Method

It is essential that interfering sample turbidity is removed using a 3-micron membrane filter. To avoid chlorine loss, the filtration is done after reacting the DPD with the chlorine in the sample. The filter used has been specifically selected to avoid retention of the colored reaction product. Sample color is compensated by zeroing the spectrophotometer on a filtered sample.

Several modifications to the normal DPD chlorine method are necessary to measure trace levels of chlorine. The 1-inch Flow-Thru or Sipper Cell must be used in the spectrophotometer. Liquid reagents are also required. The reproducible optics of the Flow-Thru and Sipper Cell gives more stable readings than is possible with movable sample cells, resulting in more stable measurements.

The reagents are packaged in ampules and sealed under argon gas to ensure stability. Use of liquid reagents eliminates any slight turbidity that might be caused by using powdered reagents. Due to the possible oxidation of the reagents (which may give a positive chlorine reading in the blank), determine a reagent blank at least once a day for each lot of reagent used. Subtract this reagent blank value from the sample result to obtain the actual chlorine concentration.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to Section 3.

CHLORINE, Total, continued

Pollution Prevention and Waste Management

Samples treated with sodium arsenite for manganese or chromium interferences will be hazardous wastes as regulated by the Federal RCRA for arsenic (D004). See Section 3 for more information on proper disposal of these materials.

REQUIRED REAGENTS AND STANDARDS

Description	Cat. No.
ULR Chlorine Reagent Set (about 20 tests)	25630-00
Includes: (1) 24930-23, (1) 24931-20, (1) 24932-20	
ULR Chlorine Quick Filter Apparatus Set	25956-00
Includes: (1) 25940-25, (1) 49660-00	

Description	Quantity Required per test	Unit	Cat. No.
ULR Chlorine Buffer Solution, 1.5-mL ampules	1 mL	20/pkg	24931-20
DPD Indicator Solution for ULR Chlorine, 1.5-mL ampules	1 mL	20/pkg	24932-20
Blanking Reagent for ULR Chlorine	1 mL	29 mL	24930-23

REQUIRED EQUIPMENT AND SUPPLIES

Beaker, 250-mL	1	each	500-46
Cylinder, graduated mixing, 50-mL	1	each	1896-41
DR/4000 Flow Cell Module Kit, 1-inch	1	each	48070-04
<i>or</i>			
DR/4000 Sipper Module Kit, 1-inch	1	each	48090-03
Membrane Filters, 3 micron, 25-mm	1	25/pkg	25940-25
Oriflo Assembly	1	each	49660-00
Pipet, TenSette, 0.1 to 1.0 mL	1	each	19700-01
Pipet Tips, for 19700-01 TenSette Pipet	2	50/pkg	21856-96

OPTIONAL REAGENTS AND STANDARDS

LR Chlorine Standard Solution, 2-mL Voluette Ampules, 20–30 mg/L	20/pkg	26300-20
Sulfuric Acid Solution, 5.25 N	1 liter	2449-53
Water, deionized	4 liter	272-56

OPTIONAL EQUIPMENT AND SUPPLIES

Ampule Breaker, PourRite	each	24846-00
Bottle, wash, 250-mL	each	620-31
Membrane Filters, 3-micron, 25-mm	100/pkg	25940-00



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Telephone: (970) 669-3050
FAX: (970) 669-2932



✓ Method 8167

DPD Method*

Powder Pillows or AccuVac® Ampuls

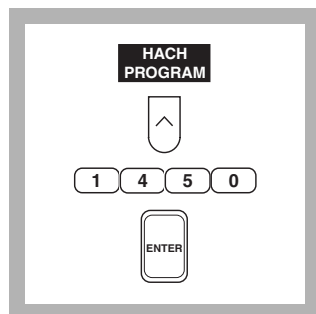
(0 to 2.00 mg/L)

Scope and Application: For testing residual chlorine and chloramines in water, wastewater, estuary water and seawater; USEPA-accepted for reporting** for drinking and wastewater analyses. The estimated detection limit for program numbers 1450 and 1460 is 0.01 mg/L Cl_2 .

* Adapted from *Standard Methods for the Examination of Water and Wastewater*

** Procedure is equivalent to USEPA method 330.5 and Standard Method 4500-Cl G for drinking water and wastewater analyses.

Using Powder Pillows



1. Press the soft key under **HACH PROGRAM**.

Select the stored program number for free chlorine (Cl_2) by pressing **1450** with the numeric keys.

Press: **ENTER**

Note: Samples must be analyzed immediately and cannot be preserved for later analysis.

Note: The Flow Cell and Sipper Modules can be used with this procedure if rinsed between samples. Use a 25-mL sample and reagents with the Flow Cell Module.



2. The display will show:
HACH PROGRAM: 1450 Chlorine, F&T

The wavelength (λ), **530 nm**, is automatically selected.

Note: For best results, determine a reagent blank for each new lot of reagent as follows. Prepare a reagent blank by repeating Steps 3 through 8, using deionized water as the sample. Zero the instrument on deionized water by pressing the soft key under **ZERO**. Insert the reagent blank and the blank value will be displayed. Correct for the reagent blank by pressing the soft keys under **OPTIONS, (MORE)**, and then **BLANK:OFF**. Enter the reagent blank value and press **ENTER**. Repeat for each new lot of reagent.



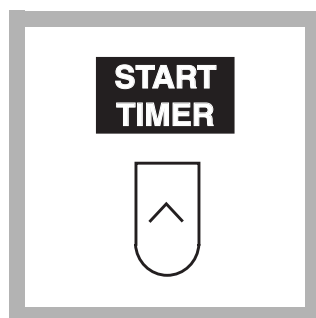
3. Fill a sample cell with 10 mL of sample.

Note: For sample with extreme pH, see *Interferences section*.



4. Add the contents of one DPD Total Chlorine Powder Pillow to the sample cell (the prepared sample). Swirl the sample cell for 20 seconds to mix.

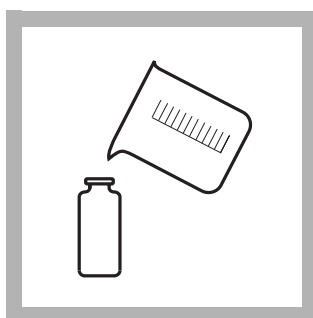
Note: A pink color will develop if chlorine is present.



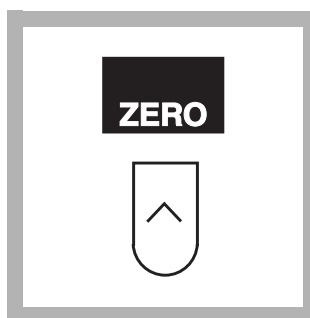
5. Press the soft key under **START TIMER**.

A 3-minute reaction period will begin.

Note: Perform steps 6 and 7 during this time period.



6. Fill another sample cell (the blank) with 10 mL of sample. Place it into the cell holder. Close the light shield.



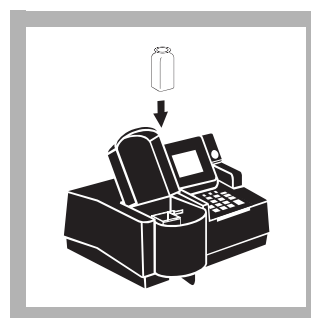
7. Press the soft key under **ZERO**.

The display will show:

0.00 mg/L Cl₂

Note: If you are using a reagent blank correction, the display will show the correction.

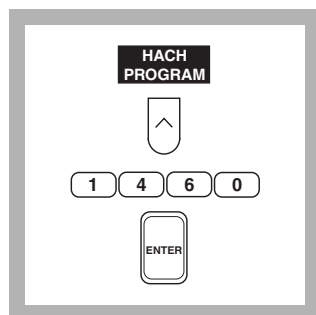
Note: For alternate concentration units press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



8. Within 3 minutes after the timer beeps, place the prepared sample into the cell holder. Close the light shield. Results in mg/L chlorine (or chosen units) will be displayed.

Note: If the sample temporarily turns yellow after reagent addition, or the display shows **OVER!**, dilute a fresh sample and repeat the test. A slight loss of chlorine may occur because of the dilution. Multiply the result by the appropriate dilution factor; see Section 1.2.6 Sample Dilution Techniques.

Using AccuVac Ampuls



1. Press the soft key under **HACH PROGRAM**.

Select the stored program number for free chlorine (Cl_2) by pressing **1460** with the numeric keys.

Press: **ENTER**

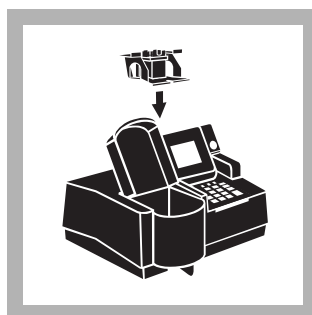
Note: Samples must be analyzed immediately and cannot be preserved for later analysis.



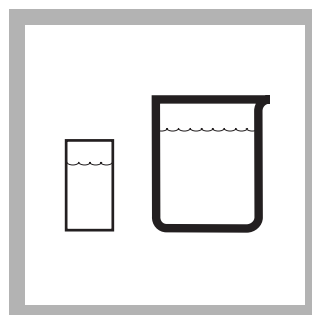
2. The display will show:
HACH PROGRAM:
1460 Chlorine, F&T AV

The wavelength (λ), **530 nm**, is automatically selected.

Note: For best results, determine a reagent blank for each new lot of reagent as follows. Prepare a reagent blank by repeating steps 3 through 10, using deionized water as the sample. Zero the instrument on deionized water by pressing the soft key under **ZERO**. Insert the reagent blank and the blank value will be displayed. Correct for the reagent blank by pressing the soft keys under **OPTIONS**, (**MORE**), and then **BLANK:OFF**. Enter the reagent blank value and press **ENTER**. Repeat for each new lot of reagent.

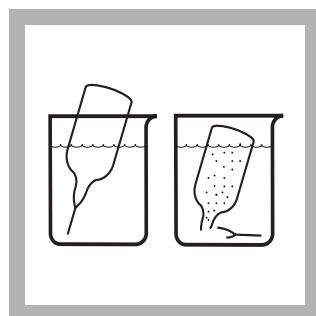


3. Insert the AccuVac Ampul Adapter into the sample cell module by sliding it under the thumb screw and into the alignment grooves. Fasten with the thumb screw.



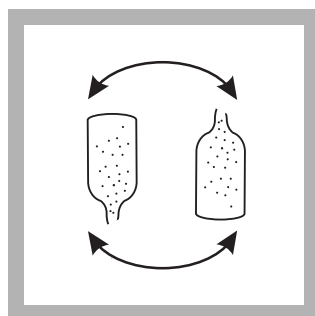
4. Fill a zeroing vial (the blank) with at least 10 mL of sample. Collect at least 40 mL of sample in a 50-mL beaker.

Note: For samples with extreme pH, see the *Interferences* section.



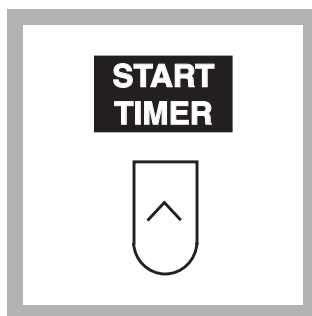
5. Fill a DPD Total Chlorine Reagent AccuVac Ampul with sample.

Note: Keep the tip immersed while the ampul fills completely.



6. Quickly invert the ampul several times to mix. Wipe off any liquid or fingerprints.

Note: A pink color will form if total chlorine is present.

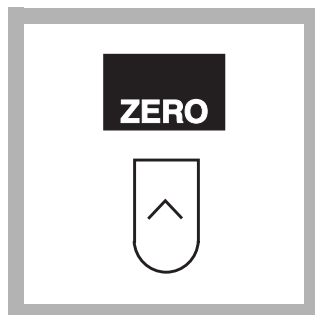


7. Press the soft key under **START TIMER**. A 3-minute reaction period will begin.

Note: Perform steps 8-9 during the three-minute period.



8. Place the blank into the cell holder. Close the light shield.



9. Press the soft key under **ZERO**.

The display will show:

0.00 mg/L Cl₂

Note: If you are using a reagent blank correction, the display will show the correction.

Note: For alternate concentration units press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



10. Within 3 minutes after the timer beeps, place the AccuVac Ampul into the cell holder. Close the light shield. Results in mg/L chlorine (or chosen units) will be displayed.

Note: If the sample temporarily turns yellow after sample addition, or the display shows **OVER!**, dilute a fresh sample and repeat the test. A slight loss of chlorine may occur due to the dilution. Multiply the result by the appropriate dilution factor; see Section 1.2.6 Sample Dilution Techniques.

Sample Collection, Storage and Preservation

Analyze samples for chlorine immediately after collection. Free chlorine is a strong oxidizing agent and it is unstable in natural waters. It reacts rapidly with various inorganic compounds and more slowly oxidizes organic compounds. Many factors, including reactant concentrations, sunlight, pH, temperature and salinity influence decomposition of free chlorine in water.

Avoid plastic containers since these may have a large chlorine demand. Pretreat glass sample containers to remove any chlorine demand by soaking in a dilute bleach solution (1 mL commercial bleach to 1 liter of deionized water) for at least 1 hour. Rinse thoroughly with deionized or distilled water. If sample containers are rinsed thoroughly with deionized or distilled water after use, only occasional pre-treatment is necessary.

Do not use the same sample cells for free and total chlorine. If trace iodide from the total chlorine reagent is carried over into the free chlorine determination, monochloramine will interfere. It is best to use separate, dedicated sample cells for free and total chlorine determinations.

A common error in testing for chlorine is obtaining a representative sample. If sampling from a tap, let the water flow for at least 5 minutes to ensure a representative sample. Let the container overflow with the sample several times, then cap the sample containers so there is no headspace (air) above the sample.

If sampling with a sample cell, rinse the cell several times with the sample, the carefully fill to the 10-mL mark. Perform the chlorine analysis immediately.

Interferences

Table 1 Interfering Substances and Suggested Treatments

Interfering Substance	Interference Levels and Treatments
Acidity	Greater than 150 mg/L CaCO ₃ . May not develop full color or color may fade instantly. Neutralize to pH 6–7 with 1 N sodium hydroxide. Determine amount to be added on separate sample aliquot, then add the same amount to the sample being tested. Correct for volume addition (See Section 1.2.2 <i>Correcting for Volume Additions</i>).
Alkalinity	Greater than 300 mg/L CaCO ₃ . May not develop full color or color may fade instantly. Neutralize to pH 6–7 with 1 N sulfuric acid. Determine amount to be added on separate sample aliquot, then add the same amount to the sample being tested. Correct for volume addition (See Section 1.2.2 <i>Correcting for Volume Additions</i>).
Bromine, Br ₂	Interferes at all levels
Chlorine Dioxide	Interferes at all levels
Chloramines, organic	May interfere
Hardness	No effect at less than 1,000 mg/L as CaCO ₃
Iodine, I ₂	Interferes at all levels
Manganese, Oxidized (Mn ⁴⁺ , Mn ⁷⁺) or Chromium, Oxidized (Cr ⁶⁺)	<ol style="list-style-type: none"> 1. Adjust sample pH to 6–7. 2. Add 3 drops potassium iodide (30-g/L) to a 25-mL sample. 3. Mix and wait one minute. 4. Add 3 drops sodium arsenite (5-g/L) and mix. 5. Analyze 10 mL of the treated sample as described in the procedure. 6. Subtract the result from this test from the original analysis to obtain the correct chlorine concentration.
Ozone	Interferes at all levels
Peroxides	May interfere
Extreme sample pH	Adjust to pH 6–7. See Section 1.3.1 <i>pH Interference</i> .
Highly Buffered Samples	Adjust to pH 6–7. See Section 1.3.1 <i>pH Interference</i> .

Accuracy Check

Standard Additions Method (using powder pillows)

- Leave the unspiked sample in the sample compartment. Verify that the units displayed are in mg/L. Select standard additions mode by pressing the soft keys under **OPTIONS, (MORE)** and then **STD ADD**.
- Press **ENTER** to accept the default sample volume (mL), 10.
- Locate the average chlorine concentration shown on the certificate enclosed with the LR Voluette Ampules. When prompted for the standard concentration, use the numeric keys to enter the certificate value. Press **ENTER**.
- Press the soft key under **ENTRY DONE**.
- Snap the neck off a LR Chlorine Voluette Ampule Standard, 25–30 mg/L Cl₂.

- f. Use the TenSette Pipet to add 0.1 mL, 0.2 mL and 0.3 mL of standard, respectively to three 10-mL samples and mix each thoroughly.
- g. Analyze each standard addition sample as described above. Accept the standard additions readings by pressing the soft key under **READ** each time. Each addition should reflect approximately 100% recovery.
- h. After completing the sequence, the display will show the extrapolated concentration value and the “best-fit” line through the standard additions data points, accounting for matrix interferences.
- i. See Section 1.4.1 *Standard Additions* for more information.

Standard Additions Method (using AccuVac Ampuls)

- a. Leave the unspiked sample in the sample compartment. Verify that the units displayed are in mg/L. Select standard additions mode by pressing the soft keys under **OPTIONS, (MORE)** and then **STD ADD**.
- b. Press **ENTER** to accept the default sample volume (mL), 25.
- c. Locate the average chlorine concentration shown on the certificate enclosed with the LR Voluette Ampules. When prompted for the standard concentration, use the numeric keys to enter the certificate value. Press **ENTER**.
- d. Press the soft key under **ENTRY DONE**.
- e. Snap the neck off a LR Chlorine Voluette Ampule Standard, 20–30 mg/L Cl₂.
- f. Use graduated cylinder to measure 25 mL of sample into each of three 50-mL beakers. Use a TenSette Pipet to add 0.2, 0.4 and 0.6 mL of standard, respectively, to each of the 25-mL samples. Swirl gently to mix.
- g. Fill a DPD Free Chlorine AccuVac Ampul completely from each beaker and analyze each standard addition sample as described above. Accept the standard additions readings by pressing the soft key under **READ** each time. Each addition should reflect approximately 100% recovery.
- h. After completing the sequence, the display will show the extrapolated concentration value and the “best-fit” line through the standard additions data points, accounting for matrix interferences.
- i. See Section 1.4.1 *Standard Additions* for more information.

Method Performance

Precision

Standard: 1.00 mg/L Cl₂

Program	95% Confidence Limits
1450	0.99-1.01 mg/L Cl ₂
1460	0.99-1.01 mg/L Cl ₂

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
1450	0.01 mg/L Cl ₂
1460	0.01 mg/L Cl ₂

For more information on derivation and use of Hach's estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, Appendix B, see Section 1.5.1.

Sensitivity

Program Number: 1450

Portion of Curve	Δ Abs	Δ Concentration
Entire Range	0.010	0.018 mg/L

Program Number: 1460

Portion of Curve	Δ Abs	Δ Concentration
Entire Range	0.010	0.020 mg/L

See Section 1.5.3 *Sensitivity Explained* for more information.

Summary of Method

Chlorine can be present in water as free chlorine and as combined chlorine. Both forms can exist in the same water and be determined together as the total chlorine. Free chlorine is present as hypochlorous acid or hypochlorite ion. Combined chlorine exists as monochloramine, dichloramine, nitrogen trichloride and other chloro derivatives. The combined chlorine oxidizes iodide in the reagent to iodine. The iodine and free chlorine reacts with DPD (N,N-diethyl-p-phenylenediamine) along with the free chlorine to form a red color which is proportional to the total chlorine concentration. To determine the concentration of combined chlorine, run a free chlorine test. Subtract the results of the free chlorine test from the total chlorine test to obtain the combined chlorine concentration.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to Section 1.

Pollution Prevention and Waste Management

Samples treated with sodium arsenite for manganese or chromium interferences will be hazardous wastes as regulated by the Federal RCRA for arsenic (D004). See Section 1 for more information on proper disposal of these materials.

CHLORINE, Total, continued

REQUIRED REAGENTS AND STANDARDS (Using Powder Pillows)

Description	Quantity Required per test	Unit	Cat. No.
DPD Total Chlorine Reagent Powder Pillows, 10-mL.....	1 pillow	100/pkg.....	21056-69

REQUIRED REAGENTS AND STANDARDS (Using AccuVac Ampuls)

DPD Total Chlorine Reagent AccuVac Ampuls.....	1 ampul.....	25/pkg.....	25030-25
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REQUIRED EQUIPMENT AND SUPPLIES (Using Powder Pillows)

DR/4000 1-Inch Cell Adapter	1	each.....	48190-00
Stopper, rubber, No. 2	1	12/pkg.....	2118-02

REQUIRED EQUIPMENT AND SUPPLIES (Using AccuVac Ampuls)

Beaker, 50-mL.....	1	each.....	500-41
DR/4000 AccuVac Ampul Adapter.....	1	each.....	48187-00
Sample Cell, 10-mL with cap (zeroing vial).....	1	each.....	21228-00

OPTIONAL REAGENTS AND STANDARDS

Chlorine Standard Solution, 2-mL Voluette Ampule, 20–30 mg/L	20/pkg.....	26300-20
Potassium Iodide Solution, 30-g/L.....	100 mL * MDB.....	343-32
Sodium Arsenite Solution, 5-g/L	100 mL * MDB.....	1047-32
Sodium Hydroxide Standard Solution, 1.00 N.....	100 mL * MDB.....	1045-32
Sulfuric Acid Standard Solution, 1.000 N.....	100 mL * MDB.....	1270-32
Water, deionized.....	4 liters.....	272-56

OPTIONAL EQUIPMENT AND SUPPLIES

AccuVac Snapper	each.....	24052-00
Ampule Breaker Kit	each.....	21968-00
Cylinder, graduated, 25-mL, poly	each.....	1081-40
DR/4000 Carousel Module Kit	each.....	48070-02
DR/4000 Flow Cell Module Kit, 1-inch (for 25-mL samples and reagents only)	each.....	48070-04
DR/4000 Flow Cell Module Kit, 1-cm.....	each.....	48070-05
DR/4000 Sipper Module Kit, 1-inch	each.....	48090-03
Graph Paper, linear.....	100/pkg.....	22313-00
pH Meter, <i>sensio</i> TM 1, portable	each.....	51700-00
Pipet, TenSette, 0.1 to 1.0 mL	each.....	19700-01
Pipet Tips, for 19700-01 TenSette Pipet	50/pkg.....	21856-96
SwifTest DPD Total Chlorine Reagent, with dispenser	250 tests.....	28024-00
SwifTest Replacement Vial	250 tests.....	21056-60

* Contact Hach for larger sizes.



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Method 10101

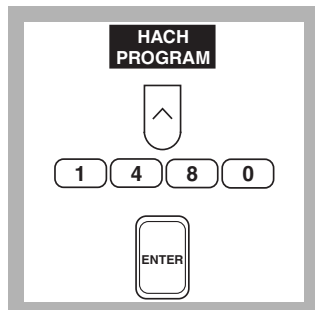
DPD Method*

Test 'N Tube™ Vials

(0 to 5.00 mg/L)

Scope and Application: For testing higher levels of free chlorine plus combined (total) chlorine in drinking water, treated wastewater, cooling water or industrial process water. The estimated detection limit for program number 1480 is 0.04 mg/L Cl₂.

* Adapted from *Standard Methods for the Examination of Water and Wastewater*



1. Press the soft key under **HACH PROGRAM**.

Select the stored program number for Test 'N Tube total chlorine (Cl₂) by pressing **1480** with the numeric keys.

Press: **ENTER**

Note: Samples must be analyzed immediately and cannot be preserved for later analysis.



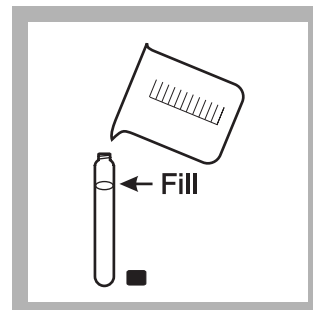
2. The display will show:

**HACH PROGRAM: 1480
Chlorine, TNT**

The wavelength (λ), **530 nm**, is automatically selected.



3. Insert the COD adapter into the sample module by sliding it under the thumbscrew and into the alignment grooves. Fasten with the thumbscrew.



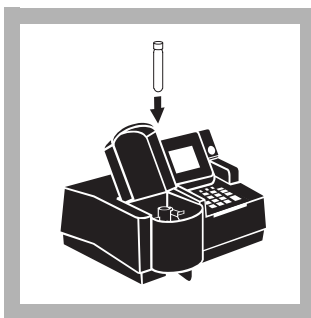
4. Fill an empty Test 'N Tube (TNT) vial with sample (the blank).

Note: Fill to the top of the label.

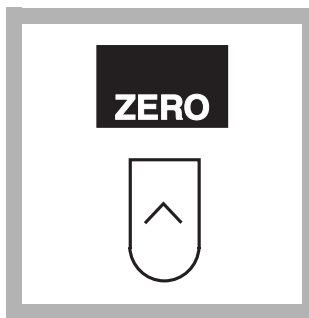


5. Clean the outside of the sample blank vial with a towel.

Note: Wiping with a damp towel followed by a dry one will remove fingerprints and other marks.



6. Place the blank into the adapter with the Hach logo facing the front of the instrument. Close the light shield.

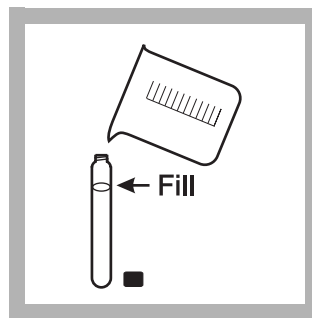


7. Press the soft key under **ZERO**.

The display will show:

0.00 mg/L Cl₂

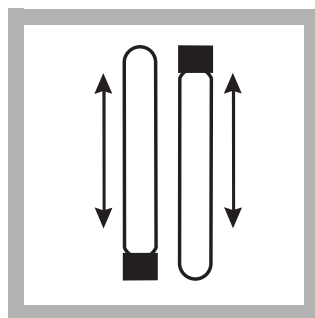
Note: For alternate concentration units press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



8. Remove the cap from a Total Chlorine DPD-TNT tube. Add 10 mL of sample.

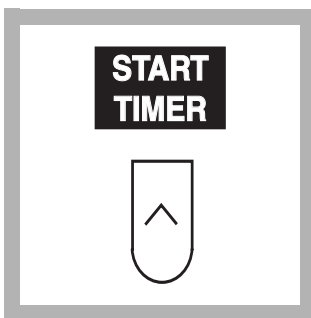
Note: Fill to the top of the label.

Note: A pink color will develop if total chlorine is present.



9. Cap and invert at least 10 times to dissolve the powder (the prepared sample).

Note: Use slow, deliberate inversion for complete recovery. Ten inversions should take at least 30 seconds. One inversion equals turning the vial upside down, then returning it to an upright position.

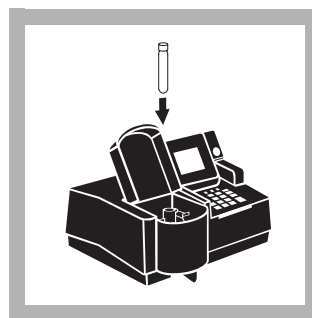


10. Press the soft key under **START TIMER**.

A 2-minute reaction period will begin.



11. When the timer beeps, wipe the vial containing the prepared sample.



12. Place the sample in the adapter with the Hach logo facing the front of the instrument. Close the light shield.

Results in mg/L chlorine (or chosen units) will be displayed.

Interferences

Table 1 Interfering Substances and Suggested Treatments

Interfering Substance	Interference Levels and Treatments
Acidity	Greater than 150 mg/L CaCO_3 . May not develop full color or color may fade instantly. Neutralize to pH 6–7 with 1 N sodium hydroxide. Determine amount to be added on separate sample aliquot, then add the same amount to the sample being tested. Correct for volume addition (see <i>Correcting for Volume Additions</i> in the <i>DR/4000 Procedures Manual</i>).
Alkalinity	Greater than 300 mg/L CaCO_3 . May not develop full color or color may fade instantly. Neutralize to pH 6–7 with 1 N sulfuric acid. Determine amount to be added on separate sample aliquot, then add the same amount to the sample being tested. Correct for volume addition (see <i>Correcting for Volume Additions</i> in the <i>DR/4000 Procedures Manual</i>).
Bromine, Br_2	Interferes at all levels
Chlorine Dioxide, ClO_2	Interferes at all levels
Chloramines, organic	May interfere
Hardness	No effect at less than 1,000 mg/L as CaCO_3
Iodine, I_2	Interferes at all levels
Manganese, oxidized (Mn^{4+} , Mn^{7+}) or Chromium, oxidized (Cr^{6+})	<ol style="list-style-type: none"> 1. Adjust sample pH to 6–7. 2. Add 3 drops potassium iodide (30-g/L) to a 25-mL sample. 3. Mix and wait 1 minute. 4. Add 3 drops sodium arsenite (5-g/L) and mix. 5. Analyze 10 mL of the treated sample as described in the procedure. 6. Subtract the result from this test from the original analysis to obtain the correct chlorine concentration.
Ozone, O_3	Interferes at all levels
Peroxides	May interfere
Extreme sample pH	Adjust to pH 6–7. See <i>pH Interference</i> in the <i>DR/4000 Procedures Manual</i> .
Highly Buffered Samples	Adjust to pH 6–7. See <i>pH Interference</i> in the <i>DR/4000 Procedures Manual</i> .

Sample Collection, Storage and Preservation

Analyze samples for chlorine immediately after collection. Free chlorine and combined chlorine are strong oxidizing agents and are unstable in natural waters. Many factors, including reactant concentrations, sunlight, pH, temperature and salinity influence decomposition of free chlorine in water.

Avoid plastic containers since these may have a large chlorine demand. Pretreat glass sample containers to remove any chlorine demand by soaking in a dilute bleach solution (1 mL commercial bleach to 1 liter of deionized water) for at least 1 hour. Rinse thoroughly with deionized or distilled water. If sample containers are rinsed thoroughly with deionized or distilled water after use, only occasional pre-treatment is necessary.

A common error in testing for chlorine is obtaining an unrepresentative sample. If sampling from a tap, let the water flow for at least 5 minutes to ensure a representative sample. Let the container overflow with the sample several times, then cap the sample containers so there is no headspace (air) above the sample. Perform the chlorine analysis immediately.

Accuracy Check

Standard Additions Method

- a. Leave the unspiked sample in the cell compartment. Verify that the units displayed are in mg/L. Select standard additions mode by pressing the soft keys under **OPTIONS, (MORE)** and then **STD ADD**.
- b. Press **ENTER** to accept the default sample volume (mL), 10.
- c. Locate the average chlorine concentration shown on the certificate enclosed with the HR Chlorine PourRite Ampules. When prompted for the standard concentration, use the numeric keys to enter the certificate value. Press **ENTER**.
- d. Press the soft key under **ENTRY DONE**.
- e. Snap the neck off a HR Chlorine PourRite® Ampule Standard, 50-75 mg/L Cl₂.
- f. Use the TenSette Pipet to add 0.1 mL of standard to a 10-mL sample and mix thoroughly.
- g. Analyze the standard addition sample as described above. Accept the standard additions readings by pressing the soft key under **READ** each time. Each addition should reflect approximately 100% recovery.
- h. See Section *Standard Additions* in the *DR/4000 Procedures Manual* for more information.

Method Performance

Precision

at mg/L Cl ₂	95% Confidence Limits
0.10	±0.01 mg/L
2.50	±0.02 mg/L
3.40	±0.02 mg/L

For more information on determining precision data and method detection limits, refer to Section 1.5 of the *DR/4000 Procedures Manual*.

Estimated Detection Limit

The estimated detection limit for program 1480 is 0.04 mg/L Cl₂. For more information on derivation and use of Hach's estimated detection limit, see Section 1.5.2 of the *DR/4000 Procedures Manual*. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, Appendix B, see Section 1.5.1 of the *DR/4000 Procedures Manual*.

Sensitivity

Portion of Curve	Δ Abs	Δ Concentration
0.10	0.010	0.032 mg/L
2.50	0.010	0.035 mg/L
3.40	0.010	0.036 mg/L

See *Sensitivity Explained* of the *DR/4000 Procedures Manual* for more information.

Summary of Method

Chlorine can be present in water as free chlorine and as combined chlorine. Both forms can exist in the same water and be determined together as the total chlorine. Free chlorine is present as hypochlorous acid or hypochlorite ion. Combined chlorine exists as monochloramine, dichloramine, nitrogen trichloride and other chloro derivatives.

Free or combined chlorine oxidizes iodide in the reagent to iodine. The iodine and chlorine react with DPD (N,N-diethyl-p-phenylenediamine) to form a magenta color, which is proportional to the total chlorine concentration. To determine the concentration of combined chlorine, run a free chlorine test. Subtract the results of the free chlorine test from the total chlorine test to obtain the combined chlorine concentration.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to Section 1 of the *DR/4000 Procedures Manual*.

Pollution Prevention and Waste Management

Samples treated with sodium arsenite for manganese or chromium interferences will be hazardous wastes as regulated by the Federal RCRA for arsenic (D004). See Section 1 of the *DR/4000 Procedures Manual* for more information on proper disposal of these materials.

CHLORINE, Total, continued

REQUIRED REAGENTS AND STANDARDS

Description	Quantity Required per test	Unit	Cat. No.
Test 'N Tube DPD Total Chlorine Reagent.....	1 vial.....	25/pkg.....	21056-45

REQUIRED EQUIPMENT AND SUPPLIES

COD/TNT Vial Adapter, DR/4000	1	each.....	48189-00
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OPTIONAL REAGENTS AND STANDARDS

Chlorine Standard Solution, 2-mL PourRite Ampule, 50-75mg/L	20/pkg.....	14268-20
Potassium Iodide Solution, 30-g/L.....	100 mL * MDB.....	343-32
Sodium Arsenite Solution, 5-g/L	100 mL * MDB.....	1047-32
Sodium Hydroxide Standard Solution, 1.00 N.....	100 mL * MDB.....	1045-32
Sulfuric Acid Standard Solution, 1.000 N.....	100 mL * MDB.....	1270-32
Water, deionized	4 liters.....	272-56

OPTIONAL EQUIPMENT AND SUPPLIES

Ampule Breaker Kit	each.....	21968-00
Beaker, 50-mL.....	each.....	500-41
pH Indicator Paper, 1 to 11 pH.....	5 rolls/pkg.....	391-33
Pipet, TenSette, 0.1 to 1.0 mL	each.....	19700-01
Pipet Tips, for 19700-01 TenSette Pipet	50/pkg.....	21856-96
Test Tube Rack	each.....	18641-00

* Contact Hach for larger sizes.



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FAX: (970) 669-2932



Method 10070

DPD method*

UHR (0.1–10.0 mg/L as Cl₂)

Scope and Application: For testing higher levels of total chlorine (free and combined) in drinking water, cooling water, industrial process waters, or treated wastewater.

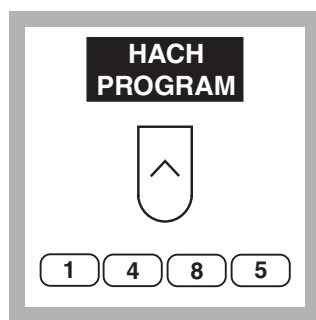
* Adapted from *Standard Methods for the Examination of Water and Wastewater*.

Tips and Techniques

- Analyze samples immediately. Do not preserve for later analysis.
- If chlorine is present, a pink color will develop after adding DPD Total Chlorine Reagent
- If the chlorine concentration is typically less than 2 mg/L, use method 8167, program number 1450.

Powder Pillow

Method 10070



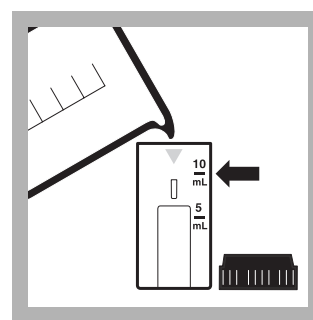
1. Press the soft key under **HACH PROGRAM**. Select the stored program number for Chlorine, HR by pressing **1485**. Use the numeric keys.



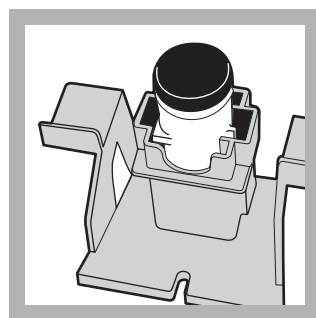
2. Press: **ENTER**.
The display will show:
HACH PROGRAM 1485 Chlorine, UHR
The wavelength (λ), **530 nm**, is automatically selected.



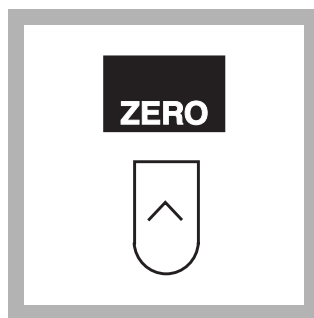
3. Insert the AccuVac[®] adapter into the sample cell module by sliding it under the thumbscrew and into the alignment grooves. Fasten with the thumbscrew.



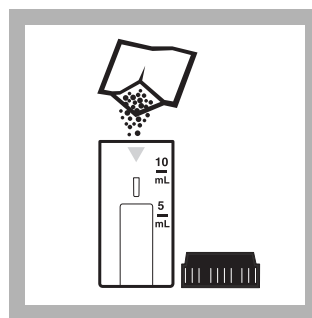
4. Fill the 10-mL/1-cm cell to the 5-mL line with sample.



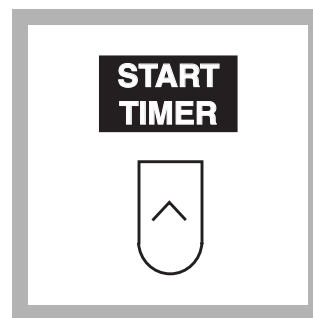
5. Place the cell into the cell holder so that the locking ridge on the cell is oriented to the left. Press the top rim of the cell on the right side to lock it in place. Close the light shield.



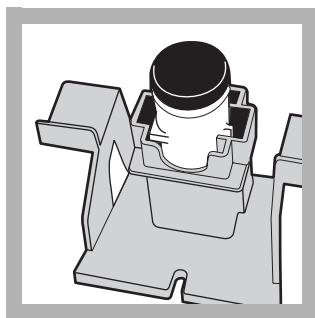
6. Press the soft key under **ZERO**.
The display will show:
0.0 mg/L Cl₂



7. Remove the cell from the cell holder and add the contents of one pillow of DPD Total Chlorine to the sample. Proceed immediately to step 8. Cap and shake the cell about 20 seconds to dissolve.



8. Press the soft key under **START TIMER**. A 3-minute reaction period will begin.



9. Place the cell into the cell holder so the locking ridge on the cell is oriented to the left. Press the top rim of the cell on the right side to lock it in place.



10. Close the light shield. Within 3 minutes after the timer beeps, read the results in mg/L chlorine (as Cl_2).

Sampling and Storage

Analyze samples for chlorine immediately after collection. Free and combined chlorine are strong oxidizing agents and react rapidly with various compounds. Many factors such as sunlight, pH, temperature, and sample composition will influence decomposition of free chlorine in water.

Avoid plastic containers, which may have a large chlorine demand. Pretreat glass containers to remove chlorine demand by soaking in a dilute bleach solution (1 mL commercial bleach to 1 L of deionized water) for at least 1 hour. Rinse thoroughly with deionized or distilled water. If sample containers are rinsed thoroughly with deionized or distilled water after use, only occasional pretreatment is necessary.

Do not use the same sample cells for free and total chlorine. If trace iodide from the total chlorine reagent is carried over to the free chlorine test, monochloramine could interfere. It is best to use separate, dedicated sample cells for free and total chlorine determinations.

A common error in testing for chlorine is obtaining a representative sample. If sampling from a tap, let the water flow for at least 5 minutes to ensure a representative sample. Let the container overflow with the sample several times, then cap the sample container so there is no air above the sample. If sampling with a sample cell, rinse the cell several times with the sample, then carefully fill to the 5-mL mark. Proceed with the chlorine test immediately.

Method Performance

Precision

Standard: 5.6 mg/L Cl_2

Program	95% Confidence Limits of Distribution
1485	5.5–5.7 mg/L Cl_2

Sensitivity

Portion of Curve	Δ Abs	Δ Concentration
Entire range	0.010	0.1 mg/L Cl ₂

Accuracy Check

Standard Additions Method (Sample Spike)

1. Leave the unspiked sample in the sample compartment. Verify that the units displayed are in mg/L. Select standard additions mode by pressing the soft keys under **OPTIONS, (MORE)** and then **STD ADD**.
2. Press **ENTER** to accept the default sample volume (mL), 10.
3. Locate the average chlorine concentration shown on the certificate enclosed with the LR Voluette® Ampules. When prompted for the standard concentration, use the numeric keys to enter the certificate value. Press **ENTER**.
4. Press the soft key under **ENTRY DONE**.
5. Snap the neck off of a Chlorine Voluette® Ampule Standard, 50–75 mg/L Cl₂.
6. Use the Tensette® Pipet to add 0.1 mL, 0.2 mL, and 0.3 mL of standard, respectively, to three 5-mL samples. Mix each thoroughly.
7. Analyze each standard addition sample as described above. Accept the standard additions readings by pressing the soft key under **READ** each time. Each additions should reflect approximately 100% recovery.
8. After completing the sequence, the display will show the extrapolated concentration value and the “best-fit” line through the standard additions data points, accounting for matrix interferences.
9. See Section 1.4.1 *Standard Additions* for more information.

Interferences

The following have been tested for interference and found to interfere at the indicated levels:

Table 1 Interfering Substances

Substance	Level	Correction
Acidity	Greater than 150 mg/L CaCO ₃ May not develop full color or color may fade instantly.	1 Neutralize to pH 6–7 with 1 N Sodium Hydroxide. 2 Determine the amount to be added on separate sample aliquot, then add the same amount to the sample being tested. 3 Correct for volume additions.
Alkalinity	Greater than 250 mg/L CaCO ₃ May not develop full color or color may fade instantly.	1 Neutralize to pH 6–7 with 1 N Sulfuric Acid. 2 Determine the amount to be added on separate sample aliquot, then add the same amount to the sample being tested. 3 Correct for volume additions.

Table 1 Interfering Substances (Continued)

Substance	Level	Correction
Bromine, Br ₂	Interferes at all levels	
Chlorine dioxide, ClO ₂	Interferes at all levels	
Chloramines, organic	May interfere	
Chromium, oxidized (Cr ⁶⁺)		1 Adjust sample pH to 6–7. 2 Add 2 drops Potassium Iodide (30 g/L) to a 5-mL sample. 3 Mix and wait 1 minute. 4 Add 2 drops Sodium Arsenite* (5 g/L) and mix. 5 Analyze the treated sample as described in the procedure. Subtract the result from this test from the original analysis to obtain the correct chlorine concentration.
Iodine, I ₂	Interferes at all levels	
Manganese, oxidized (Mn ⁴⁺ , Mn ⁷⁺)		1 Adjust sample pH to 6–7. 2 Add 2 drops Potassium Iodide (30 g/L) to a 5-mL sample. 3 Mix and wait 1 minute. 4 Add 2 drops Sodium Arsenite* (5 g/L) and mix. 5 Analyze the treated sample as described in the procedure. 6 Subtract the result from this test from the original analysis to obtain the correct chlorine concentration.
Ozone	Interferes at all levels	
Peroxides	May interfere	
pH (extreme sample pH or highly buffered samples)		Adjust to pH 6–7 using acid (1.000 N Sulfuric Acid) or base (1.00 N Sodium Hydroxide).

* Samples treated with sodium arsenite for manganese or chromium interference will be hazardous wastes as regulated by the Federal RCRA for arsenic (D004).

Summary of Method

The range of analysis using the DPD method for total chlorine can be extended by adding more indicator in proportion to sample volume. Adding a larger fill powder pillow of DPD Total Chlorine Reagent to a 5-mL sample portion will extend the range of analysis.

The combined chlorine oxidizes iodide in the reagent to iodine. The iodine reacts with the DPD (N, N-diethyl-p-phenylenediamine) along with free chlorine present in the sample to form a red color which is proportional to the total chlorine concentration. To determine the concentration of combined chlorine, run free chlorine and total chlorine tests. Subtract the results of the free chlorine test from the results of the total chlorine test to obtain combined chlorine.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the Material Safety Data Sheet (MSDS) for information specific to the reagent used.

REQUIRED REAGENTS

Description	Quantity Required		Unit	Cat. No.
	Per Test			
DPD Total Chlorine Reagent Powder Pillows.....	1		100/pkg	14064-99

REQUIRED APPARATUS

Sample Cell, 10-mL/1-cm, with cap	1		2/pkg	48643-02
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OPTIONAL REAGENTS

Sodium Hydroxide, 1 N	100 mL	MDB	1045-32
Sulfuric Acid, 1 N	100 mL	MDB	1270-32
Potassium Iodide, 30 g/L.....	100 mL	MDB	343-32
Sodium Arsenite, 5 g/L	100 mL		1047-32

OPTIONAL APPARATUS

Cylinders, graduated mixing	each	1896-40
TenSette Pipet, 0.1–1.0 mL	each	19700-01
Replacement Tips for 19700-01	50/pkg	21856-96

REQUIRED STANDARDS

Chlorine Standard Solution, PourRite™ Ampules, 50–75 mg/L.....	20/pkg	14268-20
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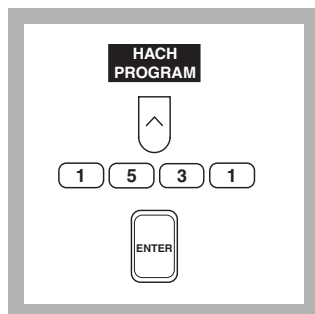
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Telephone: (970) 669-3050
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Scope and Application: For water and drinking water.
The estimated detection limit is 20 µg/L ClO_2 .

* This method is under license of Elf Atofina. Reagent sets for this method are only available in Europe.



1. Press the soft key under **HACH PROGRAM**.

Select the stored program number for Chlorine Dioxide (ClO_2) by pressing **1531** with the numeric keys.

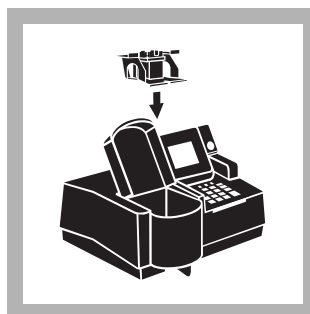
Press: **ENTER**



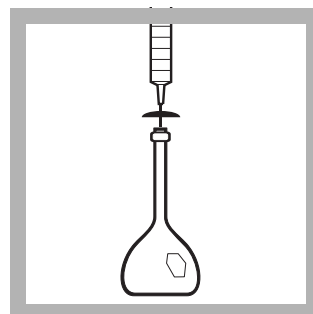
2. The display will show:

**HACH PROGRAM:
1531 ClO_2 Amaranth**

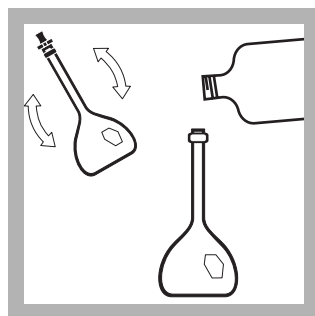
The wavelength (λ), **521 nm**, is automatically selected.



3. Place the DR/4000 1-inch Cell Adapter into the sample cell module by sliding it under the thumb screw and into the alignment grooves. Fasten with the thumb screw.



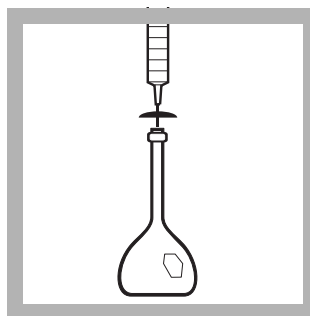
4. Using the syringe and needle provided, add 1.0 mL of Chlorine Dioxide Reagent A into a 25-mL volumetric flask.



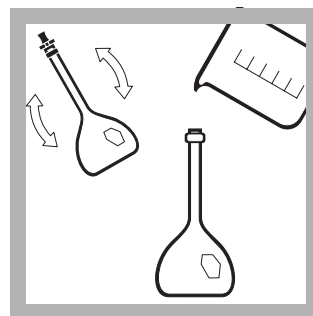
5. Fill the volumetric flask to the mark with deionized water. Stopper. Invert several times to mix.



6. Pour 10mL from the volumetric flask into a 10-mL sample cell. This is the blank.

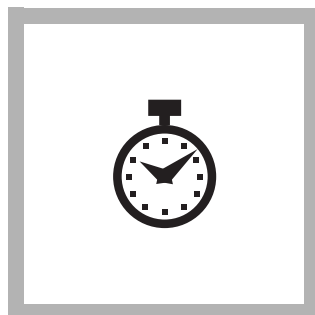


7. Using the syringe and needle provided, add 1.0 mL of Chlorine Dioxide Reagent A into a second 25-mL volumetric flask.

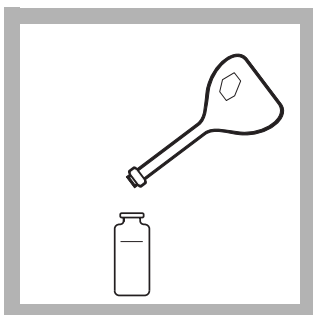


8. Fill the second volumetric flask to the mark with the sample. Stopper. Invert several times to mix. This is the sample.

CHLORINE DIOXIDE, continued



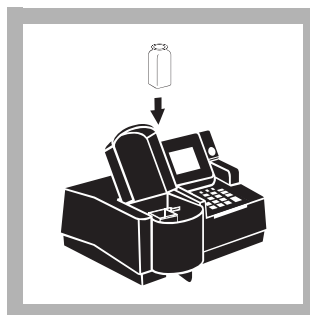
9. Begin a 1-minute reaction period.



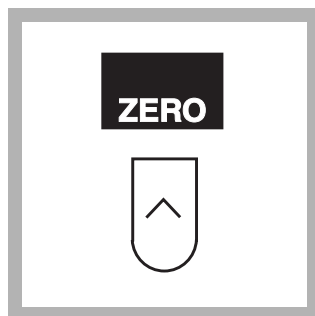
10. Pour 10 mL from the second volumetric flask into a 10-mL sample cell. This is the sample.



11. Wipe the cells with a damp towel followed by a dry one, to remove fingerprints and other marks.



12. Place the blank cell into the cell holder. Close the light shield.



13. Press the soft key under **ZERO**.

The display will show:

0 µg/L ClO₂



14. When the timer beeps, place the sample cell into the cell holder. Close the light shield. Results in µg/L ClO₂ will be displayed.

Interferences

Interfering Substance	Interference Levels
ClO ⁻	Greater than 2.0 mg/L
ClO ₂ ⁻	Greater than 2.0 mg/L
ClO ₃ ⁻	Greater than 2.0 mg/L
CrO ₄ ²⁻	Greater than 0.2 mg/L
Fe ³⁺	Greater than 0.5 mg/L
Cu ²⁺	Greater than 1 mg/L

Sample Collection, Storage and Preservation

Collect samples in clean plastic or glass bottles. Fill completely and cap tightly. Avoid excessive agitation and exposure to light, especially sunlight. Samples must be analyzed immediately upon collection and cannot be preserved or stored for later analysis.

Accuracy Check

Standard Solution Method.

Preparing chlorine dioxide standards is difficult and dangerous. In addition, these standards are both explosive and volatile! Only a trained chemist should prepare the standards using appropriate safety equipment and precautions. Hach does not recommend preparation of chlorine dioxide standards. If independent standard preparation is required, please see the instructions in *Standard Methods for the Examination of Water and Wastewater*, 20th ed., under the headings “Stock chlorine dioxide solution” and “Standard chlorine dioxide solution” (pg. 4–74). Prepare a 0.25-mg/L (250-µg/L) chlorine dioxide standard and analyze as described.

Method Performance

Precision

Standard: 250 µg/L ClO₂

Program	95% Confidence Limits
1531	192–308 µg/L ClO ₂

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
1531	20 µg/L ClO ₂

For more information on derivation and use of Hach’s estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, Appendix B, see Section 1.5.1.

Sensitivity

Program Number: 1531

Portion of Curve	ΔAbs	ΔConcentration
Entire Range	0.010	24 µg/L

See Section 1.5.3 *Sensitivity Explained* for more information.

Calibration Standard Preparation

Preparing chlorine dioxide standards is difficult and dangerous. In addition, these standards are both explosive and volatile! Only a trained chemist should prepare the standards using appropriate safety equipment and precautions. Hach does not recommend preparation of chlorine dioxide standards. If independent standard preparation is required, please see the instructions in *Standard Methods for the Examination of Water and Wastewater*, 20th ed., under the heading “Standard chlorine dioxide solution” (pg. 4–74). Using the standards prepared and the analysis procedure, generate a calibration curve.

CHLORINE DIOXIDE, continued

Summary of Method

Chlorine Dioxide (ClO₂) is determined by its combination with Amaranth. The resulting decrease in color intensity is measured at 521 nm.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to Section 1.

Pollution Prevention and Waste Management

For information on pollution prevention and waste management, refer to Section 1

REQUIRED REAGENTS AND STANDARDS

Description	Unit	Cat. No.
Chlorine Dioxide Amaranth Reagent Set*	100/pkg	LYW 240
Chlorine Dioxide Tool Kit	each	LZC 140
Includes:		
Flask, volumetric, 25-mL	2/pkg	
Syringe, 1-mL (includes needle)	each	

REQUIRED EQUIPMENT AND SUPPLIES

DR/4000 1-inch Cell Adapter	each	48190-00
Sample Cells, 1-inch, matched pair	2/pkg	26126-02
Pipet, volumetric, Class A, 1.00-mL	each	14515-35
Pipet Filler, safety bulb	each	14651-00

OPTIONAL EQUIPMENT AND SUPPLIES

DR/4000 Carousel Module Kit	each	48070-02
DR/4000 Flow Cell Module Kit, 1-inch	each	48070-04
DR/4000 Sipper Module Kit, 1-inch	each	48090-03
Pipet, TenSette, 0.1 to 1.0 mL	each	19700-01
Pipet Tips, for 19700-01 TenSette Pipet	50/pkg	21856-96

* Available in Europe only



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Method 8065

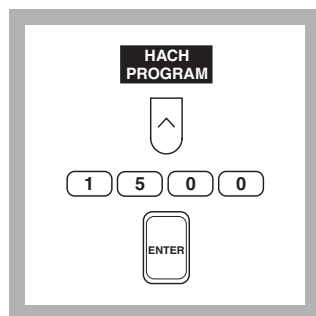
Chlorophenol Red Method*

LR (0 to 1.00 mg/L)

Scope and Application: For water and wastewater.

The estimated detection limit for program number 1500 is 0.02 mg/L ClO_2 .

* Adapted from Harp, Klein, and Schoonover, *Jour. Amer. Water Works Assn.*, 73 387-388 (1981)



1. Press the soft key under **HACH PROGRAM**.

Select the stored program number for low range chlorine dioxide (ClO_2) by pressing **1500** with the numeric keys.

Press: **ENTER**

Note: Analyze samples immediately because of the instability and volatility of chlorine dioxide. See *Sample Collection, Storage and Preservation* following these steps.

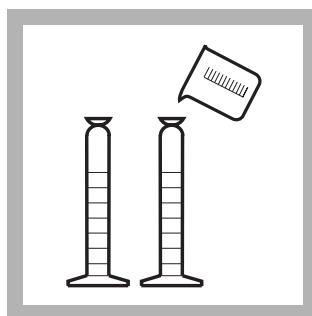
Note: For most accurate results, analyze each portion at the same sample temperature

Note: The Flow Cell and Sipper Modules can be used with this procedure if rinsed between samples with deionized water.



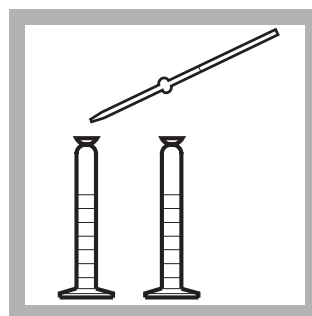
2. The display will show:
HACH PROGRAM: 1500
Chlor: Dioxide, LR

The wavelength (λ), **575 nm**, is automatically selected.



3. Fill two 50-mL mixing graduated cylinders with sample to the 50-mL mark.

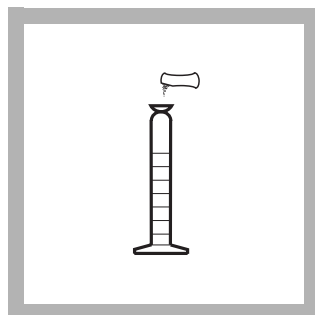
Note: For sample with extreme pH, see the *Interferences* section.



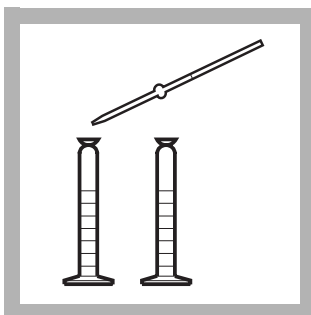
4. Add 1.0 mL of Chlorine Dioxide Reagent 1 to each cylinder. Stopper. Invert several times to mix.

Note: Use a volumetric pipet and pipet filler or a TenSette Pipet to add this reagent.

CHLORINE DIOXIDE, continued

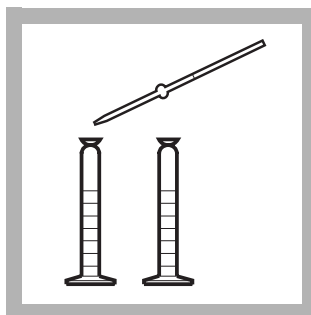


5. Add the contents of one Dechlorinating Reagent Powder Pillow to one cylinder. Stopper and invert several times until dissolved. This solution is the blank. The other solution is the prepared sample.



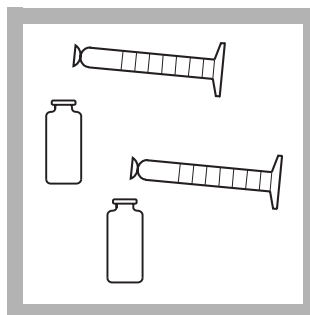
6. Add exactly 1.00 mL Chlorine Dioxide Reagent 2 to each cylinder. Stopper. Invert several times to mix.

Note: Use a Class A pipet to measure this reagent accurately.



7. Add 1.0 mL of Chlorine Dioxide Reagent 3 to each cylinder. Stopper. Invert several times to mix.

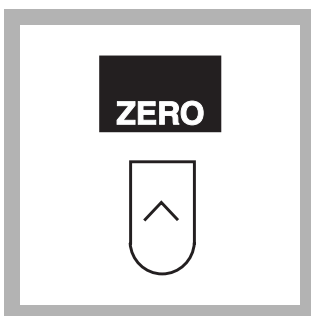
Note: Use a volumetric pipet and pipet filler or TenSette Pipet to add this reagent.



8. Pour 25 mL from each cylinder into respective sample cells.



9. Place the blank into the cell holder. Close the light shield.

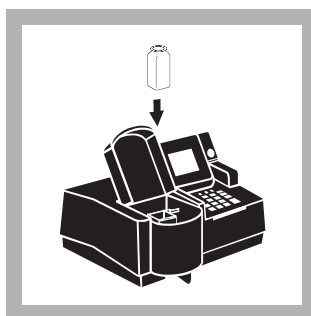


10. Press the soft key under **ZERO**.

The display will show:

0.00 mg/L ClO₂

Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



11. Place the prepared sample into the cell holder. Close the light shield. Results in mg/L ClO₂ (or chosen units) will be displayed.

Interferences

Table 1 Interfering Substances and Suggested Treatments

Interfering Substance	Interference Levels and Treatments
Highly acidic or alkaline water	May require 2.0 mL each of Chlorine Dioxide Reagent 1 and Chlorine Dioxide Reagent 3 instead of 1.0 mL
ClO^-	Greater than 5.5 mg/L
ClO_2^-	Greater than 6 mg/L
ClO_3^-	Greater than 6 mg/L
CrO_4^{2-}	Greater than 3.6 mg/L
Fe^{3+}	Greater than 5 mg/L
Hardness	Greater than 1000 mg/L
Ozone	Greater than 0.5 mg/L
Turbidity	Greater than 1000 NTU

Sample Collection, Storage and Preservation

Collect samples in clean plastic or glass bottles. Fill completely and cap tightly. Avoid excessive agitation and exposure to light, especially sunlight. Samples must be analyzed immediately upon collection and cannot be preserved or stored for later analysis.

Accuracy Check

Standard Solution Method.

Preparing chlorine dioxide standards is difficult and dangerous. In addition, these standards are both explosive and volatile! Only a trained chemist should prepare the standards using appropriate safety equipment and precautions. Hach does not recommend preparation of chlorine dioxide standards. If independent standard preparation is required, please see the instructions in *Standard Methods for the Examination of Water and Wastewater*, 18th ed., under the headings “Stock chlorine dioxide solution” and “Standard chlorine dioxide solution” (pg. 4–54). Prepare a 0.50-mg/L chlorine dioxide standard.

Method Performance

Precision

Standard: 0.50 mg/L ClO₂

Program	95% Confidence Limits
1500	0.49–0.51 mg/L ClO ₂

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
1500	0.02 mg/L ClO ₂

For more information on derivation and use of Hach's estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, Appendix B, see Section 1.5.1.

Sensitivity

Program Number: 1500

Portion of Curve	ΔAbs	ΔConcentration
0.010 Abs	0.010	0.001 mg/L
0.50 mg/L	0.010	0.006 mg/L
0.90 mg/L	0.010	0.009 mg/L

See Section 1.5.3 *Sensitivity Explained* for more information.

Calibration Standard Preparation

Preparing chlorine dioxide standards is difficult and dangerous. In addition, **these standards are both explosive and volatile!** Only a trained chemist should prepare the standards using appropriate safety equipment and precautions. Hach does not recommend preparation of chlorine dioxide standards. If independent standard preparation is required, please see the instructions in *Standard Methods for the Examination of Water and Wastewater*, 18th ed., under the heading “Standard chlorine dioxide solution” (pg. 4–54). Using the standards prepared and the analysis procedure, generate a calibration curve.

Summary of Method

Chlorine Dioxide (ClO₂) is determined by its combination with chlorophenol red at pH 5.2 to form a colorless complex. The net effect is bleaching of the color in an amount proportional to the chlorine dioxide concentration. The method is specific for ClO₂ and is unreactive to other active chlorine or moderate oxidizing compounds.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to Section 1.

Pollution Prevention and Waste Management

For information on pollution prevention and waste management, refer to Section I.

REQUIRED REAGENTS AND STANDARDS

Description	Quantity Required		Unit	Cat. No
	Per Test			
Chlorine Dioxide Reagent Set (100 Tests)				22423-00
Includes: (2) 20700-42, (2) 20701-42, (2) 20702-42, (1) 14363-69				
Chlorine Dioxide Reagent 1	2 mL	100 mL.....		20700-42
Chlorine Dioxide Reagent 2	2 mL	100 mL.....		20701-42
Chlorine Dioxide Reagent 3	2 mL	100 mL.....		20702-42
Dechlorinating Reagent Powder Pillows.....	1 pillow	100/pkg.....		14363-69

REQUIRED EQUIPMENT AND SUPPLIES

Cylinder, graduated mixing, 50-mL	2	each.....	1896-41
Pipet, volumetric, Class A, 1.00-mL	1	each.....	14515-35
Pipet Filler, safety bulb.....	1	each.....	14651-00

OPTIONAL EQUIPMENT AND SUPPLIES

DR/4000 Carousel Module Kit	each.....	48070-02
DR/4000 Flow Cell Module Kit, 1-inch.....	each.....	48070-04
DR/4000 Sipper Module Kit, 1-inch	each.....	48090-03
Pipet, TenSette, 0.1 to 1.0 mL	each.....	19700-01
Pipet Tips, for 19700-01 TenSette Pipet	50/pkg.....	21856-96



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WORLD HEADQUARTERS
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FAX: (970) 669-2932



Method 10126

DPD Method*

Powder Pillows and AccuVac® Ampuls

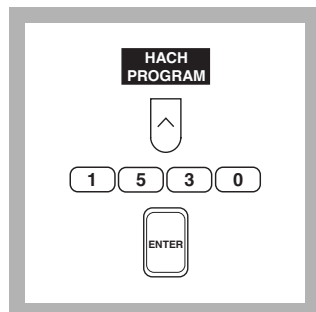
(0 to 5.00 mg/L)

Scope and Application: For testing chlorine dioxide in water; USEPA accepted for reporting for drinking water analyses**

The estimated detection limit for program numbers 1530 and 1535 is 0.04 mg/L ClO₂.

* Adapted from *Standard Methods for the Examination of Water and Wastewater*.

** Procedure is equivalent to Std. Methods, 18th Ed. 4500 ClO₂ D.



1. Press the soft key under **HACH PROGRAM**.

Select the stored program number for chlorine dioxide (ClO₂) by pressing **1530** with the numeric keys.

Press: **ENTER**

Note: Samples must be analyzed immediately and cannot be preserved for later analysis. See *Sample Collection, Storage and Preservation* following these steps.



2. The display will show:

**HACH PROGRAM:
1530 ClO₂ DPD**

The wavelength (λ), **530 nm**, is automatically selected.

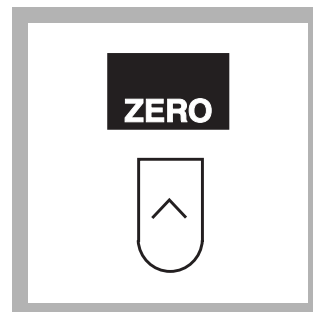
Note: For best results, determine a reagent blank for each new lot of reagent as follows. Prepare a reagent blank by repeating steps 3–8, using deionized water as the sample. Zero the instrument on deionized water by pressing the soft key under **ZERO**. Insert the reagent blank and the blank value will be displayed. Correct for the reagent blank by pressing the soft keys under **OPTIONS**, **(MORE)**, and then **BLANK:OFF**. Enter the reagent blank value and press **ENTER**. Repeat for each new lot of reagents.



3. Fill a sample cell with 10 mL of sample (the blank). Place it into the cell holder and close the light shield.

Note: For samples with extreme pH, see *Interferences*.

Note: Wipe off any liquid or fingerprints before putting the cell in the instrument.



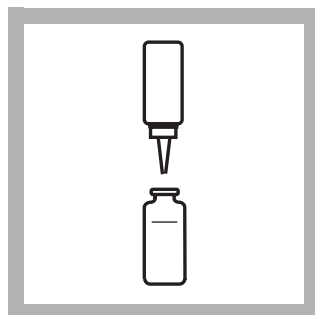
4. Press the soft key under **ZERO**.

The display will show:

0.00 mg/L ClO₂

Note: If you are using a reagent blank correction, the display will show the correction.

Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



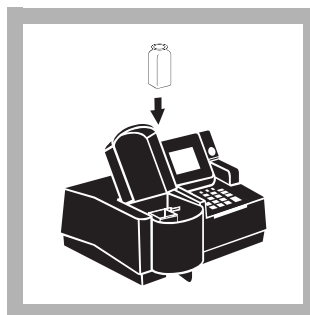
5. Add four drops of Glycine Reagent to the sample. Swirl to mix. This is the prepared sample.



6. Add the contents of one DPD Free Chlorine Powder Pillow to the sample cell (the prepared sample). Swirl the sample cell for 20 seconds to mix. Wait 30 seconds for any undissolved powder to settle. Proceed to step 7 immediately.

Note: A pink color will develop if chlorine dioxide is present.

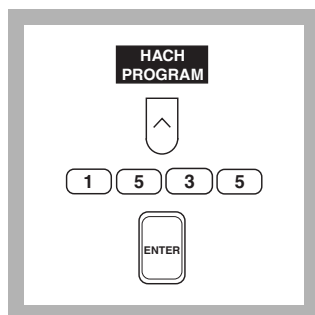
Note: Wipe off any liquid or fingerprints before putting the cell in the instrument.



7. Place the prepared sample into the cell holder. Close the light shield. Read results in mg/L chlorine dioxide (or chosen units) within one minute of reagent addition.

Note: If the chlorine dioxide concentration in the sample exceeds the upper limit of the test, the color may fade or the display may show **OVER!**. Dilute the sample with high quality water that is chlorine demand-free, and repeat the test. Some loss of chlorine dioxide may occur due to the dilution. Multiply the result by the appropriate dilution factor.

Using AccuVac® Ampuls



1. Press the soft key under **HACH PROGRAM**.

Select the stored program number for chlorine dioxide (ClO_2) by pressing **1535** with the numeric keys.

Press: **ENTER**

Note: Samples must be analyzed immediately and cannot be preserved for later analysis. See Sample Collection, Storage and Preservation following these steps.



2. The display will show:

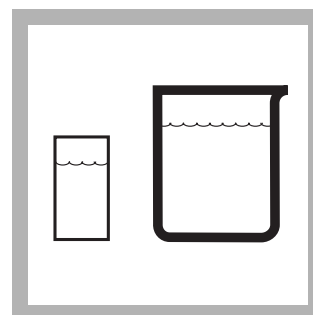
**HACH PROGRAM:
1535 ClO_2 DPD, AV**

The wavelength (λ), **530 nm**, is automatically selected.

Note: For best results, determine a reagent blank. See Step 2 in the powder pillow procedure for instructions.



3. Insert the AccuVac Ampul Adapter into the sample cell module by sliding it under the thumb screw and into the alignment grooves. Fasten with the thumb screw.

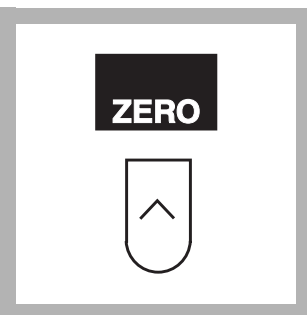


4. Fill a zeroing vial (the blank) with at least 10 mL of sample. Fill a 50-mL beaker with 40 mL of sample.



5. Place the blank into the cell holder. Close the light shield.

Note: Wipe off any liquid or fingerprints before putting the cell in the instrument.



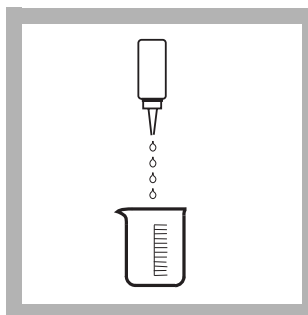
6. Press the soft key under **ZERO**.

The display will show:

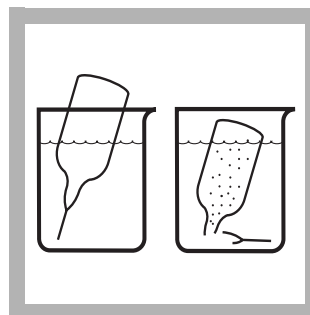
0.00 mg/L ClO₂

Note: If you are using a reagent blank correction, the display will show the correction.

Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.

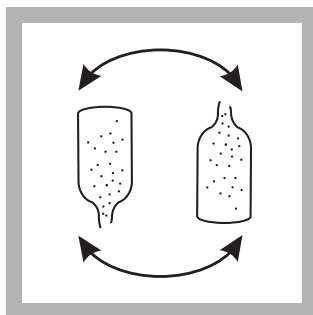


7. Add 16 drops of Glycine Reagent to the sample in the beaker. Swirl gently to mix.



8. Fill a DPD Free Chlorine Reagent AccuVac Ampul with sample.

Note: Keep the tip immersed while the ampul fills completely.



9. Quickly invert the ampul several times to mix. Wait 30 seconds for any undissolved powder to settle. Wipe off any liquid or fingerprints.

Note: A pink color will form if chlorine dioxide is present.



10. Immediately (within one minute of sample addition) place the AccuVac Ampul into the cell holder. Close the light shield. Results in mg/L chlorine dioxide (or chosen units) will be displayed.

Note: If the chlorine dioxide concentration in the sample exceeds the upper limit of the test, the color may fade or the display may show **OVER!**. Dilute the sample with high quality water that is chlorine demand-free, and repeat the test. Some loss of chlorine dioxide may occur due to the dilution. Multiply the result by the appropriate dilution factor.

CHLORINE DIOXIDE, continued

Interferences

A substance interferes if it changes the final reading by 0.1 mg/L or more.

Interfering Substance	Interference Levels and Treatments
Acidity	Greater than 150 mg/L CaCO_3 . May not develop full color or color may fade instantly. Neutralize to pH 6–7 with 1 N sodium hydroxide. Determine amount to be added on separate sample aliquot, then add the same amount to the sample being tested. Correct for volume addition (see <i>Section 1.22, Correction For Volume Additions</i>).
Alkalinity	Greater than 250 mg/L CaCO_3 . May not develop full color or color may fade instantly. Neutralize to pH 6–7 with 1 N sulfuric acid. Determine amount to be added on separate sample aliquot, then add the same amount to the sample being tested. Correct for volume addition (see <i>Section 1.2.2 Correcting for Volume Additions</i>).
Bromine, Br_2	Interferes at all levels.
Chlorine, Cl_2	May interfere at levels greater than 6 mg/L. Additional glycine may be able to compensate for this interference.
Chloramines, organic	May interfere.
Flocculating agents	High levels of most flocculating agents can be tolerated. This tolerance is decreased if chlorine is present. See the information about metals in this table. In the presence of 0.6 mg/L Cl_2 , $\text{Al}(\text{SO}_4)_3$ (< 500 mg/L) and FeCl_2 (<200 mg/L) may be tolerated.
Hardness	No effect at less than 1,000 mg/L as CaCO_3 .
Iodine, I_2	Interferes at all levels.
Manganese, oxidized (Mn^{4+} , Mn^{7+}) or Chromium, oxidized (Cr^{6+})	Oxidized manganese interferes at all levels. Oxidized chromium interferes at levels greater than 2 mg/L. To remove the interferences: <ol style="list-style-type: none">1. Adjust sample pH to 6–7.2. Add 3 drops potassium iodide (30 g/L) to a 25-mL sample.3. Mix and wait one minute.4. Add 3 drops sodium arsenite (5 g/L) and mix.5. Analyze 10 mL of the treated sample as described in the procedure.6. Subtract the result of this test from the original analysis to obtain the correct chlorine dioxide concentration.
Metals	Various metals may interfere by combining with the glycine needed to remove the chlorine interference. Metal interference is limited except when chlorine is present. In the presence of 0.6 mg/L Cl_2 , both copper (>10 mg/L) and nickel (>50 mg/L) interfere. Other metals may also interfere, depending on their ability to prevent glycine from reacting with any Cl_2 in the sample. It may be necessary to add more glycine to overcome this interference.
Monochloramine	Causes a gradual drift to higher readings. When read within 1 minute after reagent addition, 3 mg/L monochloramine causes less than a 0.1 mg/L ClO_2 increase in the reading.
Ozone	Interferes at levels greater than 1.5 mg/L.
Peroxides	May interfere.
Extreme sample pH	Adjust to pH 6-7. See <i>Section 1.3, pH Interference</i> .
Highly buffered samples	Adjust to pH 6-7. See <i>Section 1.3, pH Interference</i> .

Sample Collection, Storage and Preservation

Analyze samples for chlorine dioxide immediately after collection. Chlorine dioxide is a strong oxidizing agent and is unstable in natural waters. It reacts rapidly with various inorganic compounds, but oxidizes organic compounds more slowly. Many factors, including reactant concentrations, sunlight, pH, temperature, and salinity influence decomposition of chlorine dioxide in water.

Avoid plastic containers since these may have a large chlorine dioxide demand. Pretreat glass sample containers to remove any chlorine or chlorine dioxide demand by soaking in a dilute bleach solution (1 mL commercial bleach to 1 liter of deionized water) for at least one hour. Rinse thoroughly with deionized or distilled water. If sample containers are rinsed thoroughly with deionized or distilled water after use, only occasional pretreatment is necessary.

A common error in testing for chlorine dioxide is obtaining a representative sample. If sampling from a tap, let the water flow for at least 5 minutes to ensure a representative sample. Let the container overflow with the sample several times, then cap the sample containers so there is no headspace (air) above the sample. If sampling with a sample cell, rinse the cell several times with the sample, then carefully fill to the 10-mL mark. Perform then chlorine dioxide analysis immediately.

Accuracy Check

Because chlorine dioxide is difficult and hazardous to produce, check the DPD and glycine reagents by using chlorine standards. Proceed as follows:

1. Prepare a 1-mg/L free chlorine standard.

Method 1

- a. Obtain Free Chlorine Standards, Hach Cat. No. 14268-10.
- b. Determine the concentration of the standard from the certificate of analysis shipped with the standard (50–75 mg/L). Calculate the volume of standard needed as follows:

$$\text{mL standard needed} = 100 \div \text{standard concentration}$$

- c. Pipet the volume of standard needed into a 100-mL volumetric flask. Dilute to the line with chlorine demand-free deionized water. Invert to mix.

Method 2

Dilute 1 drop of 5% chlorine bleach in 1 liter of chlorine-demand-free deionized water. Use this as the standard.

2. Verify the standard's concentration using the Hach Free Chlorine Method, #8021.
3. Perform the chlorine dioxide test on the standard without adding glycine (*step 6* for program 1530 or *step 7* for program 1535).
4. For program 1530, the chlorine dioxide reading should be 2.38 times greater than the chlorine result. For program 1535, the chlorine dioxide reading should be 2.48 times greater than the chlorine result. If so, this verifies the DPD and the instrument are functioning properly.
5. Repeat the chlorine dioxide test on the chlorine standard, including the glycine addition (*step 6* or *7*). The reading should be less than 0.10 mg/L. This verifies that the glycine is eliminating free chlorine interference.

Method Performance

Precision

Standard: 0.35 mg/L ClO₂

Program	95% Confidence Limits
1530	0.33–0.37 mg/L ClO ₂
1535	0.32–0.38 mg/L ClO ₂

Standard: 3.70 mg/L ClO₂

Program	95% Confidence Limits
1530	3.54–3.86 mg/L ClO ₂
1535	3.59–3.81 mg/L ClO ₂

For more information on determining precision data and method detection limits, refer to *Section 1.5*.

Estimated Detection Limit

Program	EDL
1530	0.04 mg/L ClO ₂
1535	0.04 mg/L ClO ₂

For more information on derivation and use of Hach's estimated detection limit, see *Section 1.5.2*. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, Appendix B, see *Section 1.5.1*.

Sensitivity

Program Number: 1530

Portion of Curve	ΔAbs	ΔConcentration
0.40	0.010	0.042 mg/L
2.50	0.010	0.045 mg/L
4.00	0.010	0.049 mg/L

Program Number: 1535

Portion of Curve	ΔAbs	ΔConcentration
0.40	0.010	0.048 mg/L
2.50	0.010	0.051 mg/L
4.00	0.010	0.054 mg/L

See *Section 1.5.3, Sensitivity Explained*, for more information.

Summary of Method

Chlorine dioxide reacts with DPD (N, N-diethyl-p-phenylenediamine) Indicator Reagent (to the extent of one-fifth of its total available chlorine content corresponding to reduction of chlorine dioxide to chlorite) to form a pink color. The color intensity is proportional to the ClO_2 in the sample. Chlorine interference is eliminated by adding glycine, which converts free chlorine to chloroaminoascorbic acid, but has no effect on chlorine dioxide at the test pH.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to Section 1.

Pollution Prevention and Waste Management

Samples treated with sodium arsenite for interferences will be hazardous waste as regulated by Federal RCRA for arsenic (D004). See Section 1 for further information on proper disposal of these materials.

CHLORINE DIOXIDE, continued

REQUIRED REAGENTS AND STANDARDS (Using Powder Pillows)

Chlorine Dioxide DPD/Glycine Reagent Set (100 tests)27709-00
Includes: (1) 21055-69, (1) 27621-33

Description	Quantity Required		Unit	Cat. No.
	per test			
DPD Free Chlorine Reagent Powder Pillows, 10 mL	1 pillow	100/pkg	21055-69	
Glycine Reagent	4 drops	29 mL	27621-33	

REQUIRED REAGENTS AND STANDARDS (Using AccuVac Ampuls)

Chlorine Dioxide DPD/Glycine AccuVac® Ampul Reagent Set (25 tests)27710-00
Includes: (1) 25020-25, (1) 27621-33

DPD Free Chlorine Reagent AccuVac® Ampuls 1 ampul 25/pkg25020-25
Glycine Reagent16 drops 29 mL27621-33

REQUIRED EQUIPMENT AND SUPPLIES (Using Powder Pillows)

DR/4000 1-inch Cell Adapter1 each48190-00
Stopper, rubber, No. 21 12/pkg2118-02

REQUIRED EQUIPMENT AND SUPPLIES (Using AccuVac Ampuls)

Beaker, 50-mL1 each500-41
DR/4000 AccuVac Ampul Adapter1 each48187-00
Sample Cell, 10-mL with cap (zeroing vial)1 each21228-00

OPTIONAL REAGENTS AND STANDARDS

Chlorine Standard Solution, 10-mL Voluette™ Ampule, 50–75 mg/L 16/pkg 14268-10
Potassium Iodide Solution, 30-g/L 100 mL * MDB343-32
Sodium Arsenite Solution, 5-g/L 100 mL * MDB 1047-32
Sodium Hydroxide Standard Solution, 1.00 N 100 mL * MDB 1045-32
Sulfuric Acid Standard Solution, 1.000 N 100 mL * MDB 1270-32
Water, deionized 4 liters272-56

OPTIONAL EQUIPMENT AND SUPPLIES

AccuVac® Snapper each24052-00
Ampule Breaker Kit each21968-00
Cylinder, graduated, 25-mL, poly each 1081-40
DR/4000 Carousel Module Kit each48070-02
DR/4000 Flow Cell Module Kit, 1-inch each48070-04
DR/4000 Flow Cell Module Kit, 1-cm each48070-05
DR/4000 Sipper Module Kit, 1-inch each48090-03
pH Meter, *sensio*™1, portable each51700-00
Pipet, TenSette®, 0.1 to 1.0 mL each 19700-01
Pipet Tips, for 19700-01 TenSette Pipet 50/pkg21856-96

* Contact Hach for larger sizes.



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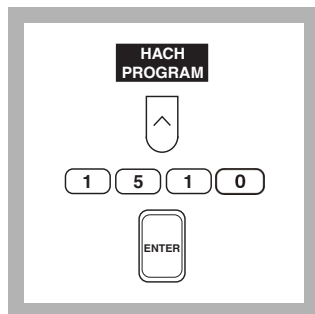
Method 8345

Direct Reading Method

MR (0 to 50 mg/L)

Scope and Application: For drinking and wastewater.

The estimated detection limit for program number 1510 is 0.6 mg/L.



1. Press the soft key under **HACH PROGRAM**.

Select the stored program number for mid range chlorine dioxide (ClO_2) by pressing **1510** with the numeric keys.

Press: **ENTER**

Note: Analyze samples immediately. See Sample Collection, Storage and Preservation following these steps.

Note: The Flow Cell and Sipper Modules can be used with this procedure. Use minimum volumes of 20 and 10 mL, respectively.



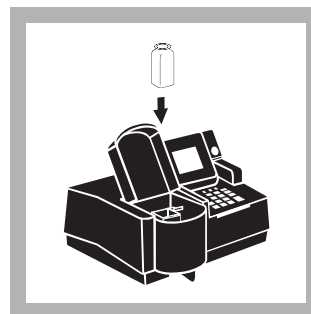
2. The display will show:

**HACH PROGRAM:
1510 Chlor: Dioxide,
MR**

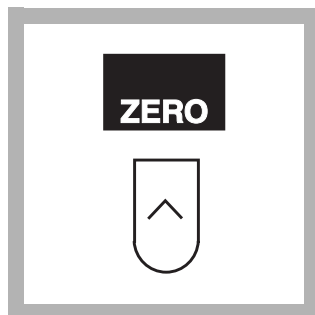
The wavelength (λ), **360 nm**, is automatically selected.



3. Fill a sample cell to the 10-mL mark with deionized water (the blank).



4. Place the blank into the cell holder. Close the light shield.



5. Press the soft key under **ZERO**.

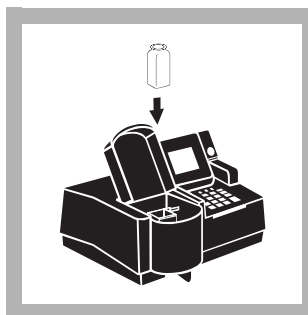
The display will show:

0.0 mg/L ClO₂

Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



6. Fill another sample cell to the 10-mL mark with sample (the prepared sample).



7. Place the prepared sample into the cell holder. Close the light shield. The results in mg/L ClO₂ (or chosen units) will be displayed.

Sample Collection, Storage and Preservation

Collect samples in clean plastic or glass bottles. Samples must be analyzed immediately. Chlorine dioxide is very volatile and unstable.

Accuracy Check

Standard Solution Method

Preparing chlorine dioxide standards is difficult and dangerous. In addition, **these standards are both explosive and volatile!** Only a trained chemist should prepare the standards using appropriate safety equipment and precautions. Hach does not recommend preparation of chlorine dioxide standards. If independent standard preparation is required, please see the instructions in *Standard Methods for the Examination of Water and Wastewater*, 18th ed., under the headings “Stock chlorine dioxide solution” and “Standard chlorine dioxide solution” (pg. 4–54). Prepare a 25.0-mg/L chlorine dioxide standard.

Method Performance

Precision

Standard: 25.0 mg/L ClO₂

Program	95% Confidence Limits
1510	24.5–25.5 mg/L ClO ₂

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
1510	0.6 mg/L ClO ₂

For more information on derivation and use of Hach's estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, Appendix B, see Section 1.5.1.

Sensitivity

Program Number: 1510

Portion of Curve	ΔAbs	ΔConcentration
0.010 Abs	0.010	0.28 mg/L
27.5 mg/L	0.010	0.25 mg/L
49.5 mg/L	0.010	0.22 mg/L

See Section 1.5.3 *Sensitivity Explained* for more information.

Calibration Standard Preparation

Preparing chlorine dioxide standards is difficult and dangerous. In addition, **these standards are both explosive and volatile!** Only a trained chemist should prepare the standards using appropriate safety equipment and precautions. Hach does not recommend preparation of chlorine dioxide standards. If independent standard preparation is required, please see the instructions in *Standard Methods for the Examination of Water and Wastewater*, 18th ed., under the headings “Stock chlorine dioxide solution” and “Standard chlorine dioxide solution” (pg. 4–54). Using the standards prepared above and the analysis procedure, generate a calibration curve.

Summary of Method

Chlorine dioxide, a yellow gas, can be measured directly in a water solution. This method uses a wavelength of 360 nm to increase the sensitivity of the test.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to Section 1.

CHLORINE DIOXIDE, continued

Pollution Prevention and Waste Management

For information on pollution prevention and waste management, refer to Section I.

REQUIRED REAGENTS AND STANDARDS

Description	Quantity Required		Unit	Cat. No
	Per Test			
Water, deionized	10 mL	4 liters272-56

REQUIRED EQUIPMENT AND SUPPLIES

DR/4000 1-Inch Cell Adapter	1	each48190-00
Sample Cells, 1-inch, matched pair.....	2	2/pkg26126-02

OPTIONAL EQUIPMENT AND SUPPLIES

DR/4000 Carousel Module Kit	each48070-02
DR/4000 Flow Cell Module Kit, 1-inch.....		each48070-04
DR/4000 Flow Cell Module Kit, 1-cm.....		each48070-05
DR/4000 Sipper Module Kit, 1-inch	each48090-03



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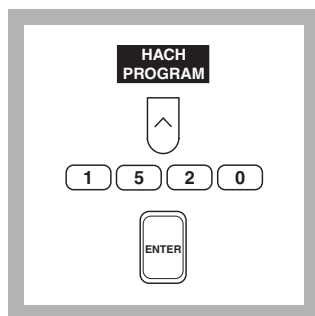
Method 8138

Direct Reading Method

HR (0 to 1000 mg/L)

Scope and Application: For water and wastewater.

The estimated detection limit for program number 1520 is 2 mg/L ClO_2 .



- 1.** Press the soft key under **HACH PROGRAM**.

Select the stored program number for high range chlorine dioxide (ClO_2) by pressing **1520** with the numeric keys.

Press: **ENTER**

Note: Analyze samples immediately. See Sample Collection, Storage and Preservation following these steps.

Note: The Flow Cell and Sipper Modules can be used with this procedure. Use minimum volumes of 20 and 10 mL, respectively.



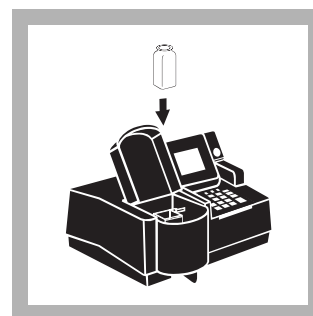
- 2.** The display will show:

**HACH PROGRAM:
1520 Chlor: Dioxide, HR**

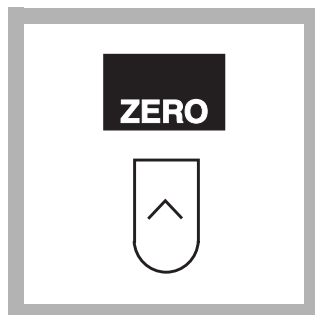
The wavelength (λ), **445 nm**, is automatically selected.



- 3.** Fill a sample cell to the 10-mL mark with deionized water (the blank).



- 4.** Place the blank into the cell holder. Close the light shield.

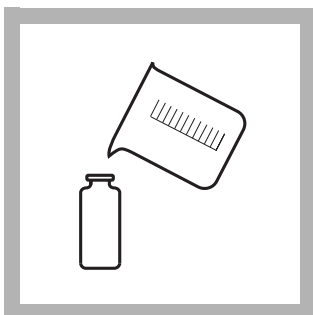


5. Press the soft key under **ZERO**.

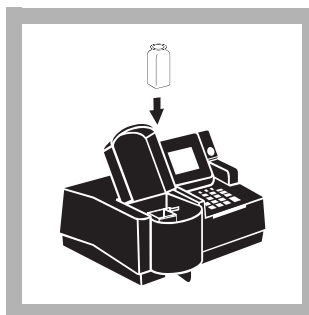
The display will show:

0 mg/L ClO₂

Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



6. Fill another sample cell to the 10-mL mark with sample (the prepared sample).



7. Place the prepared sample into the cell holder. Close the light shield. The results in mg/L ClO₂ (or chosen units) will be displayed.

Sample Collection, Storage and Preservation

Collect samples in clean plastic or glass bottles. Samples must be analyzed immediately. Chlorine dioxide is very volatile and unstable.

Accuracy Check

Standard Solution Method

Preparing chlorine dioxide standards is difficult and dangerous. In addition, **these standards are both explosive and volatile!** Only a trained chemist should prepare the standards using appropriate safety equipment and precautions. Hach does not recommend preparation of chlorine dioxide standards. If independent standard preparation is required, please see the instructions in *Standard Methods for the Examination of Water and Wastewater*, 18th ed., under the headings "Stock chlorine dioxide solution" and "Standard chlorine dioxide solution" (pg. 4–54). Prepare a 25.0-mg/L chlorine dioxide standard.

Method Performance

Precision

Standard: 500 mg/L ClO₂

Program	95% Confidence Limits
1520	499–501 mg/L ClO ₂

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
1520	2 mg/L ClO ₂

For more information on derivation and use of Hach's estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, Appendix B, see Section 1.5.1.

Sensitivity

Program Number: 1520

Portion of Curve	ΔAbs	ΔConcentration
Entire Range	0.010	4.6 mg/L

See Section 1.5.3 *Sensitivity Explained* for more information.

Calibration Standard Preparation

Preparing chlorine dioxide standards is difficult and dangerous. In addition, **these standards are both explosive and volatile!** Only a trained chemist should prepare the standards using appropriate safety equipment and precautions. Hach does not recommend preparation of chlorine dioxide standards. If independent standard preparation is required, please see the instructions in *Standard Methods for the Examination of Water and Wastewater*, 18th ed., under the headings “Stock chlorine dioxide solution” and “Standard chlorine dioxide solution” (pg. 4–54). Using the standards prepared above and the analysis procedure, generate a calibration curve.

Summary of Method

Chlorine dioxide, a yellow gas, can be measured directly in a water solution. This method uses a wavelength of 445 nm to increase the range of the test.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to Section 1.

Pollution Prevention and Waste Management

For information on pollution prevention and waste management, refer to Section 1.

CHLORINE DIOXIDE, continued

REQUIRED REAGENTS AND STANDARDS

Description	Quantity Required		Unit	Cat. No
	Per Test			
Water, deionized	10 mL	4 liters272-56

REQUIRED EQUIPMENT AND SUPPLIES

DR/4000 1-Inch Cell Adapter	1	each48190-00
Sample Cells, 1-inch, matched pair.....	2	2/pkg26126-02

OPTIONAL EQUIPMENT AND SUPPLIES

DR/4000 Carousel Module Kit			each48070-02
DR/4000 Flow Cell Module Kit, 1-inch.....			each48070-04
DR/4000 Flow Cell Module Kit, 1-cm.....			each48070-05
DR/4000 Sipper Module Kit, 1-inch			each48090-03



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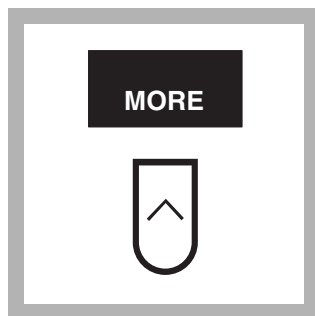


Scope and Application: For testing chlorophyll-a in water.

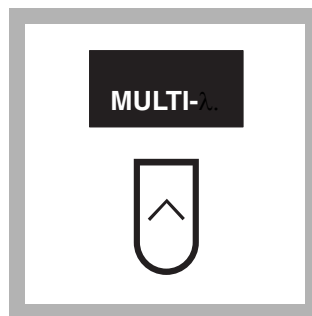
* Adapted from *Standard Methods for the Examination of Water and Wastewater*.



1. Insert the 1-cm sample cell adapter into the single cell module.



2. Press the soft key under **MORE** in the main menu.

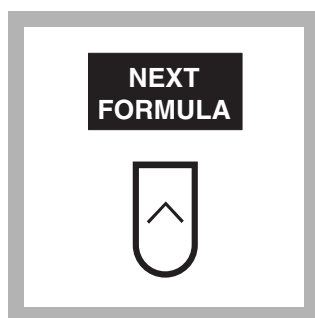


3. Press the soft key under **MULTI-λ**.

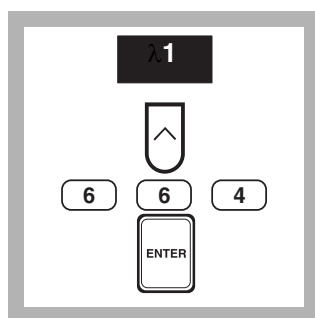


4. Press the soft key under **GOTO-λ**.

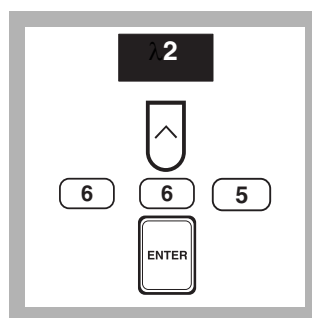
Note: This procedure requires special equipment and sample pretreatment before analysis with the DR/4000. Please see **REQUIRED APPARATUS AND REAGENTS** following these steps for more information.



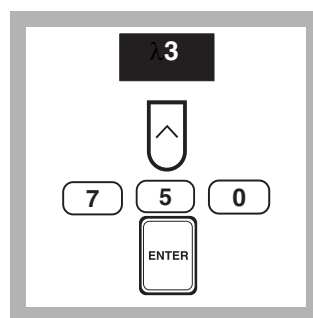
5. Press the soft key under **NEXT FORMULA** until the equation, $A = K_1A_1 + K_2A_2 + K_3A_3$, appears on the screen.



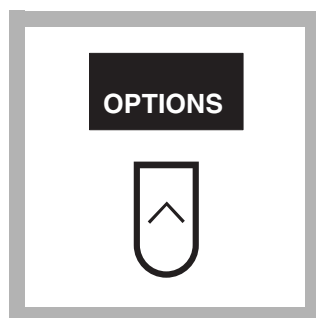
6. Press the soft key under λ_1 :
Enter **664** on the numeric pad, and press the **ENTER** key.



7. Press the soft key under λ_2 :
Enter **665** on the numeric pad, and press the **ENTER** key.

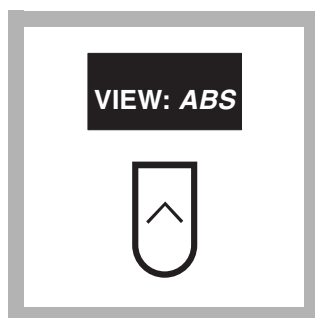


8. Press the soft key under λ_3 :
Enter **750** on the numeric pad, and press the **ENTER** key.
Press the **ENTER** key again.



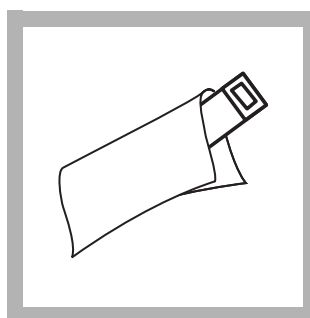
9. Press the soft key under **OPTIONS**.

Note: For software versions less than 2.01, press the soft key under **VIEW:CONC** until the display reads **VIEW:ABS**. Skip step 10.



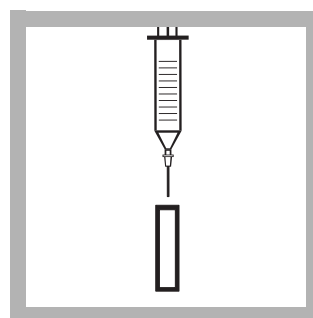
10. Press the soft key under **VIEW:ABS** to make sure the display shows **ABS**.

Press the **EXIT** key.



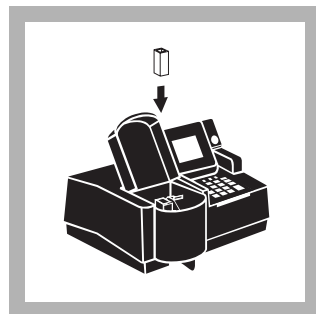
11. Clean the outside of a clean 1x1-cm glass cuvette with a lint-free non-scratching towel.

Note: Do not use plastic cuvettes. Acetone will dissolve plastic cuvettes.



12. Using the syringe containing Extraction Solvent, add 3 mL of solvent to the cuvette. This is the blank.

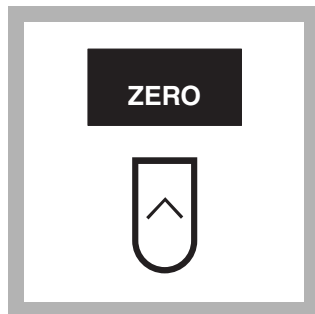
Note: Wipe the cuvette with a soft, damp towel followed by a dry towel to remove fingerprints or other marks.



13. Place the blank into the sample cell holder with the lettering on the cuvette facing to the right. Close the light shield.

Note: Do not spill acetone on the DR/4000 Spectrophotometer.

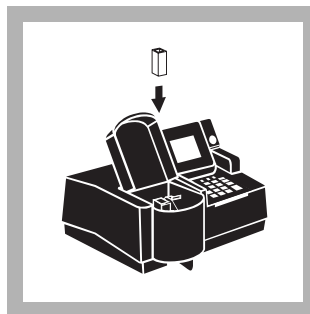
Note: Face the cuvette in the same direction for all measurements.



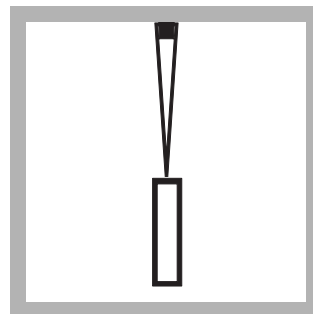
14. Press the soft key under **ZERO**.

A₁, A₂, and A₃ should all read 0.00. Disregard the large **ABS** reading.

Note: For software versions less than 2.01, disregard the 0.00 mg/L value.



15. Remove the blank from the sample cell holder.



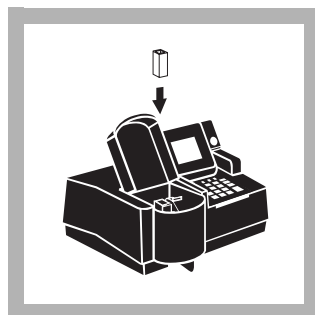
16. Pipet exactly 3 mL of sample extract into a clean 1x1-cm cuvette using a 100 to 2000 µL positive displacement pipet. Cap the cuvette

Note: The 1x1-cm cuvette should match the cuvette used for the blank (order Cat. No. 20951-00).

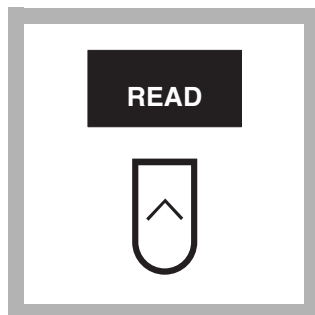
Note: Recap the sample extract tube.

Note: Acetone-based solvents are very volatile and significant pipetting error can be introduced at this step. The following pipet options are listed in order of preference:

1. Positive displacement pipet, 100 to 2000 µL, is the most accurate for acetone-based solvents. Refer to the pipet manual for instructions.
2. Syringe, 3 to 10 mL.
3. Air displacement pipet, such as a TenSette Pipet, 1.0 to 10.0 mL.



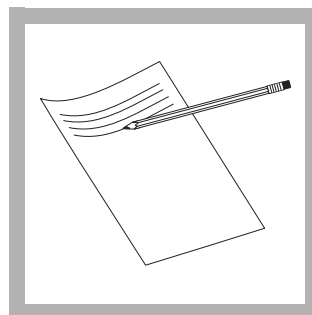
17. Place the cuvette containing sample extract into the sample cell holder. Close the light shield.



18. Press the soft key under **READ**.
The display will show Absorbance readings for A_1 , A_2 , and A_3 . Disregard the large **ABS** reading.

Note: For software versions less than 2.01, press the soft key under **START**.

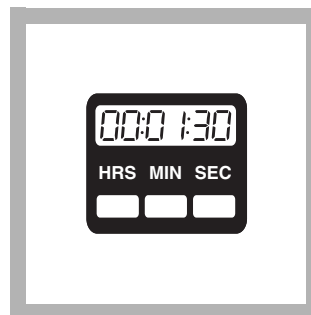
Note: The display will show **ABS** readings for A_1 , A_2 , and A_3 . Disregard the large mg/L reading.



19. Record the results for A_1 and A_3 .

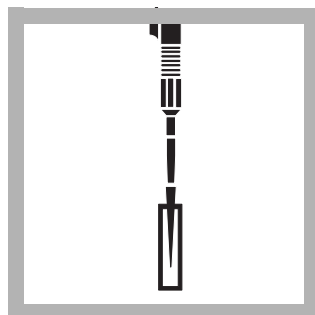
Note: The absorbance at 665 nm (A_2) is displayed on the instrument, but the reading is not recorded at this time.

Note: The absorbance at 664 nm (A_1) should be between 0.1 and 1.0. If the absorbance is too low, repeat the entire procedure and filter a larger quantity of water or use less Extraction Solvent in the preparation of the extract. If the absorbance is too high, dilute the remaining sample extract and factor the dilution into the concentration calculation.



20. Set the timer for 1 minute 30 seconds.

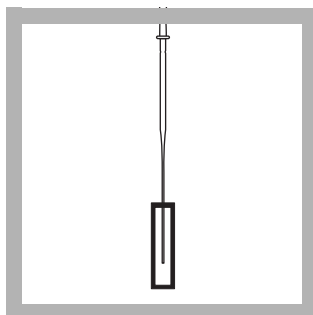
Note: To set the timer, enter 130 on the numeric keypad.



21. Using a TenSette Pipet with a clean tip, pipet 0.1 mL of 0.1 N hydrochloric acid into the sample extract cuvette. Immediately press the soft key under **START TIMER** to start the timer.

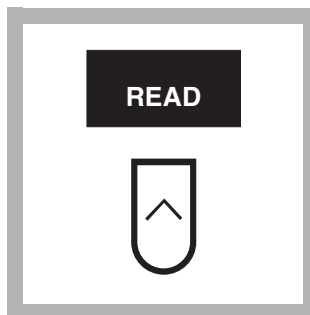
Note: Carefully pipet the hydrochloric acid while the cuvette is in the DR/4000.

Note: Tightly recap the hydrochloric acid after each use. Prolonged exposure to air could result in changes to the solution strength.



22. Quickly mix the sample extract and acid by drawing the solution into a clean Pasteur pipet and aspirating it back into the cuvette. Repeat two more times. Do not spill any extract. Recap the cuvette.

Note: Leave the pipet tip submerged in the solution while mixing.

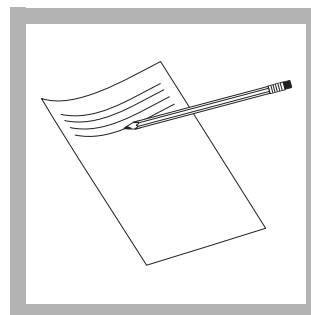


23. After 1 minute 30 seconds, press the soft key under **READ**.

The display will show Absorbance readings for A_1 , A_2 , and A_3 . Disregard the large ABS reading.

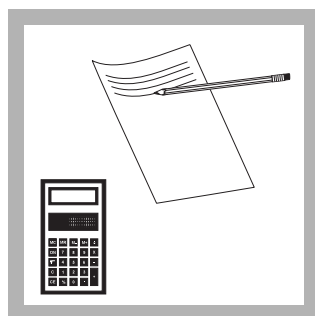
Note: For software versions less than 2.01, press the soft key under **START**.

Note: The display will show ABS readings for A_1 , A_2 , and A_3 . Disregard the large mg/L reading.



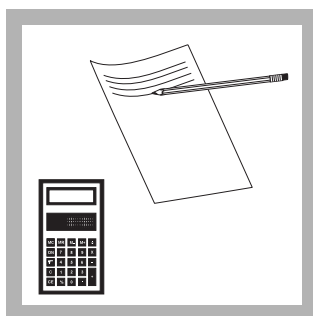
24. Record the results for A_2 and A_3 .

Note: The absorbance at 664 nm (A_1) is displayed on the instrument, but the reading is not recorded at this time.



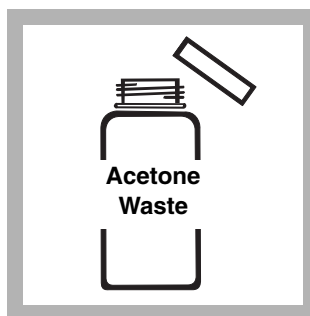
25. Subtract the first recorded absorbance at 750 nm (A_3) from the absorbance at 664 nm (A_1), see step 19. Record this result as A_{664c} .

Note: This value (A_{664c}) is a turbidity corrected absorbance at 664 nm.

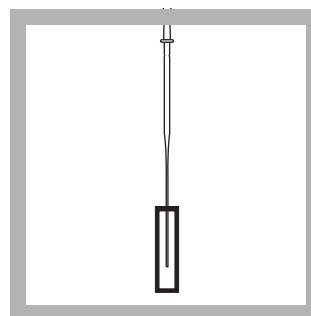


26. Subtract the second recorded absorbance at 750 nm (A_3) from the absorbance at 665 nm (A_2), see step 24. Record this result as A_{665c} .

Note: This value (A_{665c}) is a turbidity corrected absorbance at 665 nm.



27. Empty the blank cuvette and the sample extract cuvette into the acetone waste receptacle.



28. Repeatedly rinse the sample extract cuvette with acetone until two rinsings are completely clear. Empty the acetone rinsings into the acetone waste receptacle.

Clean all apparatus.

Note: See the section on using DR/4000 sample cells in the DR/4000 Procedure Manual.

Note: Do not use Extraction Solvent for rinsing.

Calculating the Results

1. Determine the chlorophyll-a concentration (in mg/L) of the sample extract:

$$[\text{Chl-a}]_{\text{EX}} = 26.7 \times (A_{664c} - A_{665c})$$

2. Determine the pheophytin-a concentration (in mg/L) of the sample extract:

$$[\text{Pheo-a}]_{\text{EX}} = 26.7 \times (1.7A_{665c} - A_{664c})$$

3. Determine the degree of pheophytinization:

$$\% \text{Pheo} = 100 \times \frac{[\text{Pheo-a}]_{\text{EX}}}{([\text{Chl-a}]_{\text{EX}} + [\text{Pheo-a}]_{\text{EX}})}$$

4. Determine the chlorophyll-a concentration (in mg/L) of the water sample.

$$[\text{Chl-a}]_{\text{W}} = [\text{Chl-a}]_{\text{EX}} \times \frac{V_{\text{EX}}}{V_{\text{W}}}$$

V_{EX} = Volume of sample extract

V_{W} = Volume of water filtered. Usually 1 liter.

5. Determine the algal biomass (in mg/L) of the water sample.

$$(\text{Algae})_{\text{W}} = 67 \times [\text{Chl-a}]_{\text{W}}$$

For Example:

$$[\text{Chl-a}]_{\text{EX}} = 26.7 \times (0.409 - 0.302) = 2.86 \text{ mg/L}$$

$$[\text{Pheo-a}]_{\text{EX}} = 26.7 \times [(1.7 \times 0.302) - 0.409] = 2.79 \text{ mg/L}$$

$$\text{Degree of pheophytinization} = 100 \times \frac{2.79}{2.86 + 2.79} = 49.5\%$$

$$[\text{Chl-a}]_{\text{W}} = (2.86) \times \frac{0.0063}{0.85} = 0.021 \text{ mg/L}$$

$$[\text{Algae}]_{\text{W}} = 67 \times 0.021 = 1.41 \text{ mg/L}$$

Where:

$A_{664} = 0.421$	Pre-acidification Readings
$A_{750} = 0.012$	
$A_{665} = 0.312$	Post-acidification Readings
$A_{750} = 0.010$	
$A_{664c} = 0.409$	
$A_{665c} = 0.302$	
$V_{\text{W}} = 0.85 \text{ L}$	
$V_{\text{EX}} = 0.0063 \text{ L} = 6.3 \text{ mL}$	

Method Performance

Range

The range of this test is determined by the sample volume collected and the extract volume. *Table 1* shows three sample collection scenarios. Notice that it is possible to have chlorophyll-a or pheophytin-a concentrations of 0.

Table 1 Sample Collection Scenarios

Volume of Extract (V _{EX})	Volume of Water (V _W)	[Chl-a] Maximum Range	[Pheo-a] Maximum Range	[Algae] Maximum Range
4 mL	5 L	0.0088 mg/L	0.0150 mg/L	0.586 mg/L
5 mL*	1 L	0.055 mg/L	0.0935 mg/L	3.67 mg/L
15 mL	0.2 L	0.825 mg/L	1.401 mg/L	55.0 mg/L

* Ideal scenario.

Precision

In a single laboratory using a research grade instrument with a 1.0-nm bandwidth, a sample was found to have a chlorophyll-a concentration of 5.61 mg/L \pm 0.0266 mg/L. A single operator using a DR/4000 Spectrophotometer obtained a standard deviation of \pm 0.0189 mg/L chlorophyll-a.

Estimated Detection Limit

The EDL is based on the ability of the DR/4000 to resolve 0.001 AU. Using the sample collection scenarios presented in *Table 1*, the following detection limit values are obtained (see *Table 2*).

Table 2 Estimated Detection Limits

Volume of Extract (V _{EX})	Volume of Water (V _W)	[Chl-a] _{EDL}	[Pheo-a] _{EDL}	[Algae] _{EDL}
4 mL	5 L	2.1×10^{-5} mg/L	3.6×10^{-5} mg/L	0.0014 mg/L
5 mL	1 L	1.3×10^{-4} mg/L	2.3×10^{-4} mg/L	0.0089 mg/L
15 mL	0.2 L	0.002 mg/L	0.0034 mg/L	0.136 mg/L

Note: The Detection Limit is based on guidelines of Initial Extract Absorbance set by Standard Methods for the Examination of Water and Wastewater, Section 10200 H-2b.

REQUIRED APPARATUS AND REAGENTS

Description	Unit	Cat. No.
Field Sampling Kit	each.....	27477-00
Includes:		
Case, Field Sampling Kit	each.....	49985-00
Algae Sampling Cup	each.....	49065-00
Algae Sampling Cup Strap	each.....	27478-00
Filter Holder, 47 mm (use with 47-mm Glass Fiber Filter, Cat. No. 2530-33)	2/pkg.....	27483-00
Intake Float Assembly	each.....	27484-00
Pump, vacuum, large hand	each.....	26977-00
Quick Reference Card	each.....	49055-44
Tubing, Tygon, R-3603	10 feet.....	20740-37
Weight, Sampling Cup	each.....	49959-00
Test Kit, Chlorophyll	each.....	27480-00
Includes:		
Acetone, ACS	500 mL.....	14429-49
Centrifuge Tube	25/pkg.....	27482-25
Extraction Solvent Bottle with magnesium carbonate solution	each.....	26956-12
Filter, glass, fiber, 47-mm (use with Filter Holder, Cat. No. 27483-00)	30/pkg.....	2530-33
Filter Cartridge	25/pkg.....	27025-00
Hydrochloric Acid, 0.1 N	15 mL.....	14812-36
Needle, disposable, blunt, 18-gauge.....	25/pkg.....	27481-25
Pipet, Pasteur, disposable, 229-mm.....	25/pkg.....	21234-01
Saturated Magnesium Carbonate Cartridge	25/pkg.....	26956-01
Spatula, micro.....	each.....	12256-00
Slurry Filtration Kit	each.....	49070-00
Includes:		
Cartridge Adapter.....	each.....	49051-00
Light Shield/Tube Holder.....	2/pkg.....	49988-00
Pipet Filler, safety bulb.....	each.....	14651-00
Tissue Grinding Kit with Drill	each.....	49961-00
Includes:		
Bulb, pipet filler, latex, 2-mL	10/pkg.....	49073-10
Cylinder, graduated, polymethylpentene, 100-mL.....	each.....	2172-42
Drill, cordless, 9.6 Vac	each.....	49072-00
Manual, Chlorophyll	each.....	49961-87
Mortar Tube Holder.....	each.....	49988-01
Syringe, 10 cc, luer slip.....	each.....	27042-00
Tissue Grinder, mortar, and pestle.....	each.....	49990-01
Tissue Grinding Kit without Drill	each.....	49961-02
Includes:		
Bulb, pipet filler, latex, 2-mL	10/pkg.....	49073-10
Cylinder, graduated, polymethylpentene, 100-mL.....	each.....	2172-42
Manual, Chlorophyll	each.....	49961-87
Mortar Tube Holder.....	each.....	49988-01
Syringe, 10 cc, luer slip.....	each.....	27042-00
Tissue Grinder, mortar, and pestle.....	each.....	49990-01

CHLOROPHYLL-A, continued

ADDITIONAL REQUIRED APPARATUS

Description	Unit	Cat. No.
DR/4000, UV, Vis, Spectrophotometer, 115 VAC.....	each.....	48000-00
DR/4000, UV, Vis, Spectrophotometer, 230 VAC.....	each.....	48000-02
DR/4000, Vis, Spectrophotometer, 115 VAC.....	each.....	48100-00
DR/4000, Vis, Spectrophotometer, 230 VAC.....	each.....	48100-02
Cuvette, 1-cm, glass, matched.....	pair.....	20951-00
Sample Transport Kit	each.....	25687-00



FOR TECHNICAL ASSISTANCE, PRICE INFORMATION AND ORDERING:

In the U.S.A. – Call toll-free 800-227-4224

Outside the U.S.A. – Contact the HACH office or distributor serving you.

On the Worldwide Web – www.hach.com; E-mail – techhelp@hach.com

HACH COMPANY

WORLD HEADQUARTERS

Telephone: (970) 669-3050

FAX: (970) 669-2932



✓ Method 8023

1,5-Diphenylcarbohydrazide Method*

Powder Pillows or AccuVac® Ampuls

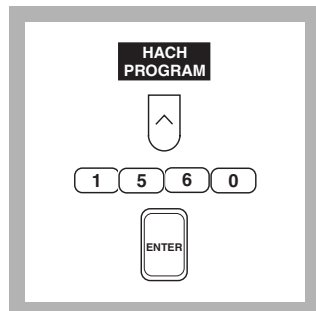
(0 to 0.700 mg/L Cr⁶⁺)

Scope and Application: For water and wastewater; USEPA accepted for reporting for wastewater analyses**. The estimated detection limits for program numbers 1560 and 1570 are 0.006 and 0.005 mg/L Cr⁶⁺, respectively.

* Adapted from Standard Methods for the Examination of Water and Wastewater.

** Procedure is equivalent to USGS method I-1230-85 for wastewater.

Using Powder Pillows



1. Press the soft key under **HACH PROGRAM**.

Select the stored program for hexavalent chromium (Cr⁶⁺) by pressing **1560** with the numeric keys.

Press: **ENTER**

Note: If samples cannot be analyzed immediately, see *Sample Collection, Preservation, and Storage* following these steps.

Note: The Flow Cell and Sipper Modules can be used for this procedure. Use a 25-mL sample volume and reagents for the Flow Cell Module.



2. The display will show: **HACH PROGRAM: 1560 Chromium, Hex.**

The wavelength (λ), **540 nm**, is automatically selected.

Note: For best results, determine a reagent blank for each new lot of reagent as follows. Prepare a reagent blank by repeating Steps 3 through 9, using chromium-free deionized water as the sample. Zero the instrument on deionized water by pressing the soft key under **ZERO**. Insert the reagent blank and the blank value will be displayed. Correct for the reagent blank by pressing the soft keys under **OPTIONS, (MORE)**, and then **BLANK:OFF**. Enter the reagent blank value and press **ENTER**. Repeat for each new lot of reagent.



3. Fill a sample cell with 10 mL of sample.

Note: For proof of accuracy, use a 0.25 mg/L hexavalent chromium standard solution (preparation given in the Accuracy Check section) in place of the sample.

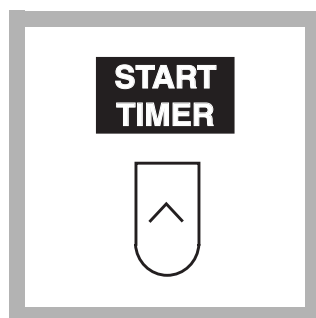
Note: For samples with extreme pH, see the Interferences section.



4. Add the contents of one ChromaVer 3 Reagent Powder Pillow to the sample cell (the prepared sample). Swirl to mix.

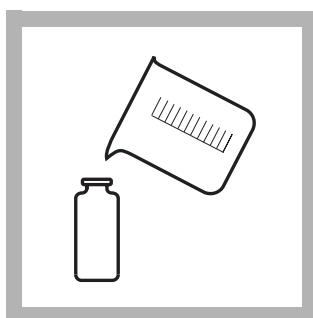
Note: A purple color will form if hexavalent chromium is present.

Note: At high chromium levels a precipitate will form. Dilute sample according to Section 1.2.6 Sample Dilution Techniques.



5. Press the soft key under **START TIMER**.

An 8-minute reaction period will begin.

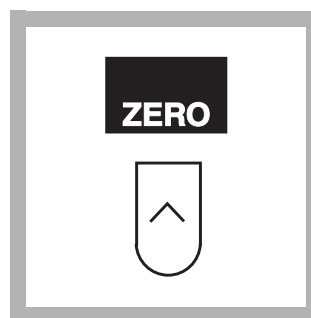


6. Fill another sample cell with 10 mL of sample (the blank).

Note: For turbid samples, treat the blank with the contents of one Acid Reagent Powder Pillow. This will ensure any turbidity dissolved by the acid in the ChromaVer 3 Chromium Reagent also will be dissolved in the blank.



7. When the timer beeps, place the blank into the cell holder. Close the light shield.



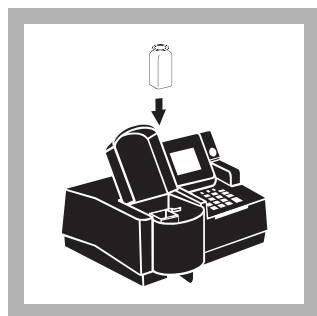
8. Press the soft key under **ZERO**.

The display will show:

0.000 mg/L Cr⁶⁺

Note: If you are using a reagent blank correction, the display will show the correction.

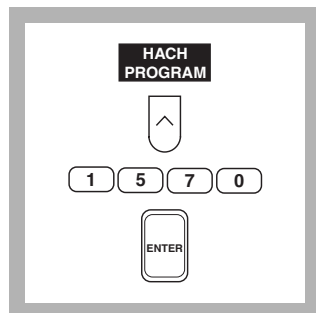
Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



9. Place the prepared sample into the cell holder. Close the light shield. Results in mg/L hexavalent chromium (Cr⁶⁺) will be displayed.

Note: Results may be expressed as chromate (CrO₄²⁻) or as sodium chromate (Na₂CrO₄), or dichromate (Cr₂O₇²⁻). Press the soft keys under **OPTIONS** and then **FORM**: to scroll through the available options. Press **ENTER** to return to the read screen.

Using AccuVac Ampuls



1. Press the soft key under **HACH PROGRAM**.

Select the stored program for hexavalent chromium (Cr^{6+}) by pressing **1570** with the numeric keys.

Press: **ENTER**

Note: If samples cannot be analyzed immediately, see *Sample Collection, Preservation, and Storage* following these steps.

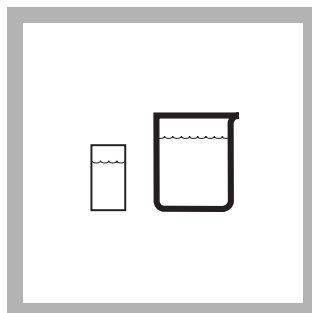


2. The display will show:
HACH PROGRAM: 1570 Chromium, Hex. AV

The wavelength (λ), **540 nm**, is automatically selected.

Note: For best results, determine a reagent blank for each new lot of reagent as follows. Prepare a reagent blank by repeating Steps 3 through 10, using chromium-free deionized water as the sample. Zero the instrument on deionized water by pressing the soft key under **ZERO**. Insert the reagent blank and the blank value will be displayed. Correct for the reagent blank by pressing the soft keys under **OPTIONS, (MORE)**, and then **BLANK:OFF**. Enter the reagent blank value and press **ENTER**. Repeat for each new lot of reagent.

Note: For sample with extreme pH, see the *Interferences* section.



3. Fill the zeroing vial with at least 10 mL of sample (the blank). Collect at least 40 mL of sample in a 50-mL beaker.

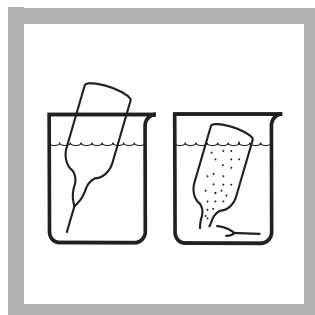
Note: For turbid samples, treat 25 mL of the blank with the contents of one Acid Reagent Powder Pillow. This will ensure any turbidity dissolved by the acid in the ChromaVer 3 Chromium Reagent also will be dissolved in the blank.

Note: For proof of accuracy, use a 0.25 mg/L hexavalent chromium standard solution (preparation given in the *Accuracy Check* section in place of the sample).



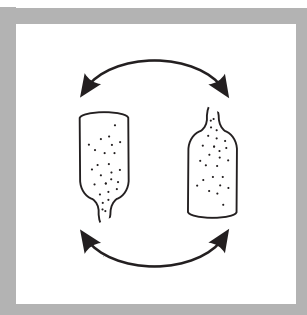
4. Insert the AccuVac Adapter into the sample cell module by sliding it under the thumb screw and into the alignment grooves. Fasten with the thumb screw.

CHROMIUM, Hexavalent, continued



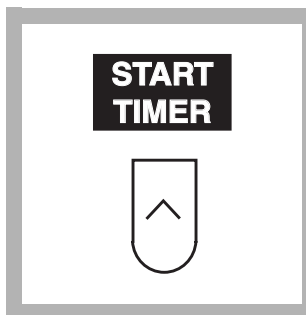
5. Fill a ChromaVer 3 Reagent AccuVac Ampul with sample (the prepared sample).

Note: Keep the tip immersed while the ampul fills completely.



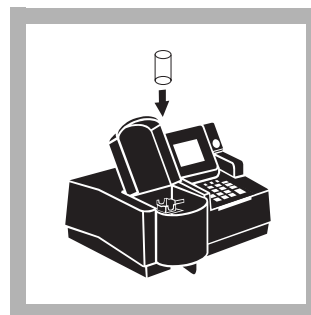
6. Quickly invert the ampul several times to mix. Wipe off any liquid or fingerprints.

Note: A purple color will form if hexavalent chromium is present.

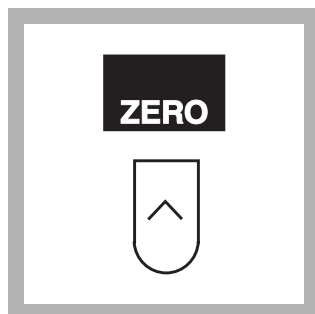


7. Press the soft key under **START TIMER**.

An 8-minute reaction period will begin.



8. When the timer beeps, place the blank into the cell holder. Close the light shield.



9. Press the soft key under **ZERO**.

The display will show:

0.000 mg/L Cr⁶⁺

Note: If you are using a reagent blank correction, the display will show the correction.

Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



10. Place the prepared sample into the cell holder. Close the light shield. Results in mg/L hexavalent chromium (Cr⁶⁺) will be displayed.

Note: Results may be expressed as chromate (CrO₄²⁻) or as sodium chromate (Na₂CrO₄), or dichromate (Cr₂O₇²⁻). Press the soft keys under **OPTIONS** and then **FORM**: to scroll through the available options. Press **ENTER** to return to the read screen.

Interferences

Table 1 Interfering Substances and Suggested Treatments

Interfering Substance	Interference Levels and Treatments
Iron	May interfere above 1 mg/L
Mercurous & Mercuric Ions	Interferes slightly
pH	Highly buffered samples or extreme sample pH may exceed the buffering capacity of the reagents and require sample pretreatment; see Section 1.3.1 <i>pH Interference</i> .
Vanadium	May interfere above 1 mg/L. Wait 10 minutes before reading.

Sample Collection, Preservation, and Storage

Collect samples in a cleaned glass or plastic container. Store at 4 °C (39 °F) up to 24 hours. Samples must be analyzed within 24 hours.

Accuracy Check

Standard Additions Method

- Leave the unspiked sample in the sample cell compartment. Verify that the units displayed are in mg/L. Select standard additions mode by pressing the soft keys under **OPTIONS, (MORE)** and then **STD ADD**.
- Press **ENTER** to accept the default sample volume (mL), 25.
- Press **ENTER** to accept the default standard concentration (mg/L), 12.5.
- Press the soft key under **ENTRY DONE**.
- Snap the neck off a Chromium Voluette Ampule Standard, 12.5-mg/L Cr⁶⁺.
- Use the TenSette Pipet to add 0.1, 0.2 and 0.3 mL of standard, respectively to three 25-mL samples and mix each thoroughly (for AccuVac Ampuls, use 50-mL beakers).
- Analyze each standard addition sample as described above. Accept the standard additions reading by pressing the soft key under **READ** each time. Each addition should reflect approximately 100% recovery.
- After completing the sequence, the display will show the extrapolated concentration value and the “best-fit” line through the standard additions data points, accounting for matrix interferences.
- See Section 1.4.1 *Standard Additions* for more information.

Standard Solution Method

Prepare a 0.25-mg/L Cr⁶⁺ standard solution by pipetting 5.00 mL of Hexavalent Chromium Standard Solution, 50-mg/L, into a 1000-mL volumetric flask. Dilute to the mark with deionized water. Prepare this solution daily. Perform the hexavalent chromium procedure as described above.

To adjust the calibration curve using the reading obtained with the 0.25-mg/L standard solution, press the soft keys under **OPTIONS, MORE** then **STD: OFF**. Press **ENTER** to accept the displayed concentration, the value of which depends on the selected units. If an alternate concentration is used, enter the actual

concentration and press **ENTER** to return to the read screen. See Section 1.5.5 *Adjusting the Standard Curve* for more information.

Method Performance

Precision

Standard: 0.25 mg/L Cr⁶⁺

Program	95% Confidence Limits
1560	0.247–0.253 mg/L Cr ⁶⁺
1570	0.247–0.253 mg/L Cr ⁶⁺

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
1560	0.006 mg/L
1570	0.005 mg/L

For more information on derivation and use of Hach's estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, Appendix B, see Section 1.5.1.

Sensitivity

Program Number: 1560

Portion of Curve	Δ Abs	Δ Concentration
Entire Range	0.010	0.0055 mg/L

Program Number: 1570

Portion of Curve	Δ Abs	Δ Concentration
Entire Range	0.010	0.0060 mg/L

See Section 1.5.3 *Sensitivity Explained* for more information.

Calibration Standard Preparation

To perform a hexavalent chromium calibration using the 1,5-Diphenylcarbohydrazide method, prepare a 5.0-mg/L chromium stock solution by pipetting 10.0 mL of a 50-mg/L Chromium Hexavalent Standard Solution into a 100-mL volumetric flask using Class A glassware. Dilute to the mark with deionized water and mix thoroughly.

Prepare calibration standards containing 0.05, 0.30, and 0.60 mg/L Cr⁶⁺ as follows:

- a. Into three different 100-mL Class A volumetric flasks, pipet 1.00, 6.00, and 12.00 mL of the 5.0 mg/L Cr⁶⁺ stock solution using Class A glassware.
- b. Dilute to the mark with deionized water and mix thoroughly.
- c. Using the 1,5-Diphenylcarbohydrazide method and the calibration procedure described in the *User-Entered Programs* section of the *DR/4000 Spectrophotometer Instrument Manual*, generate a calibration curve from the standards prepared above.

Summary of Method

Hexavalent chromium is determined by the 1,5-Diphenylcarbohydrazide method using a single dry powder formulation called ChromaVer 3 Chromium Reagent. This reagent contains an acidic buffer combined with 1,5-Diphenylcarbohydrazide, which reacts to give a purple color when hexavalent chromium is present.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to Section 1.

Pollution Prevention and Waste Management

The final samples are highly acidic. Neutralize to pH 6–9 and flush down the drain for disposal. For more information on pollution prevention and waste management, refer to Section 1.

CHROMIUM, Hexavalent, continued

REQUIRED REAGENTS AND STANDARDS (Using Powder Pillows)

Description	Quantity Required per test	Unit	Cat. No.
ChromaVer 3 Chromium Reagent Powder Pillows	1 pillow	100/pkg	12710-99

REQUIRED REAGENTS AND STANDARDS (Using AccuVac Ampuls)

ChromaVer 3 AccuVac Ampuls	1 ampul	25/pkg	25050-25
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REQUIRED EQUIPMENT AND SUPPLIES (Using Powder Pillows)

DR/4000 1-Inch Cell Adapter	1	each	48190-00
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REQUIRED EQUIPMENT AND SUPPLIES (Using AccuVac Ampuls)

Beaker, 50-mL	1	each	500-41
DR/4000 AccuVac Adapter	1	each	48187-00
Sample Cell, 10-mL with cap (zeroing vial)	1	each	21228-00

OPTIONAL REAGENTS AND SUPPLIES

Description	Unit	Cat. No.
Acid Reagent Powder Pillows	50/pkg	2126-66
Chromium, Hexavalent, Standard Solution, 50-mg/L Cr ⁶⁺	100 mL	810-42H
Chromium, Hexavalent, Standard Solution, 10-mL Voluette Ampules, 12.5-mg/L Cr ⁶⁺	16/pkg	14256-10
Chromium, Hexavalent, Standard Solution, 2-mL ampule, 5.0-mg/L Cr ⁶⁺	20/pkg	26056-20
Nitric Acid, ACS	500 mL	152-49
Nitric Acid Solution, 1:1	500 mL	2540-49
Sodium Hydroxide Standard Solution, 5.0 N	50 mL * DB	2450-26
Water, deionized	4 liters	272-56

OPTIONAL EQUIPMENT AND SUPPLIES

AccuVac Snapper Kit	each	24052-00
Ampule Breaker Kit	each	21968-00
DR/4000 Carousel Module Kit	each	48070-02
DR/4000 Flow Cell Module Kit, 1-inch	each	48070-04
DR/4000 Flow Cell Module Kit, 1-cm	each	48070-05
DR/4000 Sipper Module Kit, 1-inch	each	48090-03
Flask, volumetric, Class A, 25-mL	each	14574-40
Flask, volumetric, Class A, 1000-mL, with glass stopper	each	14574-53
pH Paper, pH 1.0 to 11.0	5 rolls/pkg	391-33
pH Meter, <i>sension</i> TM 1, portable	each	51700-00
Pipet, serological, 2-mL	each	532-36
Pipet, TenSette, 0.1 to 1.0 mL	each	19700-01
Pipet Tips, for 19700-01 TenSette Pipet	50/pkg	21856-96
Pipet, volumetric, Class A, 1.00-mL	each	14515-35
Pipet, volumetric, Class A, 5.00-mL	each	14515-37
Pipet, volumetric, Class A, 6.00-mL	each	14515-06
Pipet, volumetric, Class A, 10.00-mL	each	14515-38
Pipet Filler, safety bulb	each	14651-00
Sample Cells, 1-inch, matched pair	2/pkg	26126-02

* Contact Hach for larger sizes.



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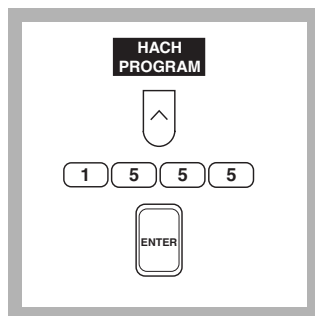


UniCell™ Vials

(0 to 1.00 mg/L Cr⁶⁺)

Scope and Application: For water and wastewater.

The estimated detection limit for program number 1555 is 0.03 mg/L Cr⁶⁺.



1. Press the soft key under **HACH PROGRAM**.

Select the stored program for UniCell Chromium by pressing **1555** with the numeric keys.

Press: **ENTER**



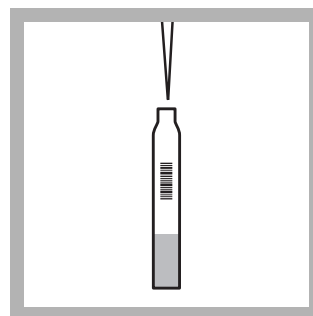
2. The display will show:

**HACH PROGRAM: 1555
Chromium, HCT 156**

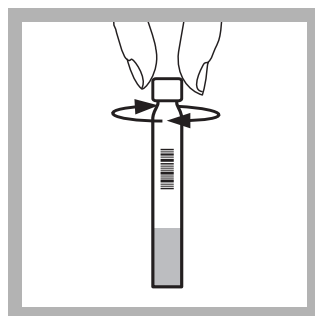
The wavelength (λ), **543 nm**, is automatically selected.



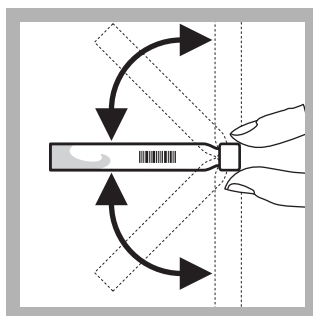
3. Insert the Test Tube Adapter into the sample cell module by sliding it under the thumb screw and into the alignment grooves. Fasten with the thumb screw.



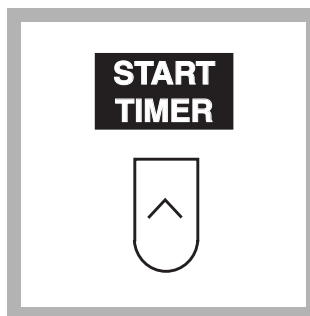
4. Pipet 4.0 mL of sample into a sample vial.



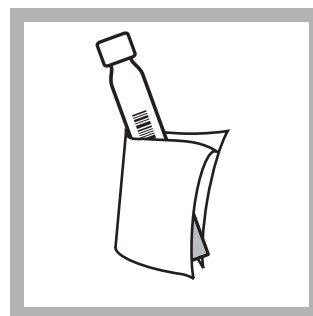
5. Cap the sample vial with the **orange** UniCap B (HCT 156 B).



6. Invert the sample vial repeatedly until the reagent in the cap is completely dissolved.



7. Press the soft key under **START TIMER**. A 10-minute reaction period will begin.

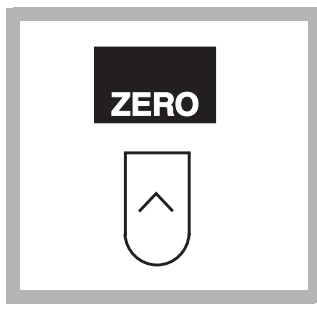


8. Wipe the outside of the zero vial (**white** cap) and the sample vial with a damp towel followed with a dry one to remove fingerprints and other marks.

CHROMIUM, Hexavalent, continued

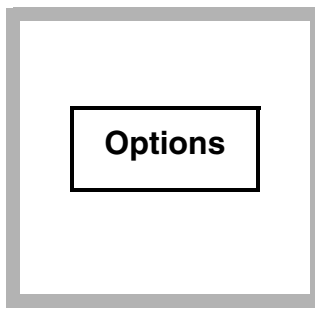


9. When the timer beeps, place the zero vial into the cell holder. Close the light shield.



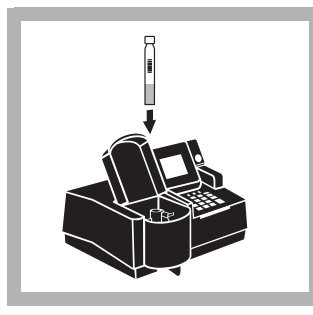
10. Press the soft key under **ZERO**.

The display will show:
0.00 mg/L Cr⁶⁺



11. Press the soft key under **OPTIONS** and then **UNITS** to select the Cr⁶⁺ display form. Press **ENTER** to return to the read screen.

Total Chromium can only be measured using the Total Chromium UniCell procedure.



12. Place the sample vial into the cell holder. Close the light shield. Results in mg/L Cr⁶⁺ will be displayed.

Interferences

The ions listed in the table have been tested up to the given concentrations. Cumulative effects and the influence of other ions have not been evaluated. There is no interference from:

Interfering Substance	Interference Levels
Cl ⁻	1000 mg/L
Ca ²⁺	125 mg/L
Mg ²⁺ , NH ₄ ⁺	100 mg/L
Zn ²⁺ , Ni ²⁺ , Co ²⁺ , Cd ²⁺	50 mg/L
Ag ⁺ , Pb ²⁺	25 mg/L
Cu ²⁺ , Fe ³⁺	10 mg/L
Sn ²⁺	1 mg/L

Higher amounts of iron, copper, and reducing or oxidizing agents will cause a negative interference. Lead, mercury, and tin will cause a positive interference.

Note: Undissolved chromium is not determined with the determination of chromium VI. Total Chromium can only be determined using the Total Chromium procedure.

Note: Concentrations above 20 mg/L produce result displays within the ranges given above. In such cases, verify the results by dilution.

Sample Collection, Storage and Preservation

Collect samples in acid-cleaned glass or plastic containers. No acid addition is necessary if analyzing the samples immediately or if the samples contain only Chromium VI ions.

To preserve the samples for Total Chromium, adjust the pH to 2 or less with concentrated nitric acid (about 2 mL per liter). Preserved samples may be stored up to six months at room temperature.

Before analysis, adjust the pH to between 3 and 9 with 5.0 N sodium hydroxide standard solution.

Accuracy Check

Standard Additions Method

- a. Select standard additions mode by pressing the soft keys under **OPTIONS, (MORE)** and then **STD ADD**.
- b. Press **ENTER** to accept the default sample volume (mL), 25.
- c. Press **ENTER** to accept the default standard concentration (mg/L), 50.0.
- d. Press the soft key under **ENTRY DONE**.
- e. Use a pipet to add 0.1, 0.2 and 0.3 mL of 50-mg/L standard, respectively to three 25-mL samples and mix each thoroughly.
- f. Analyze each standard addition sample as described above. Accept the standard additions reading by pressing the soft key under **READ** each time. Each addition should reflect approximately 100% recovery.
- g. After completing the sequence, the display will show the extrapolated concentration value and the “best-fit” line through the standard additions data points, accounting for matrix interferences.

See Section 1.4.1 *Standard Additions* for more information.

Standard Solution Method

Prepare a 0.70-mg/L Cr⁶⁺ standard solution by pipetting 1.40 mL of Hexavalent Chromium Standard Solution, 50-mg/L, into a 100-mL volumetric flask. Dilute to the mark with deionized water. Prepare this solution daily. Perform the hexavalent chromium procedure as described above.

To adjust the calibration curve using the reading obtained with the 0.70-mg/L standard solution, press the soft keys under **OPTIONS, (MORE)** then **STD: OFF**. Press **ENTER** to accept the displayed concentration, the value of which depends on the selected units. If an alternate concentration is used, enter the actual concentration and press **ENTER** to return to the read screen. See Section 1.5.5 *Adjusting the Standard Curve* for more information.

Method Performance

Precision

Standard: 0.50 mg/L Cr⁶⁺

Program	95% Confidence Limits
1555	0.38–0.62 mg/L Cr ⁶⁺

For more information on determining precision data and method detection limits, refer to Section 1.5.

CHROMIUM, Hexavalent, continued

Estimated Detection Limit

Program	EDL
1555	0.03 mg/L Cr ⁶⁺

For more information on derivation and use of Hach's estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, Appendix B, see Section 1.5.1.

Sensitivity

Program Number: 1555

Portion of Curve	Δ Abs	Δ Concentration
Entire Range	0.010	0.01 mg/L

See Section 1.5.3 *Sensitivity Explained* for more information.

Summary of Method

Chromium(VI) ions react with 1,5-diphenylcarbazide to yield 1,5-diphenylcarbazone and Chromium(III) which forms a red complex.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to Section 1.

Sample vial contains: 23% phosphoric acid.

UniCap A (HCT 156 A) contains: Sodium peroxodisulphate.

REQUIRED REAGENTS AND STANDARDS

Description	Unit	Cat. No.
Chromium - Cr (VI), UniCell™ HCT 156.....	23/pkg.....	HCT 156

OPTIONAL REAGENTS

Chromium Standard, 50-mg/L, as Cr ⁶⁺	100 mL.....	810-42
---	-------------	--------

OPTIONAL EQUIPMENT AND SUPPLIES

Graduated cylinder, mixing, 100-mL	each.....	20886-42
Flask, volumetric, 100-mL	each.....	14574-42
Pipettor, (Jencons) 1–5 mL	each.....	27951-00
Replacement tips for 27951-00	100/pkg.....	27952-00
Pipettor, (Jencons) 100–1000 μ L.....	each.....	27949-00
Replacement tips for 27949-00	400/pkg.....	27950-00
pH Paper	100/pkg.....	26013-00
Test tube rack, cooling	each.....	18641-00



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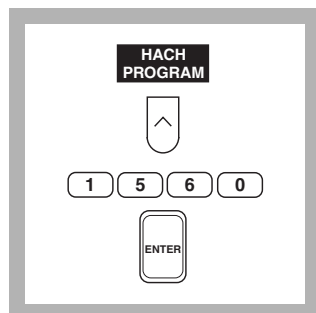


Method 10051

1,5-Diphenylcarbohydrazide Method

(0 to 0.700 mg/L Cr⁶⁺)

Scope and Application: For soil. The estimated detection limit for program number 1560 is 0.006 mg/L Cr⁶⁺.



1. Press the soft key under **HACH PROGRAM**.

Select the stored program for hexavalent chromium (Cr⁶⁺) by pressing **1560** with the numeric keys.

Press: **ENTER**

Note: If samples cannot be analyzed immediately, see *Sample Collection, Preservation, and Storage* following these steps.

Note: The Flow Cell and Sipper Modules can be used for this procedure. Use a 25-mL sample volume and reagents for the Flow Cell Module.

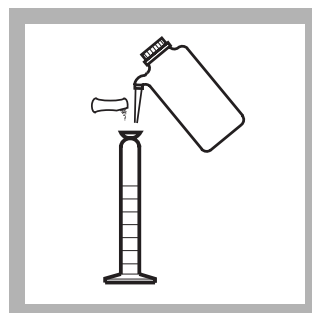


2. The display will show: **HACH PROGRAM: 1560 Chromium, Hex.**

The wavelength (λ), **540 nm**, is automatically selected.



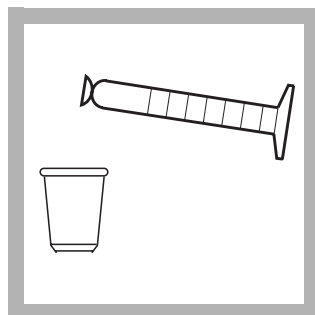
3. Accurately weigh out the required sample size based on anticipated Cr⁶⁺ levels (see *Table 1*). Place soil sample in whirl-pak bag or sample cup.



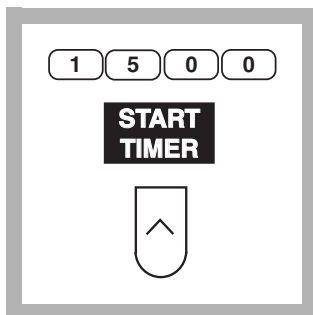
4. Prepare the extraction solution by adding the contents of one Hexavalent Chromium Extractant Pillow to a 50-mL graduated mixing cylinder. Add 40 mL of deionized water. Stopper and invert to dissolve the powder.

Table 1 Soil Sample Size

Expected Cr ⁶⁺ Concentration	Sample Size
500–5000 ppb	20 g
1.0–10 ppm	20 g
2.50–50 ppm	20 g
50–1000 ppm	1 g
500–10,000 ppm	1 g



5. Add the extraction solution to the soil in the whirl-pak bag or sample cup and close tightly. Invert and shake several times to ensure the soil is completely suspended.

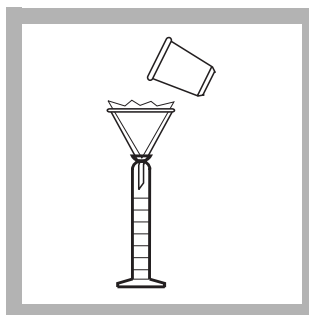


6. Press **1500** to enter a 15-minute time period. Press the soft key under **START TIMER**.

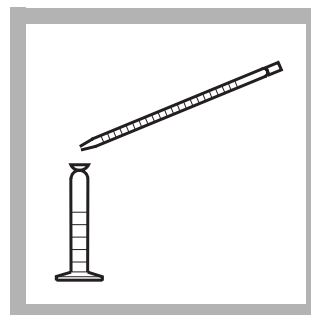
Shake the soil/soil extractant mixture for 15 seconds at 2-minute intervals during the 15-minute time period.

Note: If using a soil shaker, shake for 15 minutes at 200 rpm.

Note: The programmed 8-minute time period is not applicable for soil. It is used for the chromium in water method.



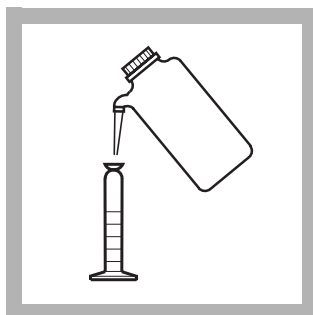
7. Set a funnel in the 50-mL graduated cylinder. Place a piece of pre-folded filter paper into the funnel. Filter the soil/soil extractant mixture into the cylinder and save the filtrate.



8. Determine the required filtrate aliquot size from *Table 2*. Pipet the required aliquot from the 50-mL graduated cylinder into a 25-mL graduated cylinder.

Table 2 Required Aliquot Size

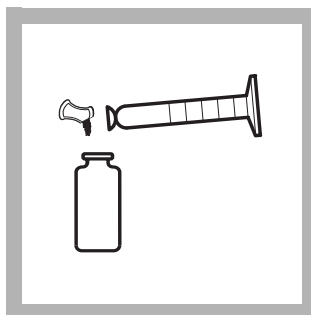
Expected Cr ⁶⁺ Concentration	Aliquot Size
500–5000 ppb	10 mL
1.0–10 ppm	5 mL
2.50–50 ppm	1 mL
50–1000 ppm	1 mL
500–10,000 ppm	0.1 mL



9. Dilute the cylinder to the 25-mL mark with deionized water. Stopper and invert to mix.



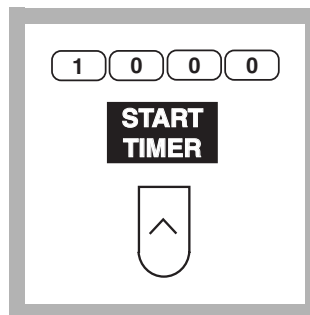
10. Fill a sample cell to the 10-mL mark with the diluted filtrate (the blank).



11. Fill another sample cell to the 10-mL mark with the diluted filtrate remaining in the cylinder. Add the contents of a ChromaVer 3 Chromium Reagent Powder Pillow to the cell and swirl to mix. This is the prepared sample.

Note: A purple color will develop if chromium is present.

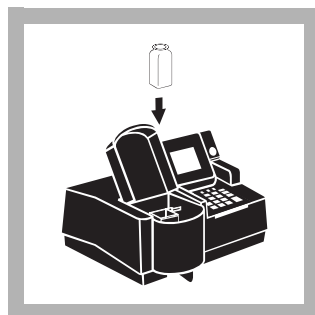
Note: For best results, determine a reagent blank for each new lot of reagent as follows. Prepare a reagent blank by repeating Steps 8-15, using deionized water as the sample. Zero the instrument on deionized water by pressing the soft key under **ZERO**. Insert the reagent blank and the blank value will be displayed. Correct for the reagent blank by pressing the soft keys under **OPTIONS, (MORE)**, and then **BLANK:OFF**. Enter the reagent blank value and press **ENTER**. Repeat for each new lot of reagent.



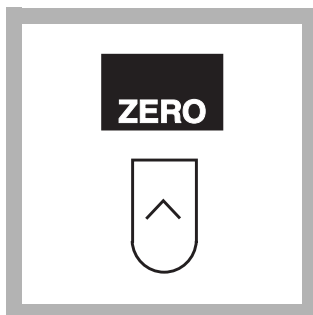
12. Press **1000** to enter a 10-minute time period. Press the soft key under **START TIMER**.

A 10-minute reaction period will begin.

Note: If the solution becomes cloudy following reagent addition and does not clear up after 10-minutes, the aliquot size must be reduced until the turbidity is no longer present after the 10-minute reaction period.



13. Place the blank into the cell holder. Close the light shield.



14. Press the soft key under **ZERO**.

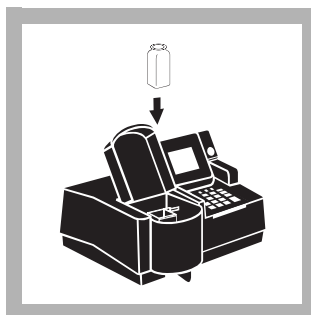
The display will show:

0.000 mg/L Cr⁶⁺

Note: If you are using a reagent blank correction, the display will show the correction.

Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.

Note: Results may be expressed as other forms of chromium. Press the soft keys under **OPTIONS** and then **FORM**: to scroll through the available options. Press **ENTER** to return to the read screen.



15. Place the prepared sample into the cell holder. Close the light shield. Results in mg/L apparent hexavalent chromium (or chosen units) will be displayed. Use the following formula to determine the actual hexavalent chromium concentration in the original soil sample:

$$\text{Cr}^{6+} \text{ (ppb)} = \frac{\text{DR/4000 reading (mg/L)} \times 10^6}{\text{Aliquot (mL)} \times \text{sample size}}$$

Interferences

Table 1 Interfering Substances and Suggested Treatments

Interfering Substance	Interference Levels and Treatments
Iron	Iron interference levels depend on the volume of the extract aliquot. To avoid iron interference, use the smallest sample and aliquot volume possible. Iron interference prevents color formation. If you get a Cr ⁶⁺ concentration of nearly 0, check the sample for iron interference by adding 0.1 mL of a 50-mg/L Cr ⁶⁺ standard to the sample and allow an additional 10-minute reaction period. If a pink color develops, iron is not present and the sample does not contain hexavalent chromium. If no color develops, interference is occurring and the aliquot and/or sample size should be decreased until the interferences stop.
Mercurous & Mercuric Ions	Interferes slightly
pH	Highly buffered samples or extreme sample pH may exceed the buffering capacity of the reagents and require sample pretreatment; see Section 1.3.1 pH Interference.
Vanadium	1 mg/L. Wait 10 minutes before reading result.

Sample Collection, Preservation, and Storage

Obtain a soil sample representative of the area being tested. If the soil is moist or sampled from depth, it will need to be air dried before sampling if low levels of hexavalent chromium are to be accurately determined. High levels of hexavalent chromium (>100 ppm) do not require as rigorous sampling/sample treatment procedures.

Accuracy Check

Standard Solution

1. Using a TenSette Pipet or a fixed volume pipetter, add 0.2 mL of a 50-mg/L Chromium, Hexavalent, Standard Solution to 20 grams of soil. The soil must not contain any Cr⁶⁺.
2. Analyze the sample as described in the above. A reading between 0.3 and 0.4 mg/L should be obtained. The calculation should give a result of 400-600 ppb, with the actual value being 500 ppb.

Method Performance

Precision

Standard: 0.25 mg/L Cr⁶⁺

Program	95% Confidence Limits
1560	0.247–0.253 mg/L Cr ⁶⁺

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
1560	0.006 mg/L Cr ⁶⁺

For more information on derivation and use of Hach's estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, Appendix B, see Section 1.5.1.

Sensitivity

Program Number: 1560

Portion of Curve:	ΔAbs	ΔConcentration
Entire Range	0.010	0.0055 mg/L

See Section 1.5.3 *Sensitivity Explained* for more information.

Summary of Method

Hexavalent chromium in soil is measured via extraction using a concentrated, alkaline extracting reagent. Color development with the 1,5 Diphenylcarbohydrazide method uses a single dry powder formulation called ChromaVer 3 Chromium Reagent. This reagent contains a buffer and 1,5 Diphenylcarbohydrazide, which gives a purple color when hexavalent chromium is present.

CHROMIUM, Hexavalent, in soil, continued

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to Section I.

Pollution Prevention and Waste Management

The final samples are highly acidic. Neutralize to pH 6–9 and flush to drain for disposal. For information on pollution prevention and waste management, refer to Section I.

REQUIRED REAGENTS AND STANDARDS

Description	Quantity Required per test	Unit	Cat. No.
ChromaVer 3 Chromium Reagent Powder Pillows	1	100/pkg	12710-99
Hexavalent Chromium Soil Extractant Powder Pillows.....	1	100/pkg	24497-99

REQUIRED EQUIPMENT AND SUPPLIES

Bags, Whirl-Pak	2	100/pkg	22331-99
or			
Sample Cups, disposable plastic, 150-mL	2	2500/pkg	22631-74
and			
Sample Cup Lids	2	3000/pkg	22632-74
Balance, laboratory, 300-g	1	each	14760-00
Clippers, large (shears)	1	each	23694-00
Cylinder, graduated mixing, 25-mL	1	each	20886-40
Cylinder, graduated, 50-mL, poly	1	each	2172-41
DR/4000 1-Inch Cell Adapter	1	each	48190-00
Filter Paper, folded, 15- cm.....	2	100/pkg	692-58
Funnel, analytical, poly., 65-mm.....	1	each	1083-67
Pipetter, 100 µL	1	each	22753-00
Pipet Tips, for 22753-00 Pipetter	2	10/pkg	22754-10
Scoop, soil, 1-g.....	1	each	26572-01
Scoop, soil, 10-g.....	1	each	26572-10

OPTIONAL REAGENTS AND SUPPLIES

Chromium, Hexavalent, Standard Solution, 50-mg/L Cr ⁶⁺	100 mL.....	810-42H
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OPTIONAL EQUIPMENT AND SUPPLIES

Demineralizer Assembly, 473-mL	each.....	21846-00
DR/4000 Carousel Module Kit	each.....	48070-02
DR/4000 Flow Cell Module Kit, 1-inch.....	each.....	48070-04
DR/4000 Flow Cell Module Kit, 1-cm.....	each.....	48070-05
DR/4000 Sipper Module Kit, 1-inch	each.....	48090-03
SOILAB Filtration Assembly.....	each.....	22624-00
Pipet TenSette, 1.0–10.0 mL.....	each.....	19700-10
Pipet Tips, for 19700-10 TenSette Pipet	50/pkg.....	21997-96



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Method 8024

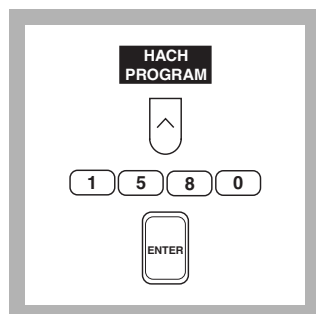
Alkaline Hypobromite Oxidation Method*

Powder Pillows

(0 to 0.700 mg/L)

Scope and Application: For water and wastewater. See Section 2 for digestion procedure, if necessary.
The estimated detection limit for program number 1580 is 0.003 mg/L Cr.

* Adapted from *Standard Methods for the Examination of Water and Wastewater*



1. Press the soft key under **HACH PROGRAM**.

Select the stored program for total chromium (Cr) by pressing **1580** with the numeric keys.

Press: **ENTER**

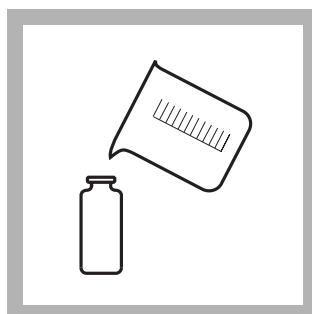
Note: If samples cannot be analyzed immediately, see *Sample Collection, Storage and Preservation* following these steps. Adjust the pH of preserved samples before analysis.

Note: The Flow Cell and Sipper Modules can be used for this procedure.



2. The display will show:
**HACH PROGRAM: 1580
Chromium, Total**

The wavelength (λ), **540 nm**, is automatically selected.



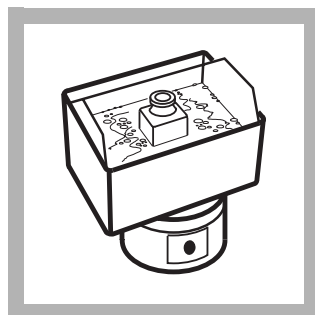
3. Fill a sample cell with 25 mL of sample.

Note: For proof of accuracy, use a 0.25 mg/L trivalent chromium standard solution (preparation given in the *Accuracy Check* section) in place of the sample.

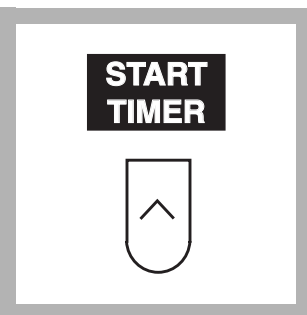
Note: For best results, determine a reagent blank for each new lot of reagent as follows. Prepare a reagent blank by repeating Steps 3 through 14, using deionized water as the sample. Zero the instrument on deionized water by pressing the soft key under **ZERO**. Insert the reagent blank and the blank value will be displayed. Correct for the reagent blank by pressing the soft keys under **OPTIONS, (MORE)**, and then **BLANK:OFF**. Enter the reagent blank value and press **ENTER**. Repeat for each new lot of reagent.



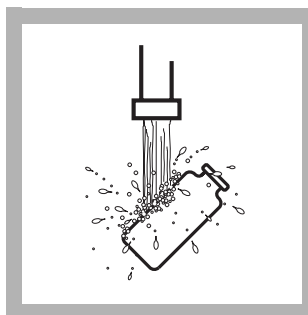
4. Add the contents of one Chromium 1 Reagent Powder Pillow (the prepared sample). Swirl to mix.



5. Place the prepared sample into a boiling water bath.



6. Press the soft key under **START TIMER**. A 5-minute reaction period will begin.



7. When the timer beeps, remove the prepared sample. Using running tap water, cool the cell to 25 °C.

***Note:** Use finger cots to handle the hot sample cell.*



8. Add the contents of one Chromium 2 Reagent Powder Pillow. Swirl to mix.

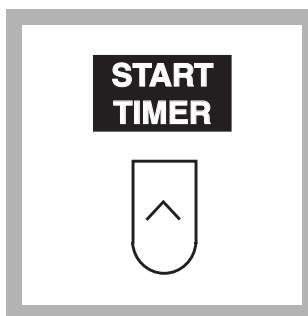


9. Add the contents of one Acid Reagent Powder Pillow. Swirl to mix.

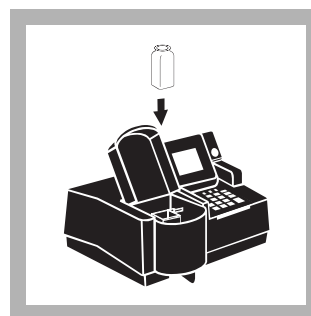


10. Add the contents of one ChromaVer 3 Chromium Reagent Powder Pillow. Swirl to mix.

***Note:** A purple color will form if chromium is present.*

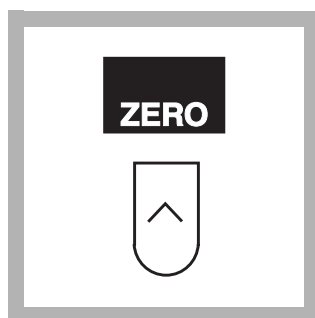


11. Press the soft key under **START TIMER**. A 5-minute reaction period will begin.



12. When the timer beeps, fill another sample cell with 25 mL of sample (the blank). Place it into the cell holder. Close the light shield.

***Note:** For turbid samples, treat the blank as described in Steps 3 through 9.*



13. Press the soft key under **ZERO**.

The display will show:

0.000 mg/L Cr

Note: If you are using a reagent blank correction, the display will show the correction.

Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



14. Place the prepared sample into the cell holder. Close the light shield. Results in mg/L chromium (or chosen units) will be displayed.

Interferences

Table 1 Interfering Substances and Suggested Treatments

Interfering Substance	Interference Levels and Treatments
Highly buffered samples or extreme sample pH	May exceed the buffering capacity of the reagents and require sample pretreatment; see Section 1.3.1 <i>pH Interference</i> .
Organic material (large amounts)	May inhibit complete oxidation of trivalent chromium. If high levels of organic material are present, see Section 2 for instructions on sample digestion. Perform the analysis as described on the digested sample.

Sample Collection, Storage and Preservation

Collect samples in acid-washed glass or plastic containers. To preserve samples, adjust the pH to 2 or less with nitric acid (about 2 mL per liter). Store preserved samples at room temperature up to six months. Adjust the pH to about 4 with 5.0 N Sodium Hydroxide before analysis. Correct the test result for volume additions; see Section 1.2.2 *Correcting for Volume Additions*.

Accuracy Check

Standard Additions Method

- a. Leave the unspiked sample in the cell compartment. Verify that the units displayed are in mg/L. Select standard additions mode by pressing the soft keys under **OPTIONS**, (**MORE**) and then **STD ADD**.

- b. Press **ENTER** to accept the default sample volume (mL), 25.
- c. Press **ENTER** to accept the default standard concentration (mg/L), 12.500.
- d. Press the soft key under **ENTRY DONE**.
- e. Snap the neck off a Trivalent Chromium Voluette Ampule Standard, 12.5-mg/L as Cr³⁺.
- f. Use the TenSette Pipet to add 0.1 mL, 0.2 mL and 0.3 mL of standard, respectively to three 25-mL samples and mix each thoroughly.
- g. Analyze each standard addition sample as described above. Accept the standard additions reading by pressing the soft key under **READ** each time. Each addition should reflect approximately 100% recovery.
- h. After completing the sequence, the display will show the extrapolated concentration value and the “best-fit” line through the standard additions data points, accounting for matrix interferences.
- i. See Section 1.4.1 *Standard Additions* for more information.

Standard Solution Method

Prepare a 0.25-mg/L trivalent chromium standard by diluting 5.00 mL of Trivalent Chromium Standard Solution, 50-mg/L as Cr³⁺, to 1000 mL with deionized water. Prepare this solution daily.

To adjust the calibration curve using the reading obtained with the 0.25-mg/L standard solution, press the soft keys under **OPTIONS, MORE** then **STD: OFF**. Press **ENTER** to accept the displayed concentration, the value of which depends on the selected units. If an alternate concentration is used, enter the actual concentration and press **ENTER** to return to the read screen. See Section 1.5.5 *Adjusting the Standard Curve* for more information.

Method Performance

Precision

Standard: 0.250 mg/L Cr

Program	95% Confidence Limits
1580	0.248–0.252 mg/L Cr

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
1580	0.003 mg/L Cr

For more information on derivation and use of Hach’s estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, Appendix B, see Section 1.5.1.

Sensitivity

Program Number: 1580

Portion of Curve	Δ Abs	Δ Concentration
Entire Range	0.010	0.006 mg/L

See Section 1.5.3 *Sensitivity Explained* for more information.

Calibration Standard Preparation

To perform a total chromium calibration using the alkaline hypobromite oxidation method, prepare a 10-mg/L chromium stock solution by pipetting 20 mL of a 50-mg/L Chromium Trivalent Standard Solution (Cat. No. 14151-42) into a 100-mL volumetric flask using Class A glassware. Dilute to the mark with deionized water and mix thoroughly.

Prepare calibration standard containing 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, and 0.7 mg/L Cr^{3+} as follows:

- a. Into seven different Class A 100-mL volumetric flasks, pipet 1, 2, 3, 4, 5, 6, and 7 mL of the 10-mg/L Cr^{3+} stock solution using Class A glassware.
- b. Dilute to the mark with deionized water and mix thoroughly.
- c. Using the alkaline hypobromite oxidation method and the procedure described in the *User-Entered Programs* section of the *DR/4000 Spectrophotometer Instrument Manual*, generate a calibration curve from the standards prepared above.

Summary of Method

Trivalent chromium in the sample is oxidized to the hexavalent form by hypobromite ion under alkaline conditions. The sample is acidified. The total chromium content is determined by the 1,5-Diphenylcarbohydrazide method. Determine trivalent chromium by subtracting the results of a separate hexavalent chromium test from the results of the total chromium test.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to Section 1.

Pollution Prevention and Waste Management

The final samples are highly acidic. Neutralize to pH 6–9 and flush to drain for disposal. For information on pollution prevention and waste management, refer to Section 1.

CHROMIUM, Total, continued

REQUIRED REAGENTS AND STANDARDS

Description	Quantity Required per test	Unit	Cat. No
Total Chromium Reagent Set (100 Tests)			22425-00
Includes: (1) 2126-99, (2) 12066-99, (1) 2043-99, (1) 2044-99			
Acid Reagent Powder Pillows	1 pillow	100/pkg	2126-99
ChromaVer 3 Chromium Reagent Powder Pillows	1 pillow	100/pkg	12066-99
Chromium 1 Reagent Powder Pillows	1 pillow	100/pkg	2043-99
Chromium 2 Reagent Powder Pillows	1 pillow	100/pkg	2044-99

REQUIRED EQUIPMENT AND SUPPLIES

DR/4000 1-Inch Cell Adapter	1	each	48190-00
Water bath and rack	1	each	1955-55

Select one based on available voltage:

Hot plate, 3½" diameter, 120 VAC, 50/60 Hz	each	12067-01
Hot plate, 4" diameter, 240 VAC, 50/60 Hz	each	12067-02

OPTIONAL REAGENTS AND STANDARDS

Chromium, Trivalent, Standard Solution, 50-mg/L Cr ³⁺	100 mL	14151-42
Chromium, Trivalent, Standard Solution, 10-mL Voluette Ampule, 12.5-mg/L Cr ³⁺	16/pkg	14257-10
Nitric Acid, ACS	500 mL	152-49
Nitric Acid Solution, 1:1	500 mL	2540-49
Sodium Hydroxide Solution 5.0 N	59 mL* DB	2450-26
Water, deionized	4 liters	272-56

OPTIONAL EQUIPMENT AND SUPPLIES

Cylinder, graduated, polypropylene, 25-mL	each	1081-40
DR/4000 Carousel Module Kit	each	48070-02
DR/4000 Flow Cell Module Kit, 1-inch	each	48070-04
DR/4000 Flow Cell Module Kit, 1-cm	each	48070-05
DR/4000 Sipper Module Kit, 1-inch	each	48090-03
Flask, volumetric, 1000-mL	each	547-53
pH Paper, pH 1.0 to 11.0	5 rolls/pkg	391-33
pH Meter, <i>sens^{ion}</i> TM 1, portable	each	51700-00
Pipet Filler, safety bulb	each	14651-00
Pipet, serological, 2-mL	each	532-36
Pipet, TenSette, 0.1 to 1.0 mL	each	19700-01
Pipet Tips for 19700-01 TenSette Pipet	50/pkg	21856-96
Pipet, volumetric, Class A, 5.00-mL	each	14515-37

* Contact Hach for larger sizes.



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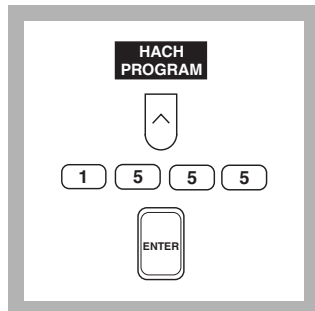


1,5-Diphenylcarbohydrazide Method

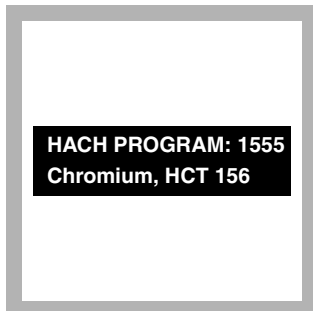
UniCell™ Vials

(0 to 1.00 mg/L Total Cr)

Scope and Application: For wastewater process control; The estimated detection limits for program number 1555 is 0.03 mg/L Cr.



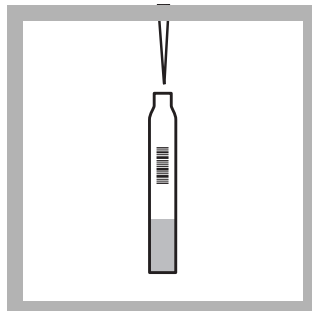
1. Press the soft key under **HACH PROGRAM**. Select the stored program for UniCell Chromium by pressing **1555** with the numeric keys. Press: **ENTER**.



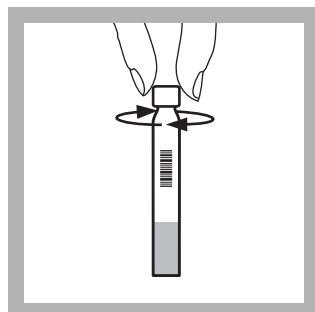
2. The display will show: **HACH PROGRAM: 1555 Chromium, HCT 156**. The wavelength (λ), **543 nm**, is automatically selected.



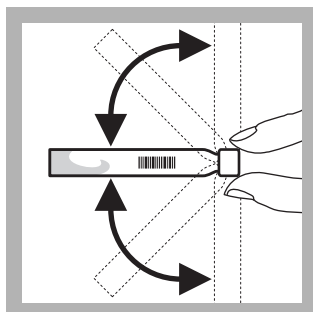
3. Insert the Test Tube Adapter into the sample cell module by sliding it under the thumb screw and into the alignment grooves. Fasten with the thumb screw.



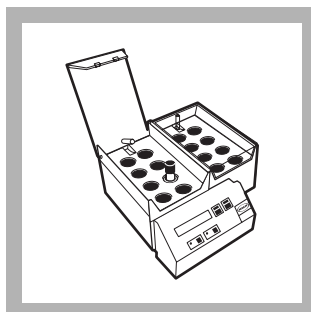
4. Pipet 4.0 mL of sample into a sample vial.



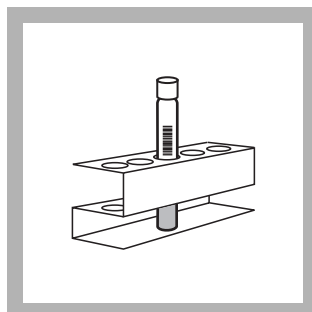
5. Screw a **blue** UniCap A (HCT 156 A) onto the sample vial.



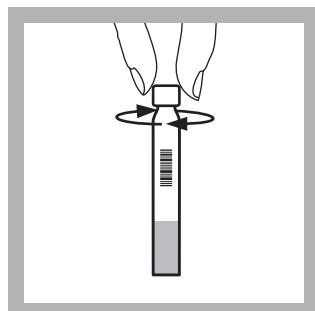
6. Invert the sample several times to mix.



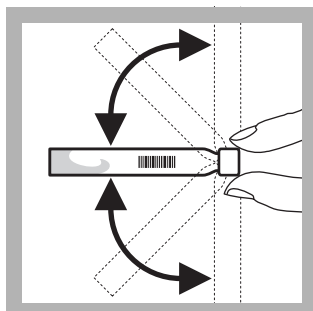
7. Heat the sample vial in the reactor block at 100 °C for 60 minutes.



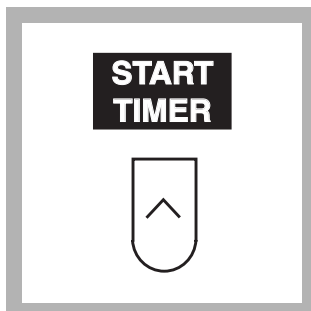
8. After the heating period, remove the vial from the reactor block and place it in a cooling rack.



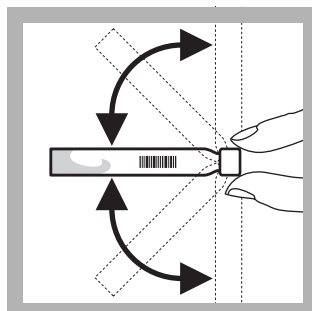
9. When the sample vial has cooled to room temperature, screw a **green** UniCap B (HCT 156 B) onto the vial.



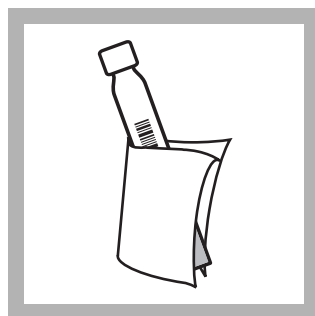
10. Invert the vial several times to mix.



11. Press the soft key under **START TIMER**. A 10-minute reaction period will begin.



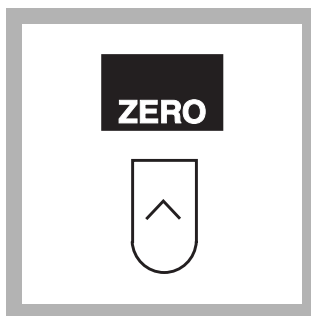
12. After the reaction period, invert the sample vial to mix.



13. Wipe the outside of the zero vial (white cap) and the sample vial with a damp towel followed with a dry one to remove fingerprints and other marks.



14. Place the zero vial into the cell holder. Close the light shield.



15. Press the soft key under **ZERO**.

The display will show:

0.00 mg/L Total Cr

Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



16. Place the sample vial into the cell holder. Close the light shield. Results in mg/L total chromium will be displayed.

Note: Press the soft keys under **OPTIONS** and then **FORM**: to scroll through the available options. Press **ENTER** to return to the read screen.

Interferences

The ions listed in the following table have been individually tested up to the given concentrations. Cumulative effects and the influence of other ions have not been evaluated.

Ion	No interference up to:
Cl ⁻	1000 mg/L
Ca ²⁺	125 mg/L
Mg ²⁺ , NH ₄ ⁺	100 mg/L
Zn ²⁺ , Ni ²⁺ , Co ²⁺ , Cd ²⁺	50 mg/L
Ag ⁺ , Pb ²⁺	25 mg/L
Cu ²⁺ , Fe ³⁺	10 mg/L
Sn ²⁺	1 mg/L

Larger amounts of iron, copper, reducing, or oxidizing agents give low results. Lead, mercury, and tin give high results.

Note: Undissolved chromium is not determined with the determination of chromium VI. Total Chromium can only be determined after digestion.

Note: Concentrations above 20 mg/L produce result displays within the ranges given above. In such cases, verify the results by dilution.

Sample Collection, Preservation, and Storage

Collect samples in acid-cleaned glass or plastic containers. No acid addition is necessary if analyzing the samples immediately or if the samples contain only Chromium(VI) ions. To preserve the sample for total Chromium adjust the pH to 2 or less with concentrated nitric acid (about 2 mL per liter). Preserved samples may be stored up to six months at room temperature.

Before analysis, adjust the pH to 3–9 with 5.0 N Sodium Hydroxide Standard Solution.

Accuracy Check

Standard Additions Method

- a. Select standard additions mode by pressing the soft keys under **OPTIONS**, **(MORE)** and then **STD ADD**.
- b. Press **ENTER** to accept the default sample volume (mL), 25.
- c. Press **ENTER** to accept the default standard concentration (mg/L), 50.0.
- d. Press the soft key under **ENTRY DONE**.
- e. Use a pipet to add 0.1, 0.2 and 0.3 mL of standard, respectively to three 25-mL samples and mix each thoroughly.
- f. Analyze each standard addition sample as described above. Accept the standard additions reading by pressing the soft key under **READ** each time. Each addition should reflect approximately 100% recovery.
- g. After completing the sequence, the display will show the extrapolated concentration value and the “best-fit” line through the standard additions data points, accounting for matrix interferences.
- h. See Section 1.4.1 *Standard Additions* for more information.

Standard Solution Method

Prepare a 0.70-mg/L Cr⁶⁺ standard solution by pipetting 1.40 mL of Hexavalent Chromium Standard Solution, 50-mg/L, into a 100-mL volumetric flask. Dilute to the mark with deionized water. Prepare this solution daily. Perform the total chromium procedure as described above.

To adjust the calibration curve using the reading obtained with the 0.70-mg/L standard solution, press the soft keys under **OPTIONS**, **(MORE)** then **STD: OFF**. Press **ENTER** to accept the displayed concentration, the value of which depends on the selected units. If an alternate concentration is used, enter the actual concentration and press **ENTER** to return to the read screen. See Section 1.5.5 *Adjusting the Standard Curve* for more information.

Method Performance

Precision

Standard: 0.50 mg/L Cr⁶⁺

Program	95% Confidence Limits
1555	0.38–0.62 mg/L Tot-Cr

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
1555	0.03 mg/L Tot-Cr

For more information on derivation and use of Hach’s estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, Appendix B, see Section 1.5.1.

CHROMIUM, Total, continued

Sensitivity

Program Number: 1555

Portion of Curve	Δ Abs	Δ Concentration
Entire Range	0.010	0.01 mg/L

See Section 1.5.3 *Sensitivity Explained* for more information.

Summary of Method

Chromium(VI) ions react with 1,5-diphenylcarbazide to yield 1,5-diphenylcarbazone and chromium(III), which forms a red complex.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to Section 1.

Sample vial contains: 23% phosphoric acid.

UniCap A (HCT 156 A) contains: Sodium peroxodisulphate.

Pollution Prevention and Waste Management

For information on pollution prevention and waste management, refer to Section 1.

REQUIRED EQUIPMENT AND SUPPLIES

Description	Unit	Cat. No.
Chromium - Cr (VI), UniCell™ HCT 156.....	23/pkg.....	HCT 156
DRB 100, Digital Reactor Block.....	each.....	DRB 100
Test tube rack, cooling	each.....	18641-00

OPTIONAL REAGENTS AND STANDARDS

Chromium Standard, 50-mg/L	100 mL.....	810-42
----------------------------------	-------------	--------

OPTIONAL APPARATUS

Graduated cylinder, mixing, 100-mL	each.....	20886-42
Flask, volumetric, 100-mL	each.....	14574-42
Pipettor, (Jencons) 1–5 mL	each.....	27951-00
Replacement tips for 27951-00	100/pkg.....	27952-00
Pipettor, (Jencons) 100–1000 μ L.....	each.....	27949-00
Replacement tips for 27949-00	400/pkg.....	27950-00
pH Paper	100/pkg.....	18641-00



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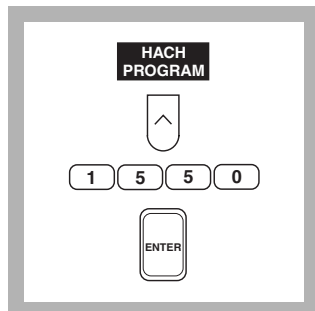


Method 8069

Colorimetric Method

(0 to 20.0 g/L Cr³⁺)

Scope and Application: For finishing baths; digestion is required for determining trivalent chromium. See Digestion Using Digesdahl following these steps. The estimated detection limit for program number 1550 is 0.1 g/L



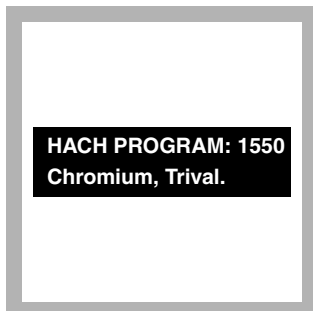
- 1.** Press the soft key under **HACH PROGRAM**.

Select the stored program number for trivalent chromium (Cr³⁺) by pressing **1550** with the numeric keys.

Press: **ENTER**

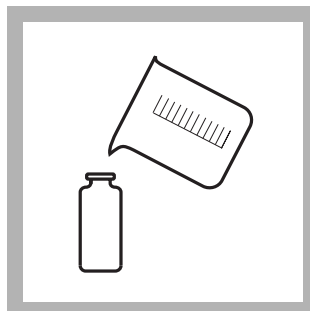
Note: This procedure requires sample digestion. See Digestion Using Digesdahl following these steps.

Note: The Flow Cell and Sipper Modules can be used for this procedure.

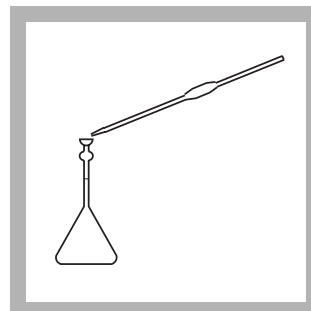


- 2.** The display will show: **HACH PROGRAM: 1550 Chromium, Trival.**

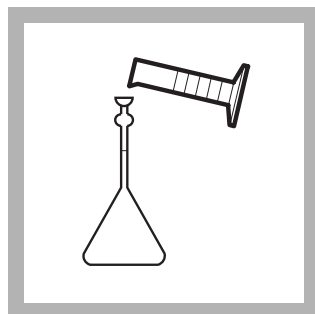
The wavelength (λ), **595 nm**, is automatically selected.



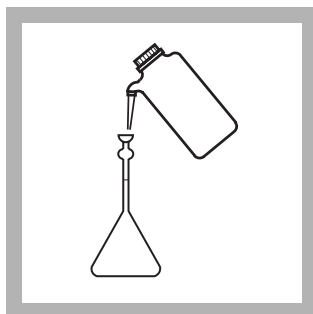
- 3.** Fill a sample cell (the blank) to the 25-mL mark with a digested sample.



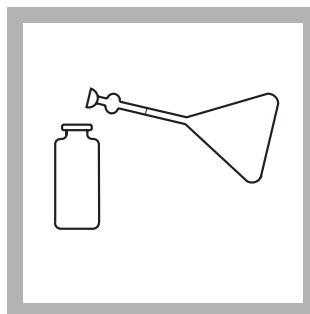
- 4.** Transfer 5.0 mL of undigested sample into a clean 100-mL volumetric flask.



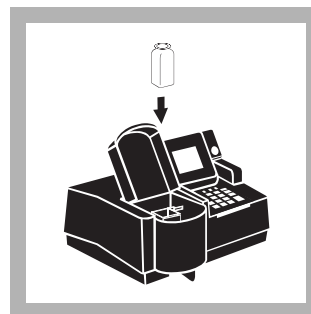
- 5.** Use a 10-mL graduated cylinder to add carefully 10.0 mL of concentrated perchloric acid to the flask.



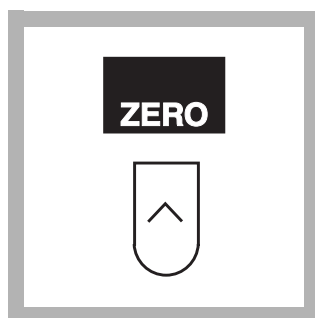
- 6.** Fill the flask to the 100-mL mark with deionized water. Stopper and invert several times to mix.



- 7.** Fill another sample cell (the prepared sample) to the 25-mL mark with the solution from Step 6.



- 8.** Place the blank from Step 3 into the cell holder. Close the light shield.



9. Press the soft key under **ZERO**.

The display will show:

0.0 g/L Cr³⁺

Note: For other concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return the read screen.



10. Place the prepared sample from Step 7 into the cell holder. Close the light shield. Results in g/L trivalent chromium (or chosen units) will be displayed.

Digestion Using Digesdahl

For safe Digesdahl operation, do not deviate from these instructions in any way. Sample size, acid volumes, heating periods and step sequence must be followed. Additional safety precautions are given in Section 2.1.3 *General Digesdahl Digestion (Not USEPA accepted)*. Always wear safety glasses and use a safety shield or operate the Digesdahl within a closed fume hood.

- a. Add 5.0 mL of sample to a 100-mL digestion flask. Add several boiling chips to prevent explosive boiling that may cause injury or sample loss).

Note: Increase the analysis sensitivity by doubling the sample volume and the acid reagents (10 mL sample, 10 mL concentrated nitric acid and 20 mL concentrated perchloric acid). Divide the result by 2. Use a 10-mL pipet to obtain and deliver the sample.

- b. Add 5.0 mL of concentrated nitric acid and 10 mL of concentrated perchloric acid.

Note: If necessary, wash down the neck of the flask with a minimal amount of water.

- c. Place the flask on the heater of the Digesdahl Digestion Apparatus. Position the heat shield. Attach the manifold and turn on the water to the vacuum aspirator.

- d. Turn temperature setting to 825 °F (440 °C). Digest until dense white fumes and yellow or red droplets appear on the sides of the flask. A reddish-orange precipitate (CrO₃) will form. Do not boil to dryness.

Note: If the flask boils to dryness, unplug the electrical cord on the digestion apparatus. Wait until the digestion apparatus cools. Then wrap a towel around the entire digestion apparatus and carefully add water. Remove the flask from the heater assembly. Dispose of the contents by flushing with water.

- e. Using the finger cots to avoid burns, remove the flask from the heater assembly. Set it on the square cooling pad to cool for two minutes. Immerse the flask bulb in cool tap water.
- f. Add 50-mL of deionized water. Swirl the contents until all precipitates have dissolved. Dilute to the 100-mL mark with deionized water. Wait for the flask to cool to the touch. Again dilute to the mark. Stopper the flask. Invert several times to mix. Analyze for trivalent chromium using the above procedure.

Method Performance

Precision

Standard: 10.0 g/L Cr³⁺

Program	95% Confidence Limits
1550	9.9–10.1 g/L Cr ³⁺

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
1550	0.1 g/L Cr ³⁺

For more information on derivation and use of Hach's estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, Appendix B, see Section 1.5.1.

Sensitivity

Program Number: 1550

Portion of Curve:	Δ Abs	Δ Concentration
Entire Range	0.010	0.2 g/L

See Section 1.5.3 *Sensitivity Explained* for more information.

Summary of Method

The concentration of trivalent chromium (Cr³⁺) is determined by oxidizing all the Cr³⁺ present in the sample to Cr⁶⁺ and spectrophotometrically comparing it with an unoxidized sample. Oxidation is performed by digesting the sample in the solution of concentrated nitric acid and perchloric acid using the Digesdahl Digestion Apparatus. The oxidized sample, having only hexavalent chromium (Cr⁶⁺), serves as a blank against which the amount of Cr³⁺ in the original sample can be measured directly.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to Section 1.

CHROMIUM, Trivalent, continued

Pollution Prevention and Waste Management

The final samples are highly acidic. Neutralize to pH 6–9 and flush to drain for disposal. For more information on pollution prevention and waste management, refer to Section I.

REQUIRED REAGENTS AND STANDARDS

Description	Quantity Required per test	Unit	Cat. No.
Nitric Acid, ACS	5 mL	500 mL.....	152-49
Perchloric Acid, ACS, 70%.....	10 mL	680 g	757-65

REQUIRED EQUIPMENT AND SUPPLIES

Boiling Chips, silicon carbide	2–3	500 g	20557-34
Cylinder, graduated, 10-mL	1	each.....	2172-38
DR/4000 1-Inch Cell Adapter	1	each.....	48190-00
Flask, volumetric, Class A, 100-mL	1	each.....	14574-42
Pipet, volumetric, Class A, 5.0-mL.....	1	each.....	14515-37
Pipet Filler, safety bulb.....	1	each.....	14651-00
Safety Shield, for Digesdahl.....	1	each.....	20974-00

Select one based on available voltage:

Digesdahl Digestion Apparatus, 115 VAC, 50/60 Hz	1	each.....	23130-20
Digesdahl Digestion Apparatus, 230 VAC, 50/60 Hz	1	each.....	23130-21

OPTIONAL EQUIPMENT AND SUPPLIES

DR/4000 Carousel Module Kit	each.....	48070-02
DR/4000 Flow Cell Module Kit, 1-inch.....	each.....	48070-04
DR/4000 Sipper Module Kit, 1-inch.....	each.....	48090-03
Pipet, volumetric, Class A, 10.00-mL	each.....	14515-38
Safety Goggles	each.....	18421-00
Sample Cells, 1-inch, matched pair.....	2/pkg.....	26126-02



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Method 8078

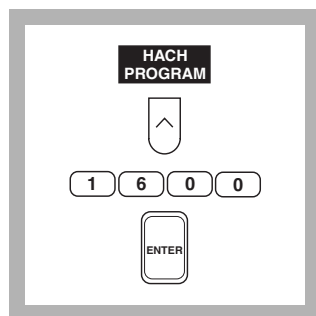
1-(2-Pyridylazo)-2-Naphthol (PAN) Method*

Powder Pillows

(0 to 2.00 mg/L)

Scope and Application: For water and wastewater; digestion is required for determining total recoverable cobalt. See Section 2 for digestion procedures; if EDTA is present use the vigorous digestion. The estimated detection limit for program number 1600 is 0.01 mg/L Co.

* Adapted from Watanbe, H., *Talanta*, 21 295 (1974)



- 1.** Press the soft key under **HACH PROGRAM**.

Select the stored program for cobalt (Co) by pressing **1600** with the numeric keys.

Press: **ENTER**

Note: If samples cannot be analyzed immediately, see Sample Collection, Storage and Preservation following these steps. Adjust the pH of preserved samples before analysis.

Note: The Flow Cell and Sipper Modules can be used for this procedure if rinsed between samples. Use a 25-mL sample and reagents with the Flow Cell Module.

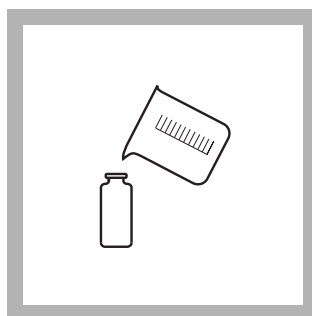


- 2.** The display will show: **HACH PROGRAM: 1600 Cobalt**

The wavelength (λ), **620 nm**, is automatically selected.

Note: For best results, determine a reagent blank for each new lot of reagent as follows. Prepare a reagent blank by repeating Steps 3 through 10, using deionized water as the sample. Zero the instrument on deionized water by pressing the soft key under **ZERO**. Insert the reagent blank and the blank value will be displayed. Correct for the reagent blank by pressing the soft keys under **OPTIONS, (MORE)**, and then **BLANK:OFF**. Enter the reagent blank value and press **ENTER**. Repeat for each new lot of reagent.

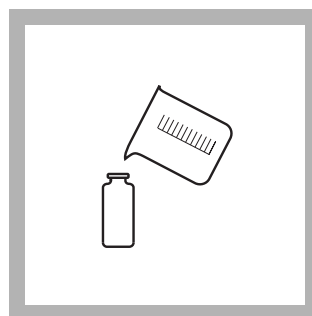
Note: When using the reagent blank feature, zero the instrument on deionized water in Step 10.



- 3.** Fill a glass-stoppered sample cell to the 10-mL mark with sample (the prepared sample).

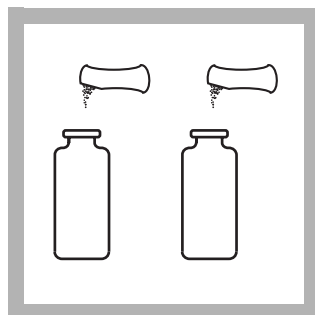
Note: If sample is less than 10 °C (50 °F), warm to room temperature prior to analysis.

Note: For proof of accuracy, use a 1.0 mg/L cobalt standard solution (preparation given in the Accuracy Check section) in place of the sample.



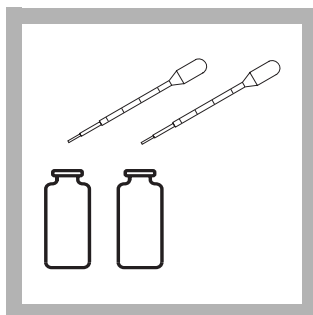
- 4.** Fill a second glass-stoppered sample cell to the 10-mL mark with deionized water (the blank).

Note: For samples with extreme pH, see the Interferences section.



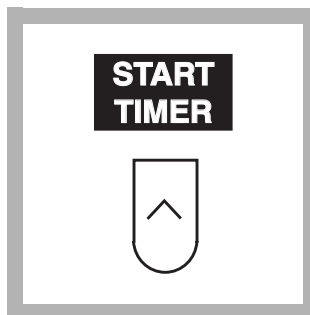
5. Add the contents of one Phthalate-Phosphate Reagent Powder Pillow to each cell. Stopper. Immediately shake to dissolve.

Note: : If sample contains iron (Fe^{3+}), it is important that all of the powder be dissolved completely before continuing with Step 6.



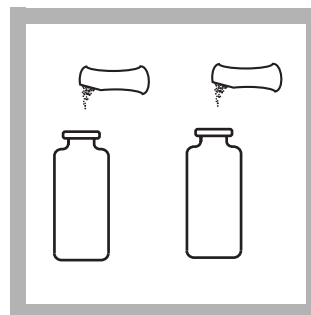
6. Add 0.5 mL of 0.3% PAN Indicator Solution to each cell. Stopper each cell. Invert several times to mix.

Note: Use plastic dropper provided.



7. Press the soft key under **START TIMER**. A three-minute reaction period will begin.

Note: During color development, the sample solution color may vary from green to dark red, depending on the chemical make-up of the sample. The deionized water blank should be yellow.



8. When the timer beeps, add the contents of one EDTA Reagent Powder Pillow to each cylinder. Stopper. Shake to dissolve.



9. Place the blank into the cell holder. Close the light shield.



10. Press the soft key under **ZERO**.

The display will show:

0.00 mg/L Co

Note: If you are using a reagent blank correction, the display will show the correction.

Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



11. Place the prepared sample into the cell holder. Close the light shield. Results in mg/L Co (or chosen units) will be displayed.

Note: Determination of nickel may be made with the same sample prepared with this method. Use program number 2370 for the nickel measurement. A reagent blank is necessary for the nickel procedure.

Interferences

Table 1 Interfering Substances and Suggested Treatments

Interfering Substance	Interference Levels and Treatments
Al ³⁺	32 mg/L
Ca ²⁺	1000 mg/L as CaCO ₃
Cd ²⁺	20 mg/L
Cl ⁻	8000 mg/L
Cr ³⁺	20 mg/L
Cr ⁶⁺	40 mg/L
Cu ²⁺	15 mg/L
F ⁻	20 mg/L
Fe ²⁺	Interferes directly and must not be present
Fe ³⁺	10 mg/L
K ⁺	500 mg/L
Mg ²⁺	400 mg/L
Mn ²⁺	25 mg/L
Mo ⁶⁺	60 mg/L
Na ⁺	5000 mg/L
Pb ²⁺	20 mg/L
Zn ²⁺	30 mg/L
Highly buffered samples or extreme sample pH	May exceed the buffering capacity of the reagents and require sample pretreatment; see Section 1.3.1 <i>pH Interference</i> .

Sample Collection, Storage and Preservation

Collect samples in acid-washed plastic bottles. Adjust the sample pH to 2 or less with nitric acid (about 5 mL per liter). Preserved samples can be stored up to six months at room temperature. Adjust the sample pH between 3 and 8 with 5.0 N Sodium Hydroxide Standard Solution just before analysis. Do not exceed pH 8 as this may cause some loss of cobalt as a precipitate. Correct test results for volume additions; see Section 1.2.2 *Correcting for Volume Additions* for more information.

Accuracy Check

Standard Solution Method

Prepare a 1.0-mg/L cobalt standard solution by diluting 10.0 mL of a 10-mg/L working stock solution to 100 mL in a volumetric flask. Prepare the 10-mg/L working stock solution daily by diluting 10.00 mL of Cobalt Standard Solution, 1000-mg/L as Co, to 1000 mL with deionized water.

To adjust the calibration curve using the reading obtained with the 1.0-mg/L standard solution, press the soft keys under **OPTIONS, (MORE)** then **STD: OFF**. Press **ENTER** to accept the displayed concentration, the value of which depends on the selected units. If an alternate concentration is used, enter the actual concentration and press **ENTER** to return to the read screen. See Section 1.5.5 *Adjusting the Standard Curve* for more information.

Method Performance**Precision**

Standard: 1.00 mg/L Co

Program	95% Confidence Limits
1600	0.99–1.01 mg/L Co

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
1600	0.01 mg/L Co

For more information on derivation and use of Hach's estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, Appendix B, see Section 1.5.1.

Sensitivity

Program Number: 1600

Portion of Curve	Δ Abs	Δ Concentration
0.010 Abs	0.010	0.0132 mg/L
1 mg/L	0.010	0.0137 mg/L
1.8 mg/L	0.010	0.0141 mg/L

See Section 1.5.3 *Sensitivity Explained* for more information.

Calibration Standard Preparation

To perform an cobalt calibration using the PAN method, prepare a 20-mg/L cobalt stock solution by pipetting 2 mL of a 1000-mg/L Cobalt Standard Solution (Cat. No. 21503-42) into a 100-mL volumetric flask using Class A glassware. Dilute to the mark with deionized water and mix thoroughly.

Prepare calibration standard containing 0.2, 0.4, 0.8, 1.20, 1.60, and 2.00 mg/L Co as follows:

- a. Into six different 100-mL volumetric flasks, pipet 1, 2, 4, 6, 8 and 10 mL of the 20-mg/L Co stock solution using Class A glassware.
- b. Dilute to the mark with deionized water and mix thoroughly.
- c. Using the PAN method and the calibration procedure described in the *User-Entered Programs* section of the *DR/4000 Spectrophotometer Instrument Manual*, generate a calibration curve from the standards prepared above.

Summary of Method

After buffering the sample and masking any Fe^{3+} with pyrophosphate, the cobalt is reacted with 1-(2-Pyridylazo)-2-Naphthol indicator. The indicator forms complexes with most metals present. After color development, EDTA is added to destroy all metal-PAN complexes except nickel and cobalt, which can both be determined on the same sample.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to Section 1.

Pollution Prevention and Waste Management

For information on pollution prevention and waste management, refer to Section 1.

REQUIRED REAGENTS AND STANDARDS

			Cat. No.
Cobalt Reagent Set, 10 mL (100 tests).....			26516-00
Includes: (2) 7005-99, (2) 26151-49 (1) 21502-32			
Description	Quantity Required per test	Unit	Cat. No.
EDTA Reagent Powder Pillows	2 pillows	100/pkg	7005-99
Phthalate-Phosphate Reagent Powder Pillows	2 pillows	100/pkg	26151-99
PAN Indicator Solution, 0.3%	1 mL	100 mL	21502-32
Water, deionized	25 mL	4 liters	272-56

REQUIRED EQUIPMENT AND SUPPLIES

Cylinder, graduated mixing, 25-mL	2	each	20886-40
DR/4000 1-Inch Cell Adapter	1	each	48190-00
Sample Cells, matched pair, 1-inch, glass, with stoppers	2	2/pkg	26126-02

OPTIONAL REAGENTS AND STANDARDS

Cobalt Standard Solution, 1000-mg/L Co.....	100 mL.....	21503-42
Nitric Acid, ACS	500 mL.....	152-49
Nitric Acid Solution, 1:1	500 mL.....	2540-49
Sodium Hydroxide Standard Solution, 5.0 N.....	100 mL MDB.....	2450-32
Sodium Hydroxide Standard Solution, 5.0 N.....	1 liter.....	2450-53

OPTIONAL EQUIPMENT AND SUPPLIES

Description	Unit	Cat. No.
DR/4000 Carousel Module Kit	each.....	48070-02
DR/4000 Flow Cell Module Kit, 1-inch.....	each.....	48070-04
DR/4000 Flow Cell Module Kit, 1-cm.....	each.....	48070-05
DR/4000 Sipper Module Kit, 1-inch	each.....	48090-03
Flask, volumetric, Class A, 100-mL	each.....	14574-42
Flask, volumetric, Class A, 1000-mL, with glass stopper.....	each.....	14574-53
pH Paper, pH 1.0 to 11.0	5 rolls/pkg.....	391-33
pH Meter, <i>sens^{ion}™1</i> , portable	each.....	51700-00
Pipet, serological, 1-mL	each.....	532-35
Pipet, serological, 5-mL	each.....	532-37
Pipet, TenSette, 0.1 to 1.0 mL	each.....	19700-01
Pipet Tips, for 19700-01 TenSette Pipet	50/pkg.....	21856-96
Pipet, volumetric, Class A, 1.0-mL	each.....	14515-35
Pipet, volumetric, Class A, 2.0-mL	each.....	14515-36
Pipet, volumetric, Class A, 4.0-mL	each.....	14515-04
Pipet, volumetric, Class A, 6.0-mL	each.....	14515-06
Pipet, volumetric, Class A, 8.0-mL	each.....	14515-08
Pipet, volumetric, Class A, 10.0-mL	each.....	14515-38
Pipet Filler, safety bulb.....	each.....	14651-00
Sample cell, 1-inch, matched pair	2/pkg.....	26126-02

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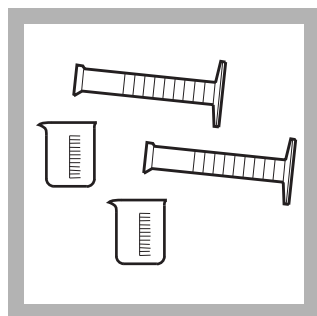
Method 10048

ADMI Weighted Ordinate Method*

(0 to 250 units Pt-Co)

Scope and Application: For colored waters and wastewaters having color characteristics significantly different from platinum-cobalt standards, as well as to those similar in hue to the standards. Turbid samples must be filtered prior to analysis. The estimated detection limit for program number 1660 is 3 ADMI (American Dye Manufacturers Institute) color value.

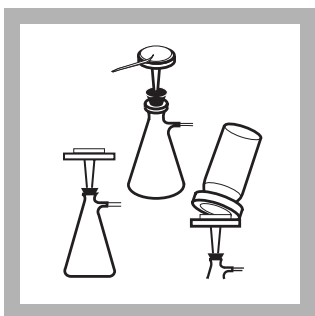
* Adapted from Allen, et. al., 1973. Determination of color of water and wastewater by means of ADMI Color Values. *Proc. 28th Ind. Waste Conf.*, Purdue Univ., Eng. Ext. Ser. No. 142:661



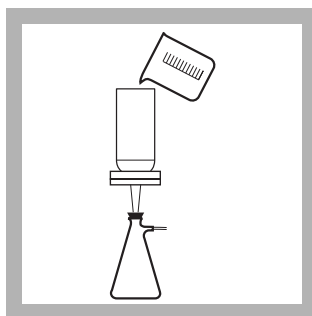
1. If the sample is not turbid, omit Steps 2–5. Pour two 100-mL aliquots of sample into separate beakers. Adjust the pH of one of the aliquots to 7.6; leave the other aliquot as is.

Note: Use 10 N sodium hydroxide or concentrated sulfuric acid to adjust the pH. Use 0.1 N acid or base near the end point.

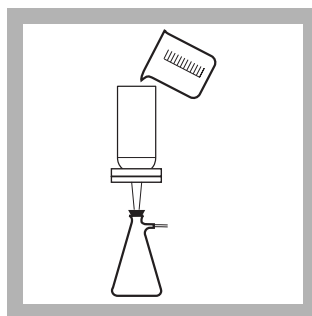
Note: If sample cannot be analyzed immediately, see *Sample Collection, Storage and Preservation* following these steps.



2. Assemble the filtering apparatus (membrane filter, filter holder, filter flask and aspirator).



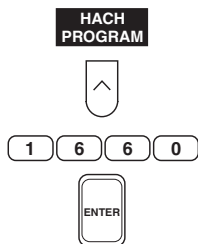
3. Rinse filter by pouring approximately 50 mL of original sample aliquot in the beaker through the filter. Discard the rinse.



4. Pour about 50 mL of original sample aliquot in the beaker through the filter. Label the flask “Original”.

Repeat Steps 2-4
for adjusted sample

5. Repeat Steps 2–4 for the pH-adjusted sample. Label the flask “pH adjusted”.



6. Press the soft key under **HACH PROGRAM**. Select the stored program for ADMI color value by pressing **1660** with the numeric keys.

Press: **ENTER**

Note: The Flow Cell and Sipper Modules can be used with this procedure. The Carousel Module cannot be used.

HACH PROGRAM:1660
Color, ADMI

7. The display will show:
HACH PROGRAM:
1660 Color, ADMI
The starting wavelength (λ), **700 nm**, is automatically selected.

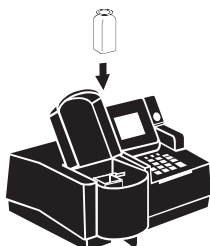


8. Fill a 1-inch square sample cell with the pH-adjusted filtered sample (the sample). Discard the excess.

Note: For proof of accuracy, use a 100-unit Co-Pt standard solution (preparation given in the Accuracy Check in place of sample).



9. Fill another sample cell with deionized water (the blank).

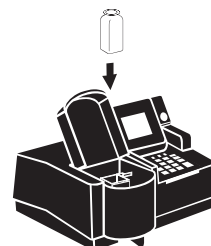


10. Place the blank into the cell holder and close the light shield.

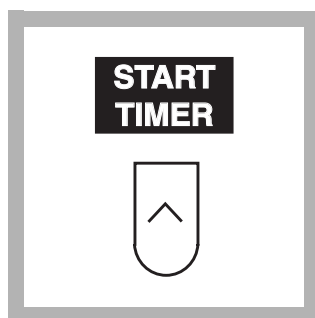
ZERO



11. Press the soft key under **ZERO**. Starting at 700 nm, the instrument will read the percent transmittance (%T) at 10 nm intervals until reaching 400 nm.



12. When prompted, place the sample in the cell holder and close the light shield.



13. Press the soft key under **START**.

Starting at 700 nm, the instrument will read the percent transmittance (%T) at 10-nm intervals until reaching 400 nm. Once finished, the instrument will display the ADMI color value of the pH adjusted sample.



14. Repeat Steps 8-13 for the original filtered sample. For USEPA reporting, report both results.

Interferences

Turbidity interferes directly and must be removed using filtration.

Sample Collection, Storage and Preservation

Collect samples in clean plastic or glass bottles. Most reliable results are obtained when samples are analyzed as soon as possible after collection. If prompt analysis is impossible, fill bottles completely and cap tightly. Avoid excessive agitation or prolonged contact with air. Samples can be stored for 24 hours by cooling to 4 °C (39 °F). Warm to room temperature before running the test.

Accuracy Check

Standard Solution Method

Prepare a 100-units Pt-Co standard solution by pipetting 20 mL of Color Standard Solution, 500 platinum-cobalt units, into a 100-mL volumetric flask. Dilute to the mark with deionized water. Perform the ADMI Color procedure as described above, omitting filtration Steps 2–5.

To adjust the calibration curve using the reading obtained with the 100-units Pt-Co standard, press the soft keys under **OPTIONS, (MORE)** then **STD: (OFF)**. Press **ENTER** to accept the default concentration, 100 units Pt-Co. If an alternate concentration is used, enter the actual concentration and press **ENTER** to return to the read screen. See Section 1.5.5 *Adjusting the Standard Curve* for more information.

Method Performance

Precision

Standard: 100 ADMI color value

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
1660	3 ADMI color value

For more information on derivation and use of Hach's estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, Appendix B, see Section 1.5.1.

Sensitivity

Program Number: 1660

Portion of Curve	Δ Abs	Δ Concentration
0.010 Abs	0.010	5.2 ADMICV
125 ADMICV	0.010	6.1 ADMICV
225 ADMICV	0.010	6.8 ADMICV

See Section 1.5.3 *Sensitivity Explained* for more information.

Summary of Method

Three properties describe color: hue, chroma and value. Hue is “color”, whether it be blue, red, green, yellow, etc. Chroma is color intensity (bright or dull). Value is the amount of color (light or dark). This method measures only the amount of color, or color value. It is independent of the hue and chroma.

This method determines the color value in a sample. Transmittance is measured from 400 to 700 nm and converted to a set of abstract numbers. These numbers describe the color as seen by an average human eye. They are converted to a single number that indicates the color value. This number is expressed on a scale used by the American Dye Manufacturers Institute to measure color value. The ADMI has adopted the Platinum-Cobalt standard of the American Public Health Association (APHA) as the standard for color value. Although this standard is yellow, the ADMI method works for all hues.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to Section 1.

Pollution Prevention and Waste Management

For information on pollution prevention and waste management, refer to Section 1.

REQUIRED REAGENTS AND STANDARDS

Description	Quantity Required per test	Unit	Cat. No.
Sodium Hydroxide Solution, 10 N.....	varies.....	500 mL.....	25450-49
Sodium Hydroxide Standard Solution, 0.100 N.....	varies.....	1000 mL.....	191-53
Sulfuric Acid, concentrated, ACS.....	varies.....	500 mL.....	979-49
Sulfuric Acid Standard Solution, 0.100 N.....	varies.....	100 mL MDB.....	202-32
Water, deionized.....	10 mL.....	4 liters.....	272-56

REQUIRED EQUIPMENT AND SUPPLIES

DR/4000 1-Inch Cell Adapter	1	each.....	48190-00
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OPTIONAL REAGENTS AND STANDARDS

Color Standard Solution, 500 Pt-Co Units	1 liter.....		1414-53
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OPTIONAL EQUIPMENT AND SUPPLIES

Aspirator, Nalgene vacuum pump	each.....		2131-00
DR/4000 Carousel Module Kit	each.....		48070-02
DR/4000 Flow Cell Module Kit, 1-inch.....	each.....		48070-04
DR/4000 Flow Cell Module Kit, 1-cm.....	each.....		48070-05
DR/4000 Sipper Module Kit, 1-inch.....	each.....		48090-03
Filter Holder, 47-mm, 300-mL graduated.....	each.....		13529-00
Filter, membrane, 47-mm, 0.45-microns.....	100/pkg.....		13530-00
Flask, filtering, 500-mL	each.....		546-49
Flask, volumetric, Class A, 100-mL	each.....		14574-42
Pipet, volumetric, Class A, 20.00-mL	each.....		14515-20
Stopper, rubber, one hole. No. 7.....	each.....		2119-07
Tubing, rubber	12 ft.....		560-19



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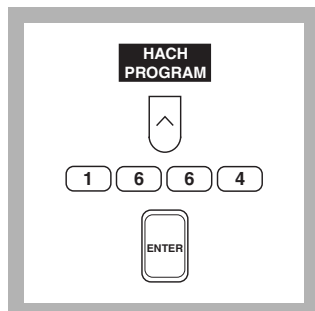
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Method 10105 For transparent liquids

ASTM Method D 6166-97
(1 to 18 Gardner Color Units)



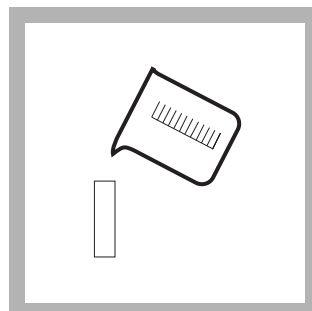
1. Press the soft key under **HACH PROGRAM**. Select the stored program number for Gardner Color by pressing **1664** with the numeric keys.

Press **ENTER**.



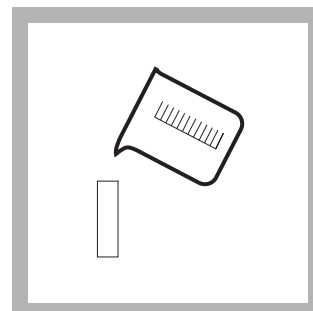
2. The display will show:
HACH PROGRAM: 1664
Color, Gardner

The starting wavelength (λ), 780 nm, is automatically selected.



3. Fill a 1-cm sample cell with the sample to be measured.

Note: Other cell sizes may be used for very light-colored samples. Insert the appropriate cell holder and press the soft keys under **OPTIONS** and then **PATH**. Enter the path length of choice and press **ENTER**. The display will indicate the selected cell path length in cm. The displayed results will be normalized to a 1-cm path length.



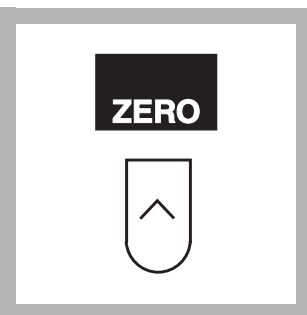
4. Fill another sample cell with the blank solution, if available.

Note: The blank solution should match the sample in composition, but without any colored components.



5. Insert a 1-cm cell adapter into the cell compartment. Place the blank into the 1-cm adapter. Close the light shield.

Note: If a colorless blank solution is not available, leave the cell holder empty and close the light shield.



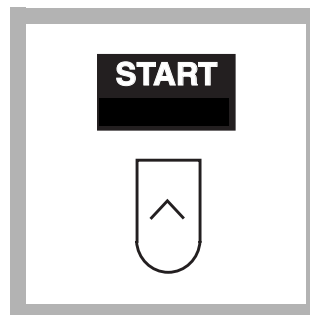
6. Press the soft key under **ZERO**. Starting at 780 nm, the instrument will establish 100% transmittance values for the blank at 5 nm intervals until it reaches 380 nm.

The display will show:

0.0 **Gardner**
 Units



7. When prompted, place the sample in the cell holder and close the light shield.



8. Press the soft key under **START**.

Starting at 780 nm, the instrument will read the percent transmittance (%T) at 5 nm intervals until it reaches 380 nm. Once finished, the instrument will display the Gardner Color value of the sample.

Note: To view tristimulus values or chromaticity coordinates, press the soft key under **OPTIONS**, then press **VIEW** repeatedly until **TRISTIM** or **CHROM** is displayed.

Interferences

Turbidity interferes directly and must be removed by filtration. Samples containing fluorescent components may interfere. Temperature and pH should be controlled for consistent results. Bubbles will interfere and should be removed.

Sample Handling

The preparation of samples can significantly affect measured results. For increased accuracy, collect the sample in such a way that it is representative of the source, and prepare it using a standard method for the material being measured.

Accuracy Check

Perform the wavelength accuracy and absorbance checks described in *DR/4000 Spectrophotometer Instrument Manual*. The wavelength and absorbance accuracy of the instrument affect the bias and precision of the method. (See ASTM Method E 308-95.)

Summary of Method

This method determines the Gardner Color of a sample. Transmittance is measured from 380 to 780 nm and converted to tristimulus values and chromaticity coordinates using ASTM Method E308-95, CIE Illuminant C, and the CIE 1931 Standard 2° Observer. ASTM Method 6166-97 (the instrumental equivalent of

D1544-80) converts the chromaticity coordinates to a single number that indicates the Gardner Color.

Use this method for clear, yellow, or yellow-brown liquid samples only. Samples which are to be compared should be similar in appearance.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to Section 1.

Pollution Prevention and Waste Management

For information on pollution prevention and waste management, refer to Section 1.

REQUIRED EQUIPMENT AND SUPPLIES

Description	Quantity required per test	Unit	Cat. No.
DR/4000 1-cm cell adapter	1	each.....	48584-00
Sample cells, 1-cm, glass	2	each.....	20951-00

OPTIONAL EQUIPMENT AND SUPPLIES

Aspirator, vacuum		each.....	2131-00
Filter Holder, 47-mm, 300-mL graduated		each.....	13529-00
Filter, membrane, 47-mm, 0.45-microns		each.....	13530-00
Flask, filtering, 500-mL		each.....	546-49
Sample cell, 1-cm, quartz, w/ stopper (for volatile samples)		each.....	27401-01
Sample cells, 5-cm, quartz, w/ stopper (for volatile samples)		each.....	27401-05
Sample cells, 10-cm, quartz, w/ stopper (for volatile samples)		each.....	27401-10
Sample cells, microcell, 1-cm, 1.5-mL, disposable	100/pkg	26295-00
Sample cell adapter, 5-cm		each.....	48186-00
Sample cell adapter, 10-cm		each.....	48118-00
Sample cell adapter, microcell, 1-cm		each.....	48588-00
Stopper, No. 7, one hole		each.....	2119-07
Temperature Control Module, 15 to 50 °C, 1-cm cell holder		each.....	48070-08
Tubing, rubber	12 ft	560-19



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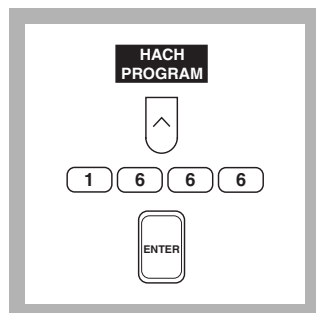
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* This method uses, as defaults, CIE Illuminant C, and the CIE 1931 Standard 2° Observer. However, CIE Illuminants A, C, D50, D55, D65, D75, F2, F7, and F11 can be used, as well as the CIE 1964 Supplementary Standard 10° Observer.

To select other Illuminants, press the soft key under **OPTIONS, MORE** and then **ILLUM**. Scroll the **ILLUM** soft key to select one of the Illuminants. The tristimulus values will be calculated using the Illuminant selected.

To select the 10° Observer, press the soft key under **OPTIONS, MORE** and then **STD OBS**. The tristimulus values will be calculated using the 10° Observer.



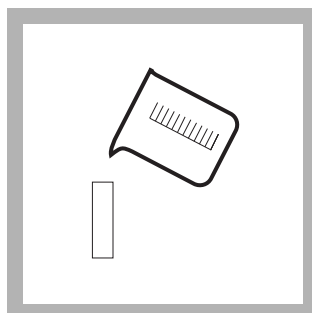
1. Press the soft key under **HACH PROGRAM**. Select the stored program number for tristimulus values by pressing **1666** with the numeric keys.

Press **ENTER**.



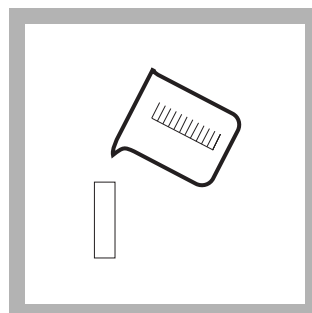
2. The display will show:
HACH PROGRAM: 1666
Color, Tristimulus

The starting wavelength (λ), 780 nm, is automatically selected.



3. Fill a 1-cm sample cell with the sample to be measured.

Note: Other cell sizes may be used for very light-colored samples. Insert the appropriate cell holder and press the soft keys under **OPTIONS** and then **PATH**. Enter the path length of choice and press **ENTER**. The display will indicate the selected cell path length in cm. The displayed results will be normalized to a 1-cm path length.



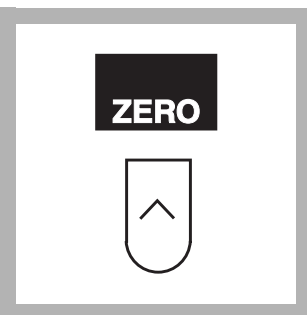
4. Fill another sample cell with the blank solution, if available.

Note: The blank solution should match the sample in composition, but without any colored components.



5. Insert a 1-cm cell adapter into the cell compartment. Place the blank into the 1-cm adapter. Close the light shield.

Note: If a colorless blank solution is not available, leave the cell holder empty and close the light shield.



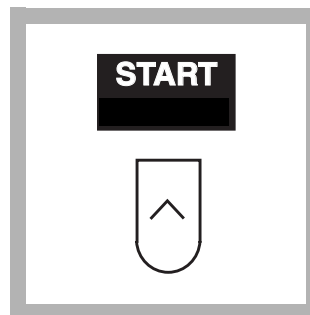
6. Press the soft key under **ZERO**. Starting at 780 nm, the instrument will establish 100% transmittance values for the blank at 5 nm intervals until it reaches 380 nm.

The display will show:

X:	Y:	Z:
0.0	0.0	0.0



7. When prompted, place the sample in the cell holder and close the light shield.



8. Press the soft key under **START**. Starting at 780 nm, the instrument will read the percent transmittance (%T) at 5 nm intervals until it reaches 380 nm. Once finished, the instrument will display the tristimulus values of the sample.

Note: To view chromaticity coordinates, press the soft key under **OPTIONS**, then press **VIEW** repeatedly until **CHROM** is displayed.

Interferences

Turbidity interferes directly and must be removed by filtration. Samples containing fluorescent components may interfere. Temperature and pH should be controlled for consistent results. Bubbles will interfere and should be removed.

The solution used for zeroing the instrument can directly affect the results. For accurate absolute results, the zeroing solution should resemble the sample as closely as possible but be absent of any color. When air is used for zeroing, the results are best used comparatively.

Sample Handling

The preparation of samples can significantly affect measured results. For increased accuracy, collect the sample in such a way that it is representative of the source, and prepare it using a standard method for the material being measured.

Accuracy Check

Perform the wavelength accuracy and absorbance checks described in the *DR/4000 Spectrophotometer Instrument Manual*. The wavelength and absorbance accuracy of the instrument affect the bias and precision of the method. (See ASTM Method E 308-95.)

Summary of Method

Tristimulus values are a set of three numbers obtained from a spectrophotometer or colorimeter that, when combined in various ways, describe how the human eye perceives a given color. Calculations using the three tristimulus values are typically used to define the color of a specimen for color matching specifications or quality control. In this program, transmittance is measured from 780 to 380 nm in 5-nm increments. These transmittance values are then used to calculate tristimulus values X, Y, and Z using ASTM Method E 308-95. The bandpass of the DR/4000 spectrophotometer matches the 5-nm measurement interval; therefore no bandpass correction is necessary.

Options exist for obtaining the tristimulus values under different viewing conditions, illuminants, and path lengths. Samples which are to be compared should be similar in appearance.

The CIE colorimetric systems are meant to provide numerical specifications to indicate whether pairs of color stimuli match when viewed by a CIE standard observer. The CIE color systems are not intended to describe visual color appearances or to provide visually uniform scales of color difference.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to Section 1.

Pollution Prevention and Waste Management

For information on pollution prevention and waste management, refer to Section 1.

REQUIRED EQUIPMENT AND SUPPLIES

Description	Quantity required per test	Unit	Cat. No.
DR/4000 1-cm Cell Adapter	1	each.....	48584-00
Sample cells, 1-cm, glass	2	each.....	20951-00

OPTIONAL EQUIPMENT AND SUPPLIES

Aspirator, vacuum	each.....	2131-00
Filter Holder, 47-mm, 300-mL graduated	each.....	13529-00
Filter, membrane, 47-mm, 0.45-microns.....	each.....	13530-00
Flask, filtering, 500-mL	each.....	546-49
Sample cell, 1-cm, quartz, w/ stopper (for volatile samples).....	each.....	27401-01
Sample cells, 5-cm, quartz, w/ stopper (for volatile samples)	each.....	27401-05
Sample cells, 10-cm, quartz, w/ stopper (for volatile samples)	each.....	27401-10
Sample cells, microcell, 1-cm, 1.5-mL, disposable	100/pkg.....	26295-00
Sample cell adapter, 5-cm	each.....	48186-00
Sample cell adapter, 10-cm	each.....	48118-00
Sample cell adapter, microcell, 1-cm	each.....	48588-00
Stopper, No. 7, one hole	each.....	2119-07
Temperature Control Module, 15 to 50 °C, 1-cm cell holder	each.....	48070-08
Tubing, rubber	12 ft.....	560-19



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Method 8025

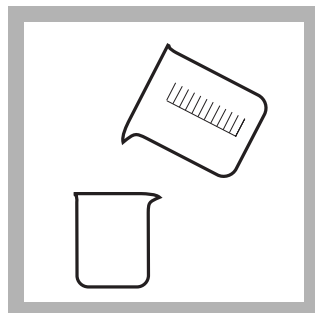
Platinum-Cobalt Standard Method* **

(0 to 500 units)

Scope and Application: For water, wastewater and seawater; equivalent to NCASI method 253 for pulp and paper effluent using 465 nm (requires pH adjustment). The estimated detection limit for program numbers 1670 and 1680 is 2 units Pt-Co.

* Adapted from *Standard Methods for the Examination of Water and Wastewater* and NCASI, Technical Bulletin No. 253, Dec., 1971

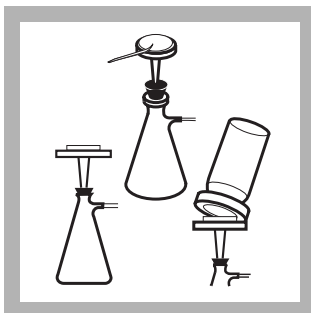
** Adapted from *Wat. Res. Vol. 30, No. 11, pp. 2771-2775. 1996*



1. Collect 200 mL of sample in a 400-mL beaker. If necessary, adjust the pH to 7.6 with 1.0 N HCl or 1.0 N NaOH.

Note: NCASI procedure requires pH adjustment.

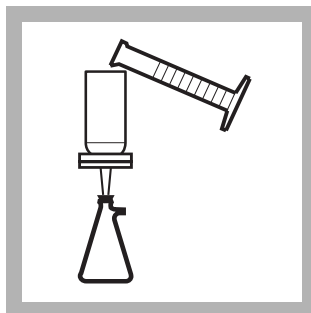
Note: When adjusting the pH, if overall volume change is greater than 1%, start over and use a stronger acid or base.



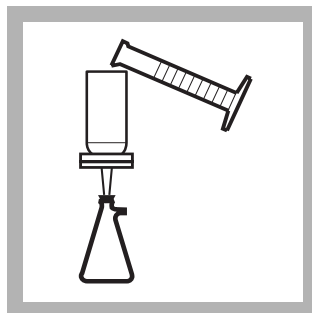
2. Assemble the filtering apparatus (0.45 micron membrane filter, filter holder, filter flask, and aspirator). NCASI prescribes a 0.8 micron filter.

Note: To test for apparent color, do not filter; omit Steps 2-4 and 8.

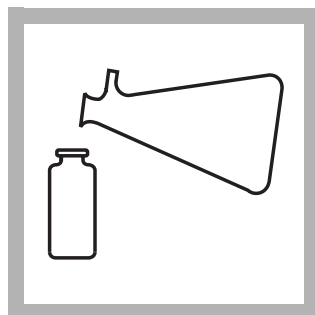
Note: If sample cannot be analyzed immediately, see *Sample Collection, Storage and Preservation*.



3. Rinse the filter by pouring about 50 mL of deionized water through the filter. Discard the rinse water.

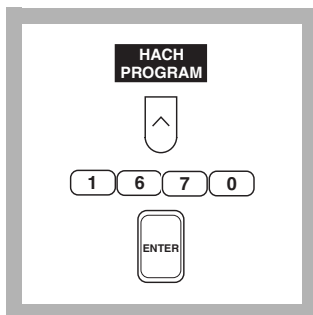


4. Pour another 50 mL of deionized water through the filter.



5. Fill a sample cell (the blank) with 10 mL of filtered deionized water. Discard the excess water in the flask.

Note: For apparent color use unfiltered deionized water.



6. Press the soft key under **HACH PROGRAM**. Select the stored program for **Color** read at 455 nm by pressing **1670**

1 **6** **7** **0**

or

Color read at 465 nm by pressing **1680** with the numeric keys.

1 **6** **8** **0**

Press: **ENTER**

Note: The Flow Cell and Sipper Modules can be used for this procedure. Use minimum volumes of 20 and 10 mL respectively.



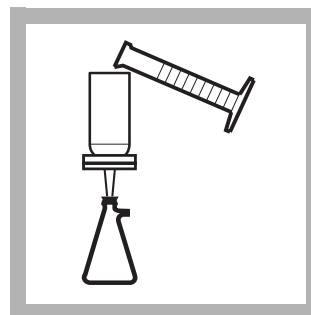
7. The display will show:
HACH PROGRAM: 1670
Color, 455 nm

or

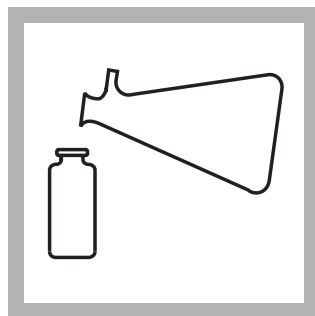
HACH PROGRAM: 1680
Color, 465 nm

HACH PROGRAM:1680
Color, 465 nm

The respective wavelength is automatically selected (455 or 465 nm).

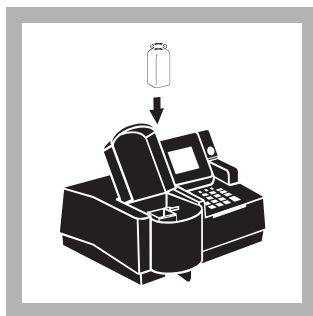


8. Pour about 50 mL of sample through the filter.

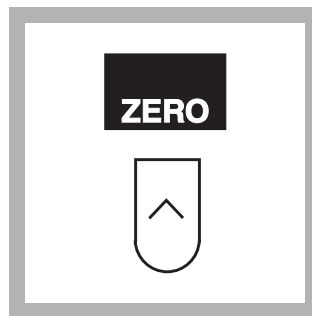


9. Fill a second sample cell (the prepared sample) with 10 mL of filtered sample.

Note: For proof of accuracy, use a 250-unit platinum-cobalt color standard solution (preparation given in the Accuracy Check section) in place of the filtered sample.



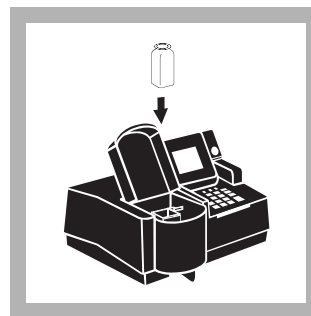
10. Place the blank into the cell holder and close the light shield.



11. Press the soft key under **ZERO**.

The display will show:

0 units Pt-Co



12. Place the prepared sample into the cell holder. Close the light shield. Results in platinum-cobalt units will be displayed.

Sample Collection, Storage and Preservation

Collect samples in clean plastic or glass bottles. Most reliable results are obtained when samples are analyzed as soon as possible after collection. If prompt analysis is impossible, fill bottles completely and cap tightly. Avoid excessive agitation or prolonged contact with air. Samples can be stored for 24 hours by cooling to 4 °C (39 °F). Warm to room temperature before analysis.

Accuracy Check

Standard Solution Method

Using Class A glassware, prepare a 250 platinum-cobalt units standard by pipetting 50.00 mL of a 500 Platinum-Cobalt Units Standard Solution into a 100-mL volumetric flask and diluting to 100 mL with deionized water.

To adjust the calibration curve using the reading obtained with the 250 Pt-Co units standard, press the soft keys under **OPTIONS, (MORE)** then **STD: (OFF)**.

Press **ENTER** to accept the displayed concentration, the value of which depends on the selected units. If an alternate concentration is used, enter the actual concentration and press **ENTER** to return to the read screen. See Section 1.5.5 *Adjusting the Standard Curve* for more information.

Method Performance

Precision

Standard: 250 units Pt-Co

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
1670	2 units Pt-Co
1680	2 units Pt-Co

For more information on derivation and use of Hach's estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, Appendix B, see Section 1.5.1.

Sensitivity

Program Number: 1670

Portion of Curve	Δ Abs	Δ Concentration
Entire Range	0.010	15.7 Pt-Co Units

Program Number: 1680

Portion of Curve	Δ Abs	Δ Concentration
Entire Range	0.010	16.2 Pt-Co Units

See Section 1.5.3 *Sensitivity Explained* for more information.

Calibration Standard Preparation

To perform a Color calibration using the platinum cobalt method, use a 500 Platinum Cobalt Units Standard Solution (Cat. No. 1414-53).

Prepare calibration standard containing 50, 100, 150, 250, 350, and 450 units Pt-Co as follows:

- a. Into six different 100-mL volumetric flasks, pipet 10.00, 20.00, 30.00, 50.00, 70.00, and 90.00 mL of the 500-units Color Standard Solution using Class A glassware. Also, use the undiluted 500-unit Pt-Co standard during calibration.
- b. Dilute to the mark with deionized water and mix thoroughly.
- c. Using the platinum-cobalt method and the calibration procedure described in the *User-Entered Programs* section of the *DR/4000 Spectrophotometer Instrument Manual*, generate a calibration curve from the standards prepared above.

Summary of Method

Color may be expressed as “apparent” or “true” color. The apparent color includes that from dissolved materials plus that from suspended matter. By filtering or centrifuging out the suspended materials, the true color can be determined. The procedure describes true color analysis. If apparent color is desired, it can be determined by measuring an unfiltered water sample. The stored program is used for both forms of color.

The stored program is calibrated in color units based on the APHA-recommended standard of 1 color unit being equal to 1 mg/L platinum as chloroplatinate ion.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to Section 1.

Pollution Prevention and Waste Management

For information on pollution prevention and waste management, refer to Section 1.

REQUIRED REAGENTS AND STANDARDS

Description	Quantity Required per test	Unit	Cat. No.
Water, deionized	50 mL	4 liters.....	272-56

REQUIRED EQUIPMENT AND SUPPLIES

Aspirator, Nalgene vacuum pump	1	each.....	2131-00
DR/4000 1-Inch Cell Adapter	1	each.....	48190-00
Filter Holder, 47 mm, 300-mL graduated	1	each.....	13529-00
Filter, membrane, 47-mm, 0.8-microns.....	1	100/pkg.....	26408-00
Filter, membrane, 47-mm, 0.45-microns.....	1	100/pkg.....	13530-00
Flask, filtering, 500-mL	1	each.....	546-49
Stopper, rubber, one hole, No. 7.....	1	6/pkg.....	2119-07
Tubing, rubber	1	12 ft.....	560-19

OPTIONAL REAGENTS AND STANDARDS

Color Standard Solution, 15 platinum-cobalt units	1 liter.....	26028-53
Color Standard Solution, 500 platinum-cobalt units	1 liter.....	1414-53
Color Standard Solution, 500 platinum-cobalt units, 10-mL Voluette Ampules	16/pkg.....	1414-10
Hydrochloric Acid Solution, 1.0 N	1 liter.....	23213-53
Hydrochloric Acid, 6.0 N.....	500 mL.....	884-49
Sodium Hydroxide, 1.00 N	900 mL.....	1045-53
Sodium Hydroxide Standard Solution, 6 N.....	1 liter.....	23324-53

OPTIONAL EQUIPMENT AND SUPPLIES

DR/4000 Carousel Module Kit	each.....	48070-02
DR/4000 Flow Cell Module Kit, 1-inch.....	each.....	48070-04
DR/4000 Flow Cell Module Kit, 1-cm.....	each.....	48070-05
DR/4000 Sipper Module Kit, 1-inch	each.....	48090-03
Flask, volumetric, Class A, 100-mL	each.....	14574-42
pH Meter, <i>sensio</i> TM 1, portable	each.....	51700-00
Pipet, volumetric, Class A, 10-mL.....	each.....	14515-38
Pipet, volumetric, Class A, 20-mL.....	each.....	14515-20
Pipet, volumetric, Class A, 50-mL.....	each.....	14515-41



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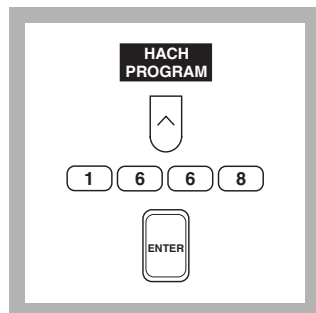
On the Worldwide Web – www.hach.com; E-mail – techhelp@hach.com

HACH COMPANY
WORLD HEADQUARTERS
Telephone: (970) 669-3050
FAX: (970) 669-2932



Scope and Application: For transparent liquids, resins, and plastics of near-colorless quality

* This method uses CIE Illuminant C and the CIE 1931 Standard 2° Observer as the default setup, but CIE Illuminant D₆₅ and the CIE 1964 Supplementary Standard 10° Observer may be used as well. To select **Illuminant D₆₅**, press the soft key under **OPTIONS, MORE** and then **ILLUM**. The displayed Yellowness Index will be calculated using CIE Illuminant D₆₅. To select the **10° Observer**, press the soft key under **OPTIONS, MORE** and then **STD OBS**. The displayed Yellowness Index will be calculated using the 10° Observer.



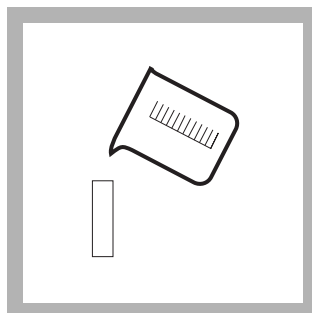
1. Press the soft key under **HACH PROGRAM**. Select the stored program number for Yellowness Index by pressing **1668** with the numeric keys.

Press **ENTER**



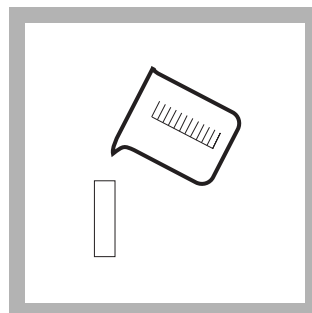
2. The display will show: **HACH PROGRAM: 1668 Color, Yellowness**

The starting wavelength (λ), 780 nm, is automatically selected.



3. Fill a 1-cm sample cell with the sample to be measured.

Note: Other cell sizes may be used for very light-colored samples. Insert the appropriate cell holder and press the soft keys under **OPTIONS** and then **PATH**. Enter the desired path length and press **ENTER**. The display will indicate the selected cell path length in cm. The displayed results will be normalized to a 1-cm path length.



4. Fill another sample cell with the blank solution, if available.

Note: The blank solution should match the sample in composition, but without any colored components.



5. Insert a 1-cm cell adapter into the cell compartment. Place the blank into the 1-cm adapter. Close the light shield.

Note: If a colorless blank solution is not available, leave the cell holder empty and close the light shield.



6. Press the soft key under **ZERO**.

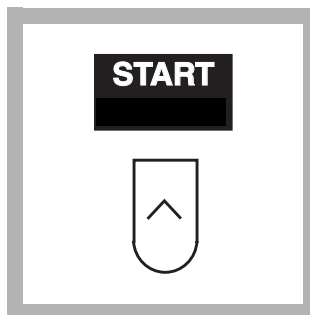
Starting at 780 nm, the instrument will establish 100% transmittance values for the blank at 5 nm intervals until it reaches 380 nm.

The display will show:

0 YI



7. When prompted, place the sample in the cell holder and close the light shield.



8. Press the soft key under **START**.

Starting at 780 nm, the instrument will read the percent transmittance (%T) at 5 nm intervals until it reaches 380 nm. Once finished, the instrument will display the Yellowness Index of the sample.

Note: To view tristimulus values or chromaticity coordinates, press the soft key under **OPTIONS** then press **VIEW** repeatedly until **TRISTIM** or **CHROM** is displayed.

Interferences

Turbidity interferes directly and must be removed by filtration. Samples containing fluorescent components may interfere. Temperature and pH should be controlled for consistent results. Bubbles will interfere and should be removed.

The solution used for zeroing the instrument can directly affect the results. For accurate absolute results, the zeroing solution should resemble the sample as closely as possible but be absent of any color. When air is used for zeroing, the results are best used comparatively.

Sample Handling

The preparation of samples can significantly affect measured results. For increased accuracy, collect the sample in such a way that it is representative of the source, and prepare it using a standard method for the material being measured.

Accuracy Check

Perform the wavelength accuracy and absorbance checks described in the *DR/4000 Spectrophotometer Instrument Manual*. The wavelength and absorbance accuracy of the instrument affect the bias and precision of the method. (See ASTM Method E 308-95.)

To adjust the Yellowness Index results using a standard, follow the procedure given above using the standard in place of the sample. Press the soft keys under **OPTIONS, (MORE)** then **STD: (OFF)**. Enter the Yellowness Index of the standard and press **ENTER**. The Yellowness Index of subsequent samples will be adjusted by a constant factor. See *Standard Curve Adjustment* in the *DR/4000 Spectrophotometer Instrument Manual* for more information.

Summary of Method

This method determines the Yellowness Index of a sample. Transmittance is measured from 380 to 780 nm and converted to tristimulus values using ASTM Method E 308-95. ASTM Method E313-96 converts these tristimulus values to a single number that indicates the Yellowness Index.

Use this method for samples which are yellow and have a dominant wavelength in the 570 to 580 nm range. Samples which are to be compared should be similar in appearance.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to Section 1.

Pollution Prevention and Waste Management

For information on pollution prevention and waste management, refer to Section 1.

COLOR, Yellowness Index, continued

REQUIRED EQUIPMENT AND SUPPLIES

Description	Quantity required per test	Unit	Cat. No.
DR/4000 1-cm Cell Adapter	1	each.....	48584-00
Sample cell, 1-cm, glass	2	each.....	20951-00

OPTIONAL EQUIPMENT AND SUPPLIES

Aspirator, vacuum	each.....	2131-00
Filter Holder, 47-mm, 300-mL graduated	each.....	13529-00
Filter, membrane, 47-mm, 0.45-microns	each.....	13530-00
Flask, filtering, 500-mL	each.....	546-49
Sample cell, 1-cm, quartz, w/ stopper (for volatile samples)	each.....	27401-01
Sample cells, 5-cm, quartz, w/ stopper (for volatile samples)	each.....	27401-05
Sample cells, 10-cm, quartz, w/ stopper (for volatile samples)	each.....	27401-10
Sample cells, microcell, 1-cm, 1.5-mL, disposable	100/pkg.....	26295-00
Sample cell adapter, 5-cm	each.....	48186-00
Sample cell adapter, 10-cm	each.....	48118-00
Sample cell adapter, microcell, 1-cm	each.....	48588-00
Stopper, No. 7, one hole	each.....	2119-07
Temperature Control Module, 15 to 50 °C, 1-cm cell holder	each.....	48070-08
Tubing, rubber	12 ft.....	560-19



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✓ Method 8506 and Method 8026

Bicinchoninate Method*

Powder Pillows or AccuVac® Ampuls

(0 to 5.000 mg/L)

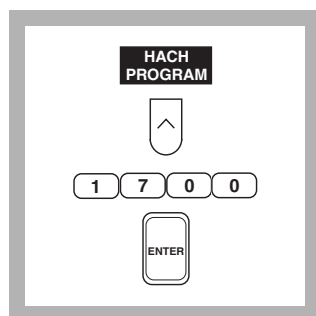
Scope and Application: For water, wastewater and seawater**; digestion is required for determining total copper; USEPA approved for reporting wastewater analyses (digestion required; See SECTION 2)***. The estimated detection limits for program numbers 1700 and 1710 are 0.021 and 0.020 mg/L Cu, respectively.

* Adapted from Nakano, S., *Yakugaku Zasshi*, 82 486-491 (1962) [Chemical Abstracts, 58 3390e (1963)]

** Pretreatment required; see *Interferences (Using Powder Pillows)*

*** Powder Pillows only: *Federal Register*, 45 (105) 36166 (May 29, 1980)

Using Powder Pillows



1. Press the soft key under **HACH PROGRAM**.

Select the stored program for bicinchoninate copper by pressing **1700** with the numeric keys.

Press: **ENTER**

Note: If samples cannot be analyzed immediately, see *Sample Collection, Storage and Preservation* following these steps. Adjust the pH of preserved samples before analysis.

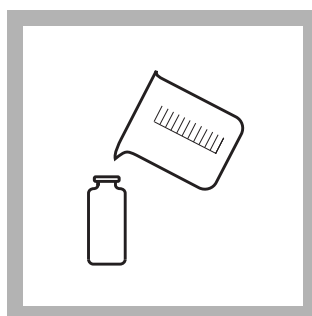
Note: The Flow Cell and Sipper Modules can be used for this procedure. Use a 25-mL sample and reagents with the Flow Cell Module.



2. The display will show: **HACH PROGRAM: 1700 Copper, Bicin**

The wavelength (λ), **560 nm**, is automatically selected.

Note: For best results, determine a reagent blank for each new lot of reagent as follows. Prepare a reagent blank by repeating Steps 3 through 8, using copper-free deionized water as the sample. Zero the instrument on deionized water by pressing the soft key under **ZERO**. Insert the reagent blank and the blank value will be displayed. Correct for the reagent blank by pressing the soft keys under **OPTIONS, (MORE)**, and then **BLANK:OFF**. Enter the reagent blank value and press **ENTER**. Repeat for each new lot of reagent.



3. Fill a sample cell with 10 mL of sample.

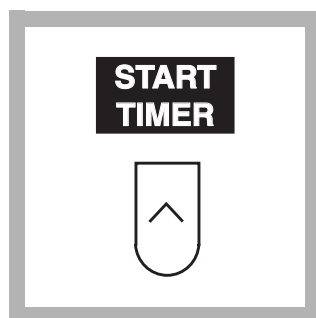
Note: For proof of accuracy, use a 4.00 mg/L copper standard solution (preparation given in the Accuracy Check section) in place of the sample.

Note: The chemistry is pH sensitive. Adjust the pH of acid-preserved samples to 4-6 with 8 N KOH before analysis.



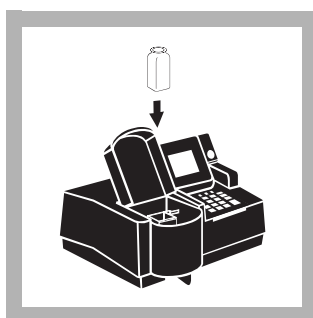
4. Add the contents of one CuVer 1 Copper Reagent Powder Pillow to the sample cell (the prepared sample). Swirl to mix.

Note: A purple color will develop if copper is present.

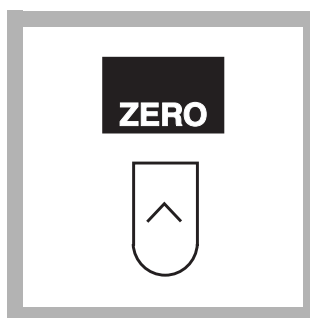


5. Press the soft key under **START TIMER**.

A 2-minute reaction period will begin.



6. When the timer beeps, fill a second sample cell (the blank) with 10 mL of sample. Place the blank into the cell holder.



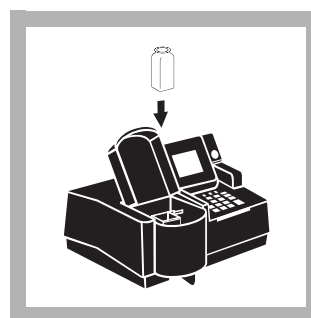
7. Press the soft key under **ZERO**.

The display will show:

0.000 mg/L Cu

Note: If you are using a reagent blank correction, the display will show the correction.

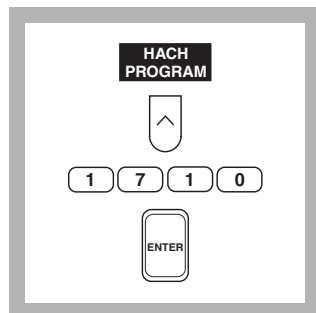
Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



8. Within 30 minutes after the timer beeps, place the prepared sample into the cell holder. Close the light shield. Results in mg/L copper (or chosen units) will be displayed.

Using AccuVac Ampuls

Method 8026



1. Press the soft key under **HACH PROGRAM**.

Select the stored program for bicinchoninate copper (AccuVac) by pressing **1710** with the numeric keys.

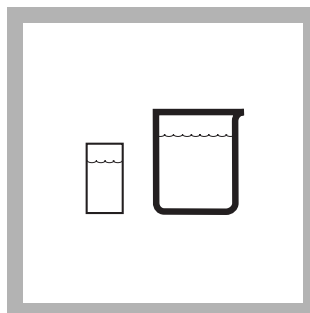
Press: **ENTER**

Note: If samples cannot be analyzed immediately, see *Sample Collection, Storage and Preservation* following these steps. Adjust the pH of preserved samples before analysis.



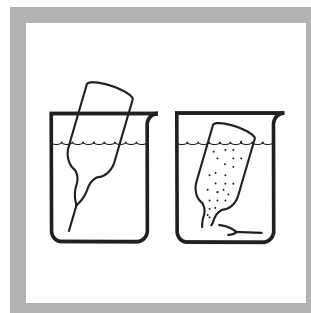
2. The display will show:
**HACH PROGRAM: 1710
Copper, Bicin. AV**

The wavelength (λ), **560 nm**, is automatically selected.



3. Fill a zeroing vial (the blank) with at least 10 mL of sample. Collect at least 40 mL of sample in a 50-mL beaker.

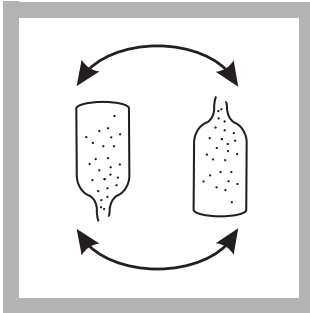
Note: For best results, determine a reagent blank by using copper-free deionized water instead of sample in Step 3. After obtaining the result in Step 10, press the soft keys under **OPTIONS (MORE)**, and then **BLANK:OFF**. Enter the reagent blank value and press **ENTER**. Repeat for each new lot of reagent.



4. Fill a CuVer 2 AccuVac Ampul with sample.

Note: Keep the tip immersed while the ampul fills completely.

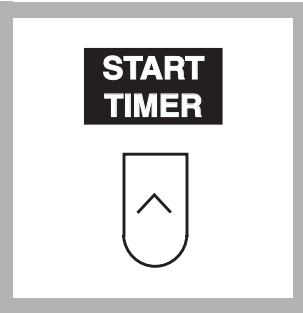
Note: For proof of accuracy, use a 4.00-mg/L copper standard solution (preparation given in the *Accuracy Check* section) in place of the sample.



5. Quickly invert the ampul several times to mix. Wipe off any liquid or fingerprints with cloth or soft paper towel.

Note: A purple color will form if copper is present.

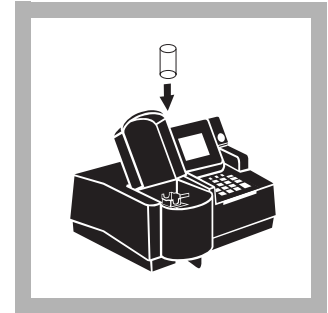
Note: Accuracy is not affected by undissolved powder.



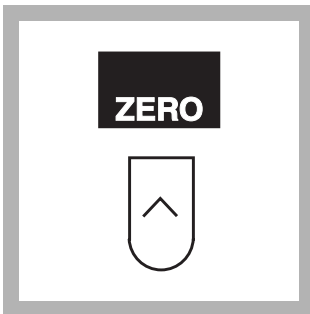
6. Press the soft key under **START TIMER**. A 2-minute reaction period will begin.



7. Insert the AccuVac Ampul Adapter into the sample cell module by sliding it under the thumb screw and into the alignment grooves. Fasten with the thumb screw.



8. When the timer beeps, place the blank into the cell holder. Close the light shield.



9. Press the soft key under **ZERO**.

The display will show:

0.000 mg/L Cu

Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



10. Within 30 minutes after the timer beeps, place the AccuVac Ampul into the cell holder. Close the light shield. Results in mg/L copper (or chosen units) will be displayed.

Interferences

Table 1 Interfering Substances and Suggested Treatments for Powder Pillows

Interfering Substance	Interference Levels and Treatments
Acidity	If the sample is extremely acidic (pH 2 or less) a precipitate may form. Add 8 N Potassium Hydroxide Standard Solution drop-wise while swirling to dissolve the turbidity. Continue with Step 3.
Aluminum, Al ³⁺	Follow the powder pillow procedure above, but substitute a CuVer 2 Copper Reagent Powder Pillow for the CuVer 1 Pillow used in Step 4. Results obtained will include total dissolved copper (free and complexed).
Cyanide, CN ⁻	Prevents full color development. Before adding the CuVer 1 Powder Pillow Reagent, add 0.2 mL of formaldehyde to the 10-mL sample. Wait 4 minutes before taking the reading. Multiply the test results by 1.02 to correct for sample dilution by the formaldehyde.
Hardness	Follow the powder pillow procedure above, but substitute a CuVer 2 Copper Reagent Powder Pillow for the CuVer 1 Pillow used in Step 4. Results obtained will include total dissolved copper (free and complexed).
Iron, Fe ³⁺	Follow the powder pillow procedure above, but substitute a CuVer 2 Copper Reagent Powder Pillow for the CuVer 1 Pillow used in Step 4. Results obtained will include total dissolved copper (free and complexed).
Silver, Ag ⁺	If a turbidity remains and turns black, silver interference is likely. Add 10 drops of saturated Potassium Chloride Solution to 75 mL of sample, followed by filtering through a fine or highly retentive filter. Use the filtered sample in the procedure.

To differentiate free copper from that complexed to EDTA or other complexing agents, analyze a 25 mL sample with a Free Copper Reagent Powder Pillow in place of the CuVer 1 Powder Pillow in Step 4. Results in *Step 10* will be free copper only. Add a Hydrosulfite Reagent Powder Pillow to the same sample and re-read the result. This result will include the total dissolved copper (free and complexed).

Table 2 Interfering Substances and suggested Treatments for AccuVac Ampuls

Interfering Substance	Interference Levels and Treatments
Acidity	If the sample is extremely acidic (pH 2 or less) a precipitate may form. Add 8 N Potassium Hydroxide Standard Solution drop-wise until sample pH is above 4. Continue with Step 3.
Aluminum, Al ³⁺	Reagents accommodate high levels.
Cyanide, CN ⁻	Prevents full color development. Add 0.5 mL of formaldehyde to a 25-mL sample before using CuVer2 Reagent AccuVac Ampul. Wait 4 minutes before taking the reading. Multiply the test results by 1.02 to correct for sample dilution by the formaldehyde.
Hardness	Reagents accommodate high levels.
Iron, Fe ³⁺	Reagents accommodate high levels.
Silver, Ag ⁺	If a turbidity remains and turns black, silver interference is likely. Add 10 drops of saturated Potassium Chloride Solution to 75 mL of sample, followed by filtering through a fine or highly retentive filter. Use the filtered sample in the procedure.

Unlike CuVer 1 Reagent, CuVer 2 Reagent reacts directly with copper which is complexed by chelants such as EDTA. If free copper is to be determined separately from complexed copper, see *Table 1* above.

Sample Collection, Storage and Preservation

Collect samples in acid-cleaned glass or plastic containers. Adjust the pH to 2 or less with concentrated nitric acid (about 2 mL per liter). Store preserved samples up to six months at room temperature. Before analysis, adjust the pH to 4–6 with 8 N Potassium Hydroxide. Do not exceed pH 6, as copper may precipitate. Correct the test result for volume additions; see Section 1.2.2 *Correcting for Volume Additions*. If only dissolved copper is to be determined, filter the sample before acid addition using the labware listed under *OPTIONAL EQUIPMENT AND SUPPLIES*.

Accuracy Check

Standard Additions Method

- a. Leave the unspiked sample in the sample cell compartment. Verify that the units displayed are in mg/L. Select standard additions mode by pressing the soft keys under **OPTIONS, (MORE)** and then **STD ADD**.
- b. Press **ENTER** to accept the default sample volume (mL), 25.
- c. Press **ENTER** to accept the default standard concentration (mg/L), 75.000.
- d. Press the soft key under **ENTRY DONE**.
- e. Snap the neck off a Copper Voluette Ampule Standard, 75-mg/L Cu.
- f. Use the TenSette Pipet to add 0.1, 0.2 mL and 0.3 mL of standard, respectively to three 25-mL samples and mix each thoroughly (for AccuVac Ampuls, use 50-mL beakers).
- g. Analyze a 10-mL portion of each standard addition sample as described above. Accept the standard additions reading by pressing the soft key under **READ** each time. Each addition should reflect approximately 100% recovery.
- h. After completing the sequence, the display will show the extrapolated concentration value and the “best-fit” line through the standard additions data points, accounting for matrix interferences.
- i. See Section 1.4.1 *Standard Additions* for more information.

Standard Solutions Method

Prepare a 4.00-mg/L Standard as follows:

Using Class A glassware, pipet 4.00 mL of Copper Standard Solution, 100-mg/L as Cu, into a 100-mL volumetric flask. Dilute to volume with deionized water, stopper and invert to mix.

To adjust the calibration curve using the reading obtained with the 4.00-mg/L standard solution, press the soft keys under **OPTIONS, MORE** then **STD: OFF**. Press **ENTER** to accept the displayed concentration, the value of which depends on the selected units. If an alternate concentration is used, enter the actual concentration and press **ENTER** to return to the read screen. See Section 1.5.5 *Adjusting the Standard Curve* for more information.

Method Performance

Precision

Standard: 4.00 mg/L Cu

Program	95% Confidence Limits
1700	3.987–4.013 mg/L Cu
1710	3.988–4.012 mg/L Cu

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
1700	0.021 mg/L Cu
1710	0.020 mg/L Cu

For more information on derivation and use of Hach's estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, Appendix B, see Section 1.5.1.

Sensitivity

Program Number: 1700

Portion of Curve	Δ Abs	Δ Concentration
Entire Range	0.010	0.0335 mg/L

Program Number 1710

Portion of Curve	Δ Abs	Δ Concentration
Entire Range	0.010	0.0376 mg/L

See Section 1.5.3 *Sensitivity Explained* for more information.

Calibration Standard Preparation

To perform a copper calibration using the bicinchoninate method, prepare a 40-mg/L copper stock solution by pipetting 4.0 mL of a 1000-mg/L Copper Standard Solution (Cat. No. 2593-42) into a 100-mL volumetric flask using Class A glassware. Dilute to the mark with deionized water and mix thoroughly.

Prepare calibration standards containing 0.400, 0.800, 1.600, 2.400, 3.200, 4.000 and 4.800 mg/L Cu as follows:

- Into seven different 100-mL Class A volumetric flasks, pipet 1.00, 2.00, 4.00, 6.00, 8.00, 10.00, and 12.00 mL of the 40-mg/L Cu stock solution using class A glassware.
- Dilute to the mark with deionized water and mix thoroughly.
- Using the bicinchoninate method and the calibration procedure described in the *User-Entered Programs* section of the *DR/4000 Spectrophotometer Instrument Manual*, generate a calibration curve from the standards prepared above.

Summary of Method

Copper in the sample reacts with a salt of bicinchoninic acid contained in CuVer 1 or CuVer 2 Copper Reagent to form a purple colored complex in proportion to the copper concentration.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to Section 1.

Pollution Prevention and Waste Management

For information on pollution prevention and waste management, refer to Section 1.

REQUIRED REAGENTS AND STANDARDS (Using Powder Pillows)

Description	Quantity Required per test	Unit	Cat. No.
CuVer 1 Copper Reagent Powder Pillows.....	1 pillow	100/pkg.....	21058-69

REQUIRED REAGENTS AND STANDARDS (Using AccuVac Ampuls)

CuVer 2 Copper Reagent AccuVac Ampuls.....	1 ampul	25/pkg.....	25040-25
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REQUIRED EQUIPMENT AND SUPPLIES (Using AccuVac Ampuls)

Beaker, 50-mL.....	1	each.....	500-41
DR/4000 AccuVac Ampul Adapter.....	1	each.....	48187-00
Sample Cell, 10-mL with cap (zeroing vial).....	1	each.....	21228-00

OPTIONAL REAGENTS AND STANDARDS

Copper, Free, Reagent Powder Pillows	100/pkg.....	21186-69
Copper Standard Solution, 100-mg/L	100 mL.....	128-42
Copper Standard Solution, 10-mL Voluette Ampule, 75 mg/L.....	16/pkg.....	14247-10
Copper Standard Solution, 1000-mg/L	100 mL.....	2593-42
CuVer 2 Reagent Powder Pillows	25/pkg.....	21882-68
Formaldehyde, 37%, ACS.....	100 mL * MDB	2059-32
Hydrochloric Acid, 6 N.....	500 mL.....	884-49
Hydrochloric Acid Standard Solution, 2.5 N.....	100 mL MDB.....	1418-32
Hydrosulfite Reagent Powder Pillows.....	100/pkg.....	21188-69
Nitric Acid, ACS	500 mL.....	152-49
Nitric Acid Solution, 1:1	500 mL	2540-49
Potassium-Chloride Solution, saturated	50 mL SCDB.....	765-26
Potassium Hydroxide Standard Solution, 8.0 N	100 mL * MDB	282-32
Sodium Hydroxide Standard Solution, 5.0 N.....	100 mL * MDB	2450-32
Water, deionized	4 liters.....	272-56

* Contact Hach for larger sizes.

OPTIONAL EQUIPMENT AND SUPPLIES

Description	Unit	Cat. No.
AccuVac Snapper Kit	each.....	24052-00
Ampule Breaker Kit	each.....	21968-00
Aspirator, Nalgene vacuum pump	each.....	2131-00
Beaker, 100-mL	each.....	500-42
Cylinder, graduated, polypropylene, 25-mL	each.....	1081-40
Cylinder, graduated, 100-mL	each.....	508-42
DR/4000 Carousel Module.....	each.....	48070-02
DR/4000 Flow Cell Module, 1-inch.....	each.....	48070-04
DR/4000 Flow Cell Module, 1-cm.....	each.....	48070-05
DR/4000 Sipper Module Kit, 1-inch	each.....	48090-03
Filter Paper, folded, 12.5-cm.....	100/pkg.....	1894-57
Flask, volumetric, 100-mL	each.....	547-42
Funnel, polypropylene, 65-mm	each.....	1083-67
Hot Plate, 3 in. diameter, 120 VAC, 50/60 Hz	each.....	12067-01
Hot Plate, 4 in.diameter, 240 VAC, 50/60 Hz	each.....	12067-02
pH Indicator Paper, pH 1.0 to 11.0.....	5 rolls/pkg.....	391-33
pH Meter, <i>sensio</i> TM 1, portable	each.....	51700-00
Pipet, TenSette, 0.1 to 1.0 mL	each.....	19700-01
Pipet Tips, for 19700-01 TenSette Pipet	50/pkg.....	21856-96
Pipet, volumetric, 1.00-mL, Class A	each.....	14515-35
Pipet, volumetric, 2.00-mL, Class A	each.....	14515-36
Pipet, volumetric, 4.00-mL, Class A	each.....	14515-04
Pipet, volumetric, 6.00-mL, Class A	each.....	14515-06
Pipet, volumetric, 8.00-mL, Class A	each.....	14515-08
Pipet, volumetric, 10.00-mL, Class A	each.....	14515-38
Pipet Filler, safety bulb.....	each.....	14651-00
Sample Cells, 1-inch, polystyrene, disposable	12/pkg.....	24102-12



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Method 8143

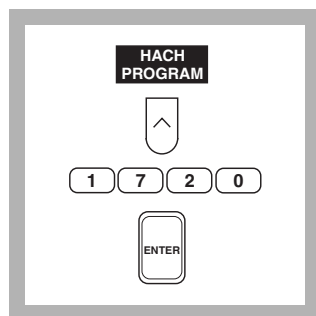
Porphyrin Method*

Powder Pillows

(0 to 210.0 µg/L)

Scope and Application: For water, wastewater and seawater; digestion is required for determining total copper. See SECTION 2 for digestion procedure. The estimated detection limit for program number 1720 is 1.4 mg/L Cu.

* Adapted from Ishii and Koh, *Bunseki Kagaku*, 28, 473 (1979)



1. Press the soft key under **HACH PROGRAM**.

Select the stored program for porphyrin copper by pressing **1720** with the numeric keys.

Press: **ENTER**

Note: If samples cannot be analyzed immediately, see Sample Collection, Storage and Preservation following these steps. Adjust the pH of preserved samples before analysis.

Note: The Flow Cell and Sipper Modules can be used for this procedure. Use a 25-mL sample and reagents with the Flow Cell Module.



2. The display will show: **HACH PROGRAM: 1720 Copper, Porphyrin**

The wavelength (λ), **425 nm**, is automatically selected.

Note: For best results, determine a reagent blank for each new lot of reagent as follows. Prepare a reagent blank by repeating Steps 3 through 10, using copper-free deionized water as the sample. Zero the instrument on deionized water by pressing the soft key under **ZERO**. Insert the reagent blank and the blank value will be displayed. Correct for the reagent blank by pressing the soft keys under **OPTIONS, (MORE)**, and then **BLANK:OFF**. Enter the reagent blank value and press **ENTER**. Repeat for each new lot of reagent.



3. Fill two sample cells with 10 mL of sample.

Note: Wash all glassware with detergent. Rinse with tap water. Rinse again with 1:1 Nitric Acid Solution. Rinse a third time with copper-free, deionized water.

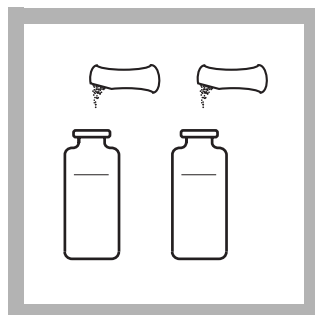
Note: For proof of accuracy, use a 150 µg/L copper standard solution (preparation given in the Accuracy Check section) in place of the sample.

Note: For non-preserved samples with extreme pH see Interferences section.



4. Add the contents of one Copper Masking Reagent Powder Pillow to one of the sample cells (the blank). Swirl to dissolve.

Note: The sample cell without masking agent is the prepared sample.



5. Add the contents of one Porphyrin 1 Reagent Powder Pillow to each sample cell.

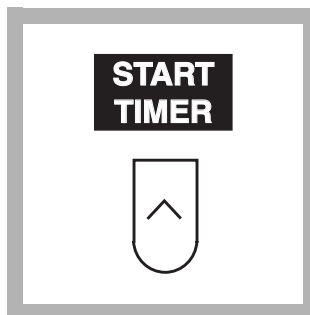
Swirl to dissolve.



6. Add the contents of one Porphyrin 2 Reagent Powder Pillow to each sample cell.

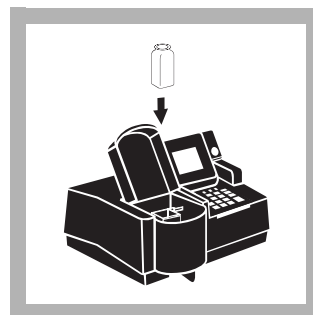
Swirl to dissolve.

Note: The yellow color will turn blue momentarily. If any copper is present, the sample will return to yellow.

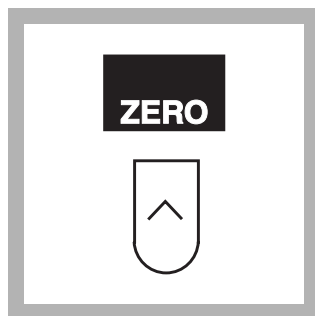


7. Press the soft key under **START TIMER**.

A 3-minute reaction period will begin.



8. When the timer beeps, place the blank into the cell holder. Close the light shield.



9. Press the soft key under **ZERO**.

The display will show:

0.0 µg/L Cu

Note: If you are using a reagent blank correction, the display will show the correction.

Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



10. Place the prepared sample into the cell holder. Close the light shield.

Results in µg/L copper (or chosen units) will be displayed.

Note: If samples with high levels of metal are analyzed, a slight metallic deposit or yellow buildup may appear on the sample cell wall. Remove by washing as recommended in Step 3.

Interferences

The following may interfere when present in concentrations exceeding the levels listed below.

Table 1 Interfering Substances and Suggested Treatments

Interfering Substance	Interference Levels and Treatments
Aluminum, Al ³⁺	60 mg/L
Cadmium, Cd ²⁺	10 mg/L
Calcium, Ca ²⁺	1500 mg/L
Chelating agents	Interfere at all levels unless either the Digesdahl or vigorous digestion is performed (see Section 2).
Chloride, Cl ⁻	90,000 mg/L
Chromium, Cr ⁶⁺	110 mg/L
Cobalt, Co ²⁺	100 mg/L
Fluoride, F ⁻	30,000 mg/L
Iron, Fe ²⁺	6 mg/L
Lead, Pb ²⁺	3 mg/L
Magnesium	10,000 mg/L
Manganese	140 mg/L
Mercury, Hg ²⁺	3 mg/L
Molybdenum	11 mg/L
Nickel, Ni ²⁺	60 mg/L
Potassium, K ⁺	60,000 mg/L
Sodium, Na ⁺	90,000 mg/L
Zinc, Zn ²⁺	9 mg/L
Highly buffered samples or extreme sample pH	May exceed the buffering capacity of the reagents and require sample pretreatment; (see Section 1.3.1 <i>pH Interferences</i> .)

Sample Collection, Storage and Preservation

Collect samples in acid-washed plastic bottles. To preserve, adjust the pH to 2 or less with nitric acid (about 5 mL per liter). Store preserved samples up to six months at room temperature. Before testing, adjust the pH of the preserved sample to between 2 and 6. If the sample is too acidic, adjust the pH with 5.0 N Sodium Hydroxide Standard Solution. Correct test results for volume additions; see Section 1.2.2 *Correcting for Volume Additions*.

Accuracy Check

Standard Additions Method

- Prepare a 4000-µg/L copper standard by adding 4.00 mL Copper Standard Solution, 100.0-mg/L, to a 100-mL volumetric flask. Dilute to 100 mL with copper-free deionized water.
- Leave the unspiked sample in the sample cell compartment. Verify that the units displayed are in µg/L. Select standard additions mode by pressing the soft keys under **OPTIONS, (MORE)** and then **STD ADD**.
- Press **ENTER** to accept the default sample volume (mL), 10.

- d. Press **ENTER** to accept the default standard concentration ($\mu\text{g/L}$), 4000.
- e. Press the soft key under **ENTRY DONE**.
- f. Fill eight sample cells with 10 mL of sample. Use the TenSette Pipet to add 0.1 mL of Copper Standard Solution, 4000- $\mu\text{g/L}$ Cu, to two of the sample cells. Then pipet 0.2 mL of the standard solution into two more cells. Finally, pipet 0.3 mL of the standard solution into two more cells.
- g. Analyze each standard addition sample as described above, using one of the two spiked samples in each set as the blank. Accept the standard additions reading by pressing the soft key under **READ** each time. The copper concentration reading should reflect approximately 100% recovery.
- h. After completing the sequence, the display will show the extrapolated concentration value and the “best-fit” line through the standard additions data points, accounting for matrix interferences.
- i. See Section 1.4.1 *Standard Additions* for more information.

Standard Solutions Method

To assure the accuracy of the test, prepare a 150- $\mu\text{g/L}$ copper standard by pipetting 15.00 mL of Copper Standard Solution, 10.0-mg/L Cu, into a 1000-mL volumetric flask. Dilute to the mark with copper-free, reagent-grade water. Prepare this solution daily. Perform the copper procedure as described above.

To adjust the calibration curve using the reading obtained with the 150 $\mu\text{g/L}$ standard solution, press the soft keys under **OPTIONS, MORE** then **STD: OFF**. Press **ENTER** to accept the displayed concentration, the value of which depends on the selected units. If an alternate concentration is used, enter the actual concentration and press **ENTER** to return to the read screen. See Section 1.5.5 *Adjusting the Standard Curve* for more information.

Method Performance

Precision

Standard: 150 $\mu\text{g/L}$ Cu

Program	95% Confidence Limits
1720	149.2–150.8 $\mu\text{g/L}$ Cu

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
1720	1.4 $\mu\text{g/L}$ Cu

For more information on derivation and use of Hach’s estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, Appendix B, see Section 1.5.1.

Sensitivity

Program Number: 1720

Portion of Curve:	Δ Abs	Δ Concentration
Entire Range	0.010	1.4 μ g/L Cu

See Section 1.5.3 *Sensitivity Explained* for more information.

Calibration Standard Preparation

To perform a copper calibration using the porphyrin method, prepare a 1000- μ g/L copper stock solution by pipetting 10 mL of a 100-mg/L Copper Standard Solution (Cat. No. 128-42) into a 1000-mL volumetric flask using Class A glassware. Dilute to the mark with deionized water and mix thoroughly.

Prepare calibration standards containing 20.0, 40.0, 80.0, 120.0, 160.0, 200.0 and 240.0 μ g/L Cu as follows:

- a. Into seven different 100-mL Class A volumetric flasks, pipet 2.00, 4.00, 8.00, 12.00, 16.00, 20.00 and 24.00 mL of the 1000- μ g/L Cu stock solution using Class A glassware.
- b. Dilute to the mark with deionized water, stopper and mix thoroughly.
- c. Using the porphyrin method and the calibration procedure described in the *User-Entered Programs* section of the *DR/4000 Spectrophotometer Instrument Manual*, generate a calibration curve from the standards prepared above.

Summary of Method

The porphyrin method is very sensitive to trace amounts of free copper. The method is free from most interferences and does not require any sample extraction or concentration before analysis. Interferences from other metals are eliminated by the copper masking reagent. The porphyrin indicator forms an intense, yellow-colored complex with any free copper present in sample.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to Section 1.

Pollution Prevention and Waste Management

For information on pollution prevention and waste management, refer to Section 1.

COPPER, continued

REQUIRED REAGENTS AND STANDARDS

	Cat. No.
Copper Reagent Set (100 Tests)	26033-00
Includes: (1) 26034-49, (2) 26035-49, (2) 26036-49	

Description	Quantity Required per test	Unit	Cat. No.
Copper Masking Reagent Powder Pillows	1 pillow	100/pkg	26034-49
Porphyrin 1 Reagent Powder Pillows	2 pillows	100/pkg	26035-49
Porphyrin 2 Reagent Powder Pillows	2 pillows	100/pkg	26036-49

REQUIRED EQUIPMENT AND SUPPLIES

DR/4000 1-Inch Cell Adapter	1	each	48190-00
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OPTIONAL REAGENTS AND STANDARDS

Copper Standard Solution, 100-mg/L Cu	100 mL	128-42
Copper Standard Solution, 10-mg/L Cu	100 mL	129-42
Hydrochloric Acid, 6 N	500 mL	884-49
Nitric Acid, ACS	500 mL	152-49
Nitric Acid Solution, 1:1	500 mL	2540-49
Sodium Hydroxide, 5 N	1 liter	2450-53
Sodium Hydroxide Standard Solution, 5 N	100 mL MDB	2450-32
Water, deionized	4 liters	272-56

OPTIONAL EQUIPMENT AND SUPPLIES

Beaker, 100-mL	each	500-42
Clipper, for opening powder pillows	each	968-00
DR/4000 Carousel Module Kit	each	48070-02
DR/4000 Flow Cell Module Kit, 1-inch	each	48070-04
DR/4000 Flow Cell Module Kit, 1-cm	each	48070-05
DR/4000 Sipper Module Kit, 1-inch	each	48090-03
Flask, volumetric, Class A, 100-mL	each	14574-42
Flask, volumetric, Class A, 1000-mL	each	14574-53
Hot Plate, 7 x 7 in., 120 VAC, 50/60 Hz	each	23441-00
Hot Plate, 7 x 7 in., 240 VAC, 50/60 Hz	each	23441-02
pH Paper, pH 1.0 to 11.0	5 rolls/pkg	391-33
pH Meter, <i>sens^{ion}</i> TM 1, portable	each	51700-00
Pipet, Mohr, 5-mL	each	20934-37
Pipet, TenSette, 0.1 to 1.0 mL	each	19700-01
Pipet Tips, for 19700-01	50/pkg	21856-96
Pipet, volumetric, Class A, 2-mL	each	14515-36
Pipet, volumetric, Class A, 4-mL	each	14515-04
Pipet, volumetric, Class A, 6-mL	each	14515-06
Pipet, volumetric, Class A, 8-mL	each	14515-08
Pipet, volumetric, Class A, 10-mL	each	14515-38
Pipet, volumetric, Class A, 20-mL	each	14515-20
Pipet Filler, safety bulb	each	14651-00
Watch Glass, Pyrex, 100-mm	each	578-70



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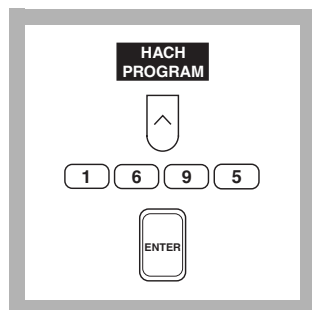
Bathocuproine Method

UniCell™ Vials

(0 to 6.00 mg/L)

Scope and Application: For water, wastewater, raw water, and process control. The Metal Prep Set (HCT 200) digestion is required for total copper. The estimated detection limit for program number 1695 is 0.10 mg/L free Cu.

UniCell™ Vials



1. Press the soft key under **HACH PROGRAM**.

Select the stored program for Copper (HCT 163) by pressing **1695** with the numeric keys.

Press: **ENTER**

Note: If samples cannot be analyzed immediately, see *Sample Collection, Storage and Preservation* following these steps. Adjust the pH of preserved samples before analysis.

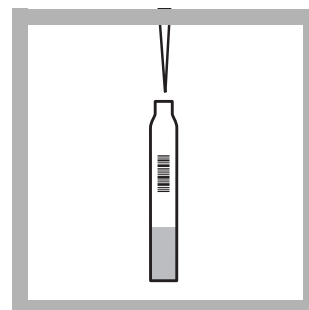


2. The display will show: **HACH PROGRAM: 1695 Copper, HCT 163**

The wavelength (λ), **478 nm**, is automatically selected.



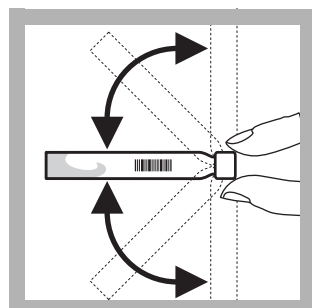
3. Insert the Test Tube Adapter into the sample cell module by sliding it under the thumb screw and into the alignment grooves. Fasten with the thumb screw.



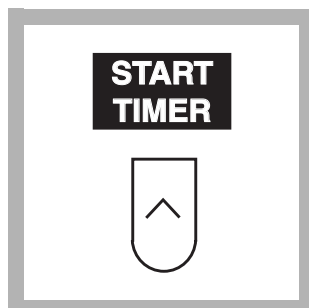
4. Pipet 4 mL of sample into a sample vial.

Note: For proof of accuracy, use a 2.00-mg/L copper standard solution (preparation given in the *Accuracy Check* section) in place of the sample.

Note: The chemistry is pH sensitive. Adjust the pH of acid-preserved samples to 3–6 with 8 N KOH before analysis.



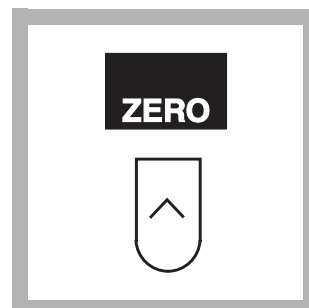
5. Cap and invert the vial until the solid in the cap is completely dissolved.



6. Press the soft key under **START TIMER**. A 3-minute reaction period will begin.



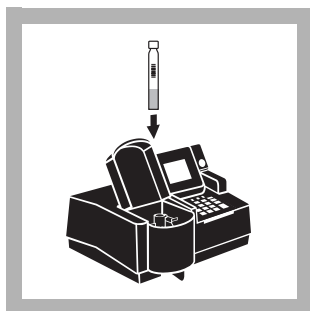
7. When the timer beeps, wipe the zero vial (**white** cap) and place it into the cell holder.



8. Press the soft key under **ZERO**.

The display will show: **0.00 mg/L Free Cu**

Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



9. Wipe the sample vial and place it into the cell holder. Close the light shield. Results in mg/L free copper (or chosen units) will be displayed.

Interference

The ions listed in the table have been tested up to the given concentrations. Cumulative effects and the influence of other ions have not been evaluated. There is no interference from:

Ion	No interference up to:
Cl^- , NO_3^- , SO_4^{2-}	1000 mg/L
Mg^{2+} , NH_4^+ , Ca^{2+} , PO_4^{3-} , CO_3^{2-} , NO_2^- , K^+ , Na^+	500 mg/L
Zn^{2+} , Cd^{2+} , Ni^{2+} , Pb^{2+}	50 mg/L
Cr^{3+} , Cr^{6+}	25 mg/L
Fe^{2+} , Fe^{3+}	15 mg/L
Sn^{2+} , Hg^{2+}	5 mg/L

Higher amounts of iron and chromium cause a positive interference.

Note: Total copper including undissolved copper and complexed copper can only be determined after digesting with the Metal Prep Set HCT 200. (Total copper measuring range 0.12 – 7.2 mg/L.)

Sample Collection, Storage and Preservation

Collect samples in acid-cleaned or plastic containers. No acid addition is necessary if analyzing the samples immediately. To preserve samples, adjust the pH to 2 or less with concentrated nitric acid (about 2 mL per liter). Preserved samples may be stored up to six months at room temperature.

Before analysis, adjust the pH to between 3 and 6 with 5.0 N Sodium Hydroxide Standard Solution. Do not exceed pH 6 or copper may precipitate.

Accuracy Check

Standard Additions Method

- a. Leave the unspiked sample in the sample cell compartment. Verify that the units displayed are in mg/L. Select standard additions mode by pressing the soft keys under **OPTIONS, (MORE)** and then **STD ADD**.
- b. Press **ENTER** to accept the default sample volume (mL), 100.
- c. Press **ENTER** to accept the default standard concentration (mg/L), 1000.0.
- d. Press the soft key under **ENTRY DONE**.
- e. Use a pipet to add 0.1, 0.2 mL and 0.3 mL of 1000-mg/L Cu standard, respectively, to three 100-mL mixing cylinders containing samples and mix each thoroughly.
- f. Analyze a 4-mL portion of each standard addition sample as described in the procedure. Accept the standard additions reading by pressing the soft key under **READ** each time. Each addition should reflect approximately 100% recovery.
- g. After completing the sequence, the display will show the extrapolated concentration value and the “best-fit” line through the standard additions data points, accounting for matrix interferences.

See Section 1.4.1 *Standard Additions* for more information.

Standard Solutions Method

Prepare a 2.0-mg/L Cu standard solution by pipetting 0.2 mL of 1000-mg/L Cu standard into a 100-mL volumetric flask. Dilute to the mark with deionized water. Stopper and invert to mix. Prepare this solution daily. Perform the copper procedure as described.

To adjust the calibration curve using the reading obtained with the 2.00-mg/L standard solution, press the soft keys under **OPTIONS, (MORE)** then **STD: OFF**. Press **ENTER** to accept the displayed concentration, the value of which depends on the selected units. If an alternate concentration is used, enter the actual concentration and press **ENTER** to return to the read screen. See Section 1.5.5 *Adjusting the Standard Curve* for more information.

Method Performance

Precision

Standard: 2.00 mg/L free Cu

Program	95% Confidence Limits
1695	1.76–2.24 mg/L free Cu

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
1695	0.10 mg/L free Cu

For more information on derivation and use of Hach's estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, Appendix B, see Section 1.5.1.

Sensitivity

Program Number: 1695

Portion of Curve	Δ Abs	Δ Concentration
Entire Range	0.010	0.041 mg/L

See Section 1.5.3 *Sensitivity Explained* for more information.

Summary of Method

Copper(I) ions form an orange complex with the disodium salt of bathocuproine disulphonic acid. Any copper(II) ions present in the water sample are reduced to copper(I) ions by ascorbic acid before the complex is formed.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the Material Safety Data Sheet for information specific to the reagents used

Pollution Prevention and Waste Management

For information on pollution prevention and waste management, refer to Section 1.

REQUIRED EQUIPMENT AND SUPPLIES

Description	Unit	Cat. No.
Copper-Cu, UniCell™ HCT 163.....	23/pkg.....	HCT 163

OPTIONAL REAGENTS AND STANDARDS

Metal-Prep-Set, UniCell™ HCT 200.....	50 digestions.....	HCT 200
Copper Standard, 1000-mg/L as Cu.....	100 mL.....	2593-42
Sodium Hydroxide, 5 N	1L.....	2450-53
Nitric Acid Solution, 1:1	500 mL.....	2540-49

OPTIONAL APPARATUS

DRB 100, Digital Reactor Block.....	each.....	DRB 100
Graduated cylinder, mixing, 100-mL	each.....	20886-42
Flask, volumetric, 100-mL	each.....	14574-42
Pipettor, (Jencons) 1–5 mL	each.....	27951-00
Replacement tips for 27951-00	pk/100.....	27952-00
Pipettor, (Jencons) 100–1000 μ L.....	each.....	27949-00
Replacement tips for 27949-00	pk/400.....	27950-00
pH Paper	pk/100.....	26013-00



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HACH COMPANY
WORLD HEADQUARTERS
Telephone: (970) 669-3050
FAX: (970) 669-2932



Method 8027

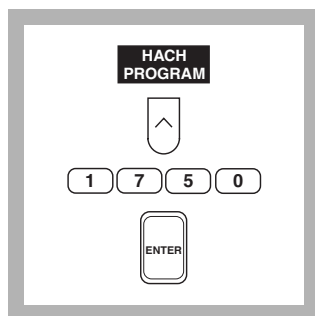
Pyridine-Pyrazalone Method*

Powder Pillows

(0 to 0.240 mg/L CN⁻)

Scope and Application: For water, wastewater and seawater. The estimated detection limit for program number 1750 is 0.003 mg/L CN⁻.

* Adapted from Epstein, Joseph, *Anal. Chem.* 19 (4), 272 (1947)



1. Press the soft key under **HACH PROGRAM**.

Select the stored program for cyanide by pressing **1750** with the numeric keys.

Press: **ENTER**

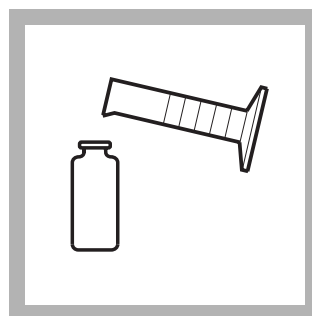
Note: If samples cannot be analyzed immediately, see *Sample Collection, Storage and Preservation* following these steps. Adjust the pH of preserved samples before analysis.

Note: The Flow Cell and Sipper Modules can be used for this procedure.



2. The display will show: **HACH PROGRAM: 1750 Cyanide**

The wavelength (λ), **612 nm**, is automatically selected.



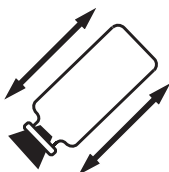
3. Using a graduated cylinder, fill a sample cell with 10 mL of sample.

Note: For proof of accuracy, use a 0.10-mg/L cyanide standard solution (preparation given in the Accuracy Check section) in place of the sample.



4. Add the contents of one CyaniVer 3 Cyanide Reagent Powder Pillow. Stopper the sample cell.

30 seconds



5. Shake the sample cell for 30 seconds.

30 seconds

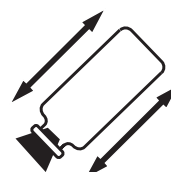


6. Wait an additional 30 seconds, leaving the sample cell undisturbed.



7. Add the contents of one CyaniVer 4 Cyanide Reagent Powder Pillow. Stopper the sample cell.

10 seconds



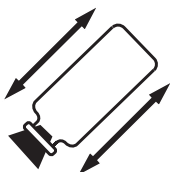
8. Shake the sample cell for 10 seconds. Immediately proceed with Step 9.

Note: Delaying the addition of the CyaniVer 5 Cyanide Reagent Powder for more than 30 seconds after the addition of the CyaniVer 4 Cyanide Reagent Powder will give lower test results.

Note: Accuracy is not affected by undissolved CyaniVer 4 Cyanide Reagent Powder.



9. Add the contents of one CyaniVer 5 Cyanide Reagent Powder Pillow. Stopper the cell.



10. Shake the cell vigorously.

Note: If cyanide is present, a pink color will develop which then turns blue after a few minutes.

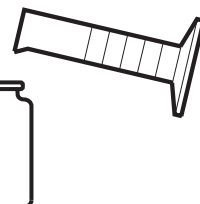
START
TIMER



11. Press the soft key under **START TIMER**.

A 30-minute reaction period will begin.

Note: Samples at less than 23 °C require longer reaction time and samples at greater than 25 °C give low test results.



12. When the timer beeps, fill another sample cell (the blank) with 10 mL of sample.



13. Place the blank into the cell holder. Close the light shield.

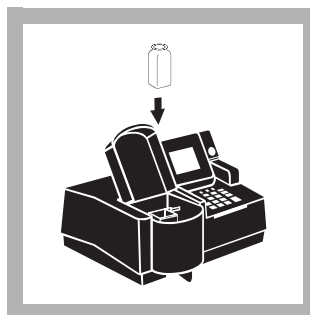


14. Press the soft key under **ZERO**.

The display will show:

0.000 mg/L Cu

Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



15. Place the prepared sample into the cell holder. Close the light shield. Results in mg/L cyanide (or chosen units) will be displayed.

Note: See Pollution Prevention and Waste Management following these steps for proper disposal of solutions containing cyanide.

Do not pour these solutions down the drain!

Interferences

Table 1 Interfering Substances and Suggested Treatments for Powder Pillows

Interfering Substance	Interference Levels and Treatments
Chlorine	Large amounts of chlorine in the sample will cause a milky white precipitate after the addition of the CyaniVer 5 Reagent. If chlorine or other oxidizing agents are known to be present, pretreat the sample before testing using the procedure in this table for oxidizing agents.
Metals	Nickel or cobalt in concentrations up to 1 mg/L do not interfere. Eliminate the interference from up to 20 mg/L copper and 5 mg/L iron by adding the contents of one HexaVer Chelating Reagent Powder Pillow to the sample and then mixing before adding the CyaniVer 3 Cyanide Reagent Powder Pillow in Step 4. Prepare a reagent blank of deionized water and reagents to zero the instrument in Step 14.
Oxidizing Agents	a) Adjust a 25-mL portion of the alkaline sample to pH 7–9 with 2.5 N Hydrochloric Acid Standard Solution. Count the number of drops of acid added. b) Add two drops of Potassium Iodide Solution and two drops of Starch Indicator Solution to the sample. Swirl to mix. The sample will turn blue if oxidizing agents are present. c) Add Sodium Arsenite Solution drop-wise until the sample turns colorless. Swirl the sample thoroughly after each drop. Count the number of drops. d) Take another 25-mL sample and add the total number of drops of Hydrochloric Acid Standard Solution counted in Step a. e) Subtract one drop from the amount of Sodium Arsenite Solution added in Step c. Add this amount to the sample and mix thoroughly. Continue with Step 4 of the cyanide procedure.
Reducing Agents	a) Adjust a 25-mL portion of the alkaline sample to pH 7–9 with 2.5 N Hydrochloric Acid Standard Solution. Count the number of drops added. b) Add four drops of Potassium Iodide Solution and four drops of Starch Indicator Solution to the sample. Swirl to mix. The sample should be colorless. c) Add Bromine Water drop-wise until a blue color appears. Swirl the sample thoroughly after each addition. Count the number of drops. d) Take another 25-mL sample and add the total number of drops of Hydrochloric Acid Standard Solution counted in Step a. e) Add the total number of drops of Bromine Water counted in Step c to the sample and mix thoroughly. f) Continue with Step 4 of the cyanide procedure.
Turbidity	Large amounts of turbidity will cause high readings. Filter highly turbid water samples before use in Steps 3 and 12, using the labware listed under <i>OPTIONAL EQUIPMENT AND SUPPLIES</i> . The test results should then be recorded as soluble cyanide.

Sample Collection, Storage and Preservation

Collect samples in glass or plastic bottles and analyze as quickly as possible.

The presence of oxidizing agents, sulfides and fatty acids can cause the loss of cyanide during sample storage. Samples containing these substances must be pretreated as described in the following procedures before preservation with sodium hydroxide. If the sample contains sulfide and is not pretreated, it must be analyzed within 24 hours.

Preserve the sample by adding 4.0 mL of 5.0 N Sodium Hydroxide Standard Solution to each liter (or quart) of sample, using a glass serological pipet and pipet filler. Check the sample pH. An addition of 4-mL of sodium hydroxide is usually enough to raise the pH of most water and wastewater samples to 12. Add more 5.0 N Sodium Hydroxide if necessary. Store the samples at 4 °C (39 °F) or less. Samples preserved in this manner can be stored for 14 days.

Before testing, samples preserved with 5.0 N Sodium Hydroxide or samples that are highly alkaline due to chlorination treatment processes or sample distillation procedures should be adjusted to approximately pH 7 with 2.5 N Hydrochloric Acid Standard Solution. Where significant amounts of preservative

are used, a volume correction should be made; see Section 1.2.2 *Correcting for Volume Additions*.

Oxidizing Agents

Oxidizing agents such as chlorine decompose cyanides during storage. To test for their presence and to eliminate their effect, pretreat the sample as follows:

- a. Take a 25-mL portion of the sample and add one drop of m-Nitrophenol Indicator Solution, 10-g/L. Swirl to mix.
- b. Add 2.5 N Hydrochloric Acid Standard Solution drop-wise until the color changes from yellow to colorless. Swirl the sample thoroughly after the addition of each drop.
- c. Add two drops of Potassium Iodide Solution, 30-g/L, and two drops of Starch Indicator Solution, to the sample. Swirl to mix. The solution will turn blue if oxidizing agents are present.
- d. If *Step c* suggests the presence of oxidizing agents, add two level 1-g measuring spoonfuls of ascorbic acid per liter of sample.
- e. Withdraw a 25-mL portion of sample treated with ascorbic acid and repeat *Steps a* to *c*. If the sample turns blue, repeat *Steps d* and *e*.
- f. If the 25-mL sample remains colorless, preserve the remaining sample to pH 12 for storage with 5 N Sodium Hydroxide Standard Solution (usually 4 mg/L).
- g. Perform the procedure given under *Interferences, Reducing Agents* to eliminate the effect of excess ascorbic acid, before following the cyanide procedure.

Sulfides

Sulfides will quickly convert cyanide to thiocyanate (SCN). To test for the presence of sulfide and eliminate its effect, pretreat the sample as follows:

- a. Place a drop of sample on a disc of hydrogen sulfide test paper that has been wetted with pH 4 Buffer Solution.
- b. If the test paper darkens, add a 1-g measuring spoon of lead acetate to the sample. Repeat *Step a*.
- c. If the test paper continues to turn dark, keep adding lead acetate until the sample tests negative for sulfide.
- d. Filter the lead sulfide precipitate through filter paper and a funnel. Preserve the sample for storage with 5 N Sodium Hydroxide Standard Solution or neutralize to a pH of 7 for analysis.

Fatty Acids

Caution: perform this operation in a hood as quickly as possible

When distilled, fatty acids will pass over with cyanide and form soaps under the alkaline conditions of the absorber. If the presence of fatty acid is suspected, do not preserve samples with sodium hydroxide until the following pretreatment is performed. The effect of fatty acids can be minimized as follows:

- a. Acidify 500 mL of sample to pH 6 or 7 with Acetic Acid Solution.
- b. Pour the sample into a 1000-mL separatory funnel and add 50 mL of hexane.

- c. Stopper the funnel and shake for one minute. Allow the layers to separate.
- d. Drain off the sample (lower) layer into a 600-mL beaker. If the sample is to be stored, add 5 N Sodium Hydroxide Standard Solution to raise the pH to above 12.

Accuracy Check

Standard Additions Method

Caution: *Cyanides and their solutions, and the hydrogen cyanide liberated by acids, are very poisonous. Both the solutions and the gas can be absorbed through the skin.*

Prepare a 100-mg/L cyanide stock solution weekly by dissolving 0.1884 grams or an equivalent amount of pure sodium cyanide in deionized water and diluting to 1000 mL.

Immediately before use prepare a 0.200-mg/L cyanide working solution by diluting 2.00 mL of the 100-mg/L stock solution to 1000 mL using deionized water.

To adjust the calibration curve using the reading obtained with the 0.200 mg/L standard solution press the soft keys under **OPTIONS, (MORE)** and then **STD:OFF**. Press **ENTER** to accept the default concentration, the value of which will depend on the selected units. If an alternate concentration is used, enter the actual concentration and press **ENTER** to return to the read screen. See Section 1.5.5 *Adjusting the Standard Curve* for more information.

Method Performance

Precision

Standard: 0.200 mg/L CN⁻

Program	95% Confidence Limits
1750	0.198–0.202 mg/L CN ⁻

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
1750	0.003 mg/L CN ⁻

For more information on derivation and use of Hach's estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, appendix B, see Section 1.5.1.

Sensitivity

Program Number: 1750

Portion of Curve	ΔAbs	ΔConcentration
Entire Range	0.010	0.0013 mg/L CN ⁻

See Section 1.5.3 *Sensitivity Explained* for more information.

Acid Distillation

All samples to be analyzed for cyanide should be treated by acid distillation except when experience has shown that there is no difference in results obtained with or without distillation. A one-hour reflux is adequate with most compounds.

If thiocyanate is present in the original sample, a distillation step is absolutely necessary because thiocyanate causes a positive interference. High concentrations of thiocyanate can yield a substantial quantity of sulfide in the distillate.

The “rotten egg” smell of hydrogen sulfide will accompany the distillate when sulfide is present. The sulfide must be removed from the distillate prior to testing.

If cyanide is not present, the amount of thiocyanate can be determined. The sample is not distilled and the final reading is multiplied by 2.2. The result is mg/L SCN^- .

The distillate can be tested and treated for sulfide after the last step of the distillation procedure by using the following lead acetate treatment procedure.

- a. Place a drop of the distillate (already diluted to 250 mL) on a disc of hydrogen sulfide test paper that has been wetted with pH 4.0 Buffer Solution.
- b. If the test paper darkens, add 2.5 N Hydrochloric Acid Standard Solution drop-wise to the distillate until a neutral pH is obtained.
- c. Add a 1-g measuring spoon of lead acetate to the distillate and mix. Repeat Step a.
- d. If the test paper continues to turn dark, keep adding lead acetate until the distillate tests negative for sulfide.
- e. Filter the black lead sulfide precipitate through filter paper and funnel. Neutralize the liquid filtrate to pH 7 and analyze for cyanide without delay.

Distillation Procedure

The following steps describe the distillation process using apparatus offered by Hach:

- a. Set up the distillation apparatus for cyanide recovery, leaving off the thistle tube. Refer to the *Hach Distillation Apparatus Manual*. Turn on the water and make certain it is flowing steadily through the condenser.
- b. Fill the distillation apparatus cylinder to the 50-mL mark with 0.25 N Sodium Hydroxide Standard Solution.
- c. Fill a clean 250-mL graduated cylinder to the 250-mL mark with sample and pour it into the distillation flask. Place a stirring bar into the flask and attach the thistle tube.
- d. Arrange the vacuum system as shown in the *Hach Distillation Apparatus Manual*, but do not connect the vacuum tubing to the gas bubbler. Turn on the water to the aspirator to full flow and adjust the flow meter to 0.5 SCFH.
- e. Connect the vacuum tubing to the gas bubbler, making certain that air flow is maintained (check the flow meter) and that air is bubbling from the thistle tube and the gas bubbler.

- f. Turn the power switch on and set the stir control to 5. Using a 50-mL graduated cylinder, pour 50 mL of 19.2 N Sulfuric Acid Standard Solution through the thistle tube and into the distillation flask.
- g. Using a water bottle, rinse the thistle tube with a small amount of deionized water.
- h. Allow the solution to mix for three minutes; then add 20 mL Magnesium Chloride Reagent through the thistle tube and rinse again. Allow the solution to mix for 3 more minutes.
- i. Verify that there is a constant flow of water through the condenser.
- j. Turn the heat control to 10.
- k. It is very important to monitor the distillation flask at this point in the procedure. Once the sample begins to boil, slowly lower the air flow to 0.3 SCFH. If the contents of the distillation flask begin to back up through the thistle tube, increase the air flow by adjusting the flow meter until the contents no longer back up through the thistle tube. Allow the sample to boil for one hour.
- l. When one hour has elapsed, turn the still off but maintain the air flow for 15 minutes.
- m. After 15 minutes, remove the rubber stopper on the 500-mL vacuum flask to break the vacuum and turn off the water to the aspirator. Turn off the water to the condenser.
- n. Remove the gas bubbler/cylinder assembly from the distillation apparatus. Separate the gas bubbler from the cylinder and pour the contents of the cylinder into a 250-mL, Class A volumetric flask. Rinse the gas bubbler, the cylinder, and J-tube connector with deionized water and add the washings to the volumetric flask.
- o. Fill the flask to the mark with deionized water and mix thoroughly. Neutralize the contents of the flask and analyze for cyanide.

Calibration Standard Preparation

To perform a cyanide calibration using the Pyridine-Pyrazalone method, prepare calibration standard containing 0.05, 0.100, and 0.200 mg/L cyanide as follows:

- a. Prepare a 100-mg/L cyanide stock solution as described in the *Accuracy Check*.
- b. Into three different 1000-mL Class A volumetric flasks, pipet 0.50, 1.00 and 2.00 mL of the 100-mg/L cyanide stock solution, respectively. Use Class A pipets.
- c. Dilute each flask to volume with deionized water. Stopper and invert several times to mix.
- d. Using the Pyridine-Pyrazalone method and the calibration procedure described in the *User-Entered Programs* section of the *DR/4000 Spectrophotometer Instrument Manual*, generate a calibration curve from the standards prepared above.

Summary of Method

The Pyridine-Pyrazalone method used for measuring cyanide gives an intense blue color with free cyanide. A sample distillation is required to determine cyanide from transition and heavy metal cyanide complexes.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to Section 1.

Pollution Prevention and Waste Management

Special Considerations for Cyanide Containing Materials

Samples analyzed by this procedure may contain cyanide, which is regulated as reactive (D003) waste by the federal RCRA. It is imperative these materials be handled safely to prevent release of hydrogen cyanide gas (an extremely toxic material with the smell of almonds). Most cyanide compounds are stable and can be safely stored for disposal in highly alkaline solutions (pH >11) such as 2 N sodium hydroxide. Never mix these wastes with other laboratory wastes which may contain lower pH materials such as acids or even water.

In the event of a spill or release, special precautions must be taken to prevent exposure to hydrogen cyanide gas. The following steps may be taken to destroy the cyanide compounds in the event of an emergency:

- a. Use a fume hood or supplied air or self contained breathing apparatus.
- b. While stirring, add the waste to a beaker containing a strong solution of sodium hydroxide and calcium hypochlorite or sodium hypochlorite (household bleach).
- c. Maintain a strong excess of hydroxide and hypochlorite. Let the solution stand for 24 hours.
- d. Neutralize and flush the solution down the drain with a large excess of water. Note: if the solution contains other regulated materials such as chloroform or heavy metals, it may still need to be collected for hazardous waste disposal. Never flush hazardous wastes down the drain.

REQUIRED REAGENTS AND STANDARDS

Cat. No.

Cyanide Reagent Set

Includes: (1) 21068-69, (1) 21069-69, (1) 21070-6924302-00

Description	Quantity Required		Cat. No.
	per test	Unit	
CyaniVer 3 Cyanide Reagent Powder Pillows	1 pillow	100/pkg	21068-69
CyaniVer 4 Cyanide Reagent Powder Pillows	1 pillow	100/pkg	21069-69
CyaniVer 5 Cyanide Reagent Powder Pillows	1 pillow	100/pkg	21070-69

REQUIRED EQUIPMENT AND SUPPLIES

Cylinder, graduated, 10-mL	1	each.....	508-38
DR/4000 1-Inch Cell Adapter	1	each.....	48190-00
Stoppers, rubber, No. 1	1	12/pkg.....	2118-01

OPTIONAL REAGENTS AND STANDARDS

Acetic Acid Solution, 10%, Alpha.....	500 mL.....	14816-49
Ascorbic Acid.....	100 g.....	6138-26
Bromine Water, 30-g/L.....	29 mL.....	2211-20
Buffer Solution, pH 4.00.....	500 mL.....	12223-49
Hexanes, ACS.....	4 liters.....	14478-17
HexaVer Chelating Reagent Powder Pillows.....	100/pkg.....	243-99
Hydrochloric Acid Standard Solution, 2.5 N.....	100 mL MDB.....	1418-32
Lead Acetate, trihydrate, ACS.....	500 g.....	7071-34
Magnesium Chloride Solution, 51%.....	1000 mL.....	14762-53
m-Nitrophenol Indicator Solution, 10-g/L, pH 7.0-8.4.....	100 mL MDB.....	2476-32
Potassium Iodide Solution, 30-g/L.....	100 mL MDB.....	343-32
Sodium Arsenite Solution, APHA, 5-g/L.....	100 mL MDB.....	1047-32
Sodium Cyanide, ACS.....	28 g.....	184-20
Sodium Hydroxide Standard Solution, 0.250 N.....	1000 mL.....	14763-53
Sodium Hydroxide Standard Solution, 5.0 N.....	1 L.....	2450-53
Starch Indicator Solution.....	100 mL MDB.....	349-32
Sulfuric Acid Standard Solution, 19.2 N.....	500 mL.....	2038-49
Water, deionized.....	4 liters.....	272-56

OPTIONAL EQUIPMENT AND SUPPLIES

Description	Unit	Cat. No.
Beaker, glass, 600-mL.....	each.....	500-52
Bottle, wash, 500-mL.....	each.....	620-11
Cylinder, graduated, 50-mL.....	each.....	508-41
Cylinder, graduated, 250-mL.....	each.....	508-46
Distillation Apparatus Set, Cyanide.....	each.....	22658-00
Distillation Apparatus Set, general purpose.....	each.....	22653-00
Distillation Apparatus Heater and Support Set, 115 VAC, 50/60 Hz.....	each.....	22744-00
Distillation Apparatus Heater and Support Set, 230 VAC, 50/60 Hz.....	each.....	22744-02
DR/4000 Carousel Module Kit.....	each.....	48090-02
DR/4000 Flow Cell Module Kit, 1-inch.....	each.....	48090-04
DR/4000 Sipper Module Kit, 1-inch.....	each.....	48090-03
Dropper, plastic.....	each.....	6080-00
Filter Paper, folded, 12.5-cm.....	100/pkg.....	1894-57
Flask, volumetric, Class A, 250-mL.....	each.....	14574-46
Flask, volumetric, Class A, 1000-mL.....	each.....	14574-53
Funnel, poly, 65-mm.....	each.....	1083-67
Funnel, separatory, 500-mL.....	each.....	520-49
Hydrogen Sulfide Test Papers.....	100/pkg.....	25377-33
pH Meter, <i>sension</i> TM 1, portable.....	each.....	51700-00
Pipet, volumetric, Class A, 0.5-mL.....	each.....	14515-34
Pipet, volumetric, Class A, 1.00-mL.....	each.....	14515-35
Pipet, volumetric, Class A, 2.00-mL.....	each.....	14515-36
Pipet, serological, 5-mL.....	each.....	532-37
Pipet Filler, safety bulb.....	each.....	14651-00
Scoop, double ended, 7.....	each.....	12257-00
Spoon, measuring, 1.0 g.....	each.....	510-00
Support Ring, 4-inch.....	each.....	580-01
Support Ring Stand, 5 x 8 in. base.....	each.....	563-00



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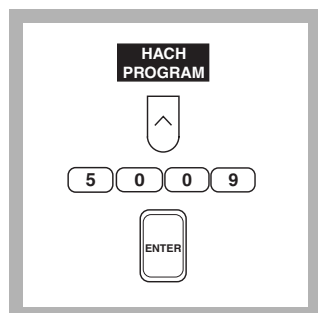
Cyanogen Chloride*

(0 to 0.50 mg/L CN⁻)

UniCell™ Vials

Scope and Application: For wastewater process control. The estimated detection limit for program number 5009 is 0.01 mg/L CN⁻.

* Reagent sets for this method are only available in Europe.



1. Press the soft key under **HACH PROGRAM**.

Select the stored program for UniCell Cyanide by pressing **5009** with the numeric keys.

Press: **ENTER**

Note: If samples cannot be analyzed immediately, see Sample Collection, Storage and Preservation following these steps. Adjust the pH of preserved samples before analysis.

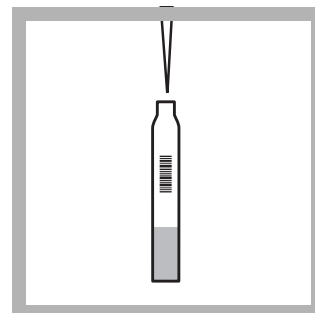


2. The display will show:
**HACH PROGRAM: 5009
Cyanide, HCT 129**

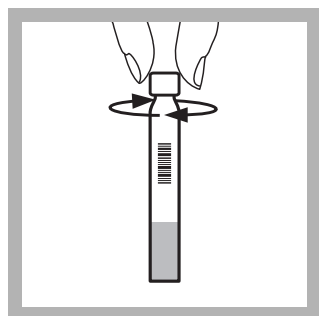
The wavelength (λ), **588 nm**, is automatically selected.



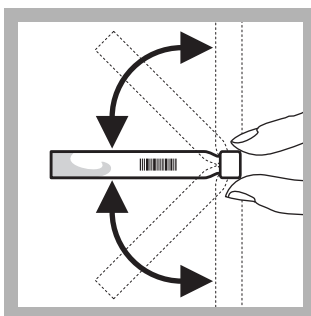
3. Insert the Test Tube Adapter into the sample cell module by sliding it under the thumb screw and into the alignment grooves. Fasten with the thumb screw.



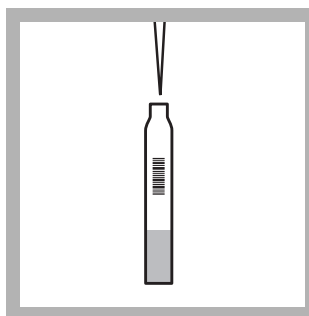
4. Pipet 2.0 mL of sample into a sample vial.



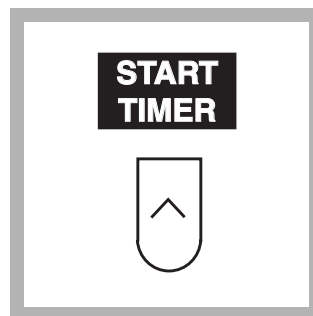
5. Immediately cap the sample vial with the **grey** UniCap A (HCT 129 A).



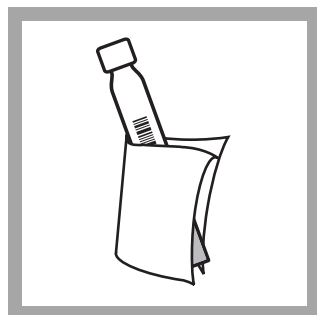
6. Invert the sample vial repeatedly until the reagent in the cap is completely dissolved.



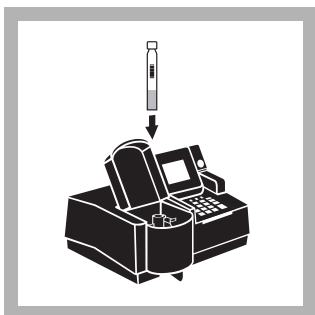
7. Remove the **grey** cap and pipet 2.0 mL of Pyridine hydrochloric acid B (HCT 129 B) into the sample vial. Recap the vial and invert several times to mix.



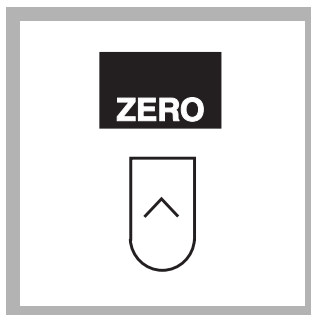
8. Press the soft key under **START TIMER**. An 8-minute reaction period will begin.



9. Wipe the zero vial (**white** cap) and the sample vial with a damp cloth followed by a dry one.



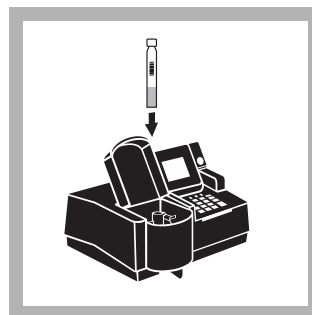
10. Place the zero vial into the cell holder. Close the light shield.



11. Press the soft key under **ZERO**.

The display will show:

0.00 mg/L CN⁻



12. Place the prepared sample into the cell holder. Close the light shield. Results in mg/L cyanide will be displayed.

Interferences

Interferences are caused by substances like formaldehyde which react with the cyanide. Reducing agents (like sulphite) and other compounds that react with the action of chlorine also interfere. Thiocyanate and cyanide react similarly with chlorine to form cyanogen chloride, thus the presence of thiocyanate will be a positive interference in the test.

Sample Collection, Storage and Preservation

Samples collected in glass or plastic containers should be analyzed as quickly as possible. Preserve the sample by adjusting the pH higher than 12 by adding 5.0 Sodium Hydroxide Standard Solution (4 mL per 1000 mL). Store the samples at 4 °C (39 °F) or less. The shelf life can be extended to 24 months if kept at 4 °C.

Accuracy Check

Caution: *Cyanides and their solutions, and the hydrogen cyanide liberated by acids, are very poisonous. Both the solutions and the gas can be absorbed through the skin.*

Prepare a 100-mg/L cyanide stock solution weekly by dissolving 0.1884 grams, or an equivalent amount of pure sodium cyanide, in deionized water and diluting to 1000 mL.

Immediately before used, prepare a 0.300-mg/L working solution by diluting 3.00 mL of the 100-mg/L stock solution to 1000 mL using deionized water.

To adjust the calibration curve using the reading obtained with the 0.30 mg/L standard solution press the soft keys under **OPTIONS, (MORE)** and then **STD:OFF**. Press **ENTER** to accept the default concentration, the value of which will depend on the selected units. If an alternate concentration is used, enter the actual concentration and press **ENTER** to return to the read screen. See Section 1.5.5 *Adjusting the Standard Curve* for more information.

Method Performance

Precision

Standard: 0.30 mg/L CN⁻

Program	95% Confidence Limits
5009	0.23–0.37 mg/L CN ⁻

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
5009	0.01 mg/L CN ⁻

For more information on derivation and use of Hach's estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, appendix B, see Section 1.5.1.

Sensitivity

Program Number: 5009

Portion of Curve	ΔAbs	ΔConcentration
Entire Range	0.010	0.003 mg/L

See Section 1.5.3 *Sensitivity Explained* for more information.

Summary of Method

Cyanides react with chlorine to form cyanogen chloride, which in turn reacts with pyridine in the presence of barbituric acid, condensing to form a violet-colored compound.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to Section 1.

CYANIDE, continued

REQUIRED REAGENTS AND STANDARDS

Description	Unit	Cat. No.
Cyanide - CN, UniCell™ HCT 129*	23/pkg	HCT 129

OPTIONAL REAGENTS

Sulfuric Acid Standard Solution, 1.00 N.....	1 L	1270-53
--	-----------	---------

OPTIONAL EQUIPMENT AND SUPPLIES

Graduated cylinder, mixing, 100-mL	each.....	20886-42
Flask, volumetric, 100-mL	each.....	14574-42
Pipettor, (Jencons) 1–5 mL	each.....	27951-00
Replacement tips for 27951-00	pkg/100.....	27952-00
Pipettor, (Jencons) 100–1000 µL.....	each.....	27949-00
Replacement tips for 27949-00	pkg/400.....	27950-00
pH Paper	pkg/100.....	26013-00

* Available in Europe only



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FAX: (970) 669-2932



Method 8028

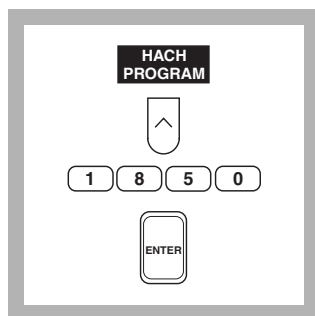
Crystal Violet Method*

(0 to 0.275 mg/L)

Scope and Application: For water, wastewater and seawater.

The estimated detection limit for program number 1850 is 0.005 mg/L LAS.

* Analytical Chemistry, 38, 791 (1966)



1. Press the soft key under **HACH PROGRAM**.

Select the stored program for anionic detergents (surfactants) by pressing **1850** with the numeric keys.

Press: **ENTER**

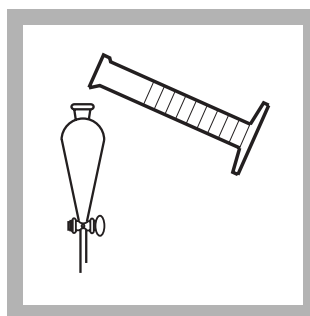
Note: If samples cannot be analyzed immediately, see *Sample Collection, Storage and Preservation* following these steps.

Note: The Flow Cell and Sipper Modules cannot be used for this procedure.

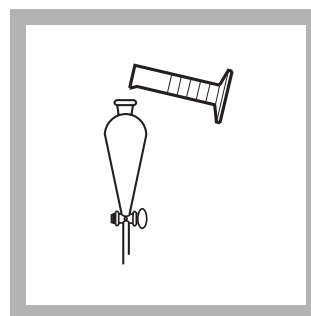


2. The display will show: **HACH PROGRAM: 1850 Detergents, Anion.**

The wavelength (λ), **605 nm**, is automatically selected.

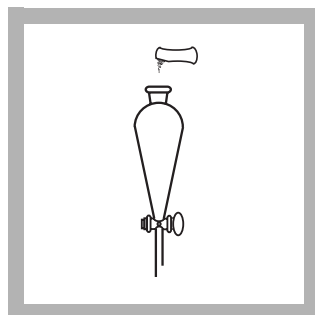


3. Fill a clean 500-mL graduated cylinder to the 300-mL mark with sample. Pour the sample into a clean 500-mL separatory funnel.

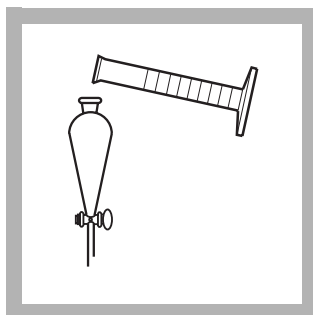


4. Add 10 mL of Sulfate Buffer Solution. Stopper the funnel. Shake the funnel for 5 seconds.

DETERGENTS, Anionic (Surfactants), continued



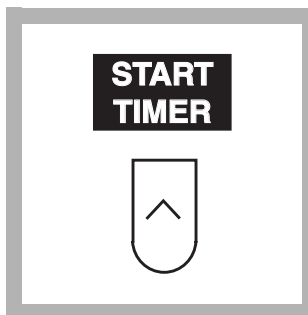
5. Add the contents of one Detergents Reagent Powder Pillow to the funnel. Stopper the funnel and shake to dissolve the powder.



6. Add 30 mL benzene to the funnel. Stopper the funnel. Gently shake the funnel for one minute.

Note: Spilled reagent will affect test accuracy and is hazardous to the skin and other materials.

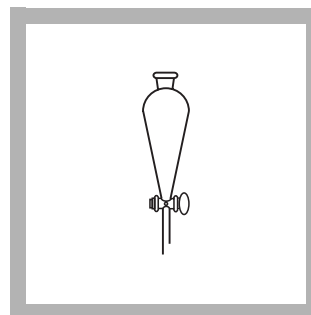
Note: Use benzene only in well-ventilated areas.



7. Place the separatory funnel in a support stand, and press the soft key under **START TIMER**.

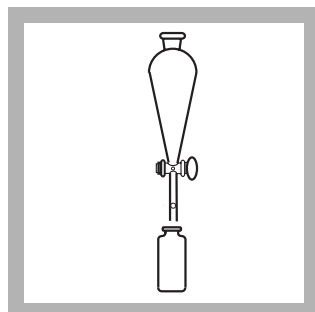
A 30-minute reaction period will begin.

Note: Excessive agitation may cause an emulsion to form, requiring a longer time for phase separation. For these samples, remove most of the water layer, then gently agitate the funnel with a clean inert object in the funnel such as a Teflon-coated magnetic stirring bar.



8. After the timer beeps, remove the stopper and drain the bottom water layer. Discard this layer into an appropriate waste collection container.

Note: Benzene solutions are a regulated waste and cannot be poured down the drain. See Pollution Prevention and Waste Management following these steps.

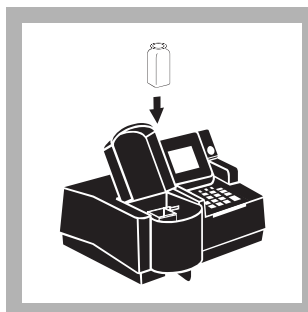


9. Drain the top benzene layer into a clean 25-mL sample cell (the prepared sample).

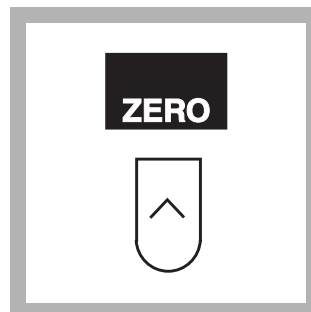
Note: The benzene layer cannot be filtered before color measurement. Filtration removes the blue color.



10. Fill another sample cell to the 25-mL mark with pure benzene (the blank).



11. Place the blank in the cell holder and close the light shield.

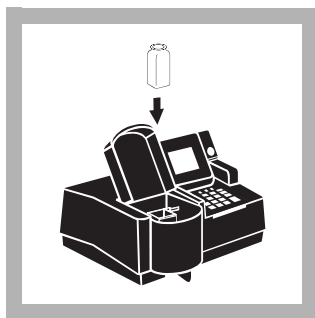


12. Press the soft key under **ZERO**.

The display will show:

0.000 mg/L LAS

Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



13. Place the prepared sample into the cell holder. Close the light shield. The result in mg/L anionic detergents as LAS (or chosen units) will be displayed.

Note: Acetone may be used to clean benzene from glassware.

Interferences

Table 1 Interfering Substances and Suggested Treatments for Powder Pillows

Interfering Substance	Interference Levels and Treatments
Chloride	High amounts of chloride, such as those levels found in brines and seawater, will cause low results.
Perchlorate ions	Interferes at all levels
Periodate ions	Interferes at all levels

Sample Collection, Storage and Preservation

Collect samples in clean plastic or glass bottles. Analyze samples as soon as possible, but they may be stored at least 24 hours by cooling to 4 °C (39 °F). Warm to room temperature before testing.

Accuracy Check

Standard Additions Method

- Leave the unspiked sample in the sample cell compartment. Verify that the units displayed are in mg/L. Select standard additions mode by pressing the soft keys under **OPTIONS, (MORE)** and then **STD ADD**.
- Press **ENTER** to accept the default sample volume (mL), 300.
- Press **ENTER** to accept the default standard concentration (mg/L LAS), 60.000.
- Press the soft key under **ENTRY DONE**.
- Snap the neck off a Detergent Voluette Ampule Standard, 60 mg/L LAS.

- f. Use the TenSette Pipet to add 0.1 mL, 0.2 mL and 0.3 mL of standard, respectively to three 300-mL samples and mix each thoroughly.
- g. Analyze each standard addition sample and as described above. Accept the standard additions readings by pressing the soft key under **READ** each time. Each addition should reflect approximately 100% recovery.
- h. After completing the sequence, the display will show the extrapolated concentration value and the “best-fit” line through the standard additions data points, accounting for matrix interferences.

See Section 1.4.1 *Standard Additions* for more information.

Method Performance

Precision

Standard: 0.150 mg/L LAS

Program	95% Confidence Limits
1850	0.147–0.153 mg/L LAS

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
1850	0.005 mg/L LAS

For more information on derivation and use of Hach’s estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, appendix B, see Section 1.5.1.

Sensitivity

Program Number: 1850

Portion of Curve:	Δ Abs	Δ Concentration
0.010 Abs	0.010	0.0026 mg/L LAS
0.16 mg/L	0.010	0.0038 mg/L LAS
0.288 mg/L	0.010	0.0047 mg/L LAS

See Section 1.5.3 *Sensitivity Explained* for more information.

Calibration Standard Preparation

To perform a surfactant calibration using the crystal violet method, use a Detergent Voluette Ampule Standard, 60-mg/L LAS (Cat. No. 14271-10).

Prepare calibration standards containing 0.012, 0.06, 0.096, 0.12, 0.18, and a 0.24 mg/L LAS as follows:

- a. Into six different Class A 500 mL volumetric flasks, pipet 0.1, 0.5, 0.8, 1.0, 1.5 and 2.0 mL of the 60-mg/L LAS stock solution using a TenSette Pipet.
- b. Dilute to the mark with deionized water. Mix thoroughly.
- c. Using the crystal violet method and the calibration procedure described in the *User-Entered Programs* section of the *DR/4000 Spectrophotometer Instrument Manual*, generate a calibration curve from the standards prepared above.

Summary of Method

Detergents, ABS (alkyl benzene sulfonate) or LAS (linear alkylate sulfonate) are determined by association with crystal violet dye and extraction of the ion-pair complex into benzene.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to Section 1.

Pollution Prevention and Waste Management

Benzene (D018) solutions are regulated as hazardous waste by the Federal RCRA. Do not pour these materials down the drain. Collect water saturated with benzene and benzene solutions for disposal with laboratory solvent wastes. See Section 1 for more information on proper disposal of these materials.

DETERGENTS, Anionic (Surfactants), continued

REQUIRED REAGENTS AND STANDARDS

Detergents Reagent Set	24468-00
Includes: (2) 452-49, (3) 1008-68, (1) 14440-17	

Description	Quantity Required		Unit	Cat. No.
	per test			
Benzene, ACS	55 mL	4 L		14440-17
Buffer Solution, sulfate-type	10 mL	500 mL		452-49
Detergent Reagent Powder Pillows	1 pillow	25/pkg		1008-68

REQUIRED EQUIPMENT AND SUPPLIES

Clippers, for opening powder pillows	1	each	968-00
Cylinder, graduated, 25-mL	1	each	508-40
Cylinder, graduated, 50-mL	1	each	508-41
Cylinder, graduated, 500-mL	1	each	508-49
DR/4000 1-Inch Cell Adapter	1	each	48190-00
Funnel, separatory, 500-mL	1	each	520-49
Support Ring, 4-inch	1	each	580-01
Support, Ring Stand, 5 x 8 in. base	1	each	563-00

OPTIONAL REAGENTS AND STANDARDS

Acetone, ACS	500 mL	14429-49
Benzene, ACS	500 mL	14440-49
Detergent Standard Solution, 10-mL Voluette Ampule, 60-mg/L LAS	16/pkg	14271-10

OPTIONAL EQUIPMENT AND SUPPLIES

Ampule Breaker Kit	each	21968-00
DR/4000 Carousel Module Kit	each	48070-02
Pipet, TenSette, 0.1 to 1.0 mL	each	19700-01
Pipet Tips, for 19700-01 Pipet	50/pkg	21856-96



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✓ Method 8029

SPADNS Method*

Reagent Solution or AccuVac® Ampuls

(0 to 2.00 mg/L F⁻)

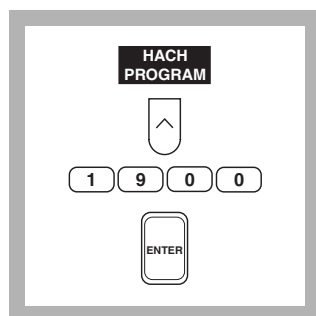
Scope and Application: For water, wastewater and seawater; USEPA accepted for reporting for drinking and wastewater analyses (distillation required; See “Distillation” on page 5.).**

The estimated detection limit for program numbers 1900 and 1910 are 0.02 and 0.04 mg/L F⁻, respectively.

* Adapted from *Standard Methods for the Examination of Water and Wastewater*, 4500-F B & D

** Procedure is equivalent to USEPA method 340.1 for drinking water and wastewater.

Using SPADNS Reagent Solution



- 1.** Press the soft key under **HACH PROGRAM**.

Select the stored program for fluoride (F⁻) by pressing **1900** with the numeric keys.

Press: **ENTER**

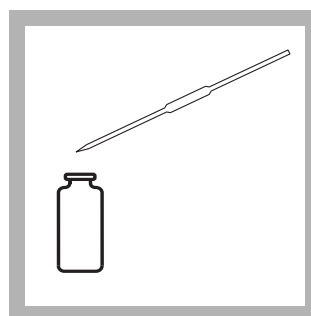
Note: If samples cannot be analyzed immediately, see *Sample Collection, Storage and Preservation* following these steps.

Note: The Flow Cell and Sipper Modules cannot be used for this procedure.



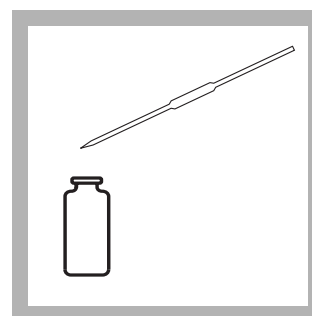
- 2.** The display will show:
HACH PROGRAM: 1900 Fluoride

The wavelength (λ), **580 nm**, is automatically selected.



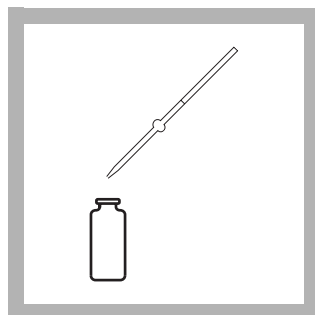
- 3.** Pipet 10.0 mL of sample into a dry sample cell (the prepared sample).

Note: For proof of accuracy, use a 1.0 mg/L Fluoride Standard Solution (listed under **OPTIONAL REAGENTS AND STANDARDS**) in place of the sample.



- 4.** Pipet 10.0 mL of deionized water into a second dry sample cell (the blank).

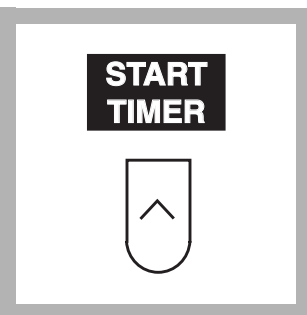
Note: The sample and deionized water should be at the same temperature (± 1 °C). Temperature adjustments may be made before or after reagent addition.



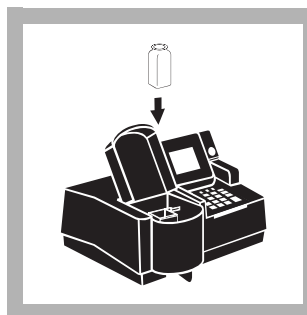
5. Use a pipet filler to pipet 2.0 mL of SPADNS Reagent into each cell. Swirl to mix.

Note: SPADNS Reagent is toxic and corrosive; use care while measuring.

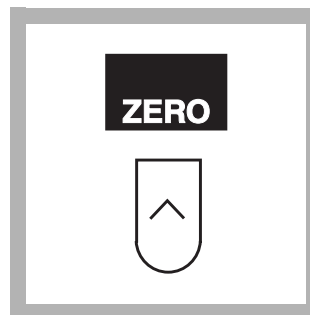
Note: The SPADNS Reagent must be measured accurately.



6. Press the soft key under **START TIMER**. A one minute reaction period will begin.



7. When the timer beeps, place the blank into the cell holder. Close the light shield.

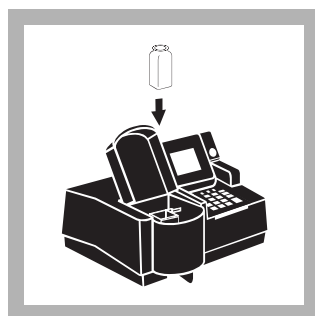


8. Press the soft key under **ZERO**.

The display will show:

0.00 mg/L F⁻

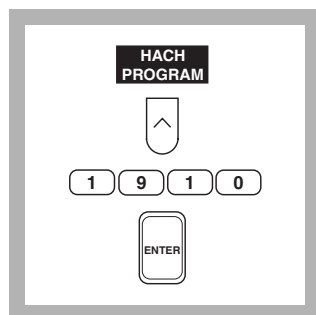
Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



9. Place the prepared sample into the cell holder. Close the light shield. Results in mg/L F⁻ (or chosen units) will be displayed.

Note: If the instrument displays **OVER!**, dilute a fresh sample with an equal volume of deionized water and repeat the test, using this solution in Step 3. Multiply the result by 2.

Using SPADNS AccuVac Ampuls



1. Press the soft key under **HACH PROGRAM**.

Select the stored program for fluoride AccuVac by pressing **1910** with the numeric keys.

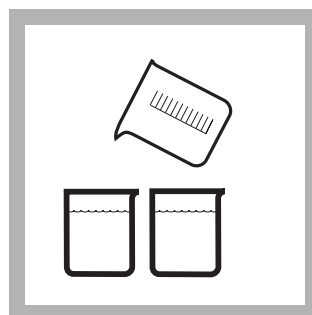
Press: **ENTER**

Note: If samples cannot be analyzed immediately, see *Sample Collection, Storage and Preservation* following these steps.

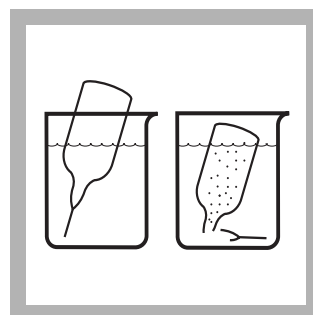


2. The display will show: **HACH PROGRAM: 1910 Fluoride, AV**

The wavelength (λ), **580 nm**, is automatically selected.



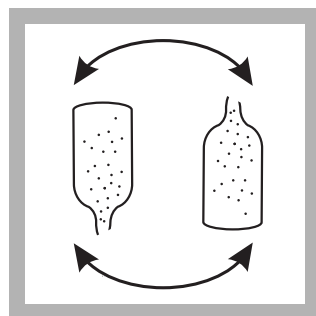
3. Collect at least 40 mL of sample in a 50-mL beaker. Pour at least 40 mL of deionized water into a second beaker.



4. Fill a SPADNS Fluoride Reagent AccuVac Ampul with sample by breaking the tip on the bottom of the beaker. Fill a second AccuVac Ampul with deionized water (the blank) in the same manner.

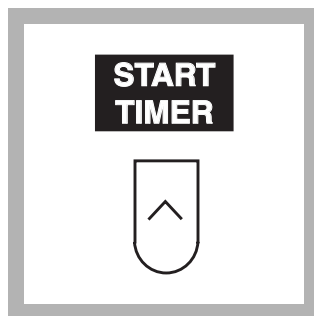
Note: Keep the tip immersed while the ampul fills completely.

Note: For proof of accuracy, use a 1.0 mg/L fluoride standard solution (listed under **OPTIONAL REAGENTS AND STANDARDS**) in place of the sample.



5. Quickly invert the ampuls several times to mix. Wipe off any liquid or fingerprints.

Note: Do not place finger over broken tip—the liquid will remain in the ampul.



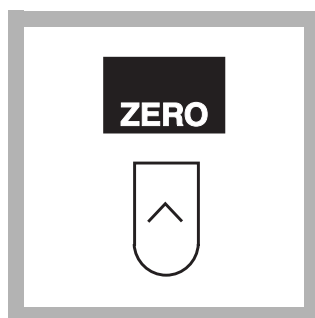
6. Press the soft key under **START TIMER**. A one-minute reaction period will begin.



7. Insert the AccuVac Ampul Adapter into the sample cell module by sliding it under the thumb screw and into the alignment grooves. Fasten with the thumb screw.



8. When the timer beeps, place the blank into the cell holder. Close the light shield.



9. Press the soft key under **ZERO**.

The display will show:

0.000 mg/L F⁻

Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



10. Place the AccuVac Ampul containing the sample into the instrument. Close the light shield. Results in mg/L F⁻ (or chosen units) will be displayed.

Note: If the instrument shows **OVER!** dilute a fresh sample with an equal volume of deionized water and repeat the test, using this solution in Step 3. Multiply the result by 2.

Interferences

This test is sensitive to small amounts of interference. Glassware must be very clean (acid rinse before each use). Repeating the test with the same glassware is recommended to ensure that results are accurate.

Table 1 Interfering Substances and Suggested Treatments

Interfering Substance	Interference Levels and Treatments
Alkalinity (as CaCO ₃)	At 5000 mg/L it causes a -0.1 mg/L F ⁻ error.
Aluminum	At 0.1 mg/L it causes a -0.1 mg/L F ⁻ error. To check for interferences from aluminum, read the concentration one minute after reagent addition, then again after 15 minutes. An appreciable increase in concentration suggests aluminum interference. Waiting 2 hours before making the final reading will eliminate the effect of up to 3.0 mg/L aluminum.
Chloride	At 7000 mg/L it causes a +0.1 mg/L F ⁻ error.
Chlorine	SPADNS Reagent contains enough arsenite to eliminate interference up to 5 mg/L chlorine. For higher chlorine levels, add one drop of Sodium Arsenite Solution to 25 mL of sample for each 2 mg/L of Chlorine.
Iron, ferric	At 10 mg/L it causes a -0.1 mg/L F ⁻ error.
Phosphate, ortho	At 16 mg/L it causes a +0.1 mg/L F ⁻ error.
Sodium Hexametaphosphate	At 1.0 mg/L it causes a +0.1 mg/L F ⁻ error.
Sulfate	At 200 mg/L it causes a +0.1 mg/L F ⁻ error.

Distillation

Most interferences can be eliminated by distilling the sample from an acid solution as described below:

1. Set up the distillation apparatus for general purpose distillation. Refer to the *Distillation Apparatus* manual for proper assembly. Use a 100-mL Erlenmeyer flask to collect the distillate.
2. Turn on the water and make certain a steady flow is maintained through the condenser.
3. Measure 100 mL of sample into the distillation flask using a 100-mL graduated cylinder. Add a magnetic stir bar and 5 glass beads.

Note: For proof of accuracy, use a 1.0-mg/L Fluoride Standard Solution (see *OPTIONAL REAGENTS AND STANDARDS*) in place of the sample.

4. Turn the stirrer power switch on. Turn the stir control to 5.
5. Using a 250-mL graduated cylinder, carefully add 150 mL of StillVer Distillation Solution into the flask.

Note: StillVer Distillation Solution is a 2:1 mixture of concentrated sulfuric acid and water. It is available already mixed from Hach.

Note: When distilling samples with high amounts of chloride, add 5 mg of Silver Sulfate to the sample for every mg/L of chloride in the sample.

6. With the thermometer in place, turn the heat control to 10. The yellow pilot lamp indicates that the heater is on.
7. When the temperature reaches 180 °C, or when 100 mL of distillate has been collected, turn the still off (requires about 1 hour).
8. Dilute the distillate to a volume of 100 mL, if necessary. The distillate may now be analyzed by the SPADNS or the fluoride ion-selective electrode method.

Sample Collection, Storage and Preservation

Samples may be stored in glass or plastic bottles for at least seven days when cooled to 4 °C (39 °F) or lower. Warm samples to room temperature before analysis.

Accuracy Check

Standard Solution Method

A variety of standard solutions covering the entire range of the test is available from Hach. Use these in place of sample to verify technique.

Minor variations between lots of reagent become measurable above 1.5 mg/L. While results in this region are usable for most purposes, better accuracy may be obtained by diluting a fresh sample 1:1 with deionized water and retesting. Multiply the result by 2.

To adjust the calibration curve using the reading obtained with a Fluoride Standard Solution, press the soft keys under **OPTIONS, MORE** then **STD: OFF**. Enter the actual value of the measured standards and press **ENTER**. See Section 1.5.5 *Adjusting the Standard Curve* for more information.

Method Performance

Precision

Standard: 1.00 mg/L F⁻

Program	95% Confidence Limits
1900	0.98–1.02 mg/L F ⁻
1910	0.97–1.03 mg/L F ⁻

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
1900	0.02 mg/L F ⁻
1910	0.04 mg/L F ⁻

For more information on derivation and use of Hach's estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, Appendix B, see Section 1.5.1.

Sensitivity

Program Number 1900

Program	ΔAbs	ΔConcentration
0.010 Abs	0.010	-0.026 mg/L
1 mg/L	0.010	-0.023 mg/L
1.8 mg/L	0.010	-0.034 mg/L

Program Number 1910

Program	ΔAbs	ΔConcentration
0.010 Abs	0.010	-0.028 mg/L
1 mg/L	0.010	-0.025 mg/L
1.8 mg/L	0.010	-0.032 mg/L

See Section 1.5.3 *Sensitivity Explained* for more information.

Calibration Standard Preparation

To perform a fluoride calibration using the SPADNS Solution AccuVac method, prepare a 20-mg/L fluoride stock solution by pipetting 20.00 mL of a 100-mg/L Fluoride Standard Solution (Cat. No. 232-49) into a 100-mL volumetric flask using Class A glassware. Dilute to the mark with deionized water and mix thoroughly.

Prepare calibration standard containing 0.20, 1.20, and 2.00 mg/L F⁻ as follows:

- a. Into three different 100-mL volumetric flasks, pipet 1.00, 6.00, and 10.00 mL of the 20 mg/L F⁻ stock solution using Class A glassware.
- b. Dilute to the mark with deionized water and mix thoroughly.
- c. Using the SPADNS Solution or AccuVac method and the calibration procedure described above, generate a calibration curve from the standards prepared above.

Summary of Method

The SPADNS Method for fluoride determination involves the reaction of fluoride with a red zirconium-dye solution. The fluoride combines with part of the zirconium to form a colorless complex, thus bleaching the red color in an amount proportional to the fluoride concentration. This method is accepted by the EPA for NPDES and NPDWR reporting purposes when the samples have been distilled. Seawater and wastewater samples require distillation. See *OPTIONAL REAGENTS AND STANDARDS* for Distillation Apparatus listing.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to Section 1.

Pollution Prevention and Waste Management

SPADNS Reagent contains sodium arsenite. Final solutions will contain arsenic (D004) in sufficient concentration to be regulated as a hazardous waste for Federal RCRA. See Section 1 for further information on proper disposal of these materials.

REQUIRED REAGENTS AND STANDARDS (Using Solution)

Description	Quantity Required		Unit	Cat. No.
	per test			
SPADNS Reagent Solution	4 mL	500 mL		444-49
Water, deionized	10 mL	4 liters		272-56

REQUIRED EQUIPMENT AND SUPPLIES (Using Solution)

DR/4000 1-Inch Cell Adapter	1	each	48190-00
Pipet Filler safety bulb	1	each	14651-00
Pipet, volumetric, Class A, 2.00-mL	1	each	14515-36
Pipet, volumetric, Class A, 10.00-mL	1	each	14515-38
Thermometer, -10 to 110 °C	1	each	1877-01

FLUORIDE, continued

REQUIRED REAGENTS AND STANDARDS (Using AccuVac Ampuls)

Description	Quantity Required per test	Unit	Cat. No.
SPADNS Fluoride Reagent AccuVac Ampuls	2 ampuls	25/pkg	25060-25
Water, deionized	varies	4 liters	272-56

REQUIRED EQUIPMENT AND SUPPLIES (Using AccuVac Ampuls)

Beaker, 50-mL	2	each	500-41
DR/4000 AccuVac Ampul Adapter	1	each	48187-00

OPTIONAL REAGENTS AND STANDARDS

Fluoride Standard Solution, 0.2 mg/L F ⁻	500 mL	405-02
Fluoride Standard Solution, 0.5 mg/L F ⁻	500 mL	405-05
Fluoride Standard Solution, 0.8 mg/L F ⁻	500 mL	405-08
Fluoride Standard Solution, 1.0 mg/L F ⁻	946 mL	291-16
Fluoride Standard Solution, 1.0 mg/L F ⁻	473 mL	291-11
Fluoride Standard Solution, 1.2 mg/L F ⁻	500 mL	405-12
Fluoride Standard Solution, 1.5 mg/L F ⁻	500 mL	405-15
Fluoride Standard Solution, 2.0 mg/L F ⁻	500 mL	405-20
Fluoride Standard Solution, 100 mg/L F ⁻	500 mL	232-49
Silver Sulfate, ACS	113 g	334-14
Sodium Arsenite Solution, 5.0 g/L	100 mL MDB	1047-32
StillVer Distillation Solution	500 mL	446-49

OPTIONAL EQUIPMENT AND SUPPLIES

AccuVac Snapper	each	24052-00
Cylinder, graduated, 100-mL	each	508-42
Cylinder, graduated, 250-mL	each	508-46
DR/4000 Carousel Module Kit	each	48070-02
Distillation Heater and Support Apparatus Set, 115 VAC, 50/60 Hz	each	22744-00
Distillation Heater and Support Apparatus Set, 230 VAC, 50/60 Hz	each	22744-02
Distillation Apparatus Set, General Purpose	each	22653-00
pH Meter, <i>sens^{ion}</i> TM 1, portable	each	51700-00
Pipet, volumetric, Class A, 1.00-mL	each	14515-35
Pipet, volumetric, Class A, 6.00-mL	each	14515-06
Pipet, volumetric, Class A, 10.00-mL	each	14515-38
Pipet, volumetric, Class A, 25.00-mL	each	14515-40



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Method 8110

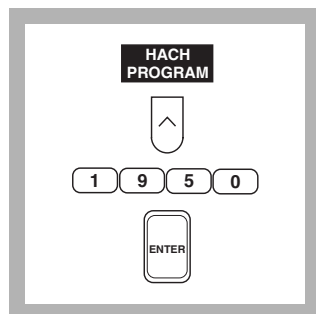
MBTH Method*

Powder Pillows

(0 to 500 µg/L)

Scope and Application: For water. The estimated detection limit for program number 1950 is 6 µg/l CH₂O.

* Adapted from Matthews, T.G. and Howell, T.C., *Journal of the Air Pollution Control Association*, 31 (11) 1181-1184 (1981)



1. Press the soft key under **HACH PROGRAM**.

Select the stored program for formaldehyde by pressing **1950** with the numeric keys.

Press: **ENTER**

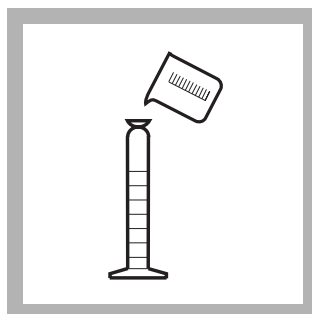
Note: Samples must be analyzed immediately and cannot be preserved for later analysis.

Note: The Flow Cell and Sipper Modules cannot be used for this procedure.



2. The display will show:
HACH PROGRAM: 1950 Formaldehyde

The wavelength (λ), **630 nm**, is automatically selected.

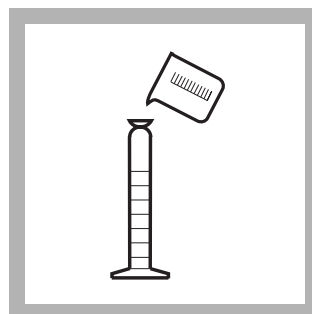


3. Accurately measure 25 mL of sample in a 50-mL mixing cylinder (the prepared sample).

Note: Wash glassware with chromic acid cleaning solution to remove trace contaminants.

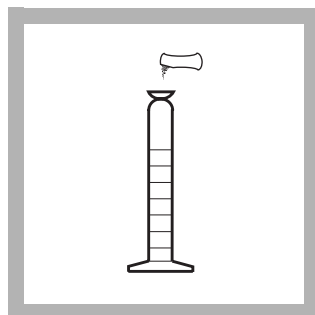
Note: Time and temperature are very important in this test. The sample should be at 25 ± 1 °C, and the times specified in steps must be followed precisely. A temperature-controlled water bath is recommended for best accuracy.

Note: For proof of accuracy, use a 320 µg/L formaldehyde standard solution (preparation given in the Accuracy Check section) in place of the sample.

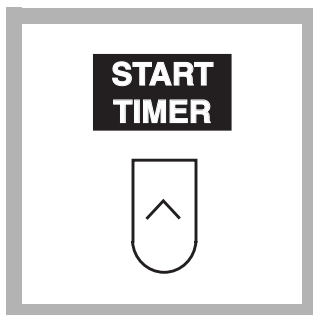


4. Accurately measure 25 mL of formaldehyde-free water in a second 50-mL mixing cylinder (the blank).

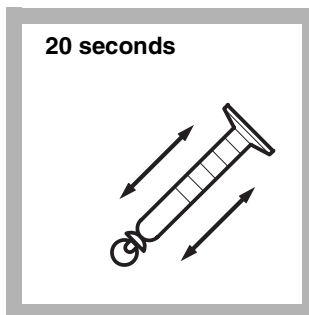
Note: Obtain formaldehyde-free water by distilling water from alkaline permanganate (4 g sodium hydroxide, 2 g potassium permanganate per 500 mL water). Discard the first 50 to 100 mL of distillate.



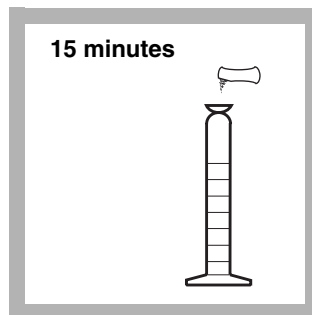
5. Add the contents of one MBTH Powder Pillow to the blank. Stopper the cylinder.



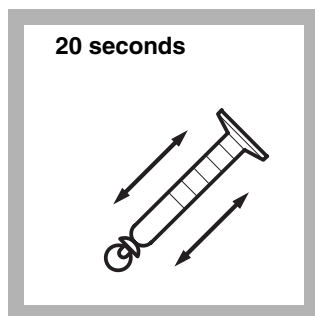
6. Immediately press the soft key under **START TIMER**. A 17-minute reaction period will begin. Proceed with Step 7 immediately. Complete Steps 8-12 during the 17-minute reaction period at the times specified.



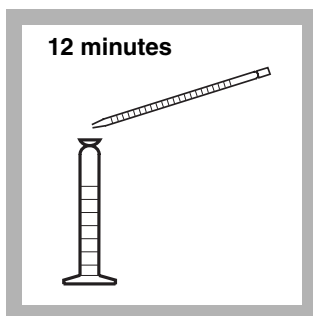
7. Immediately after the reaction period starts, shake the cylinder vigorously for 20 seconds.
Note: Do not wait for the timer to beep.



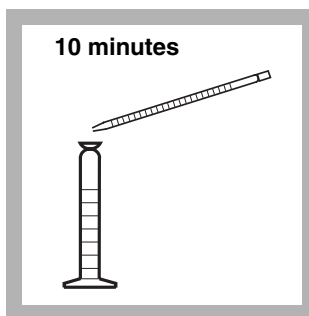
8. Add the contents of one MBTH Powder Pillow to the prepared sample when the timer displays **15:00**. Stopper the cylinder.



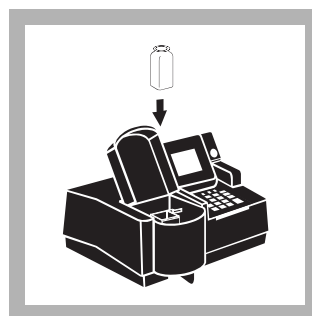
9. Shake the cylinder vigorously for 20 seconds.



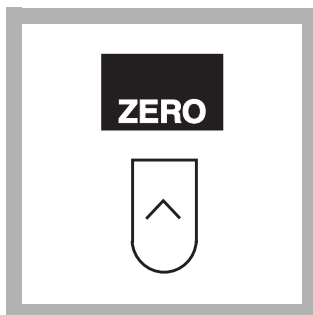
10. Add 2.5 mL of Developing Solution For Low Range Formaldehyde to the blank when the timer shows **12:00**. Stopper. Invert to mix.



11. Add 2.5 mL of Developing Solution For Low Range Formaldehyde to the prepared sample when the timer shows **10:00**. Stopper. Invert to mix.



12. Pour the blank into a sample cell just before the timer shows **2:00**. Place the blank into the cell holder. Close the light shield.
Note: Pour the solution slowly into the cell to avoid bubble formation on the cell walls. If bubbles form, swirl the cell to dislodge them.

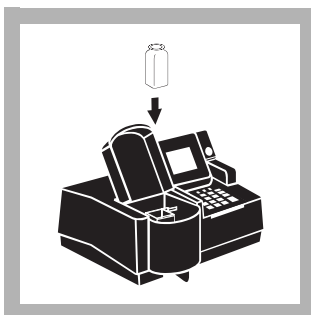


13. When the timer shows **2:00**, press the soft key under **ZERO**.

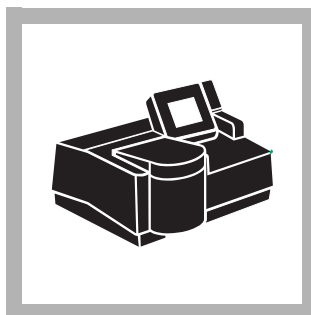
The display will show:

0 µg/L CH₂O

Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



14. Pour the prepared sample into a sample cell. Place it into the cell holder.



15. When the timer beeps, close the light shield. Results in µg/L formaldehyde (or chosen units) will be displayed.

Interferences

Table 1 Interfering Substances and Suggested Treatments

Interfering Substance	Interference Levels
Acetate	Greater than 1000 mg/L
Aldehydes (other)	Positive interference at all levels
Ammonium (as N)	Greater than 10 mg/L
Aniline	Greater than 10 mg/L
Bicarbonate	Greater than 1000 mg/L
Calcium	Greater than 3500 mg/L
Carbonate	Greater than 500 mg/L
Chloride	Greater than 5000 mg/L
Copper	Greater than 1.6 mg/L
Cyclohexylamine	Greater than 250 mg/L
Ethanolamine	Greater than 33 mg/L
Ethylenediamine	Greater than 1.5 mg/L
Glucose	Greater than 1000 mg/L
Glycine	Greater than 1000 mg/L
Iron (Fe ³⁺)	Greater than 12 mg/L
Lead	Greater than 100 mg/L
Manganese	Greater than 500 mg/L
Mercury	Greater than 70 mg/L
Morpholine	Greater than 0.36 mg/L
Nitrate	Greater than 1000 mg/L
Nitrite	Greater than 8 mg/L
Phenol	Greater than 1050 mg/L
Phosphate	Greater than 200 mg/L
Silica	Greater than 40 mg/L
Sulfate	Greater than 10,000 mg/L
Urea	Greater than 1000 mg/L
Zinc	Greater than 1000 mg/L

Accuracy Check

Standard Additions Method

- Leave the unspiked sample in the sample cell compartment. Verify that the units displayed are in µg/L. Select standard additions mode by pressing the soft keys under **OPTIONS, (MORE)** and then **STD ADD**.
- Press **ENTER** to accept the default sample volume (mL), 25.
- Press **ENTER** to accept the default standard concentration (µg/L), 8000.
- Press the soft key under **ENTRY DONE**.
- Snap the neck off a Formaldehyde Voluette Ampule Standard, 4000-mg/L CH₂O. Use a TenSette Pipet to add 0.2 mL of the standard to a 100-mL volumetric Class A flask. Dilute to volume with formaldehyde-free water and mix well. Prepare daily. This is an 8000 µg/l (8 mg/L) formaldehyde standard.

- f. Use the TenSette Pipet to add 0.1 mL, 0.2 mL and 0.3 mL of 8000-µg/L standard, respectively, to three 25-mL samples and mix each thoroughly.
- g. Analyze each standard addition sample as described above. Accept the standard additions reading by pressing the soft key under **READ** each time. Each addition should reflect approximately 100% recovery.
- h. After completing the sequence, the display will show the extrapolated concentration value and the “best-fit” line through the standard additions data points, accounting for matrix interferences.
- i. See Section 1.4.1 *Standard Additions* for more information.

Standard Solution Method

Prepare a 320-µg/L Formaldehyde Standard Solution by pipetting 1.0 mL of the 8000-µg/L solution from the *ACCURACY CHECK* into a 50-mL mixing cylinder. Dilute to 25.0 mL with formaldehyde-free water. Run the test directly on this sample.

Method Performance

Precision

Standard: 320 µg/L CH₂O

Program	95% Confidence Limits
1950	316–324 µg/L CH ₂ O

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
1950	6 µg/L CH ₂ O

For more information on derivation and use of Hach’s estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, appendix B, see Section 1.5.1.

Sensitivity

Program Number 1950

Program	ΔAbs	ΔConcentration
Entire Range	0.010	2.9 µg/L

See Section 1.5.3 *Sensitivity Explained* for more information.

Calibration Standard Preparation

To perform a formaldehyde calibration using the MBTH method, prepare a 4000- $\mu\text{g/L}$ formaldehyde stock solution by pipetting 1.00 mL of a 4000-mg/L Formaldehyde Standard Solution (Cat. No. 22573-10) into a 1000-mL volumetric flask using Class A glassware. Dilute to the mark with formaldehyde-free water and mix thoroughly.

Prepare calibration standards containing 40, 80, 400, and 480 mg/L formaldehyde as follows:

- a. Into four different 100-mL Class A volumetric flasks, pipet 1.00, 2.00, 10.00, and 12.00 mL of the 4000- $\mu\text{g/L}$ formaldehyde stock solution using Class A glassware.
- b. Dilute to the mark with formaldehyde-free water and mix thoroughly.
- c. Using the MBTH method and the calibration procedure described above, generate a calibration curve from the standards prepared above.

Summary of Method

Formaldehyde reacts with MBTH (3-methyl-2-benzothiazoline hydrazone) and a developing solution to form a blue color in proportion to the formaldehyde concentration.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to Section 3.

Pollution Prevention and Waste Management

For information on pollution prevention and waste management, refer to Section 3.

REQUIRED REAGENTS AND STANDARDS

	Cat. No.
Formaldehyde Reagent Set (100 Tests).....	22577-00
Includes: (2) 22571-69, (1) 22572-49	

Description	Quantity Required per test	Unit	Cat. No.
Developing Solution For Low Range Formaldehyde.....	5 mL	500 mL	22572-49
MBTH Powder Pillows	2 pillows	100/pkg	22571-69

REQUIRED EQUIPMENT AND SUPPLIES

Clippers, for opening powder pillow	1	each	968-00
Cylinder, graduated mixing, 50-mL	2	each	1896-41
DR/4000 1-Inch Cell Adapter	1	each	48190-00
Pipet, serological, 5-mL	1	each	532-37
Pipet Filler, safety bulb.....	1	each	14651-00

OPTIONAL REAGENTS AND STANDARDS

Chromic Acid Cleaning Solution	500 mL	1233-49
Formaldehyde Standard Solution, 10-mL Voluette Ampule, 4000-mg/L	16/pkg	22573-10
Potassium Permanganate, ACS	454 g	168-01
Sodium Hydroxide, pellets, ACS	500 g	187-34

OPTIONAL EQUIPMENT AND SUPPLIES

Ampule Breaker Kit	each	21968-00
DR/4000 Carousel Module Kit	each	48070-02
Flask, volumetric, Class A, 100-mL	each	14574-42
Flask, volumetric, Class A, 1000-mL	each	14574-53
Pipet, TenSette, 0.1 to 1.0 mL	each	19700-01
Pipet Tips, for 19700-01 TenSette Pipet	50/pkg	21856-96
Pipet, volumetric, Class A, 1.00 mg/L	each	14515-35
Pipet, volumetric, Class A, 2.00 mg/L	each	14515-36
Pipet, volumetric, Class A, 10.00 mg/L	each	14515-38
Thermometer, pocket, -10 to 110 °C	each	1877-01
Water bath, 120 VAC, 22-L	each	26163-00
Water bath, 240 VAC, 22-L	each	26163-02



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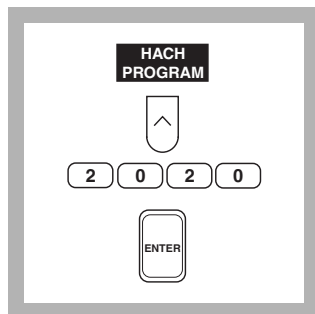


Method 8030

Calcium and Magnesium; Calmagite Colorimetric Method

(0 to 4.00 mg/L Ca and Mg as CaCO_3)

Scope and Application: For water, wastewater, and seawater. The estimated detection limit for program numbers 2010 and 2020 is 0.03 mg/L CaCO_3 .



1. Press the soft key under **HACH PROGRAM**.

Select the stored program for magnesium hardness by pressing **2020** with the numeric keys.

Press: **ENTER**

Note: If samples cannot be analyzed immediately, see *Sample Collection, Storage and Preservation* following these steps. Adjust the pH of preserved samples before analysis.

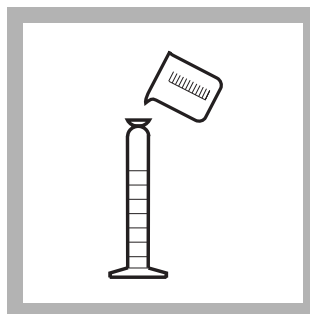
Note: The Flow Cell and Sipper Modules can be used for this procedure if rinsed with deionized water between samples.



2. The display will show:

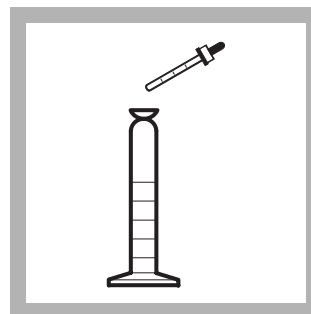
**HACH PROGRAM:
2020 Hardness, Mg**

The wavelength (λ), **522 nm**, is automatically selected.

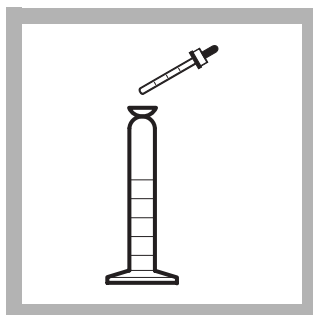


3. Pour 100 mL of sample into a 100-mL graduated mixing cylinder.

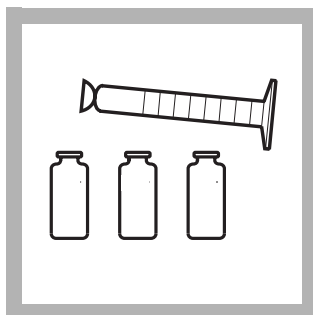
Note: For the most accurate magnesium test results the sample temperature should be 21-29 °C (70-84 °F).



4. Add 1.0 mL of Calcium and Magnesium Indicator Solution using a 1.0-mL measuring dropper. Stopper. Invert several times to mix.

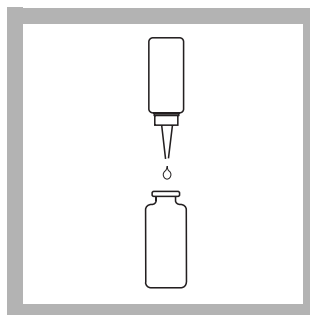


5. Add 1.0 mL of Alkali Solution for Calcium and Magnesium Test using a 1.0-mL measuring dropper. Stopper. Invert several times to mix.

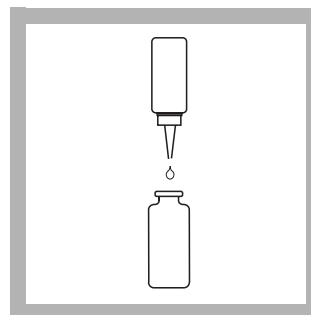


6. Pour 25 mL of the solution into each of three sample cells.

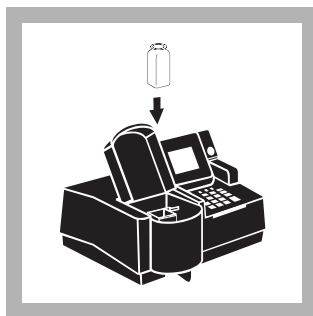
Note: The test will detect any calcium or magnesium contamination in the mixing cylinder, measuring droppers or sample cells. To test cleanliness, repeat the test multiple times until you obtain consistent results.



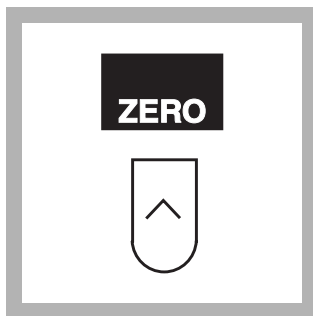
7. Add one drop of 1 M EDTA Solution to one cell (the blank). Swirl to mix.



8. Add one drop of EGTA Solution to another cell (the prepared sample). Swirl to mix.



9. Place the blank into the cell holder. Close the light shield.

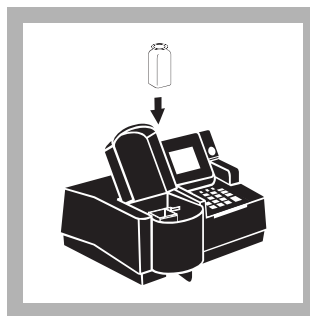


10. Press the soft key under **ZERO**.

The display will show:

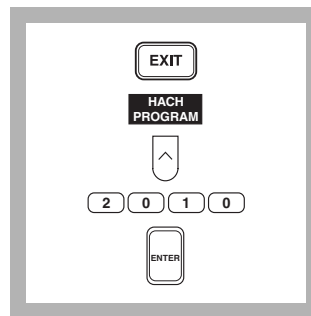
0.00 mg/L CaCO₃

Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



11. Place the prepared sample into the cell holder. Close the light shield. Results in mg/L magnesium as calcium carbonate (or chosen units) will be displayed. This value is the amount of magnesium in the sample expressed as CaCO₃.

Note: Results can be expressed as magnesium (Mg²⁺). Press the soft keys under **OPTIONS** and then **FORM**: to scroll through the available options.



12. Without removing the cell, press the **EXIT** key, followed by the soft key under **NEW PROGRAM**. At the program number prompt, select the stored program number for calcium hardness by pressing **2010** with the numeric keys.

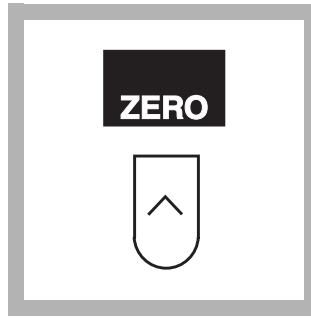
Press: **ENTER**



13. The display will show:

**HACH PROGRAM:
2010 Hardness, Ca**

The wavelength (λ),
522 nm, is automatically
selected.

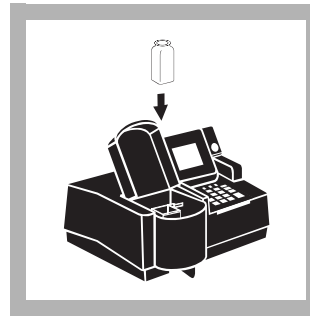


14. Press the soft key
under **ZERO**.

The display will show:

0.00 mg/L CaCO₃

Note: For alternate
concentration units,
press the soft key under
OPTIONS. Then press the
soft key under **UNITS** to scroll
through the available
options. Press **ENTER** to
return to the read screen.



15. Place the third sample
cell into the cell holder.
Close the light shield.
Results in mg/L calcium as
calcium carbonate (or
chosen units) will be
displayed. The result is the
amount of calcium in the
sample expressed as
CaCO₃.

Note: Result can be
expressed as calcium (Ca).
Press the soft keys under
OPTIONS and then **FORM**: to
scroll through the available
options. Press **ENTER** to
return to the read screen.

Note: mg/L hardness equals
mg/L Ca as CaCO₃ plus
mg/L Mg as CaCO₃.

Interferences

Table 1 Interfering Substances and Suggested Treatments

Interfering Substance	Interference Levels and Treatments
Chromium (3+)	Above 0.25 mg/L
Copper (2+)	Above 0.75 mg/L
EDTA, chelated	Above 0.2 mg/L as CaCO ₃
EDTA or EGTA	Traces remaining in sample cells from previous tests will give erroneous results. Rinse cells thoroughly before using.
Iron (2+)	Above 1.4 mg/L
Iron (3+)	Above 2.0 mg/L
Manganese (2+)	Above 0.20 mg/L
Zinc (2+)	Above 0.050 mg/L
Calcium >1.0 mg/L; Mg >0.25 mg/L	For the most accurate calcium test result, rerun the test on a diluted sample if the calcium is over 1.0 and the magnesium is over 0.25 mg/L as CaCO ₃ . No retesting is needed if either is below those respective concentrations.

Sample Collection, Storage and Preservation

Collect samples in acid-washed plastic bottles. Adjust the sample pH to 2 or less with nitric acid (about 5 mL per liter). Cool samples to 4 °C. Preserved samples can be stored up to six months. Before analysis, adjust the sample pH to between 3 and 8 with 5.0 N Sodium Hydroxide Standard Solution. Correct the test results for volume additions; see Section 1.2.2 *Correcting for Volume Additions*.

Method Performance

Precision

Standard: 1.50 mg/L as CaCO₃

Program	95% Confidence Limits
2010	1.45–1.55 mg/L as CaCO ₃
2020	1.47–1.53 mg/L as CaCO ₃

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
2010	0.03 mg/L as CaCO ₃
2020	0.03 mg/L as CaCO ₃

For more information on derivation and use of Hach's estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, Appendix B, see Section 1.5.1.

Sensitivity

Program Number: 2010

Portion of Curve	ΔAbs	ΔConcentration
0.010 Abs	0.010	0.054 mg/L as CaCO ₃
2 mg/L	0.010	0.054 mg/L as CaCO ₃
3.6 mg/L	0.010	0.079 mg/L as CaCO ₃

Program Number: 2020

Portion of Curve	ΔAbs	ΔConcentration
0.010 Abs	0.010	0.029 mg/L as CaCO ₃
2 mg/L	0.010	0.032 mg/L as CaCO ₃
3.6 mg/L	0.010	0.049 mg/L as CaCO ₃

See Section 1.5.3 *Sensitivity Explained* for more information.

Calibration Standard Preparation

To perform a hardness calibration using the Calmagite Colorimetric method, prepare calibration standards containing 0.5, 2.0, 2.5, 3.0, 3.5, and 4.0 mg/L Ca as CaCO_3 as follows:

- a. Into six different 100-mL Class A volumetric flasks, pipet 1.00, 4.00, 5.00, 6.00, 7.00, and 8.00 mL of a 50-mg/L Calcium Chloride Standard Solution as CaCO_3 (Cat. No. 21277-16) using Class A glassware.
- b. Dilute to the mark with deionized water. Mix well.

Also prepare calibration standards containing 0.41, 1.65, 2.06, 2.47, 2.88 and 3.30 mg/L Mg as CaCO_3 as follows:

- a. Into a 100-mL Class A volumetric flask, pipet 10.00 mL of 1000-mg/L Magnesium Standard Solution as Mg (Cat. No. 14794-42) using Class A glassware to prepare a 100 mg/L standard (412 mg/L Mg as CaCO_3).
- b. Into six different 1000-mL Class A volumetric flasks, pipet 1.00, 4.00, 5.00, 6.00, 7.00, and 8.00 mL of the 100-mg/L magnesium standard using Class A glassware.
- c. Dilute to the mark with deionized water. Mix well.

Using the Calmagite Colorimetric method and the instructions described in the *User-Entered Programs* section of the *DR/4000 Spectrophotometer Instrument Manual*, generate a calcium calibration curve from the calcium standards prepared above and a magnesium calibration curve from the magnesium standards prepared above.

Summary of Method

The colorimetric method for measuring hardness supplements the conventional titrimetric method because the colorimetric method can measure very low levels of calcium and magnesium. Also, some metals (those listed the table above) that interfere in the titrimetric method may be inconsequential when diluting the sample to bring it within the range of this test. The indicator dye is calmagite, which forms a purplish-blue color in a strongly alkaline solution and changes to red when contacting free calcium or magnesium. Calcium and magnesium determinations are made by chelating calcium with EGTA to destroy any red color due to calcium and then chelating the calcium and magnesium with EDTA to destroy the red color due to both calcium and magnesium. By measuring the red color in the different states, calcium and magnesium concentrations are determined.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to Section 3.

Pollution Prevention and Waste Management

For information on pollution prevention and waste management, refer to *Section 3*.

REQUIRED REAGENTS AND STANDARDS

	Cat. No.
Hardness Reagent Set (100 Tests)	23199-00
Includes: (1) 22417-32, (1) 22418-32, (1) 22419-26, (1) 22297-26	

Description	Quantity Required		Unit	Cat. No.
	per test			
Alkali Solution for Calcium and Magnesium Test.....	1 mL	100	mL MDB.....	22417-32
Calcium and Magnesium Indicator Solution.....	1 mL	100	mL MDB.....	22418-32
EDTA Solution, 1 M	1 drop	50	mL SCDB.....	22419-26
EGTA Solution	1 drop	50	mL SCDB.....	22297-26

REQUIRED EQUIPMENT AND SUPPLIES

Cylinder, 100-mL, graduated mixing.....	1	each.....	1896-42
Dropper, measuring, 0.5 and 1.0 mL.....	2	20/pkg.....	21247-20
DR/4000 1-Inch Cell Adapter	1	each.....	48190-00

OPTIONAL REAGENTS AND STANDARDS

Calcium Chloride Standard Solution, 50-mg/L as CaCO ₃	946 mL.....	21277-16
Magnesium Standard Solution, 1000-mg/L as Mg	100 mL.....	14794-42
Nitric Acid, ACS	500 mL.....	152-49
Sodium Hydroxide Standard Solution, 5.0 N.....	100 mL MDB.....	2450-32

OPTIONAL EQUIPMENT AND SUPPLIES

DR/4000 Carousel Module Kit	each.....	48070-02
DR/4000 Flow Cell Module Kit, 1-inch.....	each.....	48070-04
DR/4000 Flow Cell Module Kit, 1-cm.....	each.....	48070-05
DR/4000 Sipper Module Kit, 1-inch.....	each.....	48090-03
Flask, volumetric, Class A, 100-mL	each.....	14574-42
Flask, volumetric, Class A, 1000-mL, with stopper.....	each.....	14574-53
Pipet, volumetric, Class A, 1.00-mL	each.....	14515-35
Pipet, volumetric, Class A, 4.00-mL	each.....	14515-04
Pipet, volumetric, Class A, 5.00-mL	each.....	14515-37
Pipet, volumetric, Class A, 6.00-mL	each.....	14515-06
Pipet, volumetric, Class A, 7.00-mL	each.....	14515-07
Pipet, volumetric, Class A, 8.00-mL	each.....	14515-08
Pipet, volumetric, Class A, 10.00-mL	each.....	14515-38
Thermometer, pocket, -10 to 110 °C	each.....	1877-01



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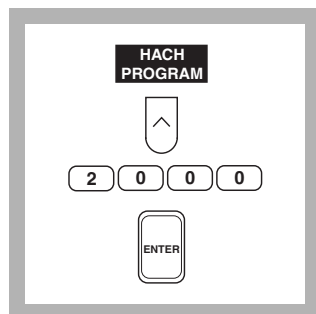
HACH COMPANY
WORLD HEADQUARTERS
Telephone: (970) 669-3050
FAX: (970) 669-2932



Method 8374

Calcium and Magnesium; Chlorophosphonazo Colorimetric Method ULR (0 to 1,000 µg/L Ca & Mg as CaCO₃)

Scope and Application: For ultra pure water. The estimated detection limit for program number 2000 is 4 µg/L.



- 1.** Press the soft key under **HACH PROGRAM**.

Select the stored program for ultra low range hardness by pressing **2000** with the numeric keys.

Press: **ENTER**

Note: If samples cannot be analyzed immediately, see *Sample Collection, Storage and Preservation* following these steps.

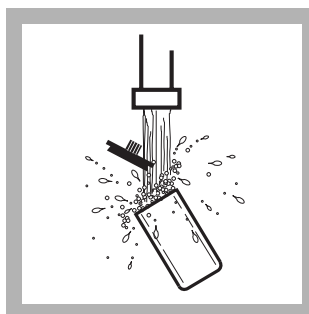
Note: The Flow Cell and Sipper Modules cannot be used for this procedure. Plastic sample cells must be used.



- 2.** The display will show:
**HACH PROGRAM: 2000
Hardness, Tot. ULR**

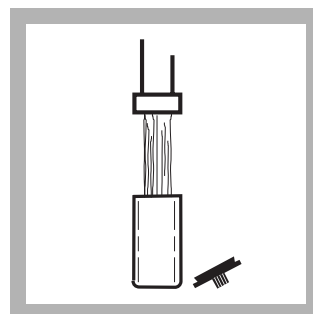
The wavelength (λ), **669 nm**, is automatically selected.

Note: For best results, determine a reagent blank for each new lot of reagent as follows. Prepare a reagent blank by repeating steps 3 through 11, using deionized water as the sample. The value displayed after Step 11 will be the reagent blank value. Correct for the reagent blank by pressing the soft keys under **OPTIONS, (MORE)**, and then **BLANK: OFF**. Enter the reagent blank value and press **ENTER**. Repeat for each new lot of reagent.



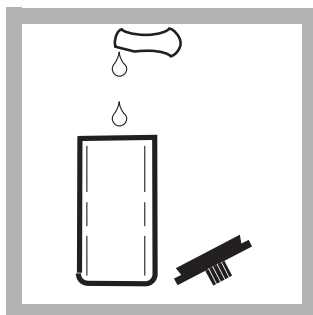
- 3.** Rinse a **plastic** sample cell and the cap three times with the water to be tested. Do not allow the underside of the cap to come in contact with surfaces that may contaminate it.

Note: Plastic sample cells must be used. Glass will contaminate the sample.



- 4.** Fill the plastic sample cell to the 25-mL mark with sample.

HARDNESS, Total, continued



5. Add the contents of one Chlorophosphonazo Solution Pillow to the sample cell.

Note: A small amount of solution may remain in the pillow. This will not affect results.

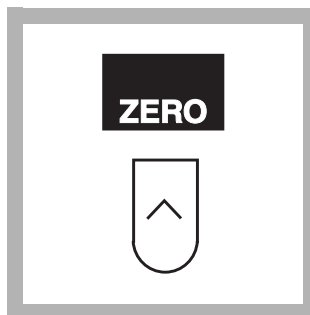
Note: One mL of Chlorophosphonazo Solution (Cat. No. 25895-49) may be used instead of the solution pillow.



6. Cap the cell and swirl to mix.



7. Place the sample cell into the cell holder. Close the light shield.



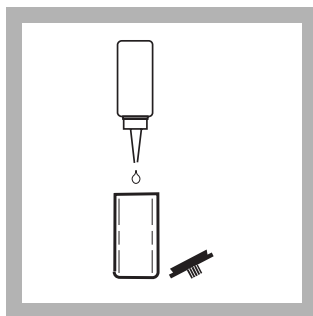
8. Press the soft key under **ZERO**.

The display will show:

0 $\mu\text{g/L CaCO}_3$

Note: If you are using a reagent blank correction, the display will show the correction.

Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



9. Remove the cell from the instrument. Add one drop of CDTA Reagent for Ultra Low Range Hardness.

Note: Complete steps 10-11 within 1-2 minutes.



10. Cap the cell and swirl to mix.



11. Place the sample cell into the cell holder. Close the light shield. The result in $\mu\text{g/L}$ as CaCO_3 (or chosen units) will be displayed.

Note: The results can be expressed as $\mu\text{g/L}$ calcium (Ca^{2+}) or magnesium (Mg^{2+}). Press the soft key under **OPTIONS**, then **FORM**: to scroll through the available options.

Interferences

Interference studies were conducted at various hardness levels between 0 and 500 µg/L as CaCO₃. Various cations and anions were evaluated at levels in the range appropriate for ultra pure water applications. An ion is said to interfere when the resulting concentration is changed by ±10%.

Table 1 Interfering Substances and Suggested Treatments

Interfering Substance	Interference Levels and Treatments
Aluminum	Negative interference above 150 µg/L
Ammonium	No interference at or below 1000 µg/L
Copper	Positive interference above 250 µg/L
Formaldehyde	No interference at or below 47,000 µg/L
Nitrate	Positive interference above 250 µg/L
Potassium	No interference at or below 1,000 µg/L
Silicon	Positive interference above 1,000 µg/L
Sodium	Negative interference above 79,000 µg/L

Sample Collection, Storage and Preservation

Do not use glass containers. Collect samples in clean plastic containers, preferably with screw-type closures. Rinse containers several times with the water to be analyzed before collecting final sample. Seal to avoid contamination during transport. Analyze as soon as possible.

Accuracy Check

Standard Additions Method

- Leave the unspiked sample in the sample cell compartment. Verify that the units displayed are in µg/L. Select standard additions mode by pressing the soft keys under **OPTIONS, (MORE)** and then **STD ADD**.
- Press **ENTER** to accept the default sample volume (mL), 25.
- Press **ENTER** to accept the default standard concentration (µg/L), 20,000.
- Press the soft key under **ENTRY DONE**.
- Obtain a Calcium Chloride Standard Solution, 20,000-µg/L as CaCO₃.
- Use the TenSette Pipet to add 0.1 mL, 0.2 mL and 0.3 mL of standard, respectively to three 25-mL samples and mix each thoroughly.
- Analyze each standard addition sample as described above. Accept the standard additions reading by pressing the soft key under **READ** each time. Each addition should reflect approximately 100% recovery.
- After completing the sequence, the display will show the extrapolated concentration value and the “best-fit” line through the standard additions data points, accounting for matrix interferences.
- See Section 1.4.1 *Standard Additions* for more information.

Standard Solution Method

Use the Calcium Chloride Standard Solution 500-µg/L (as CaCO₃) listed under **OPTIONAL REAGENTS AND STANDARDS**. Perform the total hardness procedure as described above.

HARDNESS, Total, continued

To adjust the calibration curve using the reading obtained with the 500-µg/L Calcium Chloride Standard Solution, press the soft keys under **OPTIONS, (MORE)** then **STD: OFF**. Press **ENTER** to accept the displayed concentration, the value of which depends on the selected units. If an alternate concentration is used, enter the actual concentration and press **ENTER** to return to the read screen. See Section 1.5.5 *Adjusting the Standard Curve* for more information.

Method Performance

Precision

Standard: 100 µg/L

Program	95% Confidence Limits
2000	98–102 µg/L

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
2000	4 µg/L

For more information on derivation and use of Hach's estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, appendix B, see Section 1.5.1.

Sensitivity

Program Number: 2000

Portion of Curve	ΔAbs	ΔConcentration
- 0.010 Abs	0.010	- 6.6 µg/L
500 µg/L	0.010	-7.2 µg/L
900 µg/L	0.010	-7.7 µg/L

See Section 1.5.3 *Sensitivity Explained* for more information.

Summary of Method

Calcium and magnesium combine equivalently with the chlorophosphonazo III indicator to form a complex which absorbs light very strongly at 669 nm. One drop of the CDTA reagent breaks up this complex, and the resultant decrease in absorbance is related to the amount of calcium and magnesium in the sample (as CaCO₃).

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to Section 1.

Pollution Prevention and Waste Management

For information on pollution prevention and waste management, refer to Section 1.

REQUIRED REAGENTS AND STANDARDS

Description	Cat. No.
ULR Hardness Reagent Set (100 tests)	26031-00
Includes: (1) 25895-99, (1) 25896-36, (1) 24102-01, (1) 24102-02	
ULR Hardness Reagent Set (500 tests)	26031-01
Includes; (1) 25895-49, (2) 25896-36, (1) 24102-01, (1) 24102-02	

Description	Quantity Required per test	Unit	Cat. No.
Chlorophosphonazo Indicator Solution Pillows	1 pillow	100/pkg	25895-99
CDTA Solution	1 drop	10 mL SCDB	25896-36

REQUIRED EQUIPMENT AND SUPPLIES

Clippers (Shears) for opening solution pillows	1	each	23694-00
DR/4000 1-Inch Cell Adapter	1	each	48190-00
Sample Cell, 1-inch, polystyrene w/ cap	1	12/pkg	24102-12

OPTIONAL REAGENTS AND STANDARDS

Calcium Chloride Standard Solution, 20,000-µg/L as CaCO ₃	946 mL	21246-16
Calcium Chloride Standard Solution, 500-µg/L as CaCO ₃	946 mL	20580-16
Chlorophosphonazo Indicator Solution	500 mL	25895-49

OPTIONAL EQUIPMENT AND SUPPLIES

Dispenser, 1.0-mL, Repipet Jr.	each	21113-01
DR/4000 Carousel Module Kit	each	48070-02
Pipet, TenSette, 0.1 to 1.0 mL	each	19700-01
Pipet Tips, for 19700-01 TenSette Pipet	50/pkg	21856-96



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Method 8141

p-Dimethylaminobenzaldehyde Method*

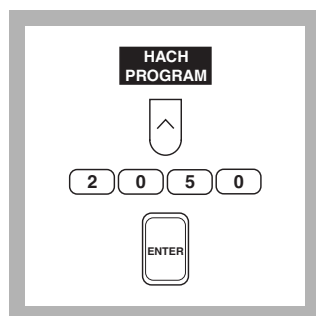
Reagent Solution or AccuVac® Ampuls

(0 to 600.0 µg/L)

Scope and Application: For boiler water/feedwater, water and seawater. The estimated detection limit for program numbers 2050 and 2060 are 2.4 and 4.3 µg/L N_2H_4 , respectively.

* Adapted from ASTM Manual of Industrial Water, D1385-78, 376 (1979)

Using Reagent Solution



- 1.** Press the soft key under **HACH PROGRAM**.

Select the stored program for hydrazine by pressing **2050** with the numeric keys.

Press: **ENTER**

Note: Samples must be analyzed immediately and cannot be preserved for later analysis.

Note: The Flow Cell and Sipper Modules can be used for this procedure. Use a 25-mL sample and reagents for the Flow Cell Module.



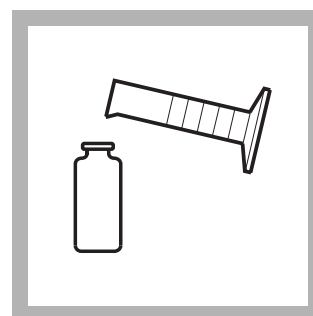
- 2.** The display will show: **HACH PROGRAM: 2050 Hydrazine**

The wavelength (λ), **455 nm**, is automatically selected.

Note: For best results, determine a reagent blank for each new lot of reagent as follows. Prepare a reagent blank by repeating steps 3 through 10, using deionized water as the sample. Zero the instrument on deionized water by pressing the soft key under **ZERO**. Insert the reagent blank and the blank value will be displayed. Correct for the reagent blank by pressing the soft keys under **OPTIONS, (MORE)**, and then **BLANK:OFF**. Enter the reagent blank value and press **ENTER**. Repeat for each new lot of reagent.



- 3.** Pour 10 mL of deionized water into a sample cell (the blank) using a graduated cylinder.



- 4.** Pour 10 mL of sample into a second sample cell (the prepared sample) using a graduated cylinder.

Note: For proof of accuracy, use a 100-µg/L hydrazine standard solution (preparation given in the Accuracy Check section) in place of the sample.

Note: Sample temperature should be 21 ± 4 °C (70 ± 7 °F).

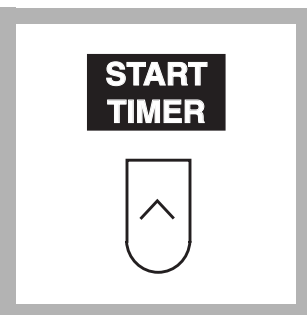
Note: For turbid samples see the Interferences section.



5. Add 0.5 mL of HydraVer 2 Hydrazine Reagent to each sample cell. Swirl to mix.

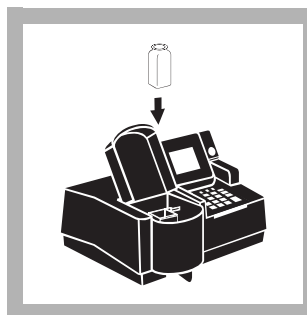
Note: A yellow color will develop if hydrazine is present.

Note: HydraVer 2 Hydrazine Reagent will cause a faint yellow color to appear in the blank.

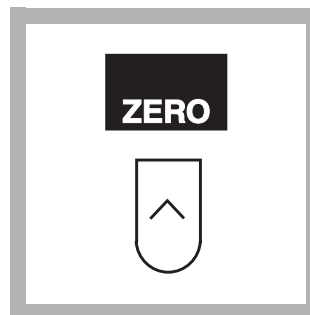


6. Immediately press the soft key under **START TIMER**.

A 12-minute reaction period will begin. Complete steps 7-9 during this period.



7. Place the blank into the cell holder. Close the light shield.



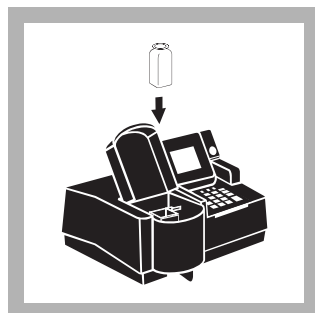
8. Press the soft key under **ZERO**.

The display will show:

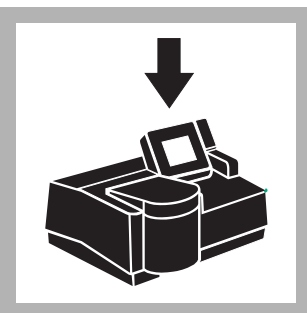
0.0 µg/L N₂H₄

Note: If you are using a reagent blank correction, the display will show the correction.

Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



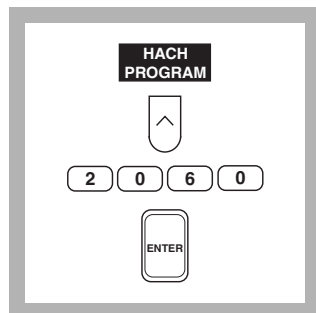
9. Place the prepared sample into the cell holder. Close the light shield.



10. Immediately after the timer beeps, read the result in µg/L hydrazine (or chosen units) that is displayed.

Note: The results can be expressed as mg/L hydrazine sulfate (N₂H₄•H₂SO₄). Press the soft keys under **OPTIONS**, then **FORM**: to scroll through the available options. Press **ENTER** to return to the read screen.

Using AccuVac Ampuls



1. Press the soft key under **HACH PROGRAM**.

Select the stored program for hydrazine by pressing **2060** with the numeric keys.

Press: **ENTER**

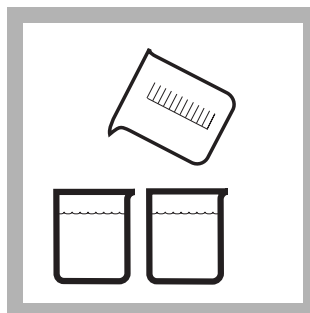
Note: Samples must be analyzed immediately and cannot be preserved for later analysis.



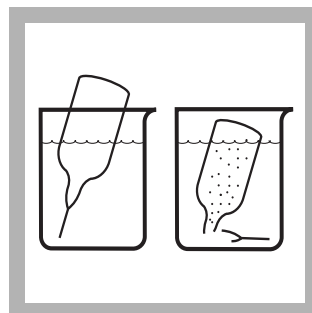
2. The display will show:
HACH PROGRAM: 2060 Hydrazine, AV

The wavelength (λ), **455 nm**, is automatically selected.

Note: For best results, determine a reagent blank for each new lot of reagent as follows. Prepare a reagent blank by repeating Steps 3 through 10, using deionized water as the sample. Zero the instrument on deionized water by pressing the soft key under **ZERO**. Insert the reagent blank and the blank value will be displayed. Correct for the reagent blank by pressing the soft keys under **OPTIONS, (MORE)**, and then **BLANK:OFF**. Enter the reagent blank value and press **ENTER**. Repeat for each new lot of reagent.



3. Collect at least 40 mL of sample in a 50-mL beaker. Pour at least 40 mL of deionized water into a second 50-mL beaker.

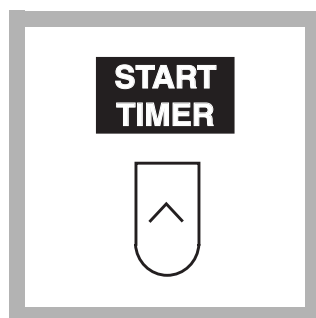


4. Fill a HydraVer Hydrazine AccuVac Ampul with sample by breaking the tip on the bottom of the beaker. Fill a second AccuVac Ampul with deionized water (the blank) in the same manner.

Note: Keep the tip immersed while the ampul fills completely.

Note: For proof of accuracy, use a 100 $\mu\text{g/L}$ hydrazine standard solution (preparation given in the Accuracy Check section) in place of the sample.

Note: Sample temperature should be $21 \pm 4^\circ\text{C}$ ($70 \pm 7^\circ\text{F}$).



5. Immediately press the soft key under **START TIMER**.

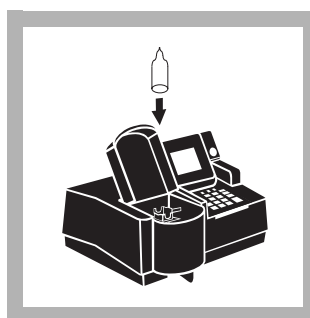
A 12-minute reaction period will begin. Complete steps 6–9 during this period.

Note: A yellow color will develop if hydrazine is present.

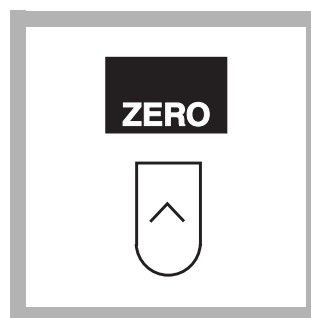
Note: HydraVer 2 Hydrazine Reagent will cause a faint yellow color to appear in the blank.



6. Insert the AccuVac Ampul Adapter into the sample cell module by sliding it under the thumb screw and into the alignment grooves. Fasten with the thumb screw.



7. Place the blank into the cell holder. Close the light shield.



8. Press the soft key under **ZERO**.

The display will show:

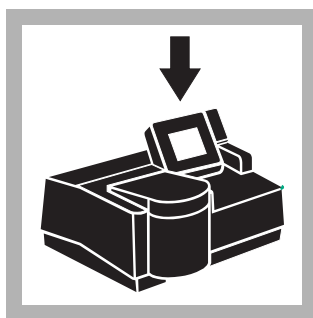
0.0 µg/L N₂H₄

Note: If you are using a reagent blank correction, the display will show the correction.

Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



9. Place the prepared sample into the cell holder. Close the light shield.



10. Immediately after the timer beeps, read the result in µg/L hydrazine (or chosen units) that is displayed.

Note: The results can be expressed as mg/L hydrazine sulfate (N₂H₄•H₂SO₄). Press the soft keys under **OPTIONS**, then **FORM**: to scroll through the available options. Press **ENTER** to return to the read screen.

Interferences

Table 1 Interfering Substances and Suggested Treatments

Interfering Substance	Interference Levels and Treatments
Highly colored or turbid samples	Prepare a blank by oxidizing the hydrazine in a portion of the sample with a 1:1 mixture of deionized water and a household bleach. Add one drop of the mixture to 25 mL of sample in a graduated mixing cylinder and invert to mix. Use this solution in Step 3, in place of deionized water, to prepare the blank.

Ammonia does not interfere up to 10 mg/L. At 20 mg/L it may cause a positive interference of up to 20%. Morpholine does not interfere up to 10 mg/L.

Sample Collection, Storage and Preservation

Samples collected in glass or plastic bottles should be filled completely and capped tightly. Avoid excessive agitation or exposure to air. Samples must be analyzed immediately after collection and cannot be preserved for later analysis.

Accuracy Check

Standard Solution Method

Use Class A glassware for standard preparation. Prepare a 25 mg/L stock solution by dissolving 0.1016 g of hydrazine sulfate in 1000 mL of oxygen-free deionized water. Prepare this stock solution daily. Prepare a 0.1-mg/L (100-μg/L) hydrazine working solution by diluting 4.00 mL of the 25-mg/L stock solution to 1000 mL with deoxygenated deionized water. Prepare just before analysis. Perform either hydrazine procedure as described above.

To adjust the calibration curve using the reading obtained with the 100-μg/L standard solution, press the soft keys under **OPTIONS, MORE** then **STD: OFF**. Press **ENTER** to accept the displayed concentration, the value of which depends on the selected units. If an alternate concentration is used, enter the actual concentration and press **ENTER** to return to the read screen. See Section 1.5.5 *Adjusting the Standard Curve* for more information.

Method Performance

Precision

Standard: 100.0 μg/L N₂H₄

Program	95% Confidence Limits
2050	98.6–101.4 μg/L N ₂ H ₄
2060	97.5–102.5 μg/L N ₂ H ₄

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
2050	2.4 µg/L N ₂ H ₄
2060	4.3 µg/L N ₂ H ₄

For more information on derivation and use of Hach's estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, appendix B, see Section 1.5.1.

Sensitivity

Program Number: 2050

Portion of Curve	ΔAbs	ΔConcentration
Entire Range	0.010	3.13 µg/L

Program Number: 2060

Portion of Curve	ΔAbs	ΔConcentration
Entire Range	0.010	3.47 µg/L

See Section 1.5.3 *Sensitivity Explained* for more information.

Calibration Standard Preparation

To perform a hydrazine calibration using the p-dimethylaminobenzaldehyde method, prepare a 100-mg/L hydrazine stock solution by adding 0.4064 g of hydrazine sulfate to a 1000-mL volumetric flask. Dilute to volume with oxygen-free deionized water and mix thoroughly. Prepare a 4000-µg/L hydrazine working solution by diluting 4.0 mL of the 100-mg/L stock solution into a 100-mL volumetric flask using Class A glassware. Dilute to volume with oxygen-free deionized water and mix thoroughly.

Prepare calibration standards containing 40.0, 80.0, 160.0, 240.0, 320.0, 400.0, and 480.0 µg/L N₂H₄ as follows:

- Into seven different Class A 100-mL volumetric flasks, pipet 1.00, 2.00, 4.00, 6.00, 8.00, 10.00, and 12.00 mL of the 4000-µg/L working solution using Class A glassware.
- Dilute to the mark with oxygen-free deionized water and mix thoroughly. These standards must be used immediately, and cannot be saved for later use.
- Using the p-dimethylaminobenzaldehyde method and the calibration procedure described in the *User-Entered Programs* section of the *DR/4000 Spectrophotometer Instrument Manual*, generate a calibration curve from the standards prepared above.

Summary of Method

Hydrazine in the sample reacts with the p-dimethylaminobenzaldehyde from the HydraVer 2 Reagent to form a yellow color which is proportional to the hydrazine concentration.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to Section 1.

Pollution Prevention and Waste Management

The final samples are highly acidic. Neutralize to pH 6–9 and flush down the drain for disposal. For information on pollution prevention and waste management, refer to Section 1.

REQUIRED REAGENTS AND STANDARDS (Using Solution)

Description	Quantity Required		Unit	Cat. No.
	per test			
HydraVer 2 Hydrazine Reagent.....	1 mL ... 100 mL*	MDB.....		1790-32
Water, deionized.....	10 mL	4 liters.....		272-56

REQUIRED EQUIPMENT AND SUPPLIES (Using Solution)

Cylinder, graduated, 25-mL	1	each.....	508-40
DR/4000 1-Inch Cell Adapter	1	each.....	48190-00

REQUIRED REAGENTS AND STANDARDS (Using AccuVac Ampuls)

Hydrazine Reagent AccuVac Ampuls	2	25/pkg.....	25240-25
Water, deionized.....	40 mL	4 liters.....	272-56

REQUIRED EQUIPMENT AND SUPPLIES (Using AccuVac Ampuls)

Beaker, 50-mL.....	2	each.....	500-41
DR/4000 AccuVac Ampul Adapter.....	1	each.....	48187-00

OPTIONAL REAGENTS AND STANDARDS

Hydrazine Sulfate, ACS	100 g.....	742-26
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OPTIONAL EQUIPMENT AND SUPPLIES

Cylinder, graduated mixing, 25-mL	each.....	1896-40
DR/4000 Carousel Module Kit	each.....	48070-02
DR/4000 Flow Cell Module Kit, 1-inch.....	each.....	48070-04
DR/4000 Sipper Module Kit, 1-inch.....	each.....	48090-03
Flask, volumetric, Class A, 100-mL	each.....	14574-42
Flask, volumetric, Class A, 1000-mL, with stopper.....	each.....	14574-53
Pipet, serological, 1-mL	each.....	532-35
Pipet, TenSette, 0.1 to 1.0 mL	each.....	19700-01
Pipet Tips, for 19700-01 TenSette Pipet	50/pkg.....	21856-96
Pipet, volumetric, Class A, 1.00-mL	each.....	14515-35
Pipet, volumetric, Class A, 2.00-mL	each.....	14515-36
Pipet, volumetric, Class A, 3.00-mL	each.....	14515-03
Pipet, volumetric, Class A, 4.00-mL	each.....	14515-04
Pipet, volumetric, Class A, 6.00-mL	each.....	14515-06
Pipet, volumetric, Class A, 8.00-mL	each.....	14515-08
Pipet, volumetric, Class A, 10.00-mL	each.....	14515-38
Pipet Filler, safety bulb.....	each.....	14651-00
Thermometer, pocket, -10 to 110 °C.....	each.....	1877-01

* Contact Hach for larger sizes.



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HACH COMPANY
WORLD HEADQUARTERS
Telephone: (970) 669-3050
FAX: (970) 669-2932



Method 8031

DPD Method*

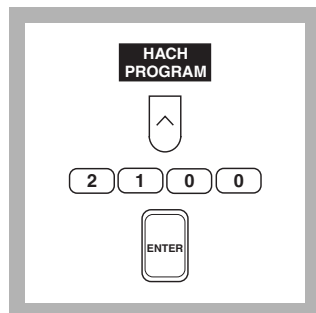
Powder Pillows or AccuVac® Ampuls

(0 to 7.00 mg/L)

Scope and Application: For testing dissolved iodine residual used as disinfectant in process water, treated water, estuary and seawater. The estimated detection limit for program numbers 2100 and 2110 is 0.04 mg/L I₂.

* Adapted from Palin, A.T., *Inst. Water Eng.*, 21 (6), 537-547 (1967)

Using Powder Pillows



1. Press the soft key under **HACH PROGRAM**.

Select the stored program number for iodine (I₂) by pressing **2100** with the numeric keys.

Press: **ENTER**

Note: Samples must be analyzed immediately and cannot be preserved for later analysis.

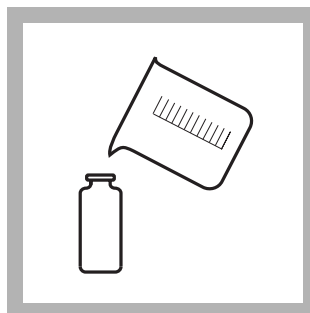
Note: The Flow Cell and Sipper Modules can be used with this procedure if rinsed with deionized water immediately after analysis. Use a 25-mL sample and reagents with the Flow Cell Module.



2. The display will show: **HACH PROGRAM: 2100 Iodine**

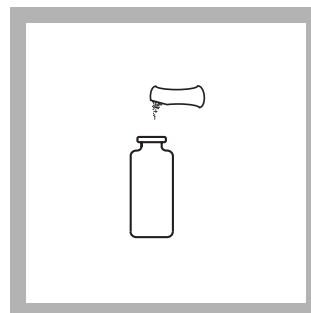
The wavelength (λ), **530 nm**, is automatically selected.

Note: For best results, determine a reagent blank for each new lot of reagent as follows. Prepare a reagent blank by repeating steps 3 through 8, using deionized water as the sample. Zero the instrument on deionized water by pressing the soft key under **ZERO**. Insert the reagent blank and the blank value will be displayed. Correct for the reagent blank by pressing the soft keys under **OPTIONS, (MORE)**, and then **BLANK:OFF**. Enter the reagent blank value and press **ENTER**. Repeat for each new lot of reagent.



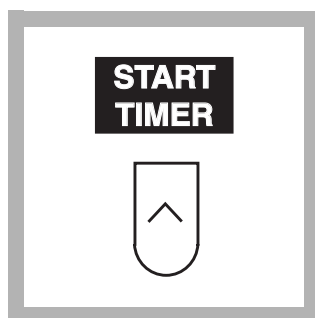
3. Fill a cell with 10 mL of sample.

Note: For samples with extreme pH, see *Interferences section*.



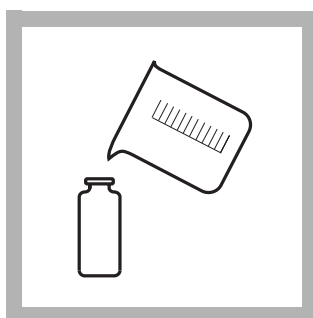
4. Add the contents of one DPD Total Chlorine Powder Pillow to the sample cell (the prepared sample). Swirl to mix.

Note: A pink color will develop if iodine is present.

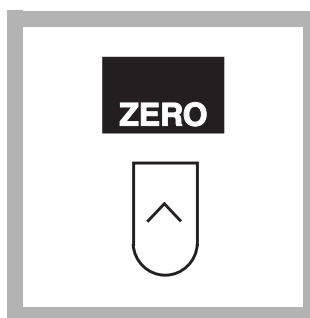


5. Press the soft key under **START TIMER**.

A 3-minute reaction period will begin.



6. Fill a second sample cell with 10 mL of sample (the blank). Place it into the cell holder.



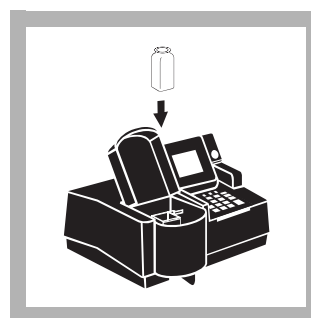
7. Press the soft key under **ZERO**.

The display will show:

0.00 mg/L I₂

Note: If you are using a reagent blank correction, the display will show the correction.

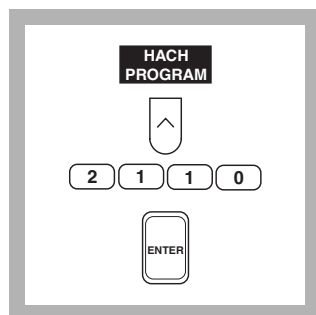
Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



8. Within 3 minutes after the timer beeps, place the prepared sample into the cell holder. Close the light shield. Result in mg/L I₂ (or chosen units) will be displayed.

Note: If the sample temporarily turns yellow after reagent addition, or the display reads **OVER!**, dilute a fresh sample. Repeat the test. A slight loss of iodine may occur because of the dilution. Apply the appropriate dilution factor; see Section 1.2.6 Sample Dilution Techniques.

Using AccuVac Ampuls



1. Press the soft key under **HACH PROGRAM**.

Select the stored program number for iodine (I_2) by pressing **2110** with the numeric keys.

Press: **ENTER**

Note: Samples must be analyzed immediately and cannot be preserved for later analysis.



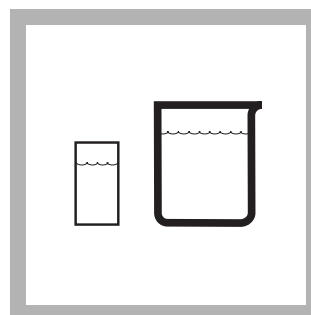
2. The display will show:
HACH PROGRAM: 2110 Iodine, AV

The wavelength (λ), **530 nm**, is automatically selected.

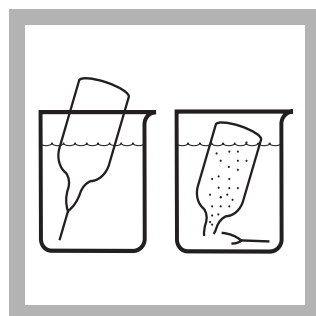
Note: For best results determine a reagent blank for each new lot of reagent. See the note under Step 2 of the powder pillow method.



3. Insert the AccuVac Ampul Adapter by sliding it under the thumb screw and into the alignment grooves. Fasten with the thumb screw.

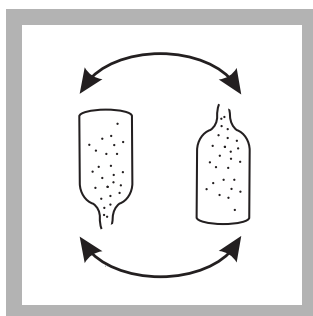


4. Fill a zeroing vial (the blank) with at least 10 mL of sample. Collect at least 40 mL of sample in a 50-mL beaker.



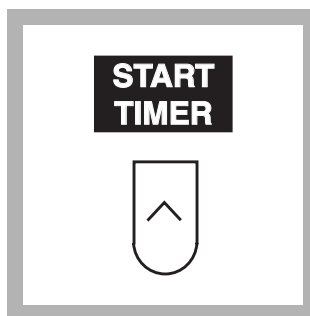
5. Fill a DPD Total Chlorine Reagent AccuVac Ampul with sample.

Note: Keep the tip immersed while the ampul fills completely.



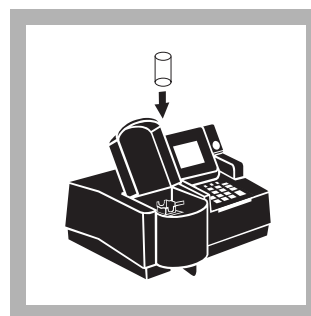
6. Quickly invert the ampul several times to mix. Wipe off any liquid or fingerprints.

Note: A pink color will form if iodine is present.

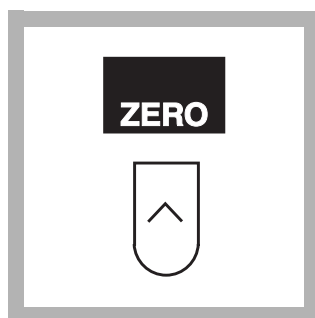


7. Press the soft key under **START TIMER**. A 3-minute reaction period will begin.

Note: Perform steps 8–9 during the three minute period.



8. Place the blank into the cell holder. Close the light shield.



9. Press the soft key under **ZERO**.

The display will show:

0.00 mg/L I₂

Note: If you are using a reagent blank correction, the display will show the correction.

Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



10. Within 3 minutes after the timer beeps, place the AccuVac Ampul into the cell holder. Close the light shield. Result in mg/L iodine will be displayed.

Note: If the sample temporarily turns yellow after sample addition, or the display shows **OVER!** dilute a fresh sample. Repeat the test. A slight loss of iodine may occur because of the dilution. Apply the appropriate dilution factor; see Section 1.2.6 Sample Dilution Techniques.

Interferences

Table 1 Interfering Substances and Suggested Treatments

Interfering Substance	Interference Level and Treatment
Acidity	Greater than 150 mg/L CaCO ₃ . May not develop full color or color may fade instantly. Neutralize to pH 6–7 with 1 N sodium hydroxide. Determine amount to be added on separate sample aliquot, then add the same amount to the sample being tested. Correct for volume addition (See Section 1.2.2 <i>Correcting for Volume Additions</i>).
Alkalinity	Greater than 250 mg/L CaCO ₃ . May not develop full color or color may fade instantly. Neutralize to pH 6–7 with 1 N sulfuric acid. Determine amount to be added on separate sample aliquot, then add the same amount to the sample being tested. Correct for volume addition (See Section 1.2.2 <i>Correcting for Volume Additions</i>).
Bromine	Interferes at all levels
Chlorine and chloramines	Causes a positive interference at all levels
Chlorine Dioxide	Interferes at all levels
Chloramines, organic	May interfere
Hardness	No effect at less than 1,000 mg/L as CaCO ₃
Manganese, Oxidized (Mn ⁴⁺ , Mn ⁷⁺) or Chromium, Oxidized (Cr ⁶⁺)	<ol style="list-style-type: none"> 1. Adjust sample pH to 6–7. 2. Add 3 drops potassium iodide (30 g/L) to a 25-mL sample. 3. Mix and wait 1 minute. 4. Add 3 drops sodium arsenite (5 g/L) and mix. 5. Analyze 10 mL of the treated sample as described in the procedure. 6. Subtract the result from this test from the original analysis to obtain the correct iodine concentration.
Ozone	Interferes at all levels
Peroxides	May interfere
Extreme sample pH	Adjust to pH 6–7. See Section 1.3.1 <i>pH Interference</i> .
Highly Buffered Samples	Adjust to pH 6–7. See Section 1.3.1 <i>pH Interference</i> .

Sample Collection, Storage and Preservation

Collect samples in clean, dry glass containers. If sampling from a tap, allow the water to flow at least 5 minutes to ensure a representative sample. Avoid excessive agitation and exposure to sunlight when sampling. Allow several volumes of water to overflow the container and cap the container so there is not headspace above the sample. If sampling with a DR cell, rinse the cell several times with the sample, then carefully fill to the 10-mL mark. Proceed with the analysis immediately.

Accuracy Check

Standard Additions Method (Using Powder Pillows)

- Leave the unspiked sample in the sample cell compartment. Verify that the units displayed are in mg/L. Select standard additions mode by pressing the soft keys under **OPTIONS, (MORE)** and then **STD ADD**.
- Press **ENTER** to accept the default sample volume (mL), 10.
- Multiply the average chlorine concentration shown on the certificate enclosed with the LR Voluettes by 3.6 to obtain the equivalent

concentration of iodine. When prompted for the standard concentration, use the numeric keys to enter the calculated iodine value. Press **ENTER**.

- d. Press the soft key under **ENTRY DONE**.
- e. Snap the neck off a LR Chlorine Voluette Ampule Standard, 20–30 mg/L Cl₂.
- f. Use the TenSette Pipet to add 0.1 mL, 0.2 mL and 0.3 mL of standard, respectively to three 10-mL samples and mix each thoroughly.
- g. Analyze each standard addition sample as described above. Accept the standard additions readings by pressing the soft key under **READ** each time. Each addition should reflect approximately 100% recovery.
- h. After completing the sequence, the display will show the extrapolated concentration value and the “best-fit” line through the standard additions data points, accounting for matrix interferences.
- i. See Section 1.4.1 *Standard Additions* for more information.

Standard Additions Method (Using AccuVac Ampuls)

- a. Leave the unspiked sample in the sample cell compartment. Verify that the units displayed are in mg/L. Select standard additions mode by pressing the soft keys under **OPTIONS, (MORE)** and then **STD ADD**.
- b. Press **ENTER** to accept the default sample volume (mL), 25.
- c. Multiply the average chlorine concentration shown on the certificate enclosed with the LR Voluettes by 3.6 to obtain the equivalent concentration of iodine. When prompted for the standard concentration, use the numeric keys to enter the calculated iodine value. Press **ENTER**.
- d. Press the soft key under **ENTRY DONE**.
- e. Snap the neck off a LR Chlorine Voluette Ampule Standard, 20–30 mg/L Cl₂.
- f. Use graduated cylinder to measure 25 mL of sample into each of three 50-mL beakers. Use a TenSette Pipet to add 0.2, 0.4 and 0.6 mL of standard, respectively, to each of the 25-mL samples. Swirl gently to mix.
- g. Fill a DPD Total Chlorine AccuVac Ampul completely from each beaker and analyze each standard addition sample as described above. Accept the standard additions readings by pressing the soft key under **READ** each time. Each addition should reflect approximately 100% recovery.
- h. After completing the sequence, the display will show the extrapolated concentration value and the “best-fit” line through the standard additions data points, accounting for matrix interferences.
- i. See Section 1.4.1 *Standard Additions* for more information.

Method Performance

Precision

Standard: 3.50 mg/L I₂

Program	95% Confidence Limits
2100	3.47–3.54 mg/L I ₂
2110	3.47–3.54 mg/L I ₂

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
2100	0.04 mg/L I ₂
2110	0.04 mg/L I ₂

For more information on derivation and use of Hach's estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, appendix B, see Section 1.5.1.

Sensitivity

Program Number: 2100

Portion of Curve:	ΔAbs	ΔConcentration
Entire Range	0.010	0.066 mg/L

Program Number: 2110

Portion of Curve:	ΔAbs	ΔConcentration
Entire Range	0.010	0.071 mg/L

See Section 1.5.3 *Sensitivity Explained* for more information.

Summary of Method

Iodine reacts with DPD (N, N-diethyl-p-phenylenediamine) to form a pink color which is proportional to the total iodine concentration.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to Section 1.

Pollution Prevention and Waste Management

Samples treated with sodium arsenite for manganese or chromium interferences will be hazardous wastes as regulated by the Federal RCRA for arsenic (D004). See Section 1 for more information on proper disposal of these materials.

IODINE, continued

REQUIRED REAGENTS AND STANDARDS (Using Powder Pillows)

Description	Quantity Required per test	Unit	Cat. No.
DPD Total Chlorine Reagent Powder Pillows.....	1 pillow	100/pkg.....	21056-69

REQUIRED REAGENTS AND STANDARDS (Using AccuVac Ampuls)

DPD Total Chlorine Reagent AccuVac Ampuls.....	1 ampul.....	25/pkg.....	25030-25
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REQUIRED EQUIPMENT AND SUPPLIES (Using Powder Pillows)

DR/4000 1-inch Cell Adapter	1	each.....	48190-00
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REQUIRED EQUIPMENT AND SUPPLIES (Using AccuVac Ampuls)

DR/4000 AccuVac Ampul Adapter.....	1	each.....	48187-00
Beaker, 50-mL.....	1	each.....	500-41
Sample Cell, 10-mL, with cap.....	1	each.....	21228-00

OPTIONAL REAGENTS AND STANDARDS

Chlorine Standard Solution, 1-mL Voluette Ampule, 20–30 mg/L Cl ₂	20/pkg.....	26300-20
Potassium Iodide Solution, 30-g/L	100 mL * MDB	343-32
Sodium Arsenite Solution, 5-g/L	100 mL * MDB	1047-32
Sodium Hydroxide Standard Solution, 1.00 N	100 mL * MDB	1045-32
Sulfuric Acid Standard Solution, 1.000 N.....	100 mL * MDB	1270-32
Water, deionized.....	4 liters.....	272-56

OPTIONAL EQUIPMENT AND SUPPLIES

AccuVac Snapper	each.....	24052-00
Ampule Breaker Kit	each.....	21968-00
Cylinder, graduated, 25-mL, poly	each.....	1081-40
Graph Paper, linear.....	100/pkg.....	22313-00
pH Meter, <i>sensio</i> TM 1, portable	each.....	51700-00
Pipet, TenSette, 0.1 to 1.0 mL	each.....	19700-01
Pipet Tips, for 19700-01 TenSette Pipet	50/pkg.....	21856-96
DR/4000 Carousel Module Kit	each.....	48070-02
DR/4000 Flow Cell Module Kit, 1-inch.....	each.....	48070-04
DR/4000 Flow Cell Module Kit, 1-cm.....	each.....	48070-05
DR/4000 Sipper Module Kit, 1-inch.....	each.....	48090-03

* Contact Hach for larger sizes.



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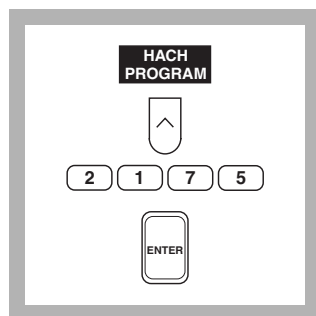
Method 8147

FerroZine Method*

(0 to 1.400 mg/L)

Scope and Application: For water and wastewater; digestion is required for determining total iron. See SECTION 2 for digestion procedure. The estimated detection limit for program number 2175 is 0.004 mg/L Fe.

* Adapted from Stookey, L.L., *Anal. Chem.*, 42 (7), 779 (1970)



1. Press the soft key under **HACH PROGRAM**.

Select the stored program number for iron (Fe), FerroZine, by pressing **2175** with the numeric keys.

Press: **ENTER**

Note: Analyze samples as soon as possible to prevent air oxidation of ferrous iron to ferric iron, which is not determined. See Sample Collection, Storage and Preservation following these steps.

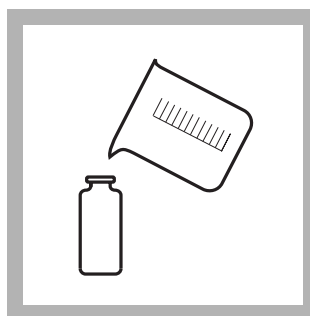
Note: The Flow Cell and Sipper Modules can be used with this procedure.



2. The display will show: **HACH PROGRAM: 2175 Iron, FerroZine**

The wavelength (λ), **562 nm**, is automatically selected.

Note: For best results, determine a reagent blank for each new lot of reagent as follows. Prepare a reagent blank by repeating steps 3 through 9, using deionized water as the sample. Zero the instrument on deionized water by pressing the soft keys under **ZERO**. Insert the reagent blank and the blank value will be displayed. Correct for the reagent blank value by pressing the soft keys under **OPTIONS, (MORE)**, and then **BLANK:OFF**. Enter the reagent blank value and press **ENTER**. Repeat for each new lot of reagent.



3. Fill a clean sample cell to the 25-mL mark with sample.

Note: Rinse glassware with a 1:1 Hydrochloric Acid Solution. Rinse again with deionized water. These two steps will remove iron deposits which can cause slightly high results.

Note: For proof of accuracy, use a 0.4 mg/L iron standard solution (preparation given in the Accuracy Check section) in place of the sample.



4. Add the contents of one FerroZine Iron Reagent Solution Pillow to the sample cell (the prepared sample). Swirl to mix.

Note: Do not allow the clippers to come into contact with the contents of the pillow.

Note: 0.5 mL of FerroZine Iron Reagent Solution can be used in place of the solution pillow if preferred.

Note: If the sample contains rust, see Interferences following these steps.

**START
TIMER**



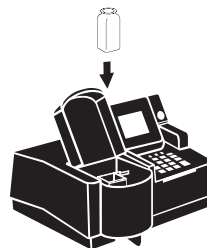
5. Press the soft key under **START TIMER**.

A 5-minute reaction period will begin.

Note: A violet color will develop if iron is present.



6. Fill another sample cell (the blank) with 25 mL of sample.



7. When the timer beeps, insert the blank into the cell holder. Close the light shield.

ZERO



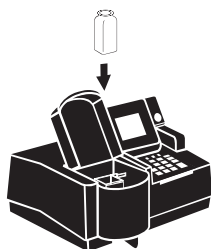
8. Press the soft key under **ZERO**.

The display will show:

0.00 mg/L Fe

Note: If you are using a reagent blank correction, the display will show the correction.

Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



9. Place the prepared sample into the cell holder. Close the light shield. The results in mg/L total iron (or chosen units) will be displayed.

Interferences

Interfering Substance	Interference Levels and Treatments
Strong chelants (EDTA)	Interfere at all levels. Use the FerroVer or TPTZ methods for these samples. Use the TPTZ method for low iron concentrations.
Cobalt	May give slightly high results
Copper	May give slightly high results
Hydroxides	Boil the sample, with the FerroZine Iron Reagent added to it from Step 4, for 1 minute in a boiling water bath. Cool to 24 °C (75 °F) before proceeding with Step 5. Return the sample volume to 25 mL with deionized water. <i>or</i> Use any of the digestions in <i>SECTION 2 SAMPLE PRETREATMENT</i> .
Magnetite (black iron oxide) or Ferrites	a) Fill a 25-mL graduated cylinder with 25 mL of sample. b) Transfer this sample into a 125-mL erlenmeyer flask. c) Add the contents of one FerroZine Iron Reagent Solution Pillow and swirl to mix. d) Place the flask on a hot plate or over a flame and bring to a boil. e) Continue boiling gently for 20 to 30 minutes. Note: Do not allow to boil dry. Note: A purple color will develop if iron is present. a) Return the boiled sample to the 25-mL graduated cylinder. Rinse the erlenmeyer flask with small amounts of deionized water and empty into the graduated cylinder. b) Return the sample volume to the 25-mL mark with deionized water. c) Pour this solution into a sample cell and swirl to mix. d) Proceed with Steps 5–9. <i>or</i> Use any of the digestions in <i>SECTION 1 SAMPLE PRETREATMENT</i> .
Rust	Boil the sample, with the FerroZine Iron Reagent from Step 4, for 1 minute in a boiling water bath. Cool to 24 °C (75 °F) before proceeding with Step 5. Return the sample volume to 25 mL with deionized water. <i>or</i> Use any of the digestions in <i>SECTION 1 SAMPLE PRETREATMENT</i> .

Sample Collection, Storage and Preservation

Collect samples in acid-washed glass or plastic bottles. To preserve samples, adjust the sample pH to 2 or less with concentrated nitric acid, ACS (about 2 mL per liter). Samples preserved in this manner can be stored up to six months at room temperature. If you are only reporting dissolved iron, filter the sample immediately after collection and before addition of nitric acid.

Before testing, adjust the sample pH to 3–5 with ammonium hydroxide, ACS. Do not exceed pH 5 as iron may precipitate. Correct test results for volume additions; see Section 1.2.2 *Correcting for Volume Additions*.

Accuracy Check

Standard Additions Method

- Leave the unspiked sample in the sample cell compartment. Verify that the units displayed are in mg/L. Select standard additions mode by pressing the soft keys under **OPTIONS, (MORE)** and then **STD ADD**.
- Press **ENTER** to accept the default sample volume (mL), 25.
- Press **ENTER** to accept the default standard concentration (mg/L), 25.
- Press the soft key under **ENTRY DONE**.
- Snap the neck off an Iron Voluette Ampule Standard, 25 mg/L Fe.

- f. Use the TenSette Pipet to add 0.1, 0.2 mL and 0.3 mL of standard, respectively to three 25-mL samples and mix each thoroughly.
- g. Analyze each standard addition sample as described above. Accept the standard additions reading by pressing the soft key under **READ** each time. Each addition should reflect approximately 100% recovery.
- h. After completing the sequence, the display will show the extrapolated concentration value and the “best-fit” line through the standard additions data points, accounting for matrix interferences.
- i. See Section 1.4.1 *Standard Additions* for more information.

Standard Solution Method

Using Class A glassware, prepare a 0.4-mg/L Fe standard solution by pipetting 1.00 mL of Iron Standard Solution, 100 mg/L, into a 250-mL volumetric flask. Dilute to the mark with deionized water. Prepare this solution daily. Perform the iron procedure as described above.

To adjust the calibration curve using the reading obtained with the 0.4-mg/L standard solution, press the soft keys under **OPTIONS, (MORE)** then **STD: OFF**. Press **ENTER** to accept the default concentration, the value of which will depend on the selected units. If an alternate concentration is used, enter the actual concentration and press **ENTER** to return to the read screen. See Section 1.5.5 *Adjusting the Standard Curve* for more information.

Method Performance

Precision

Standard: 0.400 mg/L Fe

Program	95% Confidence Limits
2175	0.380–0.420 mg/L Fe

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
2175	0.004 mg/L Fe

For more information on derivation and use of Hach’s estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, appendix B, see Section 1.5.1.

Sensitivity

Program Number: 2175

Portion of Curve	Δ Abs	Δ Concentration
Entire Range	0.010	0.0086 mg/L

See Section 1.5.3 *Sensitivity Explained* for more information.

Calibration Standard Preparation

To perform an iron calibration using the FerroZine method, use a 10-mg/L Iron Standard Solution (Cat. No. 140-49). Prepare calibration standards containing 0.200, 0.800, 1.00, 1.200, and 1.400 mg/L Fe as follows:

- a. Into five different Class A 100-mL volumetric flasks, pipet 2.00, 8.00, 10.00, 12.00 and 14.00 mL of the 10-mg/L Fe stock solution using Class A glassware.
- b. Dilute to the mark with deionized water. Mix thoroughly.
- c. Using the FerroZine method and the calibration procedure described in the *User-Entered Programs* section of the *DR/4000 Spectrophotometer Instrument Manual*, generate a calibration curve from the standards prepared above.

Summary of Method

The FerroZine Iron Reagent forms a purple-colored complex with trace amounts of iron in samples that are buffered to a pH of 3.5. This method is applicable for determining trace levels of iron in chemical reagents and glycols and can be used to analyze samples containing magnetite (black iron oxide) or ferrites.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to Section 1.

Pollution Prevention and Waste Management

For information on pollution prevention and waste management, refer to Section 1.

REQUIRED REAGENTS AND STANDARDS (Using Solution)

Description	Quantity Required per test	Unit	Cat. No.
FerroZine Iron Reagent Solution Pillows.....	1 pillow	50/pkg.....	2301-66

REQUIRED EQUIPMENT AND SUPPLIES

Clippers, for opening powder pillows	1	each.....	968-00
DR/4000 1-Inch Cell Adapter	1	each.....	48190-00

OPTIONAL REAGENTS AND STANDARDS

Ammonium Hydroxide, ACS.....	500 mL.....	106-49
Hydrochloric Acid Solution, 6.0 N	500 mL.....	884-49
FerroZine Iron Reagent Solution.....	1000 mL.....	2301-53
Iron Standard Solution, 100 mg/L Fe.....	100 mL.....	14175-42
Iron Standard Solution, 10-mL Voluette Ampule, 25 mg/L Fe.....	16/pkg.....	14253-10
Iron Standard Solution, 2-mL Voluette Ampule, 25 mg/L Fe.....	20/pkg.....	14253-20
Iron Standard Solution, 10 mg/L Fe.....	500 mL.....	140-49
Nitric Acid, ACS	500 mL.....	152-49
Nitric Acid Solution, 1:1	500 mL.....	2540-49
Water, deionized	4 liters.....	272-56

OPTIONAL EQUIPMENT AND SUPPLIES

Description	Unit	Cat. No.
Ampule Breaker Kit	each.....	21968-00
Aspirator, Nalgene vacuum pump	each.....	2131-00
Clippers, shears, 7 ¼-inch	each.....	23694-00
Cylinder, graduated, 25-mL	each.....	508-40
Dropper, calibrated, 0.5-mL & 1.0-mL mark, glass.....	5/pkg.....	14197-05
DR/4000 Carousel Module Kit	each.....	48090-02
DR/4000 Flow Cell Module Kit, 1-inch.....	each.....	48070-04
DR/4000 Sipper Module Kit, 1-inch	each.....	48090-03
Filter Disks, glass fiber, 47-mm	100/pkg.....	2530-00
Filter Holder, membrane	each.....	2340-00
Flask, Erlenmeyer, 50-mL	each.....	505-41
Flask, Erlenmeyer, 125-mL	each.....	505-43
Flask, filtering, 500-mL	each.....	546-49
Flask, volumetric, 100-mL, Class A	each.....	14574-42
Flask, volumetric, 250-mL, Class A	each.....	14574-46
Hot plate, 3 ½ in. diameter, 120 V, 50/60 Hz	each.....	12067-01
Hot plate, 3 ½ in. diameter, 240 V, 50/60 Hz	each.....	12067-02
pH Paper, pH 1.0 to 11.0	5 rolls/pkg.....	391-33
pH Meter, <i>sension</i> TM 1, portable	each.....	51700-00
Pipet, serological, 2.00 mL.....	each.....	532-36
Pipet, TenSette, 0.1 to 1.0 mL	each.....	19700-01
Pipet Tips, for 19700-01 TenSette Pipet	50/pkg.....	21856-96
Pipet, volumetric, Class A, 0.50-mL	each.....	14515-34
Pipet, volumetric, Class A, 1.00-mL	each.....	14515-35
Pipet, volumetric, Class A, 2.00-mL	each.....	14515-36
Pipet, volumetric, Class A, 8.00-mL	each.....	14515-08
Pipet, volumetric, Class A, 10.00-mL	each.....	14515-38
Pipet Filler, safety bulb.....	each.....	14651-00
Thermometer, pocket, -10 to 110 °C	each.....	1877-01



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Method 8146

1,10 Phenanthroline Method*

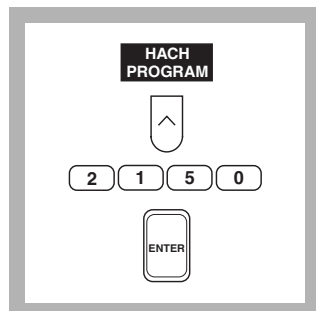
Powder Pillows or AccuVac® Ampuls

(0 to 3.000 mg/L)

Scope and Application: For water, wastewater and seawater. The estimated detection limit for program numbers 2150 and 2155 are 0.008 and 0.007 mg/L Fe^{2+} , respectively.

* Adapted from *Standard Methods for the Examination of Water and Wastewater*, 15th ed. 201 (1980)

Using Powder Pillows



1. Press the soft key under **HACH PROGRAM**.

Select the stored program number for ferrous iron (Fe^{2+}), by pressing **2150** with the numeric keys.

Press: **ENTER**

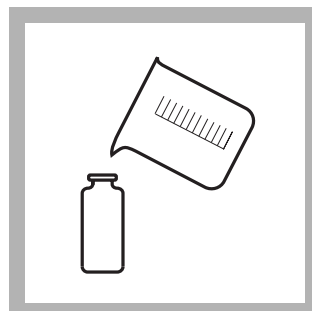
Note: Analyze samples as soon as possible to prevent air oxidation of ferrous iron to ferric iron, which is not determined. See Sample Collection, Storage and Preservation following these steps.

Note: The Flow Cell and Sipper Modules can be used with this procedure.



2. The display will show:
**HACH PROGRAM: 2150
Iron, Ferrous**

The wavelength (λ), **510 nm**, is automatically selected.



3. Fill a clean sample cell with 25 mL of sample.

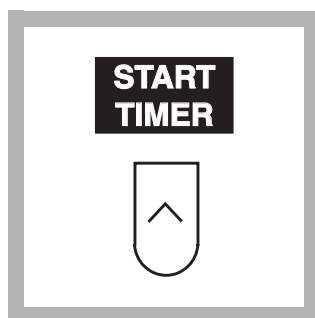
Note: For proof of accuracy, use a 1.0 mg/L ferrous iron standard solution (preparation given in the Accuracy Check section) in place of the sample.

Note: For best results, determine a reagent blank for each new lot of reagent as follows. Prepare a reagent blank by repeating steps 3 through 10, using deionized water as the sample. Zero the instrument on deionized water by pressing the soft key under **ZERO**. Insert the reagent blank and the blank value will be displayed. Correct for the reagent blank by pressing the soft keys under **OPTIONS, (MORE)**, and then **BLANK:OFF**. Enter the reagent blank value and press **ENTER**. Repeat for each new lot of reagent.



4. Add the contents of one Ferrous Iron Reagent Powder Pillow to the sample cell (the prepared sample). Swirl to mix.

Note: An orange color will form if ferrous iron is present.



5. Press the soft key under **START TIMER**.

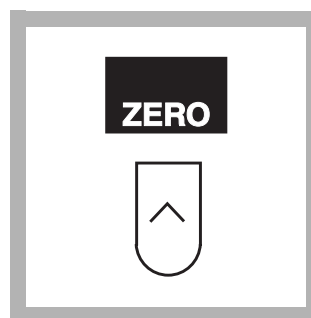
A 3-minute reaction period will begin.



6. Fill a second sample cell with 25 mL of sample (the blank).



7. When the timer beeps, place the blank into the cell holder. Close the light shield.



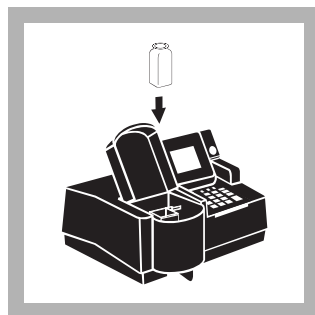
8. Press the soft key under **ZERO**.

The display will show:

0.000 mg/L Fe²⁺

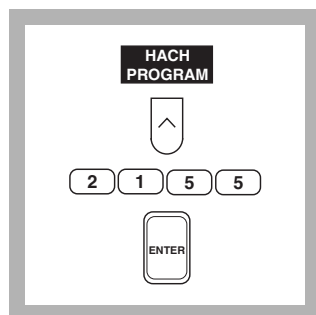
Note: If you are using a reagent blank correction, the display will show the correction.

Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



9. Place the prepared sample into the cell holder. Close the light shield. The results in mg/L Fe²⁺ (or chosen units) will be displayed.

Using AccuVac Ampuls



1. Press the soft key under **HACH PROGRAM**.

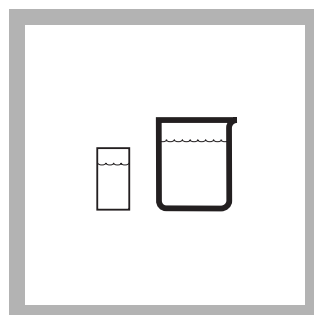
Select the stored program number for ferrous iron (Fe^{2+}) by pressing **2155** with the numeric keys.

Press: **ENTER**

Note: Analyze samples as soon as possible to prevent air oxidation of ferrous iron to ferric iron, which is not determined. See Sample Collection, Storage and Preservation following these steps.



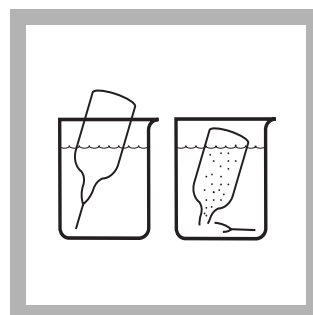
2. The display will show: **HACH PROGRAM: 2155 Iron, Ferrous AV**
The wavelength (λ), **510 nm**, is automatically selected.



3. Fill a zeroing vial (the blank) with at least 10 mL of sample. Collect at least 40 mL of sample in a 50-mL beaker.

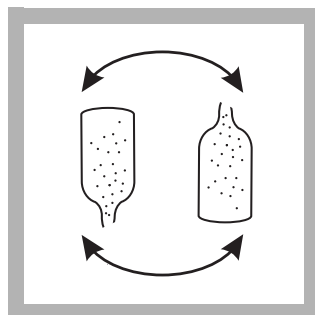
Note: For proof of accuracy, a 1.0-mg/L ferrous iron standard solution (preparation given in the Accuracy Check section) can be used in place of the sample.

Note: For best results, determine a reagent blank for each new lot of reagent as follows. Prepare a reagent blank by repeating Steps 3 through 10, using deionized water as the sample. Zero the instrument on deionized water by pressing the soft key under **ZERO**. Insert the reagent blank and the blank value will be displayed. Correct for the reagent blank by pressing the soft keys under **OPTIONS**, **(MORE)**, and then **BLANK:OFF**. Enter the reagent blank value and press **ENTER**. Repeat for each new lot of reagent.



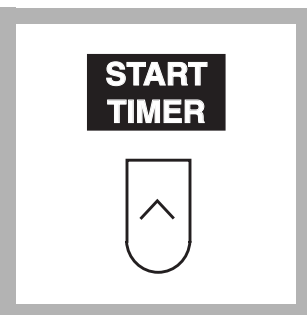
4. Fill a Ferrous Iron AccuVac Ampul with sample.

Note: Keep the tip immersed while the ampul fills completely.



5. Quickly invert the ampul several times to mix. Wipe off any liquid or fingerprints.

Note: An orange color will form if ferrous iron is present.



6. Press the soft key under **START TIMER**.

A 3-minute reaction period will begin.

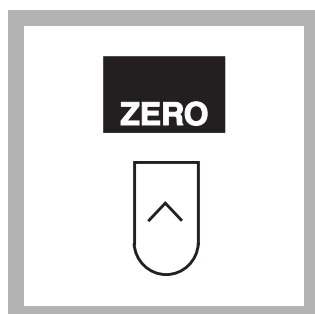
Note: Complete Step 7 during the reaction period.



7. Insert the AccuVac Ampul Adapter into the sample cell module by sliding it under the thumb screw and into the alignment grooves. Fasten with the thumb screw.



8. When the timer beeps place the blank into the cell holder. Close the light shield.



9. Press the soft key under **ZERO**.

The display will show:

0.000 mg/L Fe²⁺

Note: If you are using a reagent blank correction, the display will show the correction.

Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



10. Place the AccuVac Ampul into the cell holder. Close the light shield. Results in mg/L Fe²⁺ (or chosen units) will be displayed.

Sample Collection, Storage and Preservation

Collect samples in plastic or glass bottles. Analyze samples as soon as possible after collection.

Accuracy Check

Standard Solution Method

Prepare a ferrous iron stock solution (100-mg/L Fe²⁺) by dissolving 0.7022 grams of Ferrous Ammonium Sulfate, hexahydrate, in deionized water. Dilute to one liter in a Class A volumetric flask. In a 100-mL Class A volumetric flask, dilute 1.00 mL of this solution to 100 mL with deionized water to make a 1.0-mg/L standard solution. Prepare this solution immediately before use. Perform the iron procedure as described above.

To adjust the calibration curve using the reading obtained with the 1.0 mg/L Fe²⁺ Standard Solution, press the soft keys under **METHOD OPTIONS, (MORE)** then **STD: OFF**. Press **ENTER** to accept the default concentration, the value of which will depend on the selected units. If an alternate concentration is used, enter the actual concentration and press **ENTER** to return to the read screen. See Section 1.5.5 *Adjusting the Standard Curve* for more information.

Method Performance

Precision

Standard: 1.000 mg/L Fe

Program	95% Confidence Limits
2150	0.997–1.003 mg/L Fe
2155	0.997–1.003 mg/L Fe

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
2150	0.008 mg/L Fe
2155	0.007 mg/L Fe

For more information on derivation and use of Hach's estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, Appendix B, see Section 1.5.1.

Sensitivity

Program Number: 2150

Portion of Curve:	Δ Abs	Δ Concentration
Entire Range	0.010	0.0210 mg/L

Program Number: 2155

Portion of Curve:	Δ Abs	Δ Concentration
Entire Range	0.010	0.0226 mg/L

See Section 1.5.3 *Sensitivity Explained* for more information.

Calibration Standard Preparation

Preparing ferrous standards is difficult. These standards are very unstable and degrade rapidly. Only a trained chemist should prepare these standards.

To perform an ferrous iron calibration using the 1,10 phenanthroline method, prepare calibration standards containing 0.50, 1.00, 2.00, and 3.00 mg/L ferrous iron as follows:

- Prepare a 100 mg/L ferrous iron stock solution by dissolving 0.7022 grams of Ferrous Ammonium Sulfate, hexahydrate, in deionized water. Dilute to one liter in a Class A volumetric flask. Stopper and invert several times to mix. Prepare this solution just before use.
- Into four different 100-mL Class A volumetric flasks, pipet 0.50, 1.00, 2.00 and 3.00 mL of the 100-mg/L ferrous iron stock solution. Dilute each flask to volume with deionized water. Stopper and invert each flask to mix. Prepare these standards just before use.
- Using the 1,10 phenanthroline method and the calibration procedure described in the *User-Entered Programs* section of the *DR/4000 Spectrophotometer Instrument Manual*, generate a calibration curve from the standards prepared above.

Summary of Method

The 1,10 phenanthroline indicator in the Ferrous Iron Reagent reacts with ferrous iron in the sample to form an orange color in proportion to the iron concentration. Ferric iron does not react. The ferric iron (Fe^{3+}) concentration can be determined by subtracting the ferrous iron concentration from the results of a total iron test.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to Section 1.

Pollution Prevention and Waste Management

For information on pollution prevention and waste management, refer to Section 1.

REQUIRED REAGENTS AND STANDARDS (Using Powder Pillows)

Description	Quantity Required per test	Unit	Cat. No.
Ferrous Iron Reagent Powder Pillows.....	1 pillow	100/pkg.....	1037-69

REQUIRED REAGENTS AND STANDARDS (Using AccuVac Ampuls)

Ferrous Iron Reagent AccuVac Ampuls.....	1 ampul.....	25/pkg.....	25140-25
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REQUIRED EQUIPMENT AND SUPPLIES (Using Powder Pillows)

Clippers, for opening powder pillows	1	each.....	968-00
DR/4000 1-Inch Cell Adapter	1	each.....	48190-00

REQUIRED EQUIPMENT AND SUPPLIES (Using AccuVac Ampuls)

Beaker, 50-mL.....	1	each.....	500-41
DR/4000 AccuVac Ampul Adapter.....	1	each.....	48187-00
Sample Cell, 10-mL, with cap (zeroing vial)	1	each.....	21228-00

OPTIONAL REAGENTS AND STANDARDS

Ferrous Ammonium Sulfate, hexahydrate, ACS	113 g.....		11256-14
Water, deionized	4 liters.....		272-56

OPTIONAL EQUIPMENT AND SUPPLIES

AccuVac Snapper	each.....		24052-00
Balance, electronic, 110 VAC	each.....		26104-00
Balance, electronic, 220 VAC	each.....		26104-02
Clippers, shears, 7 ¼-inch	each.....		23694-00
DR/4000 Carousel Module Kit	each.....		48070-02
DR/4000 Flow Cell Module Kit, 1-inch.....	each.....		48070-04
DR/4000 Flow Cell Module Kit, 1-cm.....	each.....		48070-05
DR/4000 Sipper Module Kit, 1-inch.....	each.....		48070-03
Flask, volumetric, 100-mL, Class A	each.....		14574-42
Flask, volumetric, 1000-mL, Class A, with stopper.....	each.....		14574-53
pH Meter, <i>sensio</i> TM 1, portable	each.....		51700-00
pH Paper, pH 1.0 to 11.0.....	5 rolls/pkg.....		391-33
Pipet, volumetric, 0.50-mL, Class A	each.....		14515-34
Pipet, volumetric, 1.00-mL, Class A	each.....		14515-35
Pipet, volumetric, 2.00-mL, Class A.....	each.....		14515-36
Pipet, volumetric, 3.00-mL, Class A	each.....		14515-03
Pipet Filler, safety bulb.....	each.....		14651-00



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Method 8365

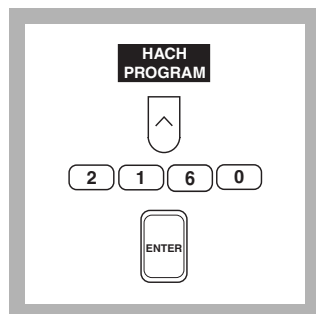
FerroMo Method*

Powder Pillows

(0 to 1.800 mg/L)

Scope and Application: For cooling water containing molybdate-based treatment; digestion is required for determining total iron. See SECTION 1 for digestion procedure. The estimated detection limit for program number 2160 is 0.025 mg/L.

* Adapted from G. Frederick Smith Chemical Co., *The Iron Reagents*, 3rd ed. (1980)



- 1.** Press the soft key under **HACH PROGRAM**.

Select the stored program number for iron, FerroMo, by pressing **2160** with the numeric keys.

Press: **ENTER**

Note: Analyze samples as soon as possible to prevent air oxidation of ferrous iron to ferric iron, which is not determined. See Sample Collection, Storage and Preservation following these steps. Adjust the pH of preserved samples before analysis.

Note: The Flow Cell and Sipper Modules can be used with this procedure.



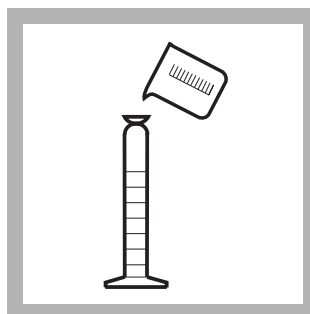
- 2.** The display will show:
**HACH PROGRAM: 2160
Iron, FerroMo**

The wavelength (λ), **590 nm**, is automatically selected.

Note: Rinse glassware with a 1:1 Hydrochloric Acid Solution. Rinse again with deionized water. These two steps will remove iron deposits which can cause slightly high results.

Note: For best results, determine a reagent blank for each new lot of reagent as follows. Prepare a reagent blank by repeating steps 3 through 11, using deionized water as the sample. Zero the instrument on deionized water by pressing the soft key under **ZERO**. Insert the reagent blank and the blank value will be displayed. Correct for the reagent blank by pressing the soft keys under **OPTIONS, (MORE)**, and then **BLANK:OFF**. Enter the reagent blank value and press **ENTER**. Repeat for each new lot of reagent.

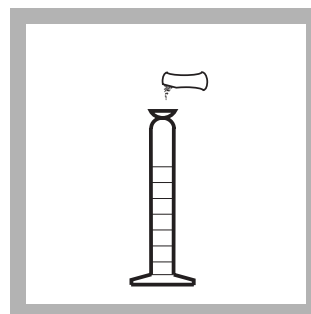
Note: Determination of total iron requires digestion; see SECTION 1 for procedures.



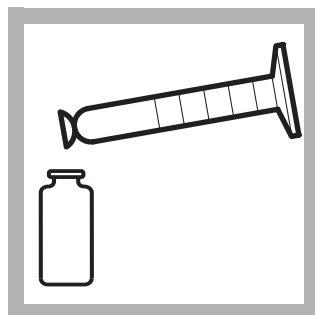
- 3.** Fill a 50-mL graduated mixing cylinder with 50 mL of water to be tested.

Note: Sample pH is important in this test and should be between 3 and 5; see pH discussion in the Interferences section.

Note: For proof of accuracy, use a 0.4-mg/L iron standard solution (preparation given in the Accuracy Check section) in place of the sample.



- 4.** Add the contents of one FerroMo Iron Reagent 1 Powder Pillow to the graduated mixing cylinder. Stopper and invert several times to dissolve the reagents. This is the prepared sample.

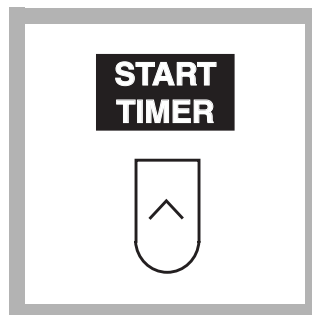


5. Fill a clean matched sample cell to the 25-mL mark with prepared sample. Save the remaining 25 mL of prepared sample for Step 8.



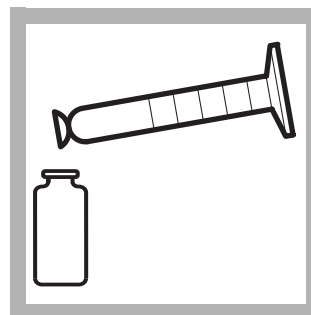
6. Add the contents of one FerroMo Iron Reagent 2 Powder Pillow to the sample cell. Swirl to dissolve the reagents. This is the developed sample.

Note: A blue color will develop if iron is present.

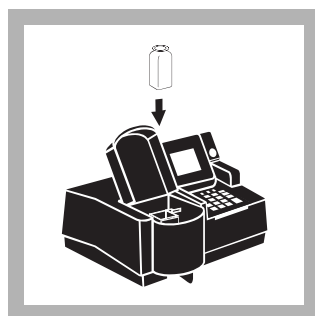


7. Press the soft key under **START TIMER**.

A 3-minute reaction period will begin.



8. Fill a second sample cell with the remaining 25 mL of prepared sample from Step 5 (the blank).



9. When the timer beeps, insert the blank into the cell holder. Close the light shield.



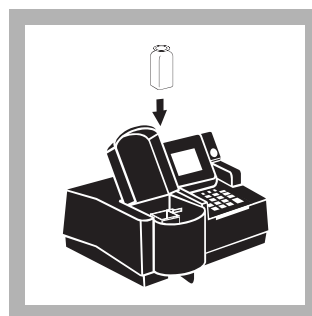
10. Press the soft key under **ZERO**.

The display will show:

0.000 mg/L Fe

Note: If you are using a reagent blank correction, the display will show the correction.

Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



11. Place the developed sample into the cell holder. Close the light shield. The results in mg/L iron (or chosen units) will be displayed.

Note: For samples containing high levels of molybdate (≥ 100 mg/L as Mo^{6+} or MoO_4), read the sample immediately after zeroing the blank.

Interferences

Interfering Substance	Interference Levels and Treatments
pH	A sample pH of less than 3 or greater than 4 after the addition of reagent may inhibit color formation, cause the developed color to fade quickly or result in turbidity. Adjust the sample pH in the graduated cylinder before the addition of reagent to between 3 and 5 by using a pH meter or pH paper and adding, dropwise, an appropriate amount of iron-free acid or base such as 1.0 N Sulfuric Acid Standard Solution or 1.0 N Sodium Hydroxide Standards Solution. Make a volume correction if significant volumes of acid or base are used; see Section 1.2.2 <i>Correcting for Volume Additions</i> .

Sample Collection, Storage and Preservation

Collect samples in acid-washed glass or plastic bottles. To preserve samples, adjust the sample pH to 2 or less with hydrochloric acid (about 2 mL per liter). Samples preserved in this manner can be stored up to six months at room temperature. If only dissolved iron is to be reported, filter sample immediately after collection through a 0.45 micron filter or equivalent medium before addition of hydrochloric acid.

Before testing, adjust the sample pH to 3–5 with 5.0 N Sodium Hydroxide Standard Solution. Do not exceed pH 5 as iron may precipitate. Correct test results for volume additions; see Section 1.2.2 *Correcting for Volume Additions*.

Accuracy Check

Standard Additions Method

- Leave the unspiked sample in the sample cell compartment. Verify that the units displayed are in mg/L. Select standard additions mode by pressing the soft keys under **OPTIONS, (MORE)** and then **STD ADD**.
- Press **ENTER** to accept the default sample volume (mL), 50.
- Press **ENTER** to accept the default standard concentration (mg/L), 100.
- Press the soft key under **ENTRY DONE**.
- Open an Iron Voluette Ampule Standard, 100-mg/L Fe.
- Use the TenSette Pipet to add 0.1 mL, 0.2 mL and 0.3 mL of standard, respectively to three 50-mL samples and mix each thoroughly.
- Analyze each standard addition sample as described above. Accept the standard additions reading by pressing the soft key under **READ** each time. Each addition should reflect approximately 100% recovery.
- After completing the sequence, the display will show the extrapolated concentration value and the “best-fit” line through the standard additions data points, accounting for matrix interferences.
- See Section 1.4.1 *Standard Additions* for more information.

Standard Solution Method

Using Class A glassware, prepare a 0.400-mg/L iron standard solution by pipetting 4.00 mL of Iron Standard Solution, 100-mg/L, into a 1-liter volumetric flask. Dilute to the mark with deionized water. Prepare this solution daily. Perform the iron procedure as described above.

To adjust the calibration curve using the reading obtained with the 0.400-mg/L Fe Standard Solution, press the soft keys under **OPTIONS, (MORE)** then **STD: OFF**. Press **ENTER** to accept the default concentration, the value of which will depend on the selected units. If an alternate concentration is used, enter the actual concentration and press **ENTER** to return to the read screen. See Section 1.5.5 *Adjusting the Standard Curve* for more information.

Method Performance

Precision

Standard: 0.40 mg/L Fe

Program	95% Confidence Limits
2160	0.387–0.413 mg/L Fe

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
2160	0.025 mg/L Fe

For more information on derivation and use of Hach's estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, appendix B, see Section 1.5.1.

Sensitivity

Program Number: 2160

Portion of Curve	Δ Abs	Δ Concentration
0.010 Abs	0.010	0.0112 mg/L
0.200 mg/L	0.010	0.0113 mg/L
1.620 mg/L	0.010	0.0121 mg/L

See Section 1.5.3 *Sensitivity Explained* for more information.

Calibration Standard Preparation

To perform an iron calibration using the FerroMo Method, use a 10-mg/L Iron Standard Solution (Cat. No. 140-49). Prepare calibration standards containing 0.30, 0.60, 0.90, 1.20, 1.50, and 1.80 mg/L Fe as follows:

- Into six different 100-mL volumetric flasks, pipet 3.00, 6.00, 9.00, 12.00, 15.00, and 18.00 mL of the 10 mg/L Iron Standard Solution using Class A glassware.
- Dilute to the mark with deionized water. Mix thoroughly.
- Using the FerroMo method and the calibration procedure described in the *User-Entered Programs* section of the *DR/4000 Spectrophotometer Instrument Manual*, generate a calibration curve from the standards prepared above.

Summary of Method

FerroMo Iron Reagent 1 contains a reducing agent combined with a masking agent. The masking agent eliminates interference from high levels of molybdate. The reducing agent converts precipitated or suspended iron, such as rust, to the ferrous state. FerroMo Iron Reagent 2 contains the indicator combined with a buffering agent. The indicator reacts with ferrous iron in the sample, buffered between pH 3 and 5, resulting in a deep blue-purple color.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to Section 1.

Pollution Prevention and Waste Management

For information on pollution prevention and waste management, refer to Section 1.

REQUIRED REAGENTS AND STANDARDS

FerroMo Iron Reagent Set (100 tests)	25448-00
Includes: (4) 25437-68, (2) 25438-66	

Description	Quantity Required		Unit	Cat. No.
	per test			
FerroMo Iron Reagent 1 Powder Pillows.....	1	pillow	25/pkg	25437-68
FerroMo Iron Reagent 2 Powder Pillows.....	1	pillow	50/pkg	25438-66

REQUIRED EQUIPMENT AND SUPPLIES

Clippers, for opening powder pillows	1	each	968-00
Cylinder, graduated mixing, 50-mL, w/ stopper	1	each	1896-41
DR/4000 1-Inch Cell Adapter	1	each	48190-00

OPTIONAL REAGENTS AND STANDARDS

Description	Unit	Cat. No.
Hydrochloric Acid, ACS	500 mL	134-49
Iron Standard Solution, 100 mg/L	100 mL	14175-42
Iron Standard Solution, 10-mL Voluette ampule, 25 mg/L Fe	16/pkg	14253-10
Iron Standard Solution, 10 mg/L Fe	500 mL	140-49
Sodium Hydroxide Standard Solution, 1.00 N	100 mL MDB	1045-32
Sodium Hydroxide Standard Solution, 5.0 N	100 test MDB	2450-32
Sulfuric Acid Standard Solution, 1.000 N	100 mL MDB	1270-32
Water, deionized	4 liters	272-56

OPTIONAL EQUIPMENT AND SUPPLIES

Ampule Breaker Kit	each	21968-00
Aspirator, Nalgene vacuum pump	each	2131-00
Digesdahl Apparatus, 115 VAC, 50/60 Hz	each	23130-20
Digesdahl Apparatus, 230 VAC, 50/60 Hz	each	23130-21
DR/4000 Carousel Module Kit	each	48070-02
DR/4000 Flow Cell Module Kit, 1-inch	each	48070-04
DR/4000 Flow Cell Module Kit, 1-cm	each	48070-05
DR/4000 Sipper Module Kit, 1-inch	each	48090-03
Filter Disks, glass fiber, 47-mm	100/pkg	2530-00
Filter Holder, membrane	each	2340-00
Flask, filtering, 500-mL	each	546-49

IRON, Total, continued

Flask, volumetric, Class A, 100-mL	each.....	14574-42
Flask, volumetric, Class A, 1000-mL, with stopper.....	each.....	14574-53
pH Paper, pH 1.0 to 11.0	5 rolls/pkg.....	391-33
pH Meter, <i>sensio</i> TM 1, portable	each.....	51700-00
Pipet Filler, safety bulb.....	each.....	14651-00
Pipet, serological, 2-mL	each.....	532-36
Pipet TenSette, 0.1 to 1.0 mL	each.....	19700-01
Pipet Tips, for 19700-01 TenSette Pipet	50/pkg.....	21856-96
Pipet, volumetric, Class A, 1.00-mL	each.....	14515-35
Pipet, volumetric, Class A, 2.00-mL	each.....	14515-36
Pipet, volumetric, Class A, 3.00-mL	each.....	14515-03
Pipet, volumetric, Class A, 4.00-mL	each.....	14515-04
Pipet, volumetric, Class A, 5.00-mL	each.....	14515-37
Pipet, volumetric, Class A, 6.00-mL	each.....	14515-06
Pipet, volumetric, Class A, 8.00-mL	each.....	14515-08
Pipet, volumetric, Class A, 9.00-mL	each.....	14515-09
Pipet, volumetric, Class A, 10.00-mL	each.....	14515-38



FOR TECHNICAL ASSISTANCE, PRICE INFORMATION AND ORDERING:

In the U.S.A. – Call toll-free 800-227-4224

Outside the U.S.A. – Contact the HACH office or distributor serving you.

On the Worldwide Web – www.hach.com; E-mail – techhelp@hach.com

HACH COMPANY

WORLD HEADQUARTERS

Telephone: (970) 669-3050

FAX: (970) 669-2932



✓ Method 8008

FerroVer Method*

Powder Pillows or AccuVac® Ampuls

(0 to 3.000 mg/L)

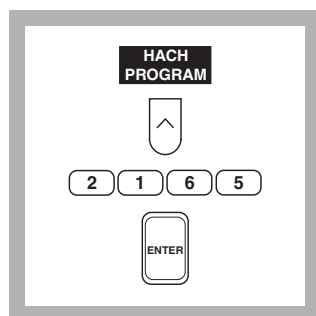
Scope and Application: For water, wastewater and seawater; digestion is required for determining total iron; USEPA approved for reporting wastewater analysis. ** See Section 1 for digestion procedure.

The estimated detection limits for program numbers 2165 and 2170 are 0.008 and 0.007 mg/L Fe, respectively.

* Adapted from *Standard Methods for the Examination of Water and Wastewater*

** Federal Register, June 27, 1980; 45 (126:43459)

Using Powder Pillows



- 1.** Press the soft key under **HACH PROGRAM**.

Select the stored program number for iron (Fe), FerroVer, method by pressing **2165** with the numeric keys.

Press: **ENTER**

Note: If samples cannot be analyzed immediately, see *Sample Collection, Storage and Preservation* following these steps. Adjust pH of preserved samples before analysis.

Note: The Flow Cell and Sipper Modules can be used with this procedure. Use a 25-mL sample and reagents with the Flow Cell Module.

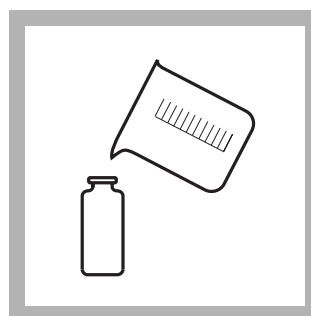


- 2.** The display will show:
HACH PROGRAM: 2165 Iron, FerroVer

The wavelength (λ), **510 nm**, is automatically selected.

Note: Determination of total iron requires digestion. See Section 1.

Note: For best results, determine a reagent blank for each new lot of reagent as follows. Prepare a reagent blank by repeating steps 3 through 9, using deionized water as the sample. Zero the instrument on deionized water by pressing the soft key under **ZERO**. Insert the reagent blank and the blank value will be displayed. Correct for the reagent blank by pressing the soft keys under **OPTIONS, (MORE)**, and then **BLANK:OFF**. Enter the reagent blank value and press **ENTER**. Repeat for each new lot of reagent.



- 3.** Fill a clean sample cell with 10 mL of sample.

Note: For proof of accuracy, use a 1.0 mg/L iron standard solution (preparation given in the *Accuracy Check* section) in place of the sample.

Note: For turbid samples, or non-preserved samples with extreme pH, see the *Interferences* section.



- 4.** Add the contents of one FerroVer Iron Reagent Powder Pillow for 10-mL sample to the sample cell (the prepared sample). Swirl to mix.

Note: An orange color will form if iron is present.

Note: Accuracy is not affected by undissolved powder.

**START
TIMER**



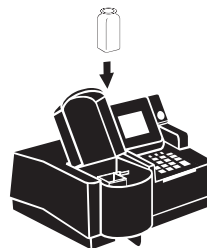
5. Press the soft key under **START TIMER**.

A 3-minute reaction period will begin.

Note: Samples containing visible rust should be allowed to react for at least 5 minutes.



6. Fill another sample cell (the blank) with 10 mL of sample.



7. When the timer beeps, place the blank into the cell holder. Close the light shield.

ZERO



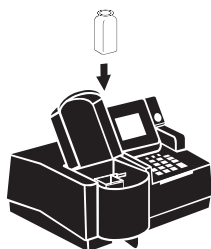
8. Press the soft key under **ZERO**.

The display will show:

0.000 mg/L Fe

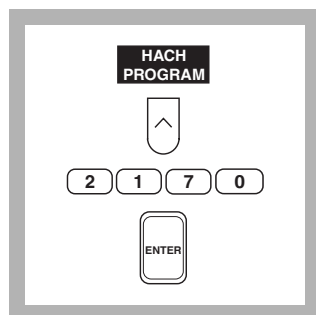
Note: If you are using a reagent blank correction, the display will show the correction.

Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



9. Place the prepared sample into the cell holder. Close the light shield. Results in mg/L iron (or chosen units) will be displayed.

Using AccuVac Ampuls



1. Press the soft key under **HACH PROGRAM**.

Select the stored program number for iron, FerroVer AccuVac method, by pressing **2170** with the numeric keys.

Press: **ENTER**

Note: If samples cannot be analyzed immediately, see *Sample Collection, Storage and Preservation* following these steps. Adjust pH of preserved samples before analysis.

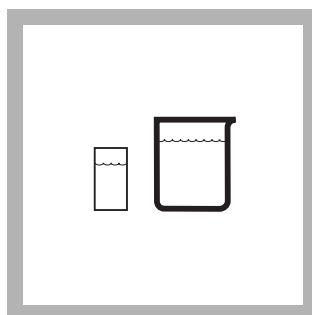


2. The display will show:
HACH PROGRAM: 2170 Iron, FerroVer AV

The wavelength (λ), **510 nm**, is automatically selected.

Note: Determination of total iron requires digestion. See Section 1.

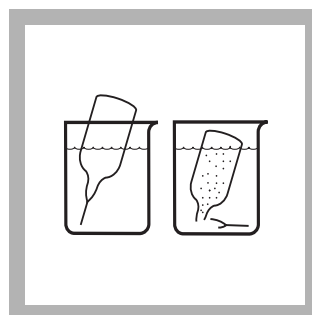
Note: For best results, determine a reagent blank for each new lot of reagent as follows. Prepare a reagent blank by repeating steps 3 through 10, using deionized water as the sample. Zero the instrument on deionized water by pressing the soft key under **ZERO**. Insert the reagent blank and the blank value will be displayed. Correct for the reagent blank by pressing the soft keys under **OPTIONS, (MORE)**, and then **BLANK:OFF**. Enter the reagent blank value and press **ENTER**. Repeat for each new lot of reagent.



3. Fill a zeroing vial (the blank) with at least 10 mL of sample. Collect at least 40 mL of sample in a 50-mL beaker.

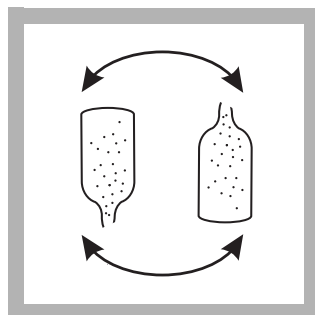
Note: For proof of accuracy, use a 1.0 mg/L iron standard solution (preparation given in the *Accuracy Check* section) in place of the sample.

Note: For turbid samples or non-preserved sample with extreme pH, see the *Interferences* section.



4. Fill a FerroVer Iron AccuVac Ampul with sample.

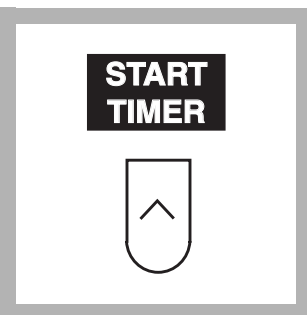
Note: Keep the tip immersed while the ampul fills completely.



5. Quickly invert the ampul several times to mix. Wipe off any liquid or fingerprints.

Note: An orange color will form if iron is present.

Note: Accuracy is not affected by undissolved powder.



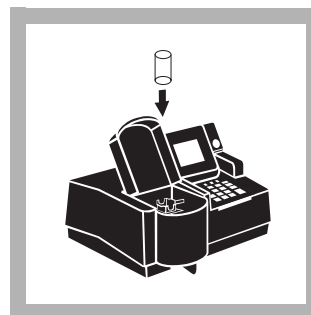
6. Press the soft key under **START TIMER**.

A 3-minute reaction period will begin.

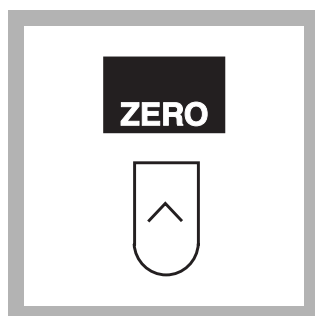
Note: Samples containing visible rust should be allowed to react for at least five minutes.



7. Insert the AccuVac Ampul Adapter into the sample cell module by sliding it under the thumb screw and into the alignment grooves. Fasten with the thumb screw.



8. When the timer beeps, place the blank into the cell holder. Close the light shield.



9. Press the soft key under **ZERO**.

The display will show:

0.000 mg/L Fe

Note: If you are using a reagent blank correction, the display will show the correction.

Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



10. Place the AccuVac Ampul into the cell holder. Close the light shield. Results in mg/L (or chosen units) iron will be displayed.

Interferences

Table 1 Interfering Substances and Suggested Treatments

Interfering Substance	Interference Level and Treatment
Calcium, Ca ²⁺	No effect at less than 10,000 mg/L as CaCO ₃
Chloride, Cl ⁻	No effect at less than 185,000 mg/L
Copper, Cu ²⁺	No effect. Masking agent is contained in FerroVer Reagent.
High Iron Levels	Inhibits color development. Dilute sample and re-test to verify results.
Iron Oxide	Requires mild, vigorous or Digesdahl digestion. After digestion, adjust sample to pH 3–5 with sodium hydroxide, then analyze.
Magnesium	No effect at 100,000 mg/L as CaCO ₃
Molybdate Molybdenum	No effect at 50 mg/L as Mo.
High Sulfide Levels, S ²⁻	<ol style="list-style-type: none"> 1. Treat in fume hood or well-ventilated area. Add 5 mL HCl to 100 mL sample in a 250-mL Erlenmeyer flask. Boil 20 minutes. 2. Cool. Adjust pH to 3–5 with NaOH. Readjust volume to 100 mL with deionized water. 3. Analyze.
Turbidity	<ol style="list-style-type: none"> 1. Add 0.1 g scoop of RoVer Rust Remover to the blank in Step 3. Swirl to mix. 2. Zero the instrument with this blank. 3. If sample remains turbid, add three 0.2 g scoops of RoVer to a 75-mL sample. Let stand 5 minutes. 4. Filter through a glass filter or centrifuge. 5. Use filtered sample in Steps 3 and 6.
Extreme Sample pH	Adjust pH to 3–5. See Section 1.3.1 <i>pH Interference</i> .
Highly Buffered Samples	Adjust pH to 3–5. See Section 1.3.1 <i>pH Interference</i> .

Sample Collection, Storage and Preservation

Collect samples in acid-cleaned glass or plastic containers. No acid addition is necessary if analyzing the sample immediately. To preserve samples, adjust the pH to 2 or less with concentrated nitric acid (about 2 mL per liter). Preserved samples may be stored up to six months at room temperature. Before analysis, adjust the pH to between 3 and 5 with 5.0 N Sodium Hydroxide Standard Solution. Correct the test result for volume additions; see Section 1.2.2 *Correcting for Volume Additions*.

If only dissolved iron is to be determined, filter the sample before acid addition using the labware listed under *OPTIONAL EQUIPMENT AND SUPPLIES*.

Accuracy Check

Standard Additions Method

- Leave the unspiked sample in the sample cell compartment. Verify that the units displayed are in mg/L. Select standard additions mode by pressing the soft keys under **OPTIONS, (MORE)** and then **STD ADD**.
- Press **ENTER** to accept the default sample volume (mL), 25.
- Press **ENTER** to accept the default standard concentration (mg/L), 50.000.
- Press the soft key under **ENTRY DONE**.
- Snap the neck off an Iron Voluette Ampule Standard, 50-mg/L.

- f. Use the TenSette Pipet to add 0.1 mL, 0.2 mL and 0.3 mL of standard, respectively to three 25-mL samples and mix each thoroughly (for AccuVac Ampuls, use 50-mL beakers).
- g. Analyze each standard addition sample as described above (use 10-mL aliquots for powder pillow method in steps 3 and 6). Accept the standard additions readings by pressing the soft key under **READ** each time. Each addition should reflect approximately 100% recovery.
- h. After completing the sequence, the display will show the extrapolated concentration value and the “best-fit” line through the standard additions data points, accounting for matrix interferences.
- i. See Section 1.4.1 *Standard Additions* for more information.

Standard Solution Method

Prepare a 1.00-mg/L Fe standard solution by pipetting 1.00 mL of Iron Standard Solution, 100-mg/L, into a 100-mL volumetric flask. Dilute to the mark with deionized water. Stopper and invert to mix. Prepare this solution daily. Perform the iron procedure as described above.

To adjust the calibration curve using the reading obtained with the 1.00 mg/L Standard Solution, press the soft keys under **OPTIONS, (MORE)** then **STD:OFF**. Press **ENTER** to accept the displayed concentration, the value of which will depend on the selected units. If an alternate concentration is used, enter the actual concentration and press **ENTER** to return to the read screen. See Section 1.5.5 *Adjusting the Standard Curve* for more information.

Method Performance

Precision

Standard: 1.000 mg/L Fe

Program	95% Confidence Limits
2165	0.950–1.050 mg/L Fe
2170	0.950–1.050 mg/L Fe

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
2165	0.008 mg/L Fe
2170	0.007 mg/L Fe

For more information on derivation and use of Hach’s estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, appendix B, see Section 1.5.1.

Sensitivity

Program Number: 2165

Portion of Curve:	Δ Abs	Δ Concentration
Entire Range	0.010	0.0209 mg/L

Program Number: 2170

Portion of Curve:	Δ Abs	Δ Concentration
Entire Range	0.010	0.0226 mg/L

See Section 1.5.3 *Sensitivity Explained* for more information.**Calibration Standard Preparation**

To perform an iron calibration using the FerroVer method, prepare a 40.0-mg/L iron stock solution by pipetting 4.00 mL of a 1000-mg/L Iron Standard Solution (Cat. No. 2271-42) into a 100-mL volumetric flask using Class A glassware. Dilute to the mark with deionized water and mix thoroughly.

Prepare calibration standards containing 0.40, 0.80, 1.20, 1.60, 2.00, 2.40, and 2.80 mg/L Fe as follows:

- a. Into seven different 100-mL Class A volumetric flasks, pipet 1.00, 2.00, 3.00, 4.00, 5.00, 6.00, and 7.00 mL of the 40 mg/L iron stock solution using Class A glassware.
- b. Dilute to the mark with deionized water. Mix thoroughly.
- c. Using the FerroVer method and the calibration procedure described in the *User-Entered Programs* section of the *DR/4000 Spectrophotometer Instrument Manual*, generate a calibration curve from the standards prepared above.

Summary of Method

FerroVer Iron Reagent converts all soluble iron and most insoluble forms of iron in the sample to soluble ferrous iron. The ferrous iron reacts with the 1,10 phenanthroline indicator in the reagent to form an orange color in proportion to the iron concentration.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to Section 1.

Pollution Prevention and Waste Management

For information on pollution prevention and waste management, refer to Section 1.

IRON, Total, continued

REQUIRED REAGENTS AND STANDARDS (Using Powder Pillows)

Description	Quantity Required per test	Unit	Cat. No.
FerroVer Iron Reagent Powder Pillows (for 10-mL sample)	1 pillow	100/pkg	21057-69

REQUIRED REAGENTS AND STANDARDS (Using AccuVac Ampuls)

FerroVer Iron Reagent AccuVac Ampuls	1 ampul	25/pkg	25070-25
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REQUIRED EQUIPMENT AND SUPPLIES (Using Powder Pillows)

DR/4000 1-Inch Cell Adapter	1	each	48190-00
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REQUIRED EQUIPMENT AND SUPPLIES (Using AccuVac Ampuls)

DR/4000 AccuVac Ampul Adapter	1	each	48187-00
Beaker, 50-mL	1	each	500-41
Sample Cell, 10-mL, with cap (zeroing vial)	1	each	21228-00

OPTIONAL REAGENTS AND STANDARDS

Ammonium Hydroxide, ACS	500 mL	106-49
Hydrochloric Acid Standard Solution, 6.0 N	500 mL	884-49
Hydrochloric Acid, ACS	500 mL	134-49
Iron Standard Solution, 100-mg/L	100 mL	14175-42
Iron Standard Solution, 1000-mg/L	100 mL	2271-42
Iron Standard Solution, 10-mL Voluette Ampule, 50-mg/L as Fe	16/pkg	14254-10
Iron Standard Solution, 10-mL Voluette Ampule, 25-mg/L as Fe	16/pkg	14253-10
Iron Standard Solution, 2-mL Voluette Ampule, 50-mg/L as Fe	20/pkg	14254-20
Nitric Acid, ACS	500 mL	152-49
Nitric Acid Solution, 1:1	500 mL	2540-49
RoVer Rust Remover	454 g	300-01
Sodium Hydroxide Standard Solution, 5.0 N	100 mL MDB	2450-32
Water, deionized	4 liters	272-56

OPTIONAL EQUIPMENT AND SUPPLIES

Description	Unit	Cat. No.
AccuVac Snapper	each.....	24052-00
Ampule Breaker Kit	each.....	21968-00
Aspirator, Nalgene vacuum pump	each.....	2131-00
Clippers, Shears 7¼-inch	each.....	23694-00
Cylinder, graduated, polypropylene, 25-mL	each.....	1081-40
Cylinder, graduated, polypropylene, 100-mL	each.....	1081-42
Digesdahl Apparatus, 115 VAC, 50/60 Hz.....	each.....	23130-20
Digesdahl Apparatus, 230 VAC, 50/60 Hz.....	each.....	23130-21
DR/4000 Carousel Module Kit	each.....	48070-02
DR/4000 Flow Cell Module Kit, 1-inch.....	each.....	48070-04
DR/4000 Flow Cell Module Kit, 1-cm.....	each.....	48070-05
DR/4000 Sipper Module Kit, 1-inch.....	each.....	48090-03
Filter Discs, glass fiber, 47-mm	100/pkg.....	2530-00
Filter Holder, membrane	each.....	2340-00
Flask, Erlenmeyer, 250-mL.....	each.....	505-46
Flask, filtering, 500-mL	each.....	546-49
Flask, volumetric, Class A, 100-mL	each.....	14574-42
Flask, volumetric, Class A, 100-mL	6/pkg.....	14574-72
Hot Plate, micro, 120 VAC, 4 in. diameter.....	each.....	12067-01
Hot Plate, micro, 240 VAC, 4 in. diameter.....	each.....	12067-02
pH Meter, <i>sens^{ion}</i> TM 1, portable.....	each.....	51700-00
pH Paper, pH 1.0 to 11.0	5 rolls/pkg.....	391-33
Pipet Filler, safety bulb.....	each.....	14651-00
Pipet, serological, 2.00-mL	each.....	532-36
Pipet, serological, 5.00-mL	each.....	532-37
Pipet, TenSette, 0.1 to 1.0 mL	each.....	19700-01
Pipet Tips, for 19700-01 TenSette Pipet	50/pkg.....	21856-96
Pipet, volumetric, Class A, 1.00-mL	each.....	14515-35
Pipet, volumetric, Class A, 2.00-mL	each.....	14515-36
Pipet, volumetric, Class A, 3.00-mL	each.....	14515-03
Pipet, volumetric, Class A, 4.00-mL	each.....	14515-04
Pipet, volumetric, Class A, 5.00-mL	each.....	14515-37
Pipet, volumetric, Class A, 6.00-mL	each.....	14515-06
Pipet, volumetric, Class A, 7.00-mL	each.....	14515-07
Spoon, measuring, 0.10-g.....	each.....	511-00
Spoon, measuring, 0.20-g.....	each.....	638-00



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WORLD HEADQUARTERS
Telephone: (970) 669-3050
FAX: (970) 669-2932



Method 8112

TPTZ Method*

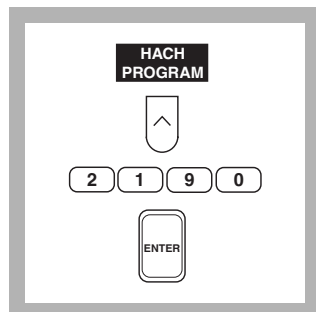
Powder Pillows or AccuVac® Ampuls

(0 to 1.800 mg/L)

Scope and Application: For water, wastewater and seawater; digestion is required for determining total iron. See Section 1 for digestion procedure. The estimated detection limits for program numbers 2190 and 2195 are 0.022 and 0.008 mg/L Fe, respectively.

* Adapted from G. Frederic Smith Chemical Co., *The Iron Reagents*, 3rd ed. (1980)

Using Powder Pillows



- 1.** Press the soft key under **HACH PROGRAM**.

Select the stored program number for iron (Fe), TPTZ method, by pressing **2190** with the numeric keys.

Press: **ENTER**

Note: If samples cannot be analyzed immediately, see *Sample Collection, Storage and Preservation*, following these steps. Adjust pH of preserved samples before analysis.

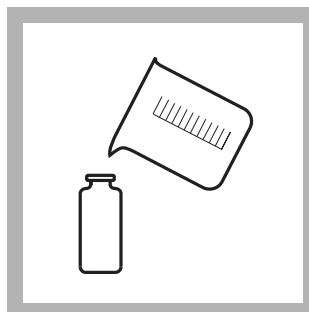
Note: The Flow Cell and Sipper Modules can be used with this procedure. Use a 25-mL sample and reagents with the Flow Cell Module.



- 2.** The display will show:
**HACH PROGRAM: 2190
Iron, Total, TPTZ**

The wavelength (λ), **590 nm**, is automatically selected.

Note: For best results, determine a reagent blank for each new lot of reagent as follows. Prepare a reagent blank by repeating steps 3 through 10, using deionized water as the sample. Zero the instrument on deionized water by pressing the soft key under **ZERO**. Insert the reagent blank and the blank value will be displayed. Correct for the reagent blank by pressing the soft keys under **OPTIONS**, **(MORE)**, and then **BLANK:OFF**. Enter the reagent blank value and press **ENTER**. Repeat for each new lot of reagent.

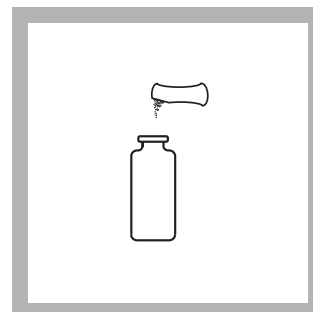


- 3.** Fill a clean sample cell with 10 mL of sample.

Note: Rinse glassware with a 1:1 Hydrochloric Acid Solution. Rinse again with deionized water. These two steps will remove iron deposits which can cause slightly high results.

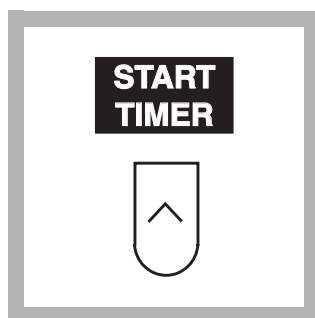
Note: For non-preserved samples with extreme pH, see the *Interferences* section.

Note: For proof of accuracy, use a 0.4-mg/L iron standard solution (preparation given in the *Accuracy Check* section) in place of the sample.



- 4.** Add the contents of one 10-mL TPTZ Iron Reagent Powder Pillow to the sample cell (the prepared sample). Stopper and shake for 30 seconds. Remove stopper.

Note: A blue color will develop if iron is present.



5. Press the soft key under **START TIMER**.

A 3-minute reaction period will begin.

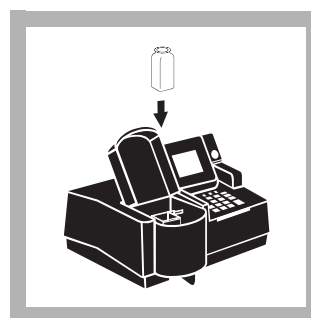
Note: Continue with steps 6 and 7 while the timer is running.



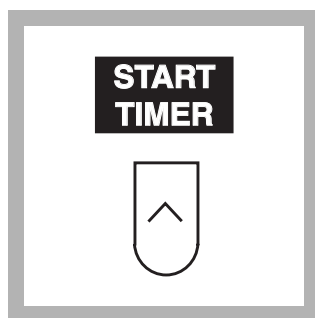
6. Fill a second sample cell with 10 mL of deionized water.



7. Add the contents of one 10-mL TPTZ Iron Reagent Powder Pillow to the deionized water. Cap and shake for 30 seconds. Remove stopper. This is the blank.



8. When the timer beeps, insert the blank into the cell holder. Close the light shield.



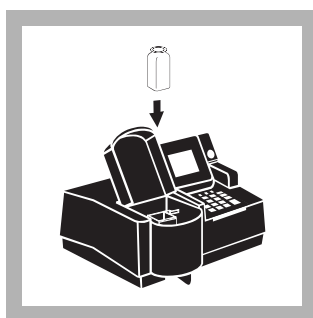
9. Press the soft key under **ZERO**.

The display will show:

0.000 mg/L Fe

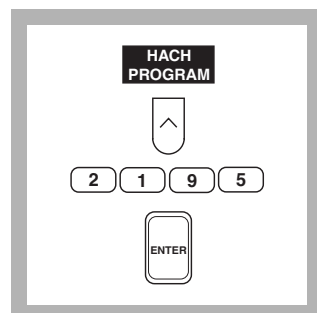
Note: If you are using a reagent blank correction, the display will show the correction.

Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



10. Place the prepared sample into the cell holder. Close the light shield. The results in mg/L Fe as total iron (or chosen units) will be displayed.

Using AccuVac Ampuls



1. Press the soft key under **HACH PROGRAM**.

Select the stored program number for iron (Fe), TPTZ AccuVac method, by pressing **2195** with the numeric keys.

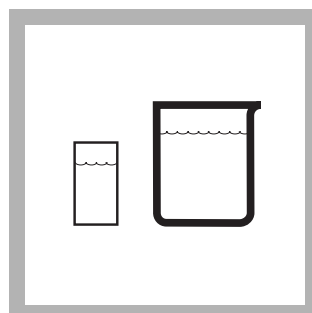
Press: **ENTER**

Note: If samples cannot be analyzed immediately, see *Sample Collection, Storage and Preservation* following these steps. Adjust pH of preserved samples before analysis.



2. The display will show: **HACH PROGRAM: 2195 Iron, TPTZAV**

The wavelength (λ), **590 nm**, is automatically selected.

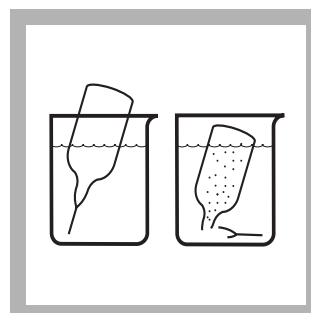


3. Collect at least 40 mL of sample in a 50-mL beaker. Fill a zeroing vial with at least 10 mL of sample.

Note: For non-preserved samples with extreme pH, see the *Interferences* section.

Note: For proof of accuracy, use a 0.4-mg/L iron standard solution (preparation given in the *Accuracy Check* section) in place of the sample.

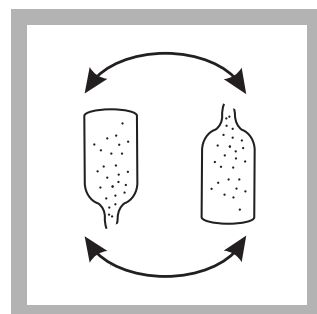
Note: Rinse glassware with a 1:1 Hydrochloric Acid Solution. Rinse again with deionized water. These two steps will remove iron deposits which can cause slightly high results.



4. Fill a TPTZ Iron AccuVac Ampul with sample.

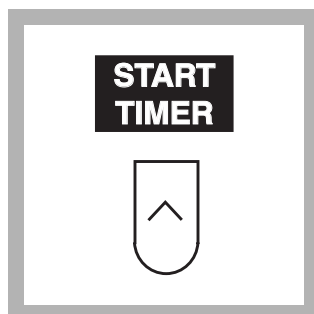
Note: Keep the tip immersed while the ampul fills completely.

Note: For best results, determine a reagent blank for each new lot of reagent as follows. Prepare a reagent blank by repeating steps 3 through 10, using deionized water as the sample. Zero the instrument on deionized water by pressing the soft key under **ZERO**. Insert the reagent blank and the blank value will be displayed. Correct for the reagent blank by pressing the soft keys under **OPTIONS, (MORE)**, and then **BLANK:OFF**. Enter the reagent blank value and press **ENTER**. Repeat for each new lot of reagent.



5. Invert the ampul (the prepared sample) repeatedly to mix. Clean the ampul and zeroing vial with a cloth.

Note: Wiping with a damp cloth followed by a dry one removes fingerprints and other marks.



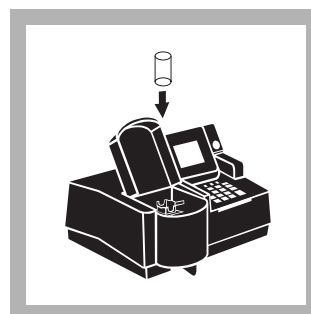
6. Press the soft key under **START TIMER**. A 3-minute reaction period will begin.

Note: A blue color will develop if iron is present.

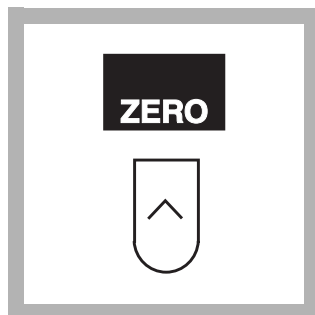
Note: Complete Step 7 during this reaction period.



7. Insert the AccuVac Ampul Adapter into the sample cell module by sliding it under the thumb screw and into the alignment grooves. Fasten with the thumb screw.



8. When the timer beeps, place the zeroing vial into the adapter. Close the light shield.



9. Press the soft key under **ZERO**.

The display will show:

0.000 mg/L Fe

Note: If you are using a reagent blank correction, the display will show the correction.

Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



10. Place the prepared sample into the adapter. Close the light shield. The results in mg/L Fe as total iron (or chosen units) will be displayed.

Interferences

Interference tests were performed using an iron concentration of 0.5 mg/L. When interferences occurred, the color formation was inhibited or a precipitate formed. The following do not interfere with the test when present up to the levels listed.

Table 1 Interfering Substances and Suggested Treatments

Interfering Substance	Interference Level and Treatment
Cadmium	Greater than 4.0 mg/L
Chromium (3+)	Greater than 0.25 mg/L
Chromium (6+)	Greater than 1.2 mg/L
Cobalt	Greater than 0.05 mg/L
Color or turbidity	In the powder pillow procedure if the sample, without a TPTZ Iron Reagent Powder Pillow, has a color or turbidity greater than the blank of Step 7 (deionized water plus TPTZ Iron Reagent), then use the sample as the blank.
Copper	Greater than 0.6 mg/L
Cyanide	Greater than 2.8 mg/L
Manganese	Greater than 50.0 mg/L
Mercury	Greater than 0.4 mg/L
Molybdenum	Greater than 4.0 mg/L
Nickel	Greater than 1.0 mg/L
Nitrite Ion	Greater than 0.8 mg/L

Table 1 Interfering Substances and Suggested Treatments (Continued)

Interfering Substance	Interference Level and Treatment
pH	A sample pH of less than 3 or greater than 4 after the addition of reagent may inhibit color formation, cause the developed color to fade quickly or result in turbidity. Adjust the sample pH in the sample cell before the addition of reagent to between 3 and 8 by using a pH meter or pH paper and adding dropwise an appropriate amount of iron-free acid or base such as 1.0 N Sulfuric Acid Standard Solution or 1.0 N Sodium Hydroxide Standards Solution. Make a volume correction if significant volumes of acid or base are used; see Section 1.2.2 <i>Correcting for Volume Additions</i> .

Sample Collection, Storage and Preservation

Collect samples in acid-washed glass or plastic bottles. To preserve samples, adjust the sample pH to 2 or less with nitric acid, ACS (about 2 mL per liter). Store preserved samples up to six months at room temperature. If reporting only dissolved iron, filter sample immediately after collection and before addition of nitric acid.

Before testing, adjust the pH of the stored sample to between 3 and 4 with 5.0 N Sodium Hydroxide Standard Solution. Do not exceed pH 5 as iron may precipitate. Correct the test result for volume additions; see Section 1.2.2 *Correcting for Volume Additions*.

Accuracy Check

Standard Additions Method (using Powder Pillows)

- Leave the unspiked sample in the sample cell compartment. Verify that the units displayed are in mg/L. Select standard additions mode by pressing the soft keys under **OPTIONS, (MORE)** and then **STD ADD**.
- Press **ENTER** to accept the default sample volume (mL), 10.
- Press **ENTER** to accept the default standard concentration (mg/L Fe), 10.
- Press the soft key under **ENTRY DONE**.
- Snap the neck off an Iron Voluette Ampule Standard, 10-mg/L Fe.
- Use the TenSette Pipet to add 0.2 mL, 0.4 mL and 0.6 mL of standard, respectively to three 10-mL samples and mix each thoroughly.
- Analyze each standard addition sample as described above. Accept the standard additions reading by pressing the soft key under **READ** each time. Each addition should reflect approximately 100% recovery.
- After completing the sequence, the display will show the extrapolated concentration value and the “best-fit” line through the standard additions data points, accounting for matrix interferences.
- See Section 1.4.1 *Standard Additions* for more information.

Standard Additions Method (using AccuVacs)

- Leave the unspiked sample in the sample cell compartment. Verify that the units displayed are in mg/L. Select standard additions mode by pressing the soft keys under **OPTIONS, (MORE)** and then **STD ADD**.
- Press **ENTER** to accept the default sample volume (mL), 25.
- Press **ENTER** to accept the default standard concentration (mg/L), 25.

- d. Press the soft key under **ENTRY DONE**.
- e. Snap the neck off an Iron Voluette Ampule Standard, 25-mg/L Fe.
- f. Use a graduated cylinder to measure 25 mL of sample into each of three 50-mL beakers. Use the TenSette Pipet to add 0.2 mL, 0.4 mL and 0.6 mL of standard, respectively to these three 25-mL samples and mix each thoroughly.
- g. Fill a TPTZ Iron AccuVac completely from each beaker and analyze each standard addition sample as described above. Accept the standard additions reading by pressing the soft key under **READ** each time. Each addition should reflect approximately 100% recovery.
- h. After completing the sequence, the display will show the extrapolated concentration value and the “best-fit” line through the standard additions data points, accounting for matrix interferences.
- i. See Section 1.4.1 *Standard Additions* for more information.

Standard Solution Method

Using Class A glassware, prepare a 0.400-mg/L iron standard solution by pipetting 1.00 mL of Iron Standard Solution, 100-mg/L, into a 250-mL volumetric flask. Dilute to the mark with deionized water. Prepare this solution daily. Perform the iron procedure as described above.

To adjust the calibration curve using the reading obtained with the 0.400-mg/L standard solution, press the soft keys under **OPTIONS, (MORE)** then **STD: (NONE)**. Press **ENTER** to accept the default concentration, the value of which will depend on the selected units. If an alternate concentration is used, enter the actual concentration and press enter to return to the read screen. See Section 1.5.5 *Adjusting the Standard Curve* for more information.

Method Performance

Precision

Standard: 0.400 mg/L Fe

Program	95% Confidence Limits
2190	0.387–0.413 mg/L Fe
2195	0.396–0.404 mg/L Fe

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
2190	0.022 mg/L Fe
2195	0.008 mg/L Fe

For more information on derivation and use of Hach’s estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, appendix B, see Section 1.5.1.

Sensitivity

Program Number: 2190

Portion of Curve	Δ Abs	Δ Concentration
0.010 Abs	0.010	0.0112 mg/L
0.200 mg/L	0.010	0.0113 mg/L
1.620 mg/L	0.010	0.0121 mg/L

Program Number: 2195

Portion of Curve	Δ Abs	Δ Concentration
Entire Range	0.010	0.0121 mg/L

See Section 1.5.3 *Sensitivity Explained* for more information.

Calibration Standard Preparation

To perform an iron calibration using the TPTZ method, use a 10-mg/L Iron Standard Solution (Cat. No. 140-49) to prepare calibration standards containing 0.30, 0.60, 0.90, 1.20, 1.50, and 1.80 mg/L iron as follows:

- Into six different 100-mL Class A volumetric flasks, pipet 3.00, 6.00, 9.00, 12.00, 15.00 and 18.00 mL of the 10-mg/L Iron Standard Solution using Class A glassware.
- Dilute to the mark with deionized water. Mix thoroughly.
- Using the TPTZ method and the calibration procedure described in the *User-Entered Programs* section of the *DR/4000 Spectrophotometer Instrument Manual*, generate a calibration curve from the standards prepared above.

Summary Of Method

The TPTZ Iron Reagent forms a deep blue-purple color with ferrous iron. The indicator is combined with a reducing agent which converts precipitated or suspended iron, such as rust, to the ferrous state. The amount of ferric iron present can be determined as the difference between the results of a ferrous iron test and the concentration of total iron.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to Section 1.

Pollution Prevention and Waste Management

For information on pollution prevention and waste management, refer to Section 1.

IRON, Total, continued

REQUIRED REAGENTS AND STANDARDS (Using Powder Pillows)

Description	Quantity Required per test	Unit	Cat. No.
TPTZ Iron Reagent Powder Pillows (for 10-mL sample).....	2 pillows	100/pkg.....	26087-99

REQUIRED REAGENTS AND STANDARDS (Using AccuVac Ampuls)

TPTZ Low Range Iron Reagent AccuVac Ampuls	1 ampul	25/pkg.....	25100-25
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REQUIRED EQUIPMENT AND SUPPLIES (Using Powder Pillows)

DR/4000 1-Inch Cell Adapter	1	each.....	48190-00
Stopper, hollow, No. 2	2	6/pkg.....	14480-01

REQUIRED EQUIPMENT AND SUPPLIES (Using AccuVac Ampuls)

Beaker, 50-mL	1	each.....	500-41
DR/4000 AccuVac Ampul Adapter.....	1	each.....	48187-00
Sample Cell, 10-mL, with cap (zeroing vial)	1	each.....	21228-00

OPTIONAL REAGENTS AND STANDARDS

Hydrochloric Acid Solution, 6.0 N	500 mL.....	884-49
Iron Standard Solution, 100-mg/L Fe	100 mL.....	14175-42
Iron Standard Solution, 10-mg/L Fe	500 mL.....	140-49
Iron Standard Solution, 10-mL Voluette Ampule, 10-mg/L Fe	16/pkg.....	140-10
Iron Standard Solution, 10-mL Voluette Ampule, 25-mg/L Fe	16/pkg.....	14253-10
Iron Standard Solution, 2-mL Voluette Ampule, 50-mg/L Fe	20/pkg.....	14253-20
Iron Standard Solution, 10-mL Voluette Ampule, 50-mg/L Fe	16/pkg.....	14254-10
Nitric Acid, ACS	500 mL.....	152-49
Nitric Acid Solution, 1:1	500 mL.....	2540-49
Sodium Hydroxide Standard Solution, 1.00 N.....	100 mL MDB.....	1045-32
Sodium Hydroxide Standard Solution, 5.0 N.....	100 mL MDB.....	2450-32
Sulfuric Acid Standard Solution, 1.000 N.....	100 mL MDB.....	1270-32
Water, deionized	4 liters.....	272-56

OPTIONAL EQUIPMENT AND SUPPLIES

Description	Unit	Cat. No.
AccuVac Snapper	each.....	24052-00
Ampule Breaker Kit	each.....	21968-00
Aspirator, Nalgene vacuum pump	each.....	2131-00
Beaker, 50-mL	each.....	500-41
Clippers, shears, 7/4-inch	each.....	23694-00
Cylinder, graduated, 25-mL, poly	each.....	1081-40
Dropper, 0.5 and 1.0 mL marks.....	10/pkg.....	21247-10
DR/4000 Carousel Module Kit	each.....	48070-02
DR/4000 Flow Cell Module Kit, 1-inch.....	each.....	48070-04
DR/4000 Flow Cell Module Kit, 1-cm.....	each.....	48070-05
DR/4000 Sipper Module Kit, 1-inch	each.....	48090-03
Digesdahl Apparatus, 115 VAC	each.....	23130-20
Digesdahl Apparatus, 230 VAC	each.....	23130-21
Flask, filtering, 500-mL	each.....	546-49
Flask, volumetric, Class A, 100-mL	each.....	14574-42
Flask, volumetric, Class A, 250-mL	each.....	14574-46
Filter Disks, glass, 47-mm.....	100/pkg.....	2530-00
Filter Holder, membrane	each.....	2340-00
pH Paper, pH 1.0 to 11.0	5 rolls/pkg.....	391-33
pH Meter, <i>sens^{ion}™1</i> , portable	each.....	51700-00
Pipet Filler, safety bulb.....	each.....	14651-00
Pipet, serological, 2-mL	each.....	532-36
Pipet TenSette, 0.1 to 1.0 mL	each.....	19700-01
Pipet Tips, for 19700-01 TenSette Pipet	50/pkg.....	21856-96
Pipet, volumetric, Class A, 1.00-mL	each.....	14515-35
Pipet, volumetric, Class A, 2.00-mL	each.....	14515-36
Pipet, volumetric, Class A, 3.00-mL	each.....	14515-03
Pipet, volumetric, Class A, 4.00-mL	each.....	14515-04
Pipet, volumetric, Class A, 5.00-mL	each.....	14515-37
Pipet, volumetric, Class A, 6.00-mL	each.....	14515-06
Pipet, volumetric, Class A, 9.00-mL	each.....	14515-09
Pipet, volumetric, Class A, 10.00-mL	each.....	14515-38
Pipet, volumetric, Class A, 15.00-mL	each.....	14515-39



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WORLD HEADQUARTERS
Telephone: (970) 669-3050
FAX: (970) 669-2932



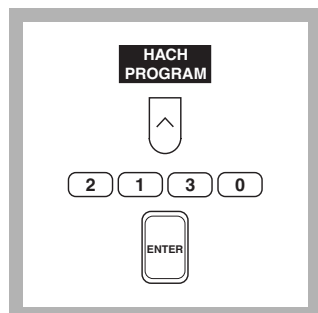
1, 10-Phenanthroline Method

UniCell™ Vials

(0 to 5.00 mg/L)

Scope and Application: For water, wastewater, and seawater. Metal Prep Set digestion is required for determining total iron. The estimated detection limit for program number 2130 is 0.010 mg/L free Fe.

Using UniCell Vials



1. Press the soft key under **HACH PROGRAM**.

Select the stored program number for UniCell Iron, HCT 159, method by pressing **2130** with the numeric keys.

Press: **ENTER**



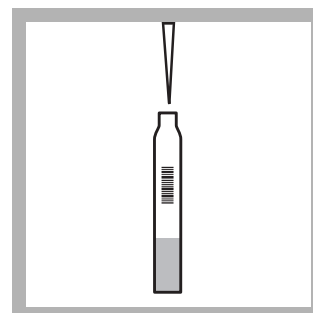
2. The display will show:
**HACH PROGRAM: 2130
Iron, HCT 159**

The wavelength (λ), **485 nm**, is automatically selected.

Note: If samples cannot be analyzed immediately, see Sample Collection, Storage and Preservation following these steps. Adjust pH of preserved samples before analysis.

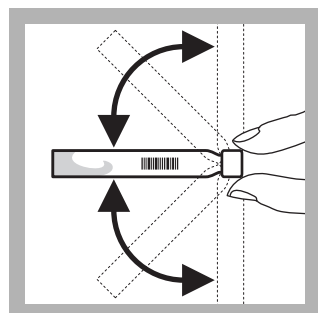


3. Insert the Test Tube Adapter into the sample cell module by sliding it under the thumb screw and into the alignment grooves. Fasten with the thumb screw.

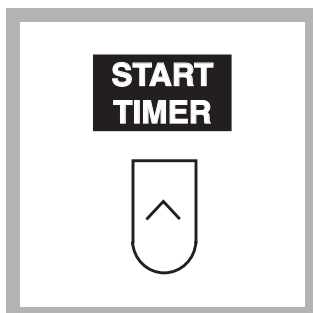


4. Pipet 4.0 mL of sample into a sample vial.

Note: For proof of accuracy, use a 3.0 -g/L iron standard solution (preparation given in the Accuracy Check section) in place of the sample.



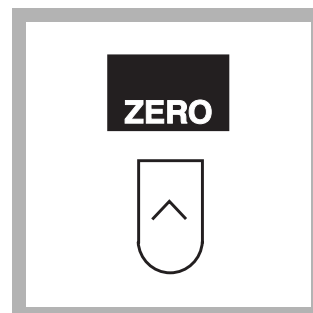
5. Cap the sample vial and invert several times to mix.



6. Press the soft key under **START TIMER**.
A 15-minute reaction period will begin.



7. When the timer beeps, place the zeroing vial (**white** cap) into the cell holder. Close the light shield.

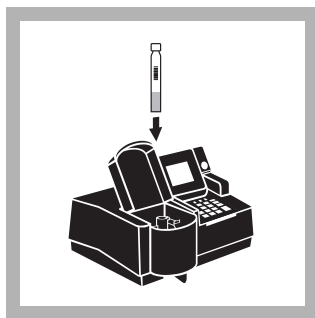


8. Press the soft key under **ZERO**.

The display will show:

0.00 mg/L Free Fe

Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



9. Place the sample vial into the cell holder. Close the light shield. Results in mg/L iron (or chosen units) will be displayed.

Interferences

The ions listed in the table have been individually checked up to the given concentrations. Cumulative effects and the influence of other ions have not been evaluated.

Ion	No interference above:
Cl ⁻	1000 mg/L
Ca ²⁺	500 mg/L
Ag ⁺	100 mg/L
Cd ²⁺	70 mg/L
Co ²⁺ , Zn ²⁺ , Pb ²⁺ , CO ₃ ²⁻ , Hg ²⁺ , Cr ³⁺ , Cr ⁶⁺	50 mg/L
Ni ²⁺	25 mg/L
Cu ²⁺	10 mg/L
Sn ²⁺	5 mg/L

Higher amounts of copper, nickel, and tin cause high-bias results.

Total iron including undissolved iron and complexed iron can only be determined after digesting with the Metal Prep Set HCT 200. (Total iron measuring range is 0.12–6.00 mg/L).

Sample Collection, Storage and Preservation

Collect samples in acid-cleaned or plastic containers. No acid addition is necessary if analyzing the samples immediately. To preserve samples, adjust the pH to 2 or less with concentrated nitric acid (about 2 mL per liter). Preserved samples may be stored up to six months at room temperature. If reporting only dissolved free iron, filter sample immediately after collection and before adding nitric acid.

Before analysis, adjust the pH to between 3 and 5 with 5.0 N sodium hydroxide standard solution. Do not exceed pH 5 or iron may precipitate. Correct the test results for volume additions.

Accuracy Check

Standard Additions Method

- a. Leave the unspiked sample in the sample cell compartment. Verify that the units displayed are in mg/L. Select standard additions mode by pressing the soft keys under **OPTIONS, (MORE)** and then **STD ADD**.
- b. Press **ENTER** to accept the default sample volume (mL), 100.0.
- c. Press **ENTER** to accept the default standard concentration (mg/L), 1000.0.
- d. Press the soft key under **ENTRY DONE**.
- e. Use a pipet to add 0.2 mL, 0.4 mL and 0.6 mL of standard, respectively, to three 100-mL samples and mix each thoroughly.
- f. Analyze each standard addition sample as described above. Accept the standard additions readings by pressing the soft key under **READ** each time. Each addition should reflect approximately 100% recovery.
- g. After completing the sequence, the display will show the extrapolated concentration value and the “best-fit” line through the standard additions data points, accounting for matrix interferences.

See Section 1.4.1 *Standard Additions* for more information.

Standard Solution Method

Prepare a 3.0-mg/L Fe standard solution by pipetting 0.3 mL of 1000-mg/L Fe into a 100-mL volumetric flask. Dilute to the mark with deionized water. Stopper and invert to mix. Prepare this solution daily. Perform the iron procedure as described.

To adjust the calibration curve using the reading obtained with the 1.00 mg/L Standard Solution, press the soft keys under **OPTIONS, (MORE)** then **STD:OFF**. Press **ENTER** to accept the displayed concentration, the value of which will depend on the selected units. If an alternate concentration is used, enter the actual concentration and press **ENTER** to return to the read screen.

See Section 1.5.5 *Adjusting the Standard Curve* for more information.

Method Performance

Precision

Standard: 3.00 mg/L free Fe

Program	95% Confidence Limits
2130	2.65–3.35 mg/L free Fe

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
2130	0.10 mg/L free Fe

For more information on derivation and use of Hach’s estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, appendix B, see Section 1.5.1.

Sensitivity

Program Number: 2130

Portion of Curve:	Δ Abs	Δ Concentration
Entire Range	0.010	0.04 mg/L free Fe

See Section 1.5.3 *Sensitivity Explained* for more information.

Summary of Method

Iron(II) ions form an orange-red complex with 1,10-phenanthroline. Any iron(III) ions present in the water sample are reduced to iron(II) ions by ascorbic acid before the complex is formed.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the Material Safety Data Sheet for information specific to the reagents used.

Pollution Prevention and Waste Management

For information on pollution prevention and waste management, refer to Section 1.

REQUIRED EQUIPMENT AND SUPPLIES

Description	Unit	Cat. No.
Iron-Fe UniCell™ HCT 159	23/pkg.....	HCT 159

OPTIONAL REAGENTS AND STANDARDS

Metal-Prep-Set, UniCell™ HCT 200.....	50 digestions.....	HCT 200
Iron Standard, 1000 mg/L as Fe	100 mL.....	2271-42
Sodium Hydroxide, 5 N	1L.....	2450-53
Nitric Acid Solution, 1:1	500 mL.....	2540-49

OPTIONAL APPARATUS

DRB 100, Digital Reactor Block.....	each.....	DRB 100
Graduated cylinder, mixing, 100-mL	each.....	20886-42
Flask, volumetric, 100-mL	each.....	14574-42
Pipettor, (Jencons) 1–5 mL	each.....	27951-00
Replacement tips for 27951-00	pkg/100.....	27952-00
Pipettor, (Jencons) 100–1000 μ L.....	each.....	27949-00
Replacement tips for 27949-00	pkg/400.....	27950-00
pH Paper	pkg/100.....	26013-00



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Telephone: (970) 669-3050

FAX: (970) 669-2932



✓ Method 8033

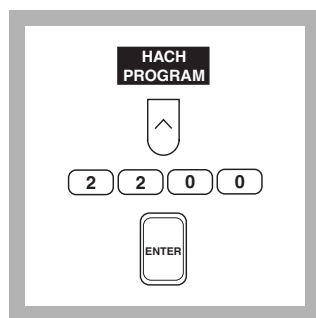
Dithizone Method*

(0 to 300 µg/L)

Scope and Application: For water and wastewater; USEPA accepted for reporting for wastewater analysis (digestion is required). ** See Section 1 for digestion procedure. The estimated detection limit for program number 2200 is 3 µg/L Pb.

* Adapted from Snyder, L. J., *Analytical Chemistry*, 19 684 (1947)

** Procedure is equivalent to Standard Method 3500-Pb D for wastewater analysis.



1. Press the soft key under **HACH PROGRAM**.

Select the stored program number for lead (Pb) by pressing **2200** with the numeric keys.

Press: **ENTER**

Note: If sample cannot be analyzed immediately, see Sample Collection, Storage and Preservation following these steps. Adjust the pH of preserved samples before analysis.

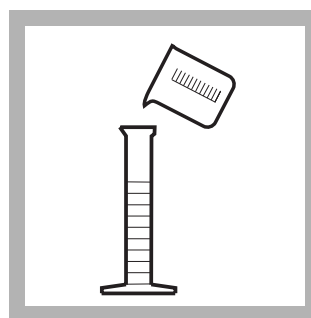
Note: The Flow Cell and Sipper Modules cannot be used with this procedure.



2. The display will show: **HACH PROGRAM: 2200 Lead, Dithizone**

The wavelength (λ), **515 nm**, is automatically selected.

Note: For best results, determine a reagent blank for each new lot of reagent as follows. Prepare a reagent blank by repeating steps 3 through 14, using deionized water as the sample. Zero the instrument on chloroform by pressing the soft key under **ZERO**. Insert the reagent blank and the blank value will be displayed. Correct for the reagent blank by pressing the soft keys under **OPTIONS**, **(MORE)**, and then **BLANK:OFF**. Enter the reagent blank value and press **ENTER**. Repeat for each new lot of reagent.

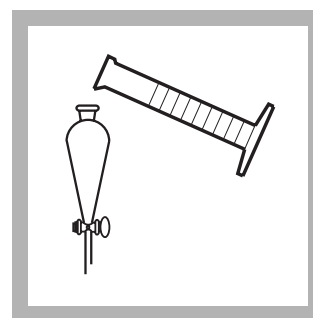


3. Fill a 250-mL graduated cylinder to the 250-mL mark with sample.

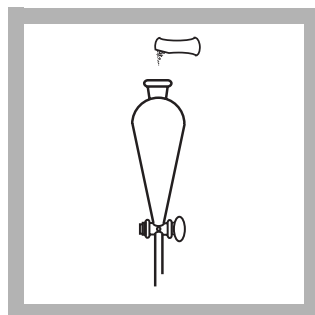
Note: Clean all glassware with a 1:1 Nitric Acid Solution. Rinse with deionized water.

Note: Cloudy and turbid samples may require filtering before running test. Results should be reported as soluble lead. Use a glass membrane filter to avoid loss of lead by adsorption on filter paper.

Note: For proof of accuracy, use a 200 µg/L lead standard solution (preparation given in the Accuracy Check section) in place of the sample.

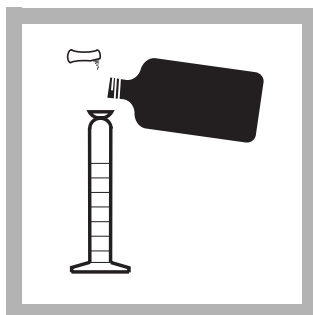


4. Transfer the sample into 500-mL separatory funnel.



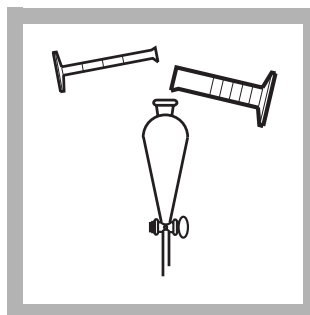
5. Add the contents of one Buffer Powder Pillow, citrate type for heavy metals. Stopper the funnel. Shake to dissolve.

Note: Spilled reagent will affect test accuracy and is hazardous to skin and other materials.



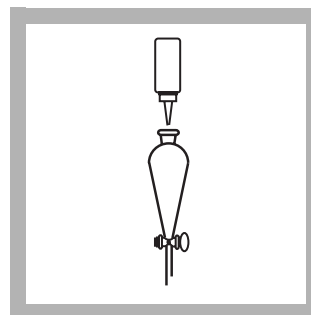
6. Add 50 mL of chloroform to a 50-mL graduated mixing cylinder. Add the contents of one DithiVer Metals Reagent Powder Pillow. Stopper. Invert repeatedly to mix. Pour 30 mL of this dithizone solution into a second graduated cylinder.

Note: Use adequate ventilation (i.e. a fume hood). The DithiVer Powder will not all dissolve in the chloroform. For further notes see DithiVer Solution Preparation, Storage and Reagent Blank.



7. Add the 30 mL of the dithizone solution to the separatory funnel. Stopper. Invert. Open stopcock to vent. Close the stopcock and add 5 mL of 5.0 N Sodium Hydroxide Standard Solution. Stopper. Invert. Open stopcock to vent. Close the stopcock and shake the funnel once or twice and vent again.

Note: Add a few drops of 5.25 N Sulfuric Acid Standard Solution if the solution turns orange on shaking. The blue-green color will reappear. To avoid higher blanks, repeat procedure on new sample and use less sodium hydroxide in this step.

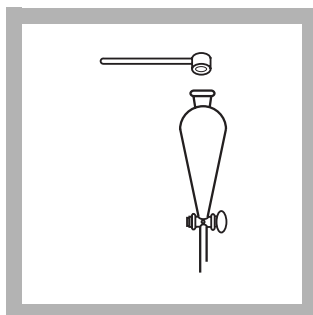


8. Continue adding 5.0 N Sodium Hydroxide Standard Solution dropwise and shaking the funnel after every few drops until the color of the solution being shaken changes from blue-green to orange. Then add 5 more drops of 5.0 N Sodium Hydroxide Standard Solution.

Note: A pink color in the bottom (chloroform) layer at this point does not necessarily indicate lead is present. Only after shaking with potassium cyanide in next step will a pink color in the chloroform layer confirm the presence of lead.

Note: Large amounts of zinc cause the color transition at the end point to be indistinct.

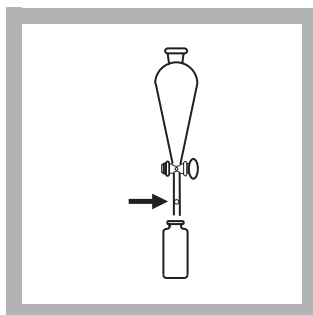
Note: For more accurate results, adjust the sample to pH 11.0-11.5 using a pH meter, omitting the five additional drops of Sodium Hydroxide Standard Solution.



9. Add 2 heaping 1.0-g scoops of potassium cyanide to the funnel. Stopper. Shake vigorously until the potassium cyanide is all dissolved (about 15 seconds).

Note: Wait one minute for the layers to separate. The bottom (chloroform) layer will be pink if lead is present.

Warning:
Cyanide is a deadly poison. Use a fume hood. Maintain cyanide solutions at pH 11 or greater to prevent formation of cyanide gas.

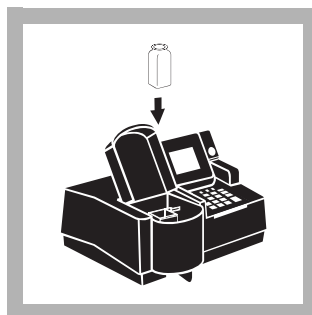


10. Insert a cotton plug the size of a pea into the delivery tube of the funnel. Slowly drain the bottom (chloroform) layer into a dry 25-mL sample cell. Stopper the sample cell.

Note: The lead-dithizone complex is stable for at least 30 minutes if the sample cell is kept tightly capped and out of direct sunlight.



11. Fill another sample cell with chloroform (the blank). Stopper.



12. Place the blank into the cell holder. Close the light shield.



13. Press the soft key under **ZERO**.

The display will show:

0.00 µg/L Pb

Note: If you are using a reagent blank correction, the display will show the correction.

Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



14. Place the prepared sample into the cell holder. Close the light shield. The results in µg/L lead (or chosen units) will be displayed.

Note: See Dithiver Solution Preparation, Storage and Reagent Blank for information on preparing a reagent blank.

Note: See Pollution Prevention and Waste Management for proper disposal of chloroform solutions.

Interference

The following do not interfere:

Aluminum	Calcium	Magnesium
Antimony	Chromium	Manganese
Arsenic	Cobalt	Nickel
Cadmium	Iron	Zinc

Interfering Substance	Interference Levels and Treatments
Bismuth	All levels. See procedure below.
Copper	All levels. See procedure below.
Mercury	All levels. See procedure below.
Silver	All levels. See procedure below.
Tin	All levels. See procedure below.
Highly buffered samples or extreme sample pH	May exceed the buffering capacity of the reagents and require sample pretreatment; see <i>Section 1.3.1 pH Interference</i> .

Eliminate interference from the metals listed above by the following treatment, beginning after procedure Step 6.

- a. Measure about 5 mL of the prepared dithizone solution into the separatory funnel. Stopper the funnel, invert and open the stopcock to vent. Close the stopcock and shake the solution vigorously for 15 seconds. Allow the funnel to stand undisturbed until the layers separate (about 30 seconds). A yellow, red, or bronze color in the bottom (chloroform) layer confirms the presence of interfering metals. Draw off and collect the bottom (chloroform) layer for proper disposal.
- b. Repeat extraction with fresh 5-mL portions of prepared dithizone solution (collecting the bottom layer each time in appropriate waste collection vessel) until the bottom layer shows a pure dark green color for three successive extracts. Extractions can be repeated a number of times without appreciably affecting the amount of lead in the sample.
- c. Extract the solution with several 2 or 3 mL portions of pure chloroform to remove any remaining dithizone, again collecting the bottom layer each time for proper disposal.
- d. Continue the procedure, substituting 28.5 mL of prepared dithizone solution for the 30 mL in Step 7.

Dithiver Solution Preparation, Storage and Reagent Blank

Store DithiVer Powder Pillows away from light and heat. A convenient way to prepare this solution is to add the contents of 10 DithiVer Metals Reagent Powder Pillows to a 500-mL bottle of chloroform and invert several times until well mixed (carrier powder may not dissolve). Store dithizone solution in an amber glass bottle. This solution is stable for 24 hours.

A reagent blank using deionized water should be carried out through the entire method to obtain the most accurate results. The amount of reagent blank determined on each lot of DithiVer Metals Reagent Powder Pillow can be subtracted automatically from the sample reading by pressing the soft keys under

OPTIONS, (MORE) and then **BLANK: (OFF)**. Enter the blank value and press **ENTER** to return to the read screen.

Sample Collection, Storage and Preservation

Collect samples in acid-cleaned glass or plastic containers. Adjust the pH to 2 or less with nitric acid (about 2 mL per liter). Preserved samples can be stored up to six months at room temperature. Before analysis, adjust the pH to 2.5–4.5 with 5.0 N Sodium Hydroxide. Correct the test result for volume additions; see Section 1.2.2 *Correcting for Volume Additions*.

Accuracy Check

Standard Additions Method

- a. Leave the unspiked sample in the sample cell compartment. Verify that the units displayed are in $\mu\text{g/L}$. Select standard additions mode by pressing the soft keys under **OPTIONS, (MORE)** and then **STD ADD**.
- b. Press **ENTER** to accept the default sample volume (mL), 250.
- c. Press **ENTER** to accept the default standard concentration ($\mu\text{g/L}$), 50,000 (equivalent to 50-mg/L).
- d. Press the soft key under **ENTRY DONE**.
- e. Snap the neck off a Lead Voluette Ampule Standard, 50-mg/L Pb.
- f. Use the TenSette Pipet (do not use a glass pipet) to add 0.1 mL, 0.2 mL and 0.3 mL of standard, respectively to three 250-mL samples and mix each thoroughly.
- g. Analyze each standard addition sample as described above. Accept the standard additions reading by pressing the soft key under **READ** each time. Each addition should reflect approximately 100% recovery.
- h. After completing the sequence, the display will show the extrapolated concentration value and the “best-fit” line through the standard additions data points, accounting for matrix interferences.
- i. See Section 1.4.1 *Standard Additions* for more information.

Standard Solution Method

Prepare a 10-mg/L lead standard solution by pipetting 10.00 mL of Lead Standard Solution, 100-mg/L, into a 100-mL volumetric flask. Add 0.2 mL of concentrated nitric acid using a TenSette Pipet to prevent the adsorption of lead onto the container walls. Dilute to the mark with deionized water and mix thoroughly. To make a 200- $\mu\text{g/L}$ standard, pipet 5.00 mL of the 10.0-mg/L standard into 245 mL of deionized water in the 500-mL separatory funnel in *Step 4* of the Dithizone procedure. Prepare these solutions daily. Perform the lead procedure as described above.

To adjust the calibration curve using the reading obtained with the 200- $\mu\text{g/L}$ standard solution, press the soft keys under **OPTIONS, MORE** then **STD: OFF**. Press **ENTER** to accept the displayed concentration, the value of which depends on the selected units. If an alternate concentration is used, enter the actual concentration and press enter to return to the read screen. See Section 1.5.5 *Adjusting the Standard Curve* for more information.

Method Performance**Precision**

Standard: 200 µg/L Pb

Program	95% Confidence Limits
2200	198–202 µg/L Pb

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
2200	3 µg/L Pb

For more information on derivation and use of Hach's estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, appendix B, see Section 1.5.1.

Sensitivity

Program Number: 2200

Portion of Curve	ΔAbs	ΔConcentration
0.010 Abs	0.010	1.8 µg/L
150 µg/L	0.010	1.7 µg/L
270 µg/L	0.010	1.6 µg/L

See Section 1.5.3 *Sensitivity Explained* for more information.

Calibration Standard Preparation

To perform a lead calibration using the Dithizone method, prepare a 10,000-µg/L lead stock solution by pipetting 10.00 mL of a 100-mg/L Lead Standard Solution (Cat. No. 12617-42) into a 100-mL volumetric flask using Class A glassware. Add 0.2 mL of concentrated nitric acid using a TenSette pipet to prevent the adsorption of lead onto the container walls. Dilute to the mark with deionized water and mix thoroughly.

Prepare calibration standards containing 40, 80, 120, 160, 200, 240, 280, and 300 µg/L lead as follows:

- a. Prepare the standards in 500-mL separatory funnels to minimize lead loss in intermediate glassware due to adsorption.
- b. Into eight different 500-mL separatory funnels, pipet 1, 2, 3, 4, 5, 6, 7, and 8 mL of the 10,000-µg/L lead stock solution using Class A glassware. Add enough deionized water to bring the final volume to exactly 250 mL. For example, use 3 mL of the 10,000-µg/L lead stock solution and 247 mL of deionized water to prepare the 120-µg/L standard.
- c. Using the Dithizone method and the calibration procedure described in the *User-Entered Programs* section of the *DR/4000 Spectrophotometer Instrument Manual*, generate a calibration curve from the standards prepared above.

Summary of Method

The DithiVer Metals Reagent is a stable powder form of dithizone. Lead ions in basic solution react with dithizone to form a pink to red lead-dithizonate complex, which is extracted with chloroform.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to Section 1.

Pollution Prevention and Waste Management

Both chloroform (D022) and cyanide (D003) solutions are regulated as hazardous wastes by the Federal RCRA. Do not pour these solutions down the drain. Chloroform solutions and the cotton plug used in the delivery tube of the separatory funnel should be collected for disposal with laboratory solvent waste. Collect the cyanide solution as a reactive waste. Be sure that cyanide solutions are stored in a caustic solution with a pH >11 to prevent potential release of hydrogen cyanide gas. See Section 3 for more information on proper disposal of these materials.

REQUIRED REAGENTS AND STANDARDS

	Cat. No.
Lead Reagent Set (100 Tests).....	22431-00
Includes: (1) 14202-99, (2) 14458-17, (1) 12616-99, (2) 767-14, (1) 2450-53, (2) 2450-26, (1) 14262-02	

Description	Quantity Required		Cat. No.
	per test	Unit	
Buffer Powder Pillows, Citrate.....	1 pillow	100/pkg	14202-99
Chloroform	50 mL	4 L.....	14458-17
DithiVer Metals Reagent Powder Pillows	1 pillow	100/pkg	12616-99
Potassium Cyanide, ACS	2 g	125 g.....	767-14
Sodium Hydroxide Solution, 5.0 N.....	5 mL	1000 mL.....	2450-53
Sodium Hydroxide Standard Solution, 5.0 N.....	varies.....	59 mL DB.....	2450-26

REQUIRED EQUIPMENT AND SUPPLIES

Description	Quantity Required		Cat. No.
	per test	Unit	
Clippers, for opening powder pillows	1	each.....	968-00
Cotton balls, absorbent	1	100/pkg.....	2572-01
Cylinder, graduated mixing, 50-mL	1	each.....	1896-41
Cylinder, graduated, 5-mL	1	each.....	508-37
Cylinder, graduated, 50-mL	1	each.....	508-41
Cylinder, graduated, 250-mL	1	each.....	508-46
DR/4000 1-Inch Cell Adapter	1	each.....	48190-00
Funnel, separatory, 500-mL	1	each.....	520-49
pH Meter, <i>sension</i> TM 1, portable.....	1	each.....	51700-00
Ring, support, 4-inch.....	1	each.....	580-01
Sample Cells, matched pair, 1-inch, glass, with stoppers	2	pair.....	26126-02
Spoon, measuring, 1.0-g.....	1	each.....	510-00
Support, Ring Stand, 5 x 8 in. base	1	each.....	563-00

OPTIONAL REAGENTS AND STANDARDS

Description	Unit	Cat. No.
Chloroform, ACS	500 mL.....	14458-49
Lead Standard Solution, 100 mg/L Pb	100 mL.....	12617-42
Lead Standard Solution, 10-mL Voluette Ampules, 50-mg/L Pb	16/pkg.....	14262-16
Nitric Acid Solution, 1:1	500 mL.....	2540-49
Nitric Acid, ACS	500 mL.....	152-49
Sodium Hydroxide Standard Solution, 5.0 N.....	59 mL SCDB.....	2450-26
Sodium Hydroxide Standard Solution, 5.0 N.....	100 mL MDB.....	2450-32
Sulfuric Acid, 5.25 N	100 mL MDB.....	2449-32
Water, deionized.....	4 liters.....	272-56

OPTIONAL EQUIPMENT AND SUPPLIES

Ampule Breaker Kit	each.....	21968-00
DR/4000 Carousel Module Kit	each.....	48090-02
Filter Discs, glass membrane, 47-mm	100/pkg.....	2530-00
Filter Holder, glass, 47-mm.....	each.....	2340-00
Flask, Erlenmeyer, 500-mL.....	each.....	505-49
Flask, filtering, 500-mL	each.....	546-49
Flask, volumetric, Class A, 100-mL	each.....	14574-42
pH Paper, pH 1.0 to 11.0	5 rolls/pkg.....	391-33
Pipet Filler, safety bulb.....	each.....	14651-00
Pipet, TenSette, 0.1 to 1.0 mL	each.....	19700-01
Pipet Tips, for 19700-01 TenSette Pipet	50/pkg.....	21856-96
Pipet, serological, 2-mL	each.....	532-36
Pipet, volumetric, Class A, 1.00-mL	each.....	14515-35
Pipet, volumetric, Class A, 2.00-mL	each.....	14515-36
Pipet, volumetric, Class A, 3.00-mL	each.....	14515-03
Pipet, volumetric, Class A, 4.00-mL	each.....	14515-04
Pipet, volumetric, Class A, 5.00-mL	each.....	14515-37
Pipet, volumetric, Class A, 6.00-mL	each.....	14515-06
Pipet, volumetric, Class A, 7.00-mL	each.....	14515-07
Pipet, volumetric, Class A, 8.00-mL	each.....	14515-08
Pipet, volumetric, Class A, 10.00-mL	each.....	14515-38



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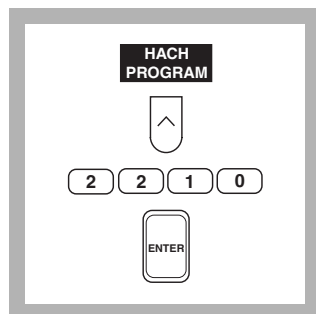
Method 8317

LeadTrak* Fast Column Extraction Method

(0 to 150 µg/L)

Scope and Application: For drinking water. The estimated detection limit for program number 2210 is 2 µg/L Pb.

* Patent number 5,019,516



1. Press the soft key under **HACH PROGRAM**.

Select the stored program number for lead (Pb) by pressing **2210** with the numeric keys.

Press: **ENTER**

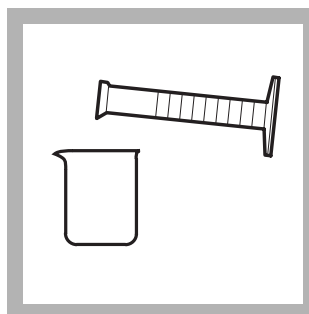
Note: The Flow Cell and Sipper Modules cannot be used with this procedure.



2. The display will show: **HACH PROGRAM: 2210 Lead, LeadTrak**

The wavelength (λ), **477 nm**, is automatically selected.

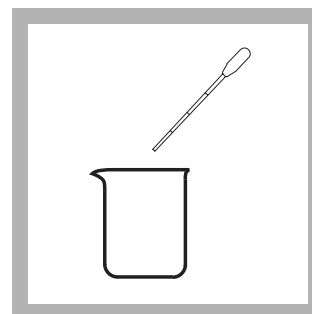
Note: For best results, determine a reagent blank for each new lot of reagent as follows. Prepare a reagent blank by repeating steps 3 through 18, using lead-free deionized water as the sample. Zero the instrument on deionized water by pressing the soft key under **ZERO**. Insert the reagent blank and the blank value will be displayed. Correct for the reagent blank by pressing the soft keys under **OPTIONS, (MORE)**, and then **BLANK:OFF**. Enter the reagent blank value and press **ENTER**. Repeat for each new lot of reagent. See Reagent Blank Adjustment following this procedure for more information.



3. Fill a 100-mL plastic graduated cylinder with 100 mL of the sample to be tested. Pour the measured sample into a 250-mL plastic beaker.

Note: The sampling requirements for "first-draw" analysis are detailed in the Sample Collection, Storage and Preservation section.

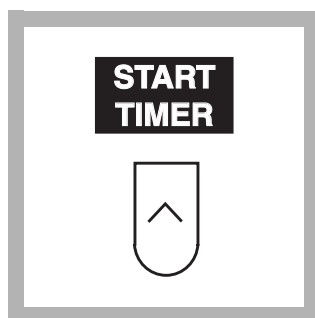
Note: For proof of accuracy, use a 100-µg/L lead standard solution (preparation given in Accuracy Check section) in place of the sample.



4. Using a plastic 1-mL dropper, add 1.0 mL of pPb-1 Acid Preservative Solution to the sample and swirl to mix.

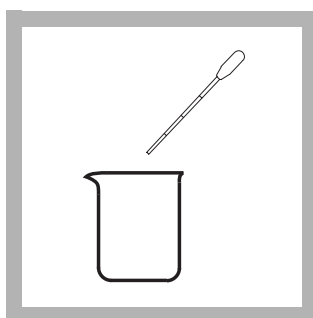
Note: If the sample has been preserved previously with pPb-1 Acid Preservative at a ratio of 1.0 mL per 100-mL sample, omit steps 4 and 5.

Note: Samples preserved with nitric acid still require steps 4 and 5.



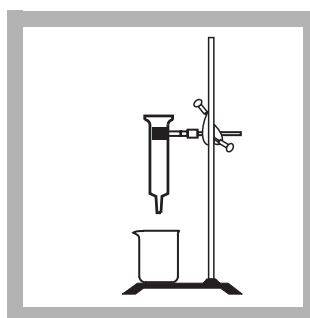
5. Press the soft key under **START TIMER**.

A 2-minute reaction period will begin.



6. When the timer beeps, use a second 1-mL plastic dropper to add 2.0 mL of pPb-2 Fixer Solution. Swirl to mix.

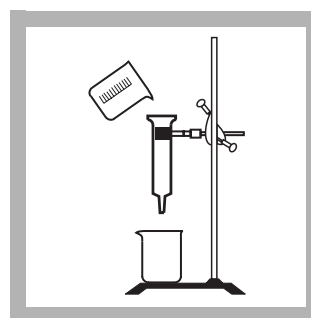
Note: Field samples that have been preserved with nitric acid or samples that have been digested may exceed the buffer capacity of the Fixer Solution. After Step 6 check the pH of these samples and adjust with 5 N sodium hydroxide to a pH of 6.7–7.1 before proceeding with Step 7.



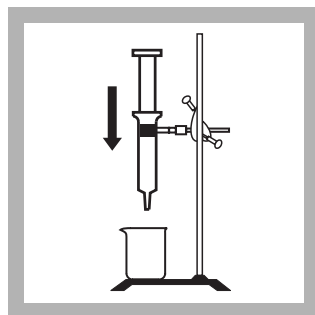
7. Mount a new Fast Column Extractor in a ring stand with a clamp. Place a 150-mL plastic beaker under the Extractor.

Note: A Fast Column Extractor is included in the LeadTrak Reagent Set.

Note: A new extractor is required for each test.

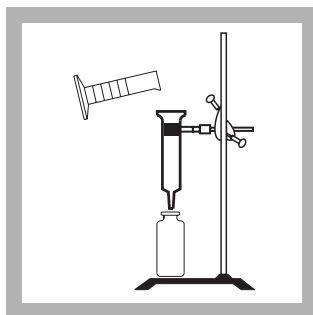


8. Pour the prepared sample slowly into the Column Extractor. Wait for the sample to flow through.

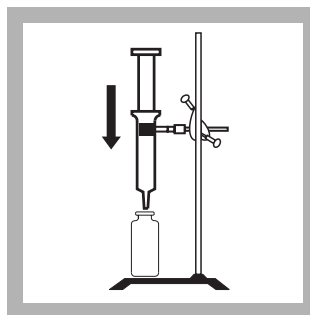


9. After the flow has stopped, fully compress the absorbent pad in the Extractor with the plunger. Discard the contents of the beaker. Withdraw the plunger slowly from the Extractor.

Note: The absorbent pad should remain at the bottom of the Extractor when the plunger is removed. Re-compress with the plunger if the pad has retracted with the plunger.



10. Place a 25-mL sample cell under the Extractor. Using a 25-mL plastic graduated cylinder, add 25 mL of pPb-3 Eluant Solution to the Extractor.



11. After the Eluant Solution has started to drip from the Extractor, insert the plunger and slowly force the remaining Eluant Solution through the Extractor. Fully compress the absorbent pad. The volume in the sample cell should be 25 mL.

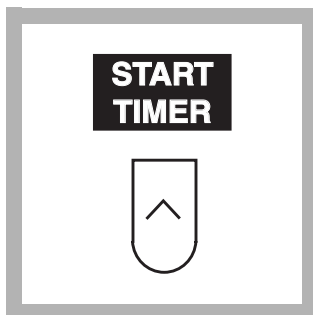


12. Using a 1-mL plastic dropper, add 1.0 mL of pPb-4 Neutralizer Solution to the cell. Swirl thoroughly to mix and proceed immediately to Step 13.

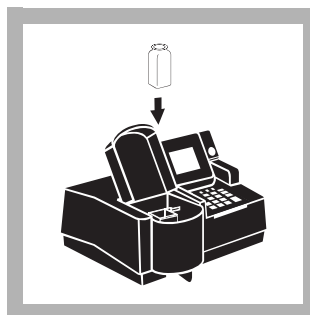


13. Add the contents of one pPb-5 Indicator Powder Pillow to the sample and swirl thoroughly to mix.

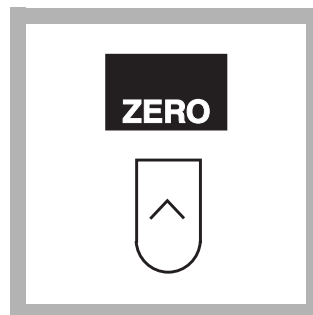
Note: The solution color will turn brown.



14. Press the soft key under **START TIMER**. A second 2-minute reaction period will begin.



15. When the timer beeps, place the sample cell into the cell holder. Close the light shield.



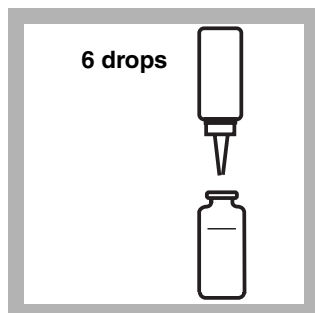
16. Press the soft key under **ZERO**.

The display will show:

-2 µg/L Pb

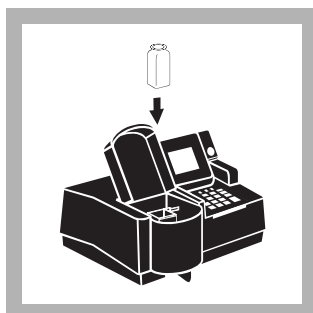
Note: Pressing **ZERO** results in a -2 since this program uses a non-zero y-intercept.

Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



17. Remove the sample cell and add 6 drops of pPb-6 Decolorizer Solution to the cell. Swirl to mix thoroughly.

Note: There will be little visual difference between the prepared and decolorized sample.



18. Place the sample back into the cell holder. Close the light shield. The results in µg/L lead (or chosen units) will be displayed.

Interference

Interference studies were conducted by preparing a known lead solution of approximately 25 µg/L as well as the potential interfering ion. The ion was said to interfere when the resulting lead concentration changed by $\pm 10\%$. Samples containing levels exceeding these concentration values may be diluted 1:1 and re-analyzed. Multiply the value obtained by the factor of 2 to determine the lead present in the original sample.

Interfering Substance	Interference Levels and Treatments
Aluminum, Al^{3+}	0.5 mg/L
Ammonium, NH_4^+	500 mg/L
Barium, Ba^{2+}	6 mg/L
Calcium, Ca^{2+}	500 mg/L
Chloride, Cl^-	1000 mg/L
Copper, Cu^{2+}	2 mg/L
Fluoride, F^-	10 mg/L
Iron, Fe^{2+}	2 mg/L
Magnesium, Mg^{2+}	500 mg/L
Manganese, Mn^{2+}	0.5 mg/L
Nitrate, NO_3^-	1000 mg/L
Sulfate, SO_4^{2-}	1000 mg/L
Zinc, Zn^{2+}	1 mg/L

Every effort has been made to prevent contamination in packaging the reagents. Use of black rubber stoppers, black dropper bulbs and droppers with inked graduations may contaminate the sample and should be avoided. Use the plastic droppers provided in the reagent set.

Treat glassware and plasticware to prevent sample contamination, especially if the previous sample had a high lead level (see the *Apparatus and Sample Preparation* section).

The Extractor plunger may be used for more than one test and should be rinsed as well.

Apparatus and Sample Preparation

Because lead is very common to our environment, care must be taken to prevent sample contamination. Follow these steps for greatest test accuracy:

- a. Lead-free water is necessary to minimize sample contamination when rinsing apparatus or diluting sample. The water may be either distilled or deionized. If the water is obtained from a grocery store, verify the lead concentration is zero from the label. If the lead concentration is uncertain, determine the lead concentration with the LeadTrak test.
- b. Plastic or glass sample containers and lids may be checked for contamination by rinsing with 1 mL of pPb-1 Acid Preservative Reagent. Add 100 mL of lead-free water. After 24 hours, analyze this solution using the LeadTrak test to confirm the absence of lead.

- c. Rinse glassware used in this test with a small amount of dilute lead-free 0.1 N nitric acid or pPb-1 Acid Preservative Reagent followed by rinsing with lead-free water.

Note: The pPb-5 Indicator may be rinsed from the glass sample cells with a few drops of pPb-1 Acid Preservative Reagent or a small amount of dilute lead-free nitric acid.

Note: Acidify solutions containing lead with nitric acid or pPb-1 to below pH 2 to prevent adsorption of lead onto the container walls. See Sample Collection, Storage and Preservation.

Sample Collection, Storage and Preservation

Samples may be collected either from household pipes (point-of-use) or from water sources. Preserved samples may be stored up to six months.

Sampling for lead contamination in household pipes for point-of-use drinking water

- a. The sample should be collected after sitting in pipes with no flow for 8 to 18 hours.
- b. Add 10 mL of pPb-1 Acid Preservative to a one-liter bottle.
- c. Turn on tap and collect exactly the first liter of water in the bottle containing acid preservative.
- d. Cap and invert several times to mix.
- e. After two minutes the sample is ready for analysis. Steps 4 and 5 are skipped in the analysis procedure. Use 100 mL of this preserved sample directly in Step 6.

Sampling for lead contamination from drinking water sources such as well water or water from main supply lines

- a. Add 10 mL of pPb-1 Acid Preservative to a one-liter bottle.
- b. Turn on the tap for 3–5 minutes or until the water temperature has been stable for 3 minutes.
- c. Collect exactly one-liter of water into the bottle containing the acid preservative.
- d. Cap and invert several times to mix.
- e. After two minutes the sample is ready for analysis. Steps 4 and 5 are skipped in the analysis procedure. Use 100 mL of this preserved sample directly in Step 6.

Note: At least one liter should be collected to obtain a representative sample. If less than one liter is collected, use 1 mL of pPb-1 Acid Preservative per 100 mL of sample.

Note: If nitric acid is to be substituted for pPb-1 as a preservative or the sample is digested, the buffering capacity of the pPb-2 Fixer Solution may be exceeded. Adjust the sample pH to 6.7–7.1 pH with 5 N sodium hydroxide after Step 7.

Note: Each sample type typically requires different sampling procedures. Consult with the appropriate regulatory agency in your area for more information about your specific sampling requirements.

Reagent Blank Adjustment

The LeadTrak program will allow a reagent blank value from -5 to +5 µg/L Pb to be automatically subtracted from the test result. The factory calibration uses a y-intercept of approximately -2 µg/L. When using the reagent blank adjustment feature, the concentration value displayed after zeroing will not be exactly what was entered, rather that value minus the default value of -2 µg/L.

Accuracy Check

Standard Additions Method

- a. Leave the unspiked sample in the sample cell compartment. Verify that the units displayed are in µg/L. Select standard additions mode by pressing the soft keys under **OPTIONS, (MORE)** and then **STD ADD**.
- b. Press **ENTER** to accept the default sample volume (mL), 100.
- c. Press **ENTER** to accept the default standard concentration (µg/L), 10,000 (equivalent to 10-mg/L).
- d. Press the soft key under **ENTRY DONE**.
- e. Open a 10-mg/L Lead Standard Solution Standard.
- f. Use the TenSette Pipet (do not use a glass pipet) to add 0.1 mL, 0.2 mL and 0.3 mL of standard, respectively to three 100-mL samples and mix each thoroughly.
- g. Analyze each standard addition sample as described above. Accept the standard additions reading by pressing the soft key under **READ** each time. Each addition should reflect approximately 100% recovery.
- h. After completing the sequence, the display will show the extrapolated concentration value and the “best-fit” line through the standard additions data points, accounting for matrix interferences.
- i. See Section 1.4.1 *Standard Additions* for more information.

Standard Solution Method

Using Class A glassware, prepare a 100-µg/L lead working standard solution by pipetting 1.0 mL of Lead Standard Solution, 1000-mg/L, into a 100-mL volumetric flask. Use a TenSette Pipet to add 0.2 mL of concentrated nitric acid to the flask. Dilute to the mark with lead-free deionized water. This makes a 10-mg/L working standard.

Pipet 10.00 mL of this working solution into a one-liter plastic volumetric flask. Add 2.0 mL of concentrated nitric acid to the flask. Dilute to the mark with lead-free water. This 100-µg/L standard solution should be prepared immediately before use. Perform the LeadTrak procedure as described above.

Alternatively, prepare a 100-µg/L lead standard solution by using a TenSette Pipet to pipet 0.2 mL from a Lead Voluette Ampule Standard Solution, 50-mg/L as Pb, into a 100-mL plastic volumetric flask. Add 0.2 mL of concentrated nitric acid, and dilute to volume with deionized water. Prepare this solution immediately before use.

To adjust the calibration curve using the reading obtained with the 100- $\mu\text{g/L}$ standard solution, press the soft keys under **OPTIONS, MORE** then **STD: OFF**. Press **ENTER** to accept the displayed concentration, the value of which depends on the selected units. If an alternate concentration is used, enter the actual concentration and press enter to return to the read screen. See Section 1.5.5 *Adjusting the Standard Curve* for more information.

Method Performance

Precision

Standard: 100 $\mu\text{g/L}$ Pb

Program	95% Confidence Limits
2210	99–101 $\mu\text{g/L}$ Pb

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
2210	2 $\mu\text{g/L}$ Pb

For more information on derivation and use of Hach's estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, appendix B, see Section 1.5.1.

Sensitivity

Program Number: 2210

Portion of Curve:	ΔAbs	$\Delta\text{Concentration}$
0.010 Abs	0.010	-1.2 $\mu\text{g/L}$
75 $\mu\text{g/L}$	0.010	-1.4 $\mu\text{g/L}$
135 $\mu\text{g/L}$	0.010	-1.2 $\mu\text{g/L}$

See Section 1.5.3 *Sensitivity Explained* for more information.

Calibration Standard Preparation

To perform a lead calibration using the LeadTrak method, prepare a 10,000- $\mu\text{g/L}$ lead stock solution by pipetting 10 mL of a 100-mg/L Lead Standard Solution (Cat. No. 12617-42) into a 100-mL volumetric flask using Class A glassware. Add 0.2 mL of concentrated nitric acid and dilute to the mark with deionized water and mix thoroughly.

Prepare calibration standards containing 20, 40, 60, 80, 100, 120, 140 and 160 $\mu\text{g/L}$ Pb as follows:

- a. Into eight different 500-mL volumetric flasks, pipet 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0 and 8.0 mL of the 10,000- $\mu\text{g/L}$ Pb stock solution using Class A glassware.

- b. Dilute to the mark with deionized water mixed with pPb-1 Acid Preservative at the ratio of 1.0 mL of preservative per 100 mL of water. Mix thoroughly.
- c. Using the LeadTrak method (omit steps 4 and 5) and the calibration procedure described in the *User-Entered Programs* section of the *DR/4000 Spectrophotometer Instrument Manual*, generate a calibration curve from the standards prepared above.

Summary of Method

Acid soluble lead, as Pb^{2+} , in a potable water sample is first concentrated on a Fast Column Extractor. The lead is then eluted from the Extractor and determined colorimetrically with an indicator.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to Section 1.

Pollution Prevention and Waste Management

For information on pollution prevention and waste management, refer to Section 1.

REQUIRED REAGENTS AND STANDARDS

Description	Quantity Required per test	Unit	Cat. No.
LeadTrack Reagent Set	1	20 tests/pkg	23750-00
Includes: (1) 23685-31, (1) 23686-55, (1) 23687-49, (1) 23688-55, (1) 23689-64, (1) 23747-55, (1) 23748-20			

REQUIRED EQUIPMENT AND SUPPLIES

Beaker, polypropylene, 150-mL.....	1	each.....	1080-44
Beaker, polypropylene, 250-mL.....	1	each.....	1080-46
Clamp, two-prong extension	1	each.....	21145-00
Clamp holder.....	1	each.....	326-00
Clippers, for opening powder pillows	1	each.....	936-00
Cylinder, graduated, polypropylene, 25-mL	1	each.....	1081-40
Cylinder, graduated, polypropylene, 100-mL	1	each.....	1081-42
DR/4000 1-Inch Cell Adapter	1	each.....	48190-00
Dropper, 0.5 & 1.0 mL marks		20/pkg.....	21247-20
Sample Cells, glass-stoppered, matched pair, 1-inch.....	2	2/pkg.....	26126-02
Support, ring stand	1	each.....	563-00

OPTIONAL REAGENTS AND STANDARDS

Lead Standard Solution, 1000-mg/L as Pb.....	100 mL.....	12796-42
Lead Standard Solution, 100-mg/L	100 mL.....	12617-42
Lead Standard Solution, 50-mg/L, 10-mL Voluette Ampules	16/pkg.....	14262-10
Lead Standard Solution, 10-mg/L	25 mL.....	23748-20
Nitric Acid, ACS	500 mL.....	152-49
Nitric Acid Standard Solution, 0.1 N	100 mL.....	23328-42
pPb-1 Acid Preservative Reagent.....	236 mL.....	23685-31
Sodium Hydroxide Solution, 5.0 N.....	1 liter.....	2450-53
Water, deionized	4 liters.....	272-56

OPTIONAL EQUIPMENT AND SUPPLIES

Description	Unit	Cat. No.
Ampule Breaker Kit	each.....	21968-00
Bottle, sampling, 125-mL.....	each.....	23240-43
Bottle, sampling, 125-mL.....	48/pkg.....	23240-73
Bottle, sampling, 1000-mL.....	each.....	23242-53
Bottle, sampling, 1000-mL.....	24/pkg.....	23242-83
DR/4000 Carousel Module Kit	each.....	48090-02
Flask, volumetric, plastic, 100-mL.....	each.....	20995-42
Flask, volumetric, plastic, 500-mL.....	each.....	20995-49
Flask, volumetric, plastic, 1000-mL.....	each.....	20995-53
pH Meter, <i>sens^{ion}</i> TM 1, portable.....	each.....	51700-00
Pipet Filler, safety bulb.....	each.....	14651-00
Pipet, serological, 5-mL	each.....	532-37
Pipet, TenSette, 0.1 to 1.0 mL	each.....	19700-01
Pipet Tips, for 19700-01 TenSette Pipet	50/pkg.....	21856-96
Pipet, volumetric, Class A, 1.00-mL	each.....	14515-35
Pipet, volumetric, Class A, 2.00-mL	each.....	14515-36
Pipet, volumetric, Class A, 3.00-mL	each.....	14515-03
Pipet, volumetric, Class A, 4.00-mL	each.....	14515-04
Pipet, volumetric, Class A, 5.00-mL	each.....	14515-37
Pipet, volumetric, Class A, 6.00-mL	each.....	14515-06
Pipet, volumetric, Class A, 7.00-mL	each.....	14515-07
Pipet, volumetric, Class A, 8.00-mL	each.....	14515-08
Pipet, volumetric, Class A, 10.00-mL	each.....	14515-38
Pipetter, 100-μL.....	each.....	22753-00
Stopper, hollow, size #1.....	6/pkg.....	14480-00



FOR TECHNICAL ASSISTANCE, PRICE INFORMATION AND ORDERING:

In the U.S.A. – Call toll-free 800-227-4224

Outside the U.S.A. – Contact the HACH office or distributor serving you.

On the Worldwide Web – www.hach.com; E-mail – techhelp@hach.com

HACH COMPANY
WORLD HEADQUARTERS
Telephone: (970) 669-3050
FAX: (970) 669-2932

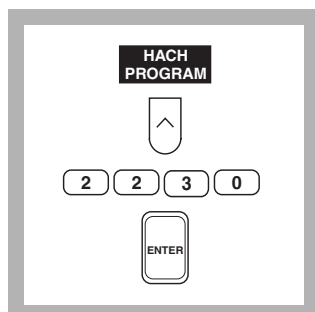


PAR Method

UniCell™ Vials

(0 to 2.00 mg/L free Pb)

Scope and Application: For wastewater process control. The estimated detection limit for program number 2230 is 0.20 mg/L Pb.



1. Press the soft key under **HACH PROGRAM**.

Select the stored program number for UniCell lead by pressing **2230** with the numeric keys.

Press: **ENTER**



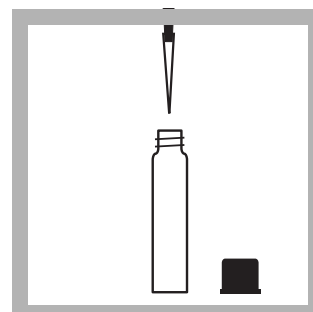
2. The display will show:

**HACH PROGRAM:
2230 Lead, HCT 152**

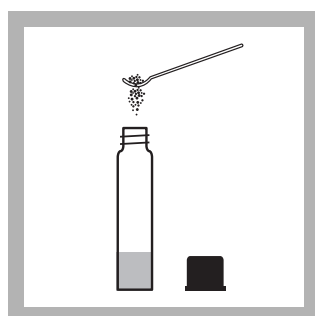
The wavelength (λ), **520 nm**, is automatically selected.



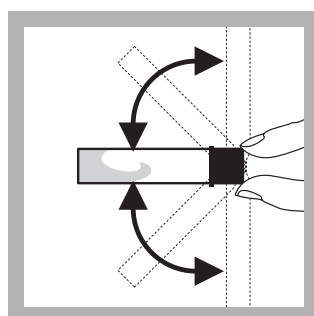
3. Insert the Test Tube Adapter into the sample cell module by sliding it under the thumb screw and into the alignment grooves. Fasten with the thumb screw.



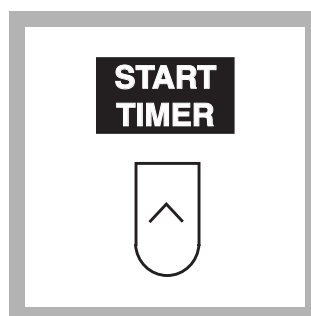
4. Pipet 10.0 mL of sample into the reaction tube (red cap).



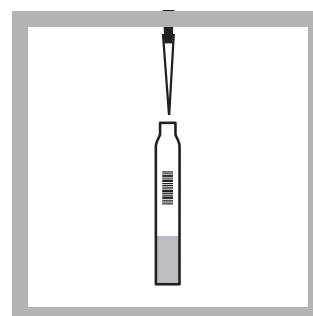
5. Use the spoon to add one level spoonful of Masking Agent A (HCT 152 A) to the reaction tube.



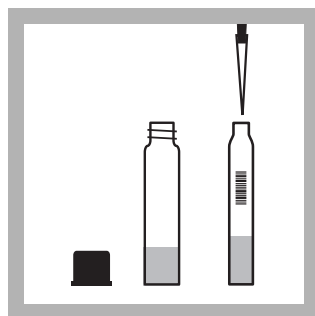
6. Close the reaction tube and invert several times to mix.



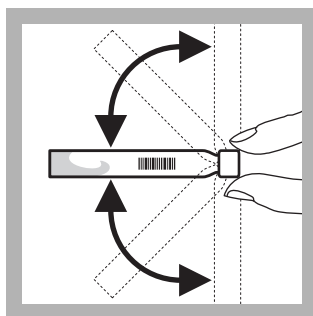
7. Press the soft key under **START TIMER**. A 2-minute reaction period will begin.



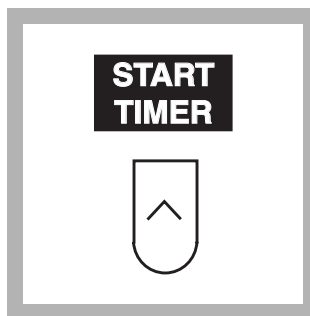
8. When the timer beeps, pipet 1.5 mL of Buffer Solution B (HCT 152 B) into a sample vial (light red cap).



9. Pipet 4.0 mL of sample from the reaction tube into the sample vial.



10. Close the sample vial and invert several times to mix.



11. Press the soft key under **START TIMER**.

A 2-minute reaction period will begin.



12. Clean the outside of the vial with a towel.

Note: Wipe with a damp towel, followed by a dry one, to remove fingerprints and other marks.



13. When the timer beeps, place the sample vial into the cell holder. Close the light shield.

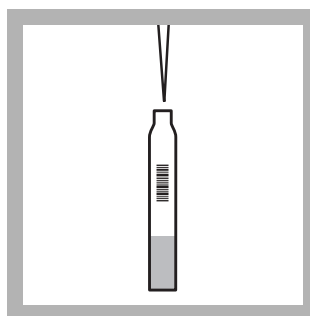


14. Press the soft key under **ZERO**.

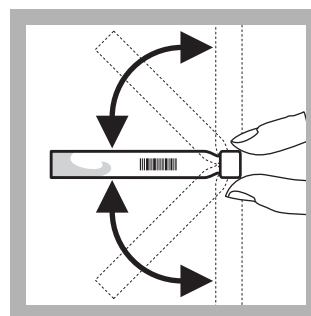
The display will show:

0.00 mg/L free Pb

Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



15. Pipet 0.3 mL of Masking Solution C (HCT 152 C) into the sample vial.



16. Close the vial and invert several times to mix.



17. Wipe the sample vial and place it into the cell holder. Close the light shield. The results in mg/L free Pb (or chosen units) will be displayed.

Interference

The ions listed in the table have been individually checked up to the given concentrations. Cumulative effects and the influence of other ions have not been evaluated.

Ion	No interference above:
Ca^{2+} , Mg^{2+} , NO_3^- , Cl^- , PO_4^{3-} , CO_3^{2-}	500 mg/L
F^- , NH_4^+ , Sr^{2+}	50 mg/L
Ag^+ , Cd^{2+} , Cr^{6+} , Zn^{2+} , Cu^{2+} , Co^{2+} , Ni^{2+}	25 mg/L
Cr^{3+} , Al^{3+} , Fe^{2+} , Fe^{3+}	10 mg/L
Mn^{2+} , Hg^{2+}	5 mg/L
Sn^{2+}	0.5 mg/L

Total lead, including undissolved lead hydroxide and complexed lead, can only be determined after digesting with the Metal Prep Set, HCT 200.

Note: The total lead measuring range is 0.24–2.40 mg/L.

Sample Collection, Storage and Preservation

Collect samples in acid-cleaned glass or plastic containers. No acid addition is necessary if analyzing the samples immediately. To preserve samples, adjust the pH to 2 or less with concentrated nitric acid (about 2 mL per liter). Preserved samples may be stored up to six months at room temperature.

Before analysis, adjust the pH to between 3 and 5 with 5.0 N Sodium Hydroxide Standard Solution. Water samples which are free from complexing agents and organic compounds can be analyzed directly. Other water samples have to be digested with the Metal Prep Set in order to bring undissolved lead hydroxide or complex lead compounds into solution.

Accuracy Check

Standard Additions Method

- a. Prepare a 1.00-mg/L Pb standard solution by pipetting 1.0 mL of 100-mg/L Pb into a 100-mL volumetric flask. Dilute to the mark with deionized water. Stopper and invert to mix. Prepare this solution daily.
- b. Select standard additions mode by pressing the soft keys under **OPTIONS, (MORE)** and then **STD ADD**.
- c. Press **ENTER** to accept the default sample volume (mL), 100.
- d. Press **ENTER** to accept the default standard concentration (mg/L), 100.
- e. Press the soft key under **ENTRY DONE**.
- f. Use a pipet to add 0.2 mL, 0.4 mL and 0.6 mL of 100-mg/L Pb standard, respectively, to three 100-mL mixing cylinders containing three 100-mL samples. Mix each thoroughly.
- g. Analyze each standard addition sample as described above. Accept the standard additions reading by pressing the soft key under **READ** each time. Each addition should reflect approximately 100% recovery.
- h. After completing the sequence, the display will show the extrapolated concentration value and the “best-fit” line through the standard additions data points, accounting for matrix interferences.
- i. See Section 1.4.1 *Standard Additions* for more information.

Standard Solution Method

Prepare a 1.00-mg/L Pb standard solution by pipetting 1.0 mL of 100-mg/L Pb into a 100-mL volumetric flask. Dilute to the mark with deionized water. Stopper and invert to mix. Prepare this solution daily. Perform the Lead procedure as described above.

To adjust the calibration curve using the reading obtained with the 100-mg/L standard solution, press the soft keys under **OPTIONS, (MORE)** then **STD: OFF**. Press **ENTER** to accept the displayed concentration, the value of which depends on the selected units. If an alternate concentration is used, enter the actual concentration and press **ENTER** to return to the read screen. See Section 1.5.5 *Adjusting the Standard Curve* for more information.

Method Performance

Precision

Standard: 1.00 mg/L Free Pb

Program	95% Confidence Limits
2230	0.76–1.24 mg/L Free Pb

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
2230	0.20 mg/L Free Pb

For more information on derivation and use of Hach's estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, appendix B, see Section 1.5.1.

Sensitivity

Program Number: 2230

Δ Abs	Δ Concentration
0.010	0.08 mg/L

See Section 1.5.3 *Sensitivity Explained* for more information.

Summary of Method

Lead(II) ions react at pH 9 with 4-(2-pyridylazo)-resorcinol (PAR) to form a red complex.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used.

The sample vial contains potassium cyanide. Masking Substance A (HCT 152 A) contains sodium salicylate.

Pollution Prevention and Waste Management

For information on pollution prevention and waste management, refer to Section 1.

REQUIRED EQUIPMENT AND SUPPLIES

Description	Unit	Cat. No.
Lead - Pb, UniCell™ HCT 152.....	24/pkg.....	HCT 152

OPTIONAL REAGENTS AND STANDARDS

Lead Standard, 100-mg/L as Pb	100 mL.....	12617-42
Metal Prep Set	50 digestions.....	HCT 200

OPTIONAL APPARATUS

DRB 100, Digital Reactor Block.....	each.....	DRB 100
Graduated cylinder, mixing, 100-mL	each.....	20886-42
Flask, volumetric, 100-mL	each.....	14574-42
Pipettor, (Jencons) 1–5 mL	each.....	27951-00
Replacement tips for 27951-00	100/pkg.....	27952-00
Pipettor, (Jencons) 100–1000 μ L.....	each.....	27949-00
Replacement tips for 27949-00	400/pkg.....	27950-00
pH Paper	100/pkg.....	26013-00



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FAX: (970) 669-2932



Method 8149

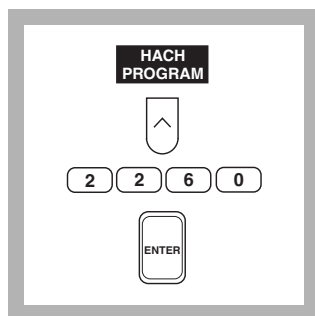
PAN Method*

Powder Pillows

LR (0 to 0.700 mg/L)

Scope and Application: For water and wastewater; digestion is required for determining total manganese. See Section 1 for digestion procedure. The estimated detection limit for program number 2260 is 0.005 mg/L total Mn.

* Adapted from Goto, K., et al., *Talanta*, 24, 752-3 (1977)



1. Press the soft key under **HACH PROGRAM**.

Select the stored program number for low range manganese by pressing **2260** with the numeric keys.

Press: **ENTER**

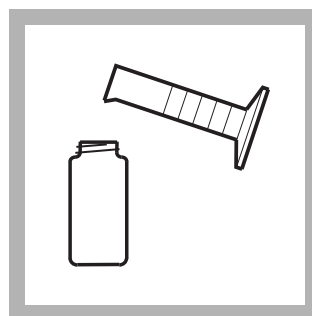
Note: If samples cannot be analyzed immediately, see *Sample Collection, Storage and Preservation* following these steps. Adjust the pH of preserved samples before analysis.

Note: The Flow Cell and Sipper Modules can be used with this procedure if rinsed well with deionized water between the blank and prepared sample.



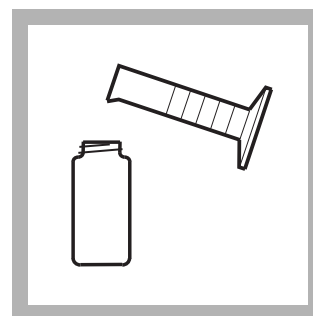
2. The display will show: **HACH PROGRAM: 2260 Manganese, LR**

The wavelength (λ), **560 nm**, is automatically selected.



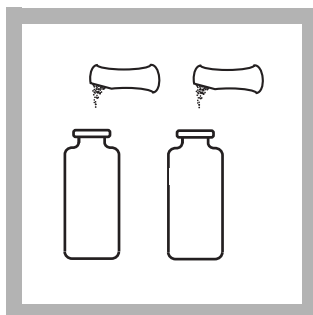
3. Pour 10.0 mL of deionized water into a sample cell (the blank).

Note: Rinse all glassware with 1:1 Nitric Acid Solution. Rinse again with deionized water.



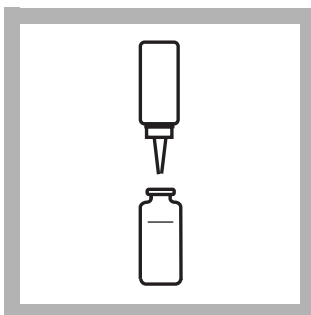
4. Pour 10.0 mL of sample into another sample cell (the prepared sample).

Note: For proof of accuracy, use a 0.500-mg/L manganese standard solution (see the *Accuracy Check* section) in place of the sample.



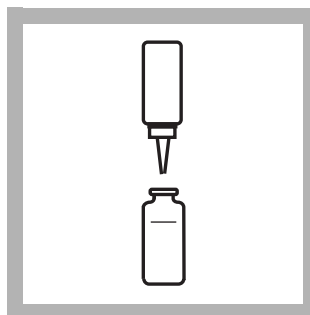
5. Add the contents of one Ascorbic Acid Powder Pillow to each cell. Swirl to mix.

Note: For samples containing hardness greater than 300-mg/L CaCO_3 , add ten drops of Rochelle Salt Solution to the sample after addition of the Ascorbic Acid Powder Pillow.



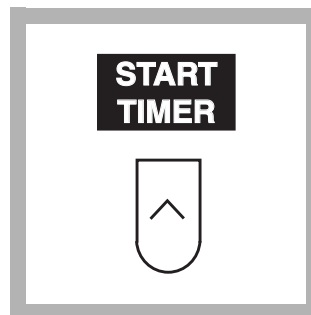
6. Add 12 drops of Alkaline-Cyanide Reagent Solution to each cell. Swirl to mix.

Note: A cloudy or turbid solution may form in some samples after addition of the Alkaline-Cyanide Reagent Solution. The turbidity should dissipate after Step 7.



7. Add 12 drops of PAN Indicator Solution, 0.1%, to each sample cell. Swirl to mix.

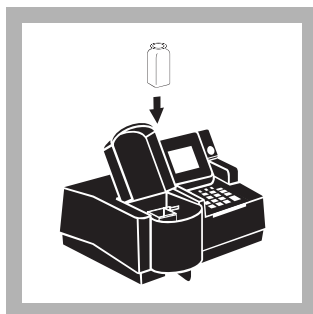
Note: An orange color will develop in the sample if manganese is present.



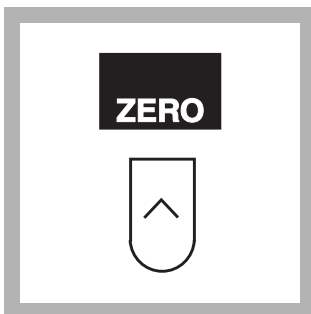
8. Press the soft key under **START TIMER**.

A 2-minute reaction period will begin.

Note: If the sample contains high amounts of iron (greater than 5 mg/L), allow ten minutes for complete color development.



9. When the timer beeps, place the blank into the cell holder. Close the light shield.

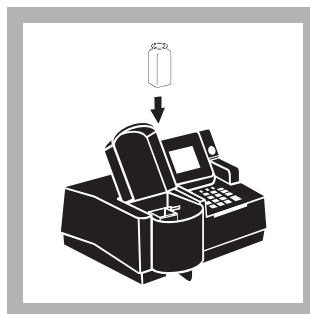


10. Press the soft key under **ZERO**.

The display will show:

0.000 mg/L Mn

Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



11. Place the prepared sample into the cell holder. Close the light shield. Results in mg/L manganese (or chosen units) will be displayed.

Interferences

The following do not interfere up to the indicated concentrations:

Interfering Substance	Interference Levels and Treatments
Aluminum	20 mg/L
Cadmium	10 mg/L
Calcium	1000 mg/L as CaCO ₃
Cobalt	20 mg/L
Copper	50 mg/L
Iron	25 mg/L
Lead	0.5 mg/L
Magnesium	300 mg/L as CaCO ₃
Nickel	40 mg/L
Zinc	15 mg/L

Sample Collection, Storage and Preservation

Collect samples in a clean glass or plastic container. Adjust the pH to 2 or less with concentrated nitric acid (about 2 mL per liter). Preserved samples can be stored up to six months at room temperature. Adjust the pH to between 4.0 to 5.0 with 5.0 N Sodium Hydroxide before analysis. Correct the test result for volume additions; see Section 1.2.2 *Correcting for Volume Additions*.

Accuracy Check

Standard Additions Method

- Leave the unspiked sample in the sample compartment. Verify that the units displayed are in mg/L. Select standard additions mode by pressing the soft keys under **OPTIONS, (MORE)** and then **STD ADD**.
- Press **ENTER** to accept the default sample volume (mL), 10.
- Press **ENTER** to accept the default standard concentration (mg/L), 10.0.
- Press the soft key under **ENTRY DONE**.
- Snap the neck off a Manganese Voluette Ampule Standard, 10 mg/L Mn.
- Use the TenSette Pipet to add 0.1, 0.2 mL and 0.3 mL of standard, respectively to three 10-mL samples and mix each thoroughly.
- Analyze each standard addition sample as described above. Accept the standard additions reading by pressing the soft key under **READ** each time. Each addition should reflect approximately 100% recovery.
- After completing the sequence, the display will show the extrapolated concentration value and the “best-fit” line through the standard additions data points, accounting for matrix interferences.
- See Section 1.4.1 *Standard Additions* for more information.

Standard Solution Method

Prepare a 0.5-mg/L manganese standard solution by pipetting 2.0 mL of Manganese Voluette Standard Solution, 250-mg/L Mn, into a 1000-mL volumetric flask. Dilute to the mark with deionized water. This solution should be prepared daily. Perform the manganese procedure as described above.

To adjust the calibration curve using the reading obtained with the 0.5-mg/L standard solution, press the soft keys under **OPTIONS, (MORE)** then **STD: OFF**. Press **ENTER** to accept the default concentration, the value of which will depend on the selected units. If an alternate concentration is used, enter the actual concentration and press enter to return to the read screen. See Section 1.5.5 *Adjusting the Standard Curve* for more information.

Method Performance

Precision

Standard: 0.500 mg/L Mn

Program	95% Confidence Limits
2260	0.498–0.502 mg/L Mn

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
2260	0.005 mg/L Mn

For more information on derivation and use of Hach's estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, appendix B, see Section 1.5.1.

Sensitivity

Program Number: 2260

Portion of Curve:	Δ Abs	Δ Concentration
Entire Range	0.010	0.0057 mg/L

See Section 1.5.3 *Sensitivity Explained* for more information.

Calibration Standard Preparation

To perform a manganese calibration using the PAN method, prepare a 5.00-mg/L Mn stock solution by pipetting 5.00 mL of a 1000-mg/L Manganese Standard Solution (Cat. No. 12791-42) into a 1000-mL volumetric flask using Class A glassware. Dilute to the mark with deionized water and mix thoroughly.

Prepare calibration standards containing 0.100, 0.200, 0.300, 0.400, 0.500, 0.600, and 0.700-mg/L Mn as follows:

- a. Into seven different 100-mL Class A volumetric flasks, pipet 2.00, 4.00, 6.00, 8.00, 10.00, 12.00, and 14.00 mL of the 5.00-mg/L Mn stock solution using Class A glassware.

- b. Dilute to the mark with deionized water. Mix thoroughly.
- c. Using the PAN method and the calibration procedure described in the *User-Entered Programs* section of the *DR/4000 Spectrophotometer Instrument Manual*, generate a calibration curve from the standards prepared above.

Summary of Method

The PAN method is a highly sensitive and rapid procedure for detecting low levels of manganese. An ascorbic acid reagent is used initially to reduce all oxidized forms of manganese to Mn^{2+} . An alkaline-cyanide reagent is added to mask any potential interferences. PAN Indicator is then added to combine with the Mn^{2+} to form an orange-colored complex.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to Section 1.

Pollution Prevention and Waste Management

The alkaline cyanide solution contains cyanide. Cyanide solutions should be collected for disposal as a reactive (D001) waste. Be sure cyanide solutions are stored in a caustic solution with $\text{pH} > 11$ to prevent release of hydrogen cyanide gas. See Section 1 for more information on proper disposal of these materials.

MANGANESE, continued

REQUIRED REAGENTS AND STANDARDS

	Cat. No.
Manganese Reagent Set, 10 mL (50 tests)	26517-00
Includes: (1) 14577-99, (1) 21223-26, (1) 21224-26	

Description	Quantity Required per test	Unit	Cat. No.
Alkaline Cyanide Reagent.....	30 drops ..	50 mL SCDB.....	21223-26
Ascorbic Acid Powder Pillows	2 pillows	100/pkg.....	14577-99
PAN Indicator Solution, 0.1%	42 drops ..	50 mL SCDB.....	21224-26
Water, deionized	10 mL	4 liters.....	272-56

REQUIRED EQUIPMENT AND SUPPLIES

DR/4000 1-Inch Cell Adapter	1	each.....	48190-00
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OPTIONAL REAGENTS AND STANDARDS

Hydrochloric Acid Solution	500 mL.....	884-49
Manganese Standard Solution, 1000-mg/L Mn	100 mL.....	12791-42
Manganese Standard Solution, 2-mL ampule, 10-mg/L Mn.....	20/pkg.....	26058-20
Manganese Standard Solution, 2-mL Voluette Ampule, 25-mg/L Mn	20/pkg.....	21128-20
Manganese Standard Solution, 10-mL Voluette ampule, 250-mg/L Mn	16/pkg.....	14258-10
Nitric Acid Solution, 1:1	500 mL.....	2540-49
Rochelle Salt Solution	29 mL DB.....	1725-33
Sodium Hydroxide Solution, 50%	500 mL.....	2180-49
Nitric Acid, ACS	500 mL.....	152-49

OPTIONAL EQUIPMENT AND SUPPLIES

Ampule Breaker Kit	each.....	21968-00
Beaker, 1000-mL	each.....	500-53
DR/4000 Carousel Module Kit	each.....	48070-02
DR/4000 Flow Cell Module Kit, 1-inch.....	each.....	48070-04
DR/4000 Flow Cell Module Kit, 1-cm	each.....	48070-05
DR/4000 Sipper Module Kit, 1-inch	each.....	48090-03
Dropper, 0.5 and 1 mL marks.....	20/pkg.....	21247-20
Flask, volumetric, Class A, 100-mL	each.....	14574-42
Flask, volumetric, Class A, 1000-mL	each.....	14574-53
Pipet, TenSette, 0.1 to 1.0 mL	each.....	19700-01
Pipet Tips, for 19700-01 TenSette Pipet	50/pkg.....	21856-96
Pipet, volumetric, Class A, 2.00-mL.....	each.....	14515-36
Pipet, volumetric, Class A, 4.00-mL	each.....	14515-04
Pipet, volumetric, Class A, 5.0-mL	each.....	14515-37
Pipet, volumetric, Class A, 6.0-mL	each.....	14515-06
Pipet, volumetric, Class A, 7.0-mL	each.....	14515-07
Pipet, volumetric, Class A, 8.0-mL	each.....	14515-08
Pipet, volumetric, Class A, 10.0-mL	each.....	14515-38
Pipet Filler, safety bulb	each.....	14651-00



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✓ Method 8034

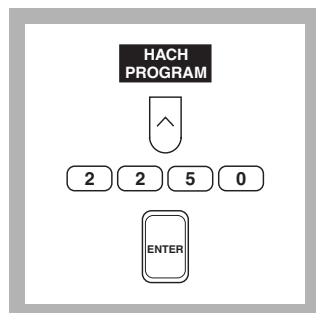
Periodate Oxidation Method*

HR (0 to 20.0 mg/L)

Scope and Application: For soluble manganese in water and wastewater; USEPA approved for reporting wastewater analyses (digestion is required)**. See Section 1 for digestion procedure. The estimated detection limit for program number 2250 is 0.1 mg/L as Mn.

* Adapted from *Standard Methods for the Examination of Water and Wastewater*

** *Federal Register*, 44 (116) 34193 (June 14, 1979)



1. Press the soft key under **HACH PROGRAM**.

Select the stored program number for high range manganese (Mn) by pressing **2250** with the numeric keys.

Press: **ENTER**

Note: If samples cannot be analyzed immediately, see *Sample Collection, Storage and Preservation*, following these steps. Adjust pH of preserved samples before analysis.

Note: The Flow Cell and Sipper Modules can be used with this procedure. Use a 25-mL sample and reagents with the Flow Cell Module.



2. The display will show:
HACH PROGRAM: 2250 Manganese, HR

The wavelength (λ), **525 nm**, is automatically selected.



3. Fill a cell with 10 mL of sample.

Note: For best results, determine a reagent blank for each new lot of reagent as follows. Prepare a reagent blank by repeating steps 3 through 10, using deionized water as the sample. Zero the instrument on deionized water by pressing the soft key under **ZERO**. Insert the reagent blank and the blank value will be displayed. Correct for the reagent blank by pressing the soft keys under **OPTIONS, (MORE)**, and then **BLANK:OFF**. Enter the reagent blank value and press **ENTER**. Repeat for each new lot of reagent.

Note: For proof of accuracy, use a 5.0 mg/L manganese standard solution (preparation given in the Accuracy Check section) in place of the sample.

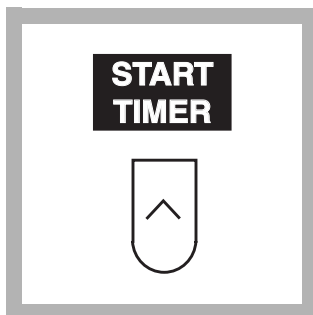


4. Add the contents of one Buffer Powder Pillow, Citrate Type for Manganese. Swirl to mix.



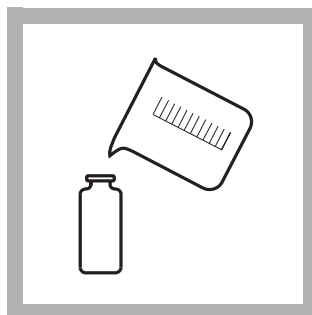
5. Add the contents of one Sodium Periodate Powder Pillow to the sample cell (the prepared sample). Swirl to mix.

Note: A violet color will develop if manganese is present.

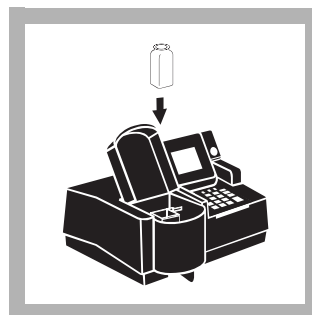


6. Press the soft key under **START TIMER**.

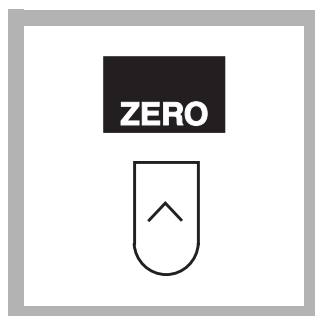
A 2-minute reaction period will begin.



7. Fill another sample cell (the blank) with 10 mL of sample.



8. When the timer beeps, place the blank into the cell holder. Close the light shield.



9. Press the soft key under **ZERO**.

The display will show:

0.0 mg/L Mn

Note: If you are using a reagent blank correction, the display will show the correction.

Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



10. Within eight minutes after the timer beeps, place the prepared sample into the cell holder. Close the light shield. The result in mg/L Mn (or chosen units) will be displayed.

Note: Results may be expressed as permanganate (MnO_4^-) or as potassium permanganate (KMnO_4). Press the soft keys under **OPTIONS** and then **FORM**: to scroll through the available options.

Interferences

The following may interfere when present in concentrations exceeding those listed below:

Interfering Substance	Interference Levels and Treatments
Calcium	700 mg/L
Chloride	70,000 mg/L
Iron	5 mg/L
Magnesium	100,000 mg/L
pH	Highly buffered samples or extreme sample pH may exceed the buffering capacity of the reagents and require sample pretreatment; see Section 1.3.1 <i>pH Interference</i> .

Sample Collection, Storage and Preservation

Collect samples in acid-washed plastic bottles. Do not use glass containers due to possible adsorption of Mn to glass. If samples are acidified, adjust the pH to 4–5 with 5.0 N Sodium Hydroxide before analysis. Do not exceed pH 5, as manganese may precipitate. Correct the test result for volume additions; see Section 1.2.2 *Correcting for Volume Additions*.

If only dissolved manganese is to be determined, filter the sample before acid addition.

Accuracy Check

Standard Additions Method

- Leave the unspiked sample in the sample compartment. Verify that the units displayed are in mg/L. Select standard additions mode by pressing the soft keys under **OPTIONS, (MORE)** and then **STD ADD**.
- Press **ENTER** to accept the default sample volume (mL), 10.
- Press **ENTER** to accept the default standard concentration (mg/L), 100.
- Press the soft key under **ENTRY DONE**.
- Prepare a 10.0-mg/L manganese standard solution. (See *Calibration Standard Preparation*)
- Use the TenSette Pipet to add 0.1, 0.2 mL and 0.3 mL of standard, respectively to three 10-mL samples and mix each thoroughly.
- Analyze each standard addition sample as described above. Accept the standard additions reading by pressing the soft key under **READ** each time. Each addition should reflect approximately 100% recovery.
- After completing the sequence, the display will show the extrapolated concentration value and the “best-fit” line through the standard additions data points, accounting for matrix interferences.
- See Section 1.4.1 *Standard Additions* for more information.

Standard Solution Method

Prepare a 10.0-mg/L manganese standard solution by pipetting 10.0 mL of Manganese Standard Solution, 1000-mg/L, into a 1000-mL volumetric flask. Dilute to the mark with deionized water. Prepare this solution daily. Perform the manganese periodate oxidation procedure as described above.

The calibration curve can be adjusted to account for variability in laboratory technique. To adjust the calibration curve using the reading obtained with the 10.0Hmg/L standard solution, press the soft keys under **OPTIONS, (MORE)** then **STD: OFF**. Press **ENTER** to accept the default concentration, the value of which will depend on the selected units. If an alternate concentration is used, enter the actual concentration and press **ENTER** to return to the read screen. See Section 1.5.5 *Adjusting the Standard Curve* for more information.

Method Performance

Precision

Standard: 10.0 mg/L Mn

Program	95% Confidence Limits
2250	9.9–10.1 mg/L Mn

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
2250	0.1 mg/L Mn

For more information on derivation and use of Hach's estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, appendix B, see Section 1.5.1.

Sensitivity

Program Number: 2250

Portion of Curve	Δ Abs	Δ Concentration
0.010 Abs	0.010	0.11 mg/L
10 mg/L	0.010	0.13 mg/L
18 mg/L	0.010	0.14 mg/L

See Section 1.5.3 *Sensitivity Explained* for more information.

Calibration Standard Preparation

To perform a manganese calibration using the periodate oxidation method, prepare a 100-mg/L Mn stock solution by pipetting 10.00 mL of a 1000-mg/L Manganese Standard Solution (Cat. No. 12791-42) into a 100-mL volumetric flask using Class A glassware. Dilute to the mark with deionized water and mix thoroughly.

Prepare calibration standards containing 2.0, 4.0, 8.0, 12.0, 16.0 and 20.0 mg/L Mn as follows:

- a. Into six different 100-mL volumetric flasks, pipet 2.00, 4.00, 8.00, 12.00, 16.00 and 20.00 mL of the 100-mg/L Mn stock solution using Class A glassware.
- b. Dilute to the mark with deionized water. Mix thoroughly.
- c. Using the periodate oxidation method and the calibration procedure described above, generate a calibration curve from the standards prepared above.

Summary of Method

Manganese in the sample is oxidized to the purple permanganate state by sodium periodate, after buffering the sample with citrate. The purple color is directly proportional to the manganese concentration.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to Section 1.

Pollution Prevention and Waste Management

For information on pollution prevention and waste management, refer to Section 1.

MANGANESE, continued

REQUIRED REAGENTS AND STANDARDS

	Cat. No.
High Range Manganese Reagent Set (100 Tests*)	24300-00
Includes: (1) 21076-69, (1) 21077-69	

Description	Quantity Required		Cat. No.
	per test	Unit	
Buffer Powder Pillows, citrate type for manganese	1 pillow	100/pkg	21076-69
Sodium Periodate Powder Pillows, for manganese	1 pillow	100/pkg	21077-69

REQUIRED EQUIPMENT AND SUPPLIES

DR/4000 1-Inch Cell Adapter	1	each	48190-00
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OPTIONAL REAGENTS AND STANDARDS

Hydrochloric Acid, 6.0 N	500 mL	884-49
Manganese Standard Solution, 1000-mg/L Mn	100 mL	12791-42
Nitric Acid, ACS	500 mL	152-49
Nitric Acid Solution, 1:1	500 mL	2540-49
Sodium Hydroxide Solution, 5.0 N	100 mL MDB	2450-32
Water, deionized	4 liters	272-56

OPTIONAL EQUIPMENT AND SUPPLIES

Ampule Breaker Kit	each	21968-00
Dropper, 0.5 and 1.0 mL marks	20/pkg	21247-20
DR/4000 Carousel Module Kit	each	48070-02
DR/4000 Flow Cell Module Kit, 1-inch	each	48070-04
DR/4000 Flow Cell Module Kit, 1-cm	each	48070-05
DR/4000 Sipper Module Kit, 1-inch	each	48090-03
Flask, Erlenmeyer, 250-mL	each	505-46
Flask, volumetric, Class A, 50-mL	each	14574-41
Flask, volumetric, Class A, 100-mL	each	14574-42
Flask, volumetric, Class A, 1000-mL	each	14574-53
pH Paper, pH 1.0 to 11.0	5 rolls/pkg	391-33
pH Meter, <i>sension</i> TM 1, portable	each	51700-00
Pipet, serological, 1-mL	each	532-35
Pipet, serological, 5-mL	each	532-37
Pipet, volumetric, Class A, 2.00-mL	each	14515-36
Pipet, volumetric, Class A, 4.00-mL	each	14515-04
Pipet, volumetric, Class A, 5.0-mL	each	14515-37
Pipet, volumetric, Class A, 6.0-mL	each	14515-06
Pipet, volumetric, Class A, 8.0-mL	each	14515-08
Pipet, volumetric, Class A, 10.0-mL	each	14515-38
Pipet, volumetric, Class A, 20.0-mL	each	14515-20
Pipet, TenSette, 0.1 to 1.0 mL	each	19700-01
Pipet Tips, for 19700-01 TenSette Pipet	50/pkg	21856-96
Pipet Filler, safety bulb	each	14651-00

* 100 tests equal 100 samples and 100 blanks.



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Method 10065

Cold Vapor Mercury Preconcentration Method*

(0.1 to 2.5 µg/L)

Scope and Application: For water, wastewater and seawater.

* Adapted from *Analytical Chemistry*, 25 (9) 1363 (1953)

Phase 1:

Sample Digestion—must be done in a fume hood! Toxic gases may be produced.



1. Transfer one liter of the sample to a 2000-mL erlenmeyer flask. Add a 50-mm magnetic stir bar to the sample. Place the flask on a magnetic stirring hot plate and begin stirring.

Note: This procedure must be done in a fume hood. Toxic chlorine or other gases may be produced!

Note: Hach recommends using dedicated digestion glassware and sample cells for this procedure.

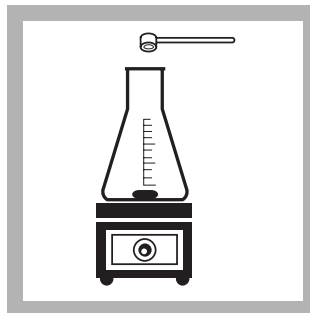


2. Add 50 mL concentrated sulfuric acid to the sample.

Note: Determine a reagent blank for each new lot of reagent by running the entire procedure, including the digestion, using one liter of deionized water instead of sample. Add the same amount of potassium permanganate as required by the sample. Subtract the reagent blank from each test result.

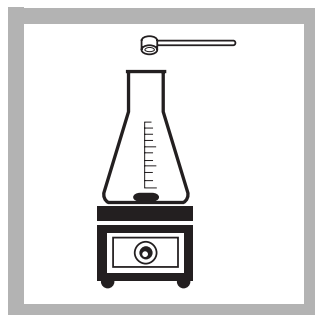


3. Add 25 mL concentrated nitric acid to the sample.



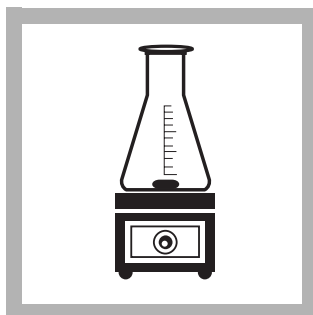
4. Add 4.0 g potassium persulfate to the sample. Stir until dissolved.

Note: Alternatively, add one 5-gram measuring scoop of potassium persulfate to the sample.



5. Add 7.5 g potassium permanganate to the sample. Stir until dissolved.

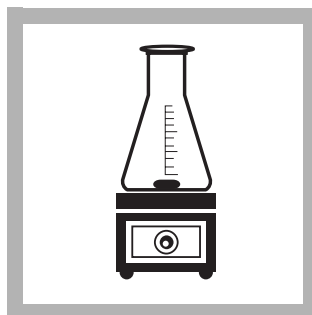
Note: Alternatively, add a 10-gram measuring scoop of potassium permanganate to the sample.



6. Cover the flask with a watch glass. Begin heating the sample to a temperature of 90 °C **after** the reagents have dissolved.

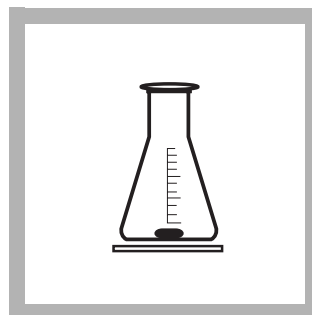
AVOID BOILING.

Note: For a mercury standard or reagent blank in distilled water the heat step is not necessary.



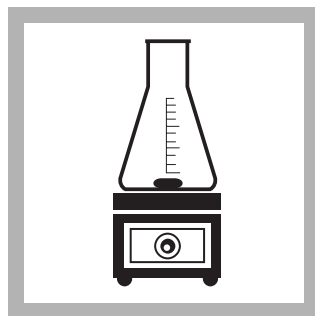
7. Continue to stir and heat the sample at 90 °C for two hours.

Note: A dark purple color must persist throughout the two hour digestion. Some samples, such as sea waters, industrial effluents or other samples high in organic matter or chloride concentration, require additional permanganate. It may be difficult to see a dark purple color if the sample contains a black/brown manganese dioxide precipitate. You may add more potassium permanganate if the solution is not dark purple.



8. Cool the digested sample to room temperature.

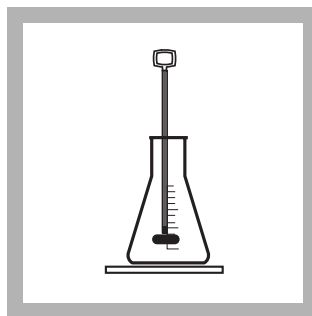
A brown/black precipitate of manganese dioxide may settle during cooling. If the digested sample does not have a purple color, the digestion may be incomplete. Add more potassium permanganate. Return the sample to the magnetic stirring hot plate and continue digestion until a purple color persists.



9. Return the cool, digested sample to the cool, magnetic stirring hot plate. Turn the stirrer on.



10. Using a 0.5-gram measuring spoon, add 0.5g-additions of hydroxylamine-hydrochloride until the purple color disappears. Wait 30 seconds after each addition to see if the purple disappears. Add hydroxylamine-hydrochloride until all the manganese dioxide is dissolved.

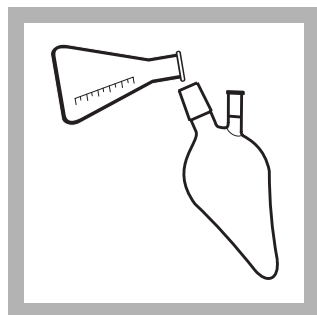


11. Remove the stir bar.



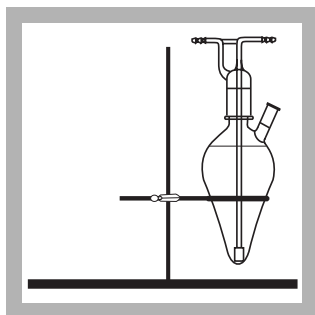
12. The digested sample is now ready for processing by cold vapor separation and preconcentration. Proceed to Phase 2.

Phase 2: Cold Vapor Separation and Preconcentration of Mercury

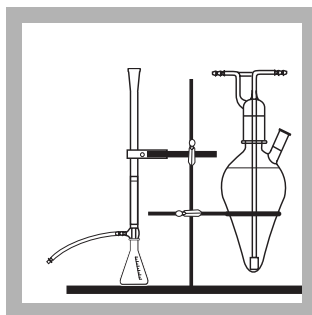


1. Transfer the digested sample to the Cold Vapor Gas Washing Bottle.

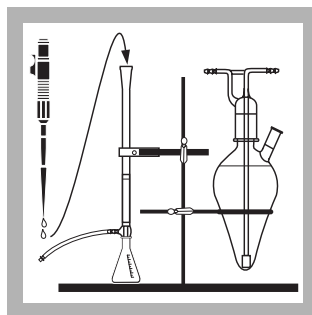
Note: The volume of digested sample should contain 0.1 to 2.5 μg Hg.



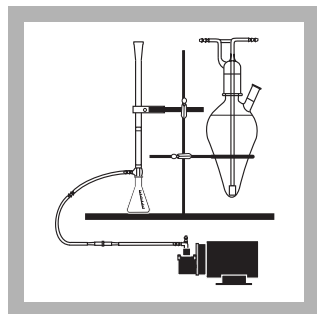
2. Set the Gas Washing Bottle in the support ring. Place the top on the Gas Washing Bottle. Wait until Step 9 to connect the mercury absorber column to the Gas Washing Bottle.



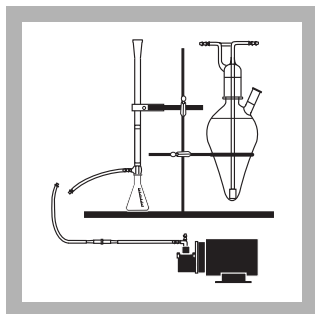
3. Connect the 100-mL Erlenmeyer flask to the mercury absorber column.



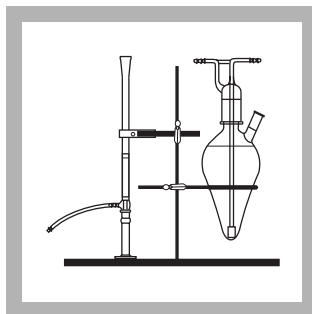
4. Pipet 8 mL of HgEx Reagent B into the Mercury Absorber column.



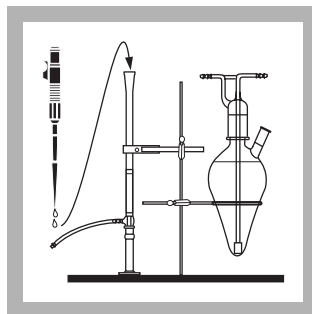
5. Connect the power to the vacuum pump and apply vacuum to the Mercury Absorber Column. Draw most of the HgEx Reagent B into the 100-mL Erlenmeyer flask.



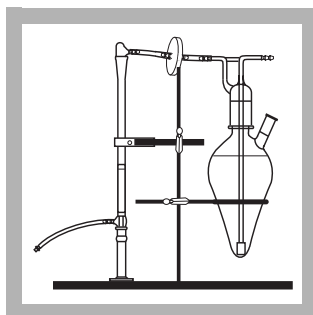
6. Disconnect the vacuum using the quick disconnect when HgEx Reagent B begins to drip from the inner delivery tube on the Mercury Absorber Column (about 10 seconds after starting the vacuum). Do not draw enough air through the column to begin drying the packing.



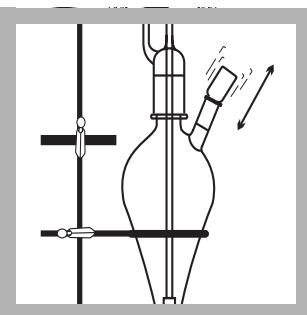
7. Remove the 100-mL Erlenmeyer flask from the Mercury Absorber Column. Replace it with the 10-mL Distilling Receiver.



8. Pipet 2 mL of HgEx Reagent C into the Mercury Absorber Column.

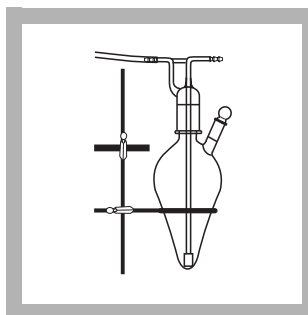


9. Connect the Mercury Absorber Column to the Gas Washing Bottle using the glass elbow.

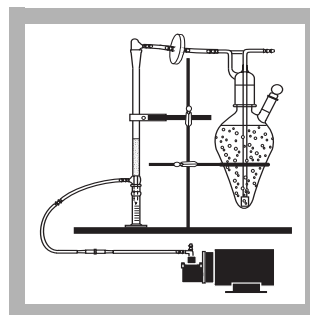


10. Shake an ampule of HgEx Reagent A to suspend the undissolved reagent. Open the ampule and gently shake the contents into the Gas Washing Bottle through the side neck.

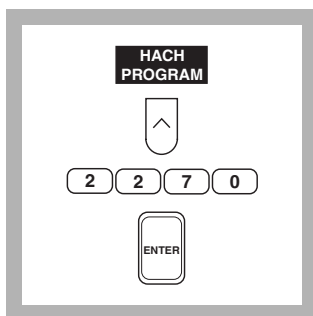
Note: Shaking the ampule is not necessary if there is no undissolved reagent in the ampule.



11. Stopper the side neck on the Gas Washing Bottle.



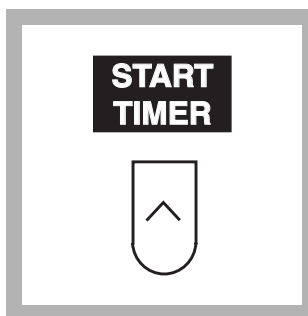
12. Reconnect the vacuum to the Mercury Absorber Column using the quick disconnect. The vacuum will pull HgEx Reagent C through the Mercury Absorber Column packing into the 10-mL receiver. Air bubbles should be produced at the gas dispersion tube in the Gas Washing Bottle. Perform steps 13–15 immediately.



13. Press the soft key under **HACH PROGRAM**. Select the stored program number for cold vapor mercury by pressing **2270** with the numeric keys. Press: **ENTER**

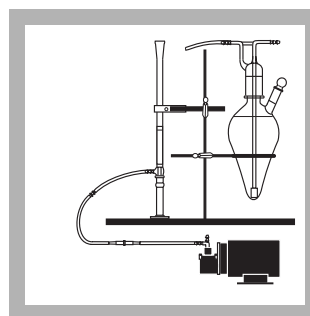


14. The display will show:
HACH PROGRAM: 2270 Cold Vapor Mercury
The wavelength (λ), **412 nm**, is automatically selected.

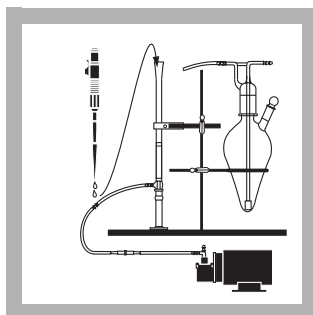


15. Press the soft key under **START TIMER**. A 5-minute reaction period will begin. Let the solution bubble for this period.

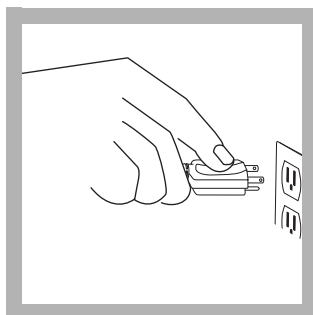
Note: Air flow rate through the Gas Washing Bottle should be between 1–5 liters per minute. Allow more bubbling time for lower air flow rates. For example, if the air flow rate is 1 liter per minute, let the solution bubble for 10 minutes.



16. After the timer beeps, remove the glass elbow from the top of the Mercury Absorber Column. Keep the vacuum pump on.

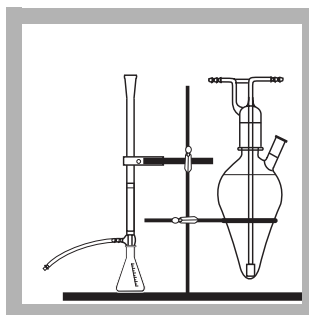


17. Pipet 8 mL of HgEx Reagent B into the Mercury Absorber Column to elute the captured mercury. Continue to apply vacuum to pull the HgEx Reagent B into the Distilling Receiver.

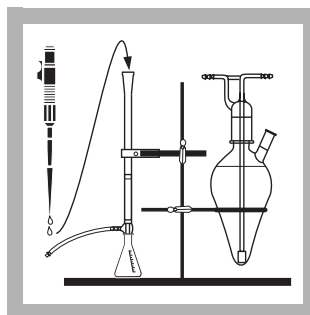


18. Turn off or disconnect power to the vacuum pump when the volume in the Distilling Receiver reaches the 10 mL mark.

***Note:** If necessary, the volume in the Distilling Receiver may be brought up to 10 mL with HgEx Reagent B. To avoid low volumes in the future, disconnect the vacuum a little sooner in Step 6. This leaves more HgEX Reagent B in the packing of the Mercury Absorber Column.*



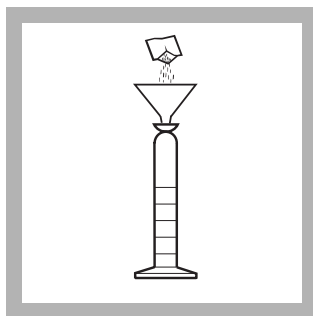
19. Remove the Distilling Receiver from the Mercury Absorber Column. Reconnect the 100-mL Erlenmeyer flask to the column.



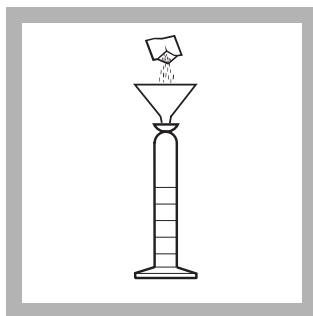
20. Pipet 3 mL of HgEx Reagent B into the Mercury Absorber Column without applying vacuum. This keeps the absorber packing wet between tests.

The Mercury Absorber Column eluate in the Distilling Receiver is ready for analysis. Proceed to Phase 3.

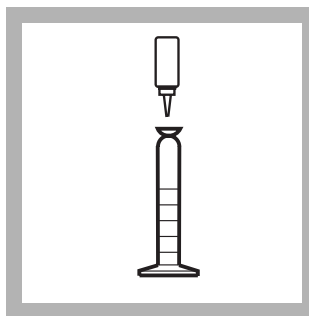
Phase 3: Colorimetric Analysis



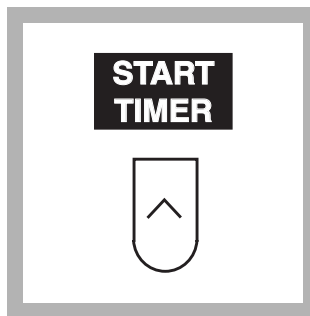
1. Using the funnel provided, add the contents of one HgEx Reagent 3 foil pillow to the eluate in the Distilling Receiver. Stopper the receiver. Invert to dissolve the reagent.



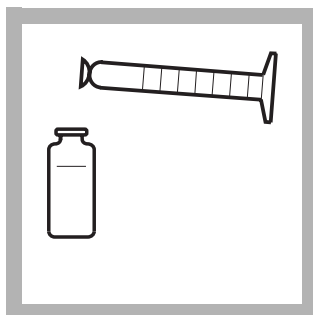
2. Add the contents of one HgEx Reagent 4 foil pillow to the Distilling Receiver using the funnel provided. Stopper the receiver. Invert to dissolve the reagent.



3. Add 8 drops of HgEx Reagent 5 to the Distilling Receiver. Stopper the Receiver. Invert to mix.



4. Press the soft key under **START TIMER**. A 2-minute reaction period will begin.

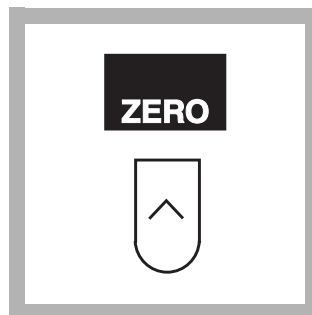


5. During the reaction period, transfer the solution to a sample cell. Wipe the sample cell sides with a clean tissue.

Note: The Flow-Thru Cell cannot be used with this procedure.



6. After the timer beeps, place the prepared sample into the cell holder and close the light shield.

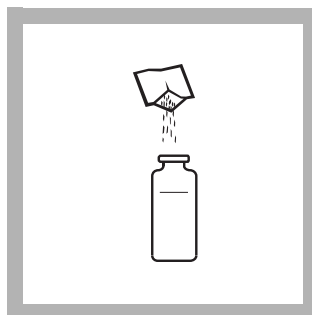


7. Press the soft key under **ZERO**.

The display will show:

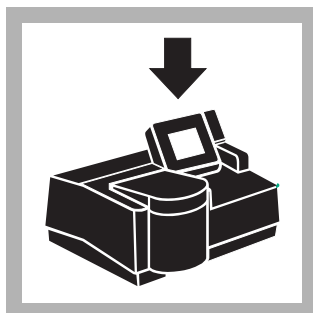
0.1 µg/L Hg

Note: This program uses a non-zero intercept.



8. Remove the cell from the cell holder. Add the contents of one HgEx Reagent 6 foil pillow to the solution. Swirl the cell until the reagent is completely dissolved. Immediately go to Step 9.

Note: Do not use the funnel to add HgEx Reagent 6 to the sample cell. Any HgEx Reagent 6 in the funnel will make mercury undetectable in subsequent tests.



9. Return the sample cell to the cell holder. Close the light shield. Results in µg/L mercury will be displayed. This is the concentration of mercury in the original sample.

Interferences

Standards were used to prepare a single test solution with the following matrix. A second test solution containing only mercury at the same concentration was prepared as the control. The two solutions were digested then analyzed concurrently. There was no interference from the matrix of the test solution at the concentrations listed.

Table 1 Interference Test Solution Matrix

Ion or Substance	Concentration
Ag ⁺	7 mg/L Ag ⁺
Al ⁺³	10 mg/L Al ⁺³
Au ⁺³	500 µg/L Au ⁺³
Cd ⁺²	10 mg/L Cd ⁺²
Co ⁺²	10 mg/L Co ⁺²
Cr ⁺⁶	10 mg/L Cr ⁺⁶
Cu ⁺²	10 mg/L Cu ⁺²
F ⁻	1.0 mg/L F ⁻
Fe ⁺²	100 mg/L Fe ⁺²
Hg ⁺²	1 µg/L Hg ⁺²
Mo ⁺⁶	10 mg/L Mo ⁺⁶
Ni ⁺²	10 mg/L Ni ⁺²
NO ₃ ⁻ -N	50 mg/L NO ₃ ⁻ -N
Pb ⁺²	10 mg/L Pb ⁺²
SiO ₂	100 mg/L SiO ₂
Zn ⁺²	10 mg/L Zn ⁺²

In addition, no interference occurred with a test solution containing 1000 mg/L Na⁺, 1000 mg/L K⁺, 1000 mg/L Mg²⁺, and 400 mg/L Ca²⁺.

Sample Collection and Preservation

Collect 1000 mL of sample in an analytically clean, glass or polyethylene terephthalate (PET) container. Add 10 mL of concentrated hydrochloric acid to preserve the sample before sample collection. Fill the container completely full to minimize air space when closed. Close a glass container with a ground glass stopper. Close a PET container with a PET cap or a polypropylene cap (no liner).

Store aqueous samples at 2–6 °C. Acid-preserved samples are stable for at least 6 months.

Accuracy Check

Standard Additions Method

- Prepare a 10.0-mg/L Mercury Standard Solution according to *Standard Solution Method*, Step C-1.
- Use a TenSette Pipet to add 0.10 mL of the 10.0-mg/L Mercury Standard Solution to the purged solution in the Gas Washing Bottle after an analysis has been performed. Immediately stopper the Gas Washing Bottle.
- Begin at Step 3 of Phase 2. Follow the procedure steps.
- Test the eluate as described in Phase 3. The displayed concentration should be 0.9–1.1 µg/L Hg.

Standard Solution Method

- a. Transfer 800 mL of deionized water into the Gas Washing Bottle.
- b. Add 50 mL of concentrated sulfuric acid and 25 mL of concentrated nitric acid to the water. Swirl to mix.
- c. Prepare a 0.1-mg/L mercury standard solution by serially diluting a 1000-mg/L Mercury Standard Solution:
 1. To make a 10.0-mg/L standard, add 1.0 mL of concentrated nitric acid to a 500-mL volumetric flask. Dilute 5.00 mL of a 1000-mg/L standard to 500 mL with deionized water. Mix well.
 2. To make a 1.0-mg/L standard solution, add 0.2 mL of concentrated nitric acid to a 100-mL volumetric flask. Dilute 10.0 mL of the 10.0-mg/L standard to 100 mL with deionized water. Mix well.
 3. To make a 0.1-mg/L standard solution, add 0.2 mL of concentrated nitric acid to a 100-mL volumetric flask. Dilute 10.00 mL of the 1.0-mg/L solution to 100 mL with deionized water. Mix well.
- d. Pipet 10.0 mL of the 0.1-mg/L mercury standard solution into the Gas Washing Bottle. Swirl to mix.
- e. Begin at Step 2 of Phase 2. Follow the procedure steps.
- f. Test the eluate as described in Phase 3. The displayed concentration should be 0.9–1.1 µg/L Hg.

System Start Up

Hach recommends that the analyst perform a few analyses on mercury standards and blanks for system equilibration before beginning sample testing. This allows the system to stabilize before processing samples.

Startup Standard

Test a mercury standard solution by following the procedure under *Accuracy Check* using the *Standard Solution Method*. Continue with step g (below) if the value is not within specified limits.

- g. Pipet 10.0 mL of the 0.1-mg/L mercury standard solution into the purged solution in the Gas Washing Bottle. Immediately stopper the Gas Washing Bottle.
- h. Begin at Step 3 of Phase 2. Follow the procedure steps.
- i. Test the eluate as described in Phase 3. The displayed concentration should be 0.9–1.1 µg/L Hg. Repeat steps g–i if the value is not within these limits.

Startup Blank

Run a system blank by using the purged solution in the Gas Washing Bottle after a satisfactory test of the Startup Standard has been completed.

- a. Leave the purged solution in the Gas Washing Bottle. Do not add an aliquot of mercury standard.
- b. Begin at Step 3 of Phase 2. Follow the procedure steps.

- c. Test the eluate as described in Phase 3. The displayed concentration should be $\leq 0.2 \mu\text{g/L Hg}$. Repeat the *Startup Blank* procedure until a reproducible value is obtained.

Method Performance

Precision

Standard: $1.00 \mu\text{g/L Hg}$

Program	95% Confidence Limits
2270	$0.92\text{--}1.02 \mu\text{g/L Hg}$

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
2270	$0.1 \mu\text{g/L Hg}$

For more information on derivation and use of Hach's estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, Appendix B, see Section 1.5.1.

Sensitivity

Program Number: 2270

Portion of Curve	ΔAbs	$\Delta\text{Concentration}$
Entire Range	0.010	$0.03 \mu\text{g/L}$

See Section 1.5.3 *Sensitivity Explained* for more information.

Storage and Maintenance of the Cold Vapor Mercury Apparatus

Storage

Store the apparatus as follows for fastest system stabilization and greatest sensitivity:

- Store the Gas Washing Bottle filled with deionized water containing 15 mL of concentrated sulfuric acid. Seal the bottle with the Gas Washing Bottle stopper and top.
- Store the Mercury Absorber Column with the packing wetted with HgEx Reagent B. The erlenmeyer flask should be kept attached underneath the column. The top of the Mercury Absorber column should be attached to the Gas Washing Bottle with the glass elbow as in the procedure.

Glassware Care

Hach recommends using dedicated glassware and sample cells because of the sensitivity of this procedure. Thoroughly clean the glassware and sample cells between tests. After washing, rinse with 1:1 hydrochloric acid solution, then rinse several times with deionized water.

Maintaining the System

- With proper care and storage, the Mercury Absorber Column may be used an unlimited number of times.
- Replace the Mercury Scrubber in the air trap housing at least once for every reagent set used.
- Moisture build up on the Gas Washing Bottle side of the Acro 50 Vent Filter will reduce the purging air flow rate. If this occurs replace the filter or dry it in an oven at 110 °C.

Summary of Method

The sample is digested to convert all forms of mercury in the sample to mercuric (Hg^{2+}) ions. The mercuric ions in the digested sample are converted to mercury vapor in a semi-closed system. The vapor is carried into a chemically activated absorber column by ambient air where the mercury vapor is converted to mercuric chloride.

The mercuric chloride is eluted off the column and a sensitive indicator is added. The instrument is zeroed using the absorbance peak of the unreacted indicator. A complexing agent is added to break the mercury:indicator complex. The increase in unreacted indicator causes an increase in absorbance which is proportional to the amount of mercury in the original sample.

Safety

Wear personal protective equipment such as safety glasses with side shields, or a face shield to protect your eyes. Use other protective equipment as necessary (such as a fume hood) to avoid chemical exposure. Perform all steps exactly as prescribed in the procedure.

Pollution Prevention and Waste Management

Proper management and disposal of waste is the responsibility of the waste generator. Hach Company provides waste disposal information as a guideline only. It is up to the generator to arrange for proper disposal and comply with applicable local, state, and federal regulations governing waste disposal. Hach Company makes no guarantees or warranties, express or implied, for the waste disposal information represented in this procedure.

1. Dispose of the solution in the Gas Washing Bottle by neutralizing the solution to a pH of 6–9 and flushing to the sanitary sewer with water for several minutes.
2. The mercury contained in one liter of sample is concentrated by a factor of 100 by the Mercury Absorber Column. Mercury analysis within the range of the test may produce a solution in the sample cell that is above the RCRA Toxicity Characteristic limit of 0.20 mg/L Hg. The sample cell will contain 0.25 mg/L mercury if the original sample was at 2.5 µg/L mercury (the upper limit of the test range). Dispose of the solution in the sample cell as a hazardous waste if the test result was over 2 µg/L mercury in the original sample. Otherwise, pour the solution into the sanitary sewer and flush with water for several minutes.
3. The mercury scrubber will capture mercury vapor if the Mercury Absorber Column is not properly activated using HgEx Reagent B and HgEx Reagent C.

In addition, mercury is also captured if the capacity of the Absorber Column is exceeded. If the Mercury Scrubber has captured mercury vapor, it must be disposed of according to applicable regulations.

REQUIRED REAGENTS

			Cat. No.
Cold Vapor Mercury Reagent Set (25 tests).....			26583-00
Description	Quantity Required		Cat. No.
	Per Test	Unit	
HgEx Reagent A, Stannous Sulfate Solution, 20-mL ampules.....	1	25/pkg	26588-25
HgEx Reagent B, Sulfuric Acid Solution.....	19 mL	500 mL	26589-49
HgEx Reagent C, Sodium Hypochlorite Solution.....	2 mL	55 mL	26590-59
HgEx Reagent 3, Alkaline Reagent Powder Pillows	1 pillow	25/pkg	26584-48
HgEx Reagent 4, Indicator Powder Pillows	1 pillow	25/pkg	26585-48
HgEx Reagent 5, Sodium Hydroxide Solution	8 drops	10 mL SCDB	26586-36
HgEx Reagent 6, Complexing Reagent Powder Pillows.....	1 pillow	25/pkg	26587-48
Mercury Scrubber.....	2/reagent set	2/pkg	26558-00

Digestion Reagents

Hydroxylamine Hydrochloride	varies	113 g	246-14
Nitric Acid, ACS	25 mL	500 mL	152-49
Potassium Permanganate, ACS	varies	454 g	168-01
Potassium Persulfate, ACS	4.0 g	454 g	26175-01
Sulfuric Acid, ACS, concentrated	50 mL	4 kg	979-09

REQUIRED EQUIPMENT AND SUPPLIES

Cold Vapor Mercury Apparatus Set			26744-00
Acro 50 Vent Filter.....	1	18/pkg	26833-18
Air Trap Holder Assembly	1	each	26639-00
Ampule Breaker	1	each	25640-00
Breaker/Capper Tool for Mercury Scrubber	1	each	26640-00
C-flex Tubing, 0.25" ID, white.....	4 ft	25 ft	23273-67
Clamp for Mercury Absorber Column	1	each	26562-00
Clamp Holder	2	each	326-00
Cylinder, graduated, 50-mL	1	each	508-41
Distilling Receiver, 10-mL	1	each	26554-38
Flask, Erlenmeyer, 100-mL.....	1	each	26553-42
Funnel, micro	1	each	25843-35
Gas Washing Bottle, 1200-mL	1	each	26622-00
Glass Elbow, 90-degree, with hose adapter.....	1	each	26552-00
Mercury Absorber Column	1	each	26555-10
Pipet, TenSette, 0.1 to 1.0 mL		each	19700-01
Pipet, TenSette, 1.0 to 10.0 mL		each	19700-10
Pipet Tips, for 19700-01 TenSette Pipet		50/pkg	21856-96
Pipet Tips, for 19700-10 TenSette Pipet		50/pkg	21997-96
Support Ring for Gas Washing Bottle	1	each	26563-00
Stopper, for Distilling Receiver.....	1	each	26559-00
Stopper, for Gas Washing Bottle	1	each	26623-00
Support, Base and Rod	1	each	329-00
Tubing Quick Disconnect, HDPE	1	12/pkg	14810-00
Vacuum Pump, with fittings, 115 VAC		each	26557-00
Vacuum Pump, with fittings, 230 VAC		each	26557-02

MERCURY, continued

REQUIRED EQUIPMENT AND SUPPLIES, continued

Description	Quantity Required Per Test	Unit	Cat. No.
Digestion Apparatus			
Flask, Erlenmeyer, 2000-mL	1	each.....	24894-54
Hot Plate/Stirrer, 120 VAC	1	each.....	23442-00
Hot Plate/Stirrer, 240 VAC	1	each.....	23442-02
Spoon, measuring, 0.5-g.....	1	each.....	907-00
Stir Bar	1	each.....	20953-55
Thermometer, -20 to 110 °C.....	1	each.....	566-01
Watch Glass, Pyrex, 65-mm	1	each.....	578-67

OPTIONAL REAGENTS

Hydrochloric Acid, ACS	500 mL.....	134-49
Mercury Standard Solution, 1000 mg/L Hg (NIST)	100 mL.....	14195-42
Water, deionized	4 L.....	272-56

OPTIONAL EQUIPMENT AND SUPPLIES

Balance, analytical, 115 VAC.....	each.....	26103-00
Balance, analytical, 230 VAC.....	each.....	26103-02
Cylinder, graduated, 1000-mL, with handle.....	each.....	26129-53
Flask, volumetric, Class A, 500-mL	each.....	14574-49
Flask, volumetric, Class A, 100-mL	each.....	14574-42
Incoming Air Filtration Apparatus.....	each.....	26846-00
Pipet, volumetric, Class A, 10.00-mL	each.....	14515-38
Pipet, volumetric, Class A, 5.00-mL	each.....	14515-37
Pipet, volumetric, Class A, 1.00-mL	each.....	14515-35
Pipet Filler	each.....	14651-00
Soil Scoop, 5-g	each.....	26572-05
Soil Scoop, 10-g	each.....	26572-10
Stir Bar Retriever, magnetic	each.....	15232-00



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Outside the U.S.A. – Contact the HACH office or distributor serving you.

On the Worldwide Web – www.hach.com; E-mail – techhelp@hach.com

HACH COMPANY

WORLD HEADQUARTERS

Telephone: (970) 669-3050

FAX: (970) 669-2932



Scope and Application: For water

* This test is semi-quantitative. Results are expressed as greater or less than the threshold value used.

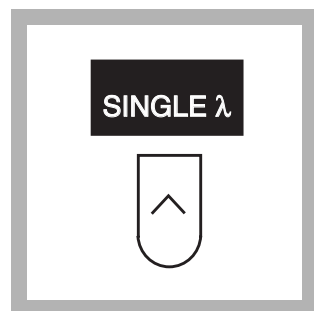
This method analyzes for Metolachlor in water. Sample calibrators and reagents are added to cuvettes coated with Metolachlor-specific antibodies. The color that develops is then measured and compared with the color measurements of the calibrators. The test requires about 30 minutes for complete analysis. As many as 20 cuvettes (18 samples and 2 calibrators) can be run simultaneously.

Tips and Techniques

- **Read the entire procedure before starting.** Identify and have ready all the necessary reagents, cuvettes, and other apparatus before beginning the analysis.
- **Timing is critical;** follow instructions carefully.
- **A consistent technique when mixing the cuvettes is critical to this test.** The best results come from using the cuvette rack and mixing as described in *Using the 1-cm MicroCuvette Rack*. Cuvettes can be mixed individually, but test results may not be as consistent.
- Handle the cuvettes carefully. Scratches on the inside or outside may cause erroneous results. Carefully clean the outside of the cuvettes with a clean absorbent cloth or tissue before placing them into the instrument. (Kimwipe® tissues are provided with the kit.)
- Antibody cuvettes and enzyme conjugate are made in matched lots. Do not mix reagent lots.
- To avoid damaging the Color Developing Solution, do not expose it to direct sunlight.
- The cuvette rack is designed to be inverted with the cuvettes in place. This is especially helpful when running many samples at once; the cuvettes can remain in the rack and be processed together until they are read in the Immunoassay Pocket Colorimeter.
- Twenty Antibody Cuvettes are provided with each reagent set. One Antibody Cuvette will be used for each calibrator and each sample. Cuvettes are not reusable.
- Ensure the 1cm MicroCell adapter is installed in the DR/4000.

Note: Hach Company recommends wearing protective nitrile gloves for this procedure.

Immunoassay



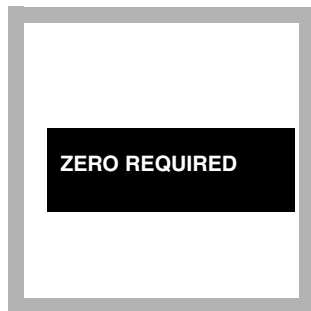
1. Press the soft key under **SINGLE λ**.

Press the soft key under **GO TO λ**.

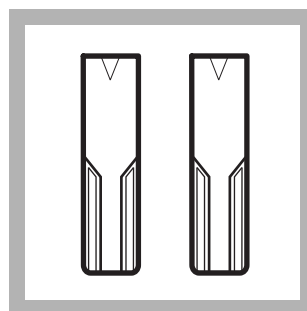
Select 450 nm by pressing the numeric keys **450**.

Press: **ENTER**

Note: The Flow Cell and Sipper Modules cannot be used with this procedure.

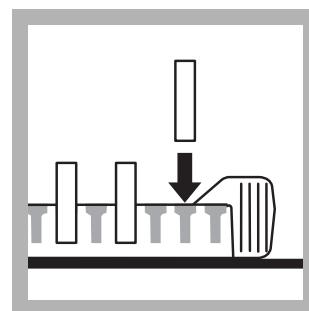


2. The display will show:
ZERO REQUIRED

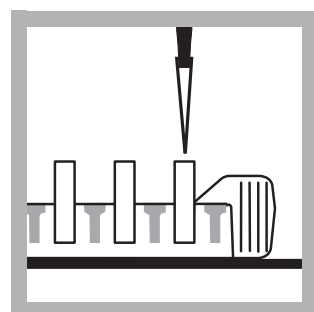


3. Label an Antibody Cuvette for each calibrator and each sample to be tested.

Note: As many as 20 cuvettes may be tested at one time and may comprise any combination of samples and calibrators.

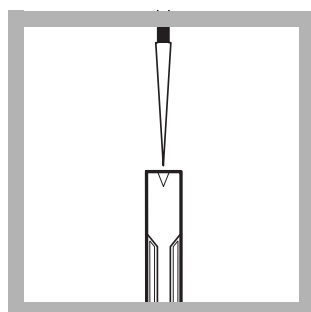


4. Place the cuvettes into the rack snugly.



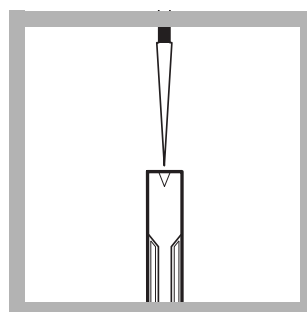
5. Pipet 0.5 mL of each calibrator into the appropriately labeled cuvette.

Note: Use a new pipette tip for each sample.

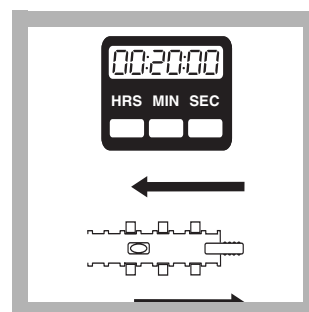


6. Pipet 0.5 mL of each sample to be tested into the appropriately labeled cuvette.

Note: Use a new pipette tip for each sample.



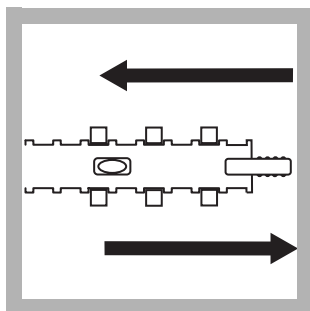
7. Immediately pipet 0.5 mL of Metolachlor Enzyme Conjugate into each cuvette.



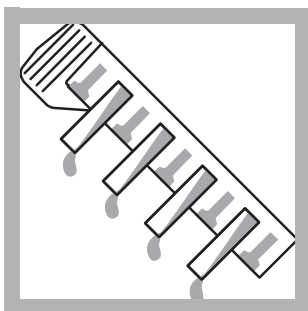
8. Key **2000**. Press the soft key under **START TIMER**.

A 20-minute reaction time will begin.

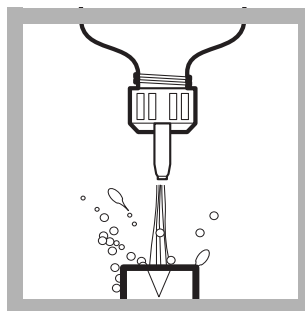
Immediately mix the contents of the cuvettes for 30 seconds using the technique described in *Using the 1-cm MicroCuvette Rack*.



9. After 10 minutes mix the contents of the rack for 30 seconds using the technique described in “Using the 1-cm MicroCuvette Rack” on page 5.



10. At the end of the 20-minute period, discard the contents of all the cuvettes into an appropriate waste container.

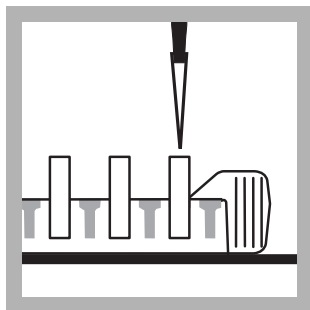


11. Wash each cuvette forcefully and thoroughly four times with deionized water. Empty the rinse water into the waste container.

Note: Ensure most of the water is drained from the cuvettes by turning the cuvettes upside down and tapping them lightly on a paper towel

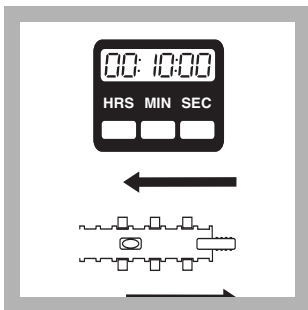
Color Development

Note: Timing is critical; follow instructions carefully.



12. With the cuvettes still held snugly in the rack, pipet 0.5 mL of Color Developing Solution into each Antibody Cuvette.

Note: Use a new pipette tip for each cuvette.



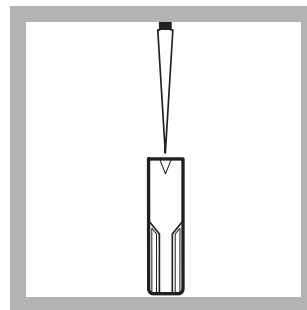
13. Key 1000. Press the soft key under **START TIMER**.

A reaction period will begin. Mix following the instructions in *Using the 1-cm MicroCuvette Rack*.



14. After 5 minutes, mix the contents of the rack a second time for a period of 30 seconds using the same technique.

Note: Solutions will turn blue in some or all of the cuvettes.



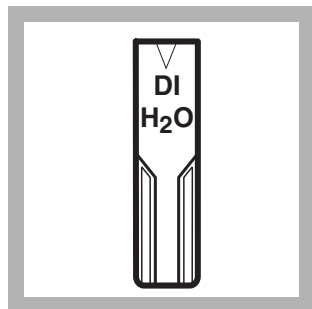
15. At the end of the 10-minute reaction period, pipette 0.5 mL of Stop Solution into each cuvette in the same order as the Color Developing Solution was added in step 12.

Slide the rack for 20 seconds using the technique described in *Using the 1-cm MicroCuvette Rack*.

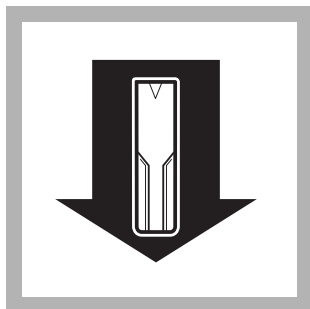
Note: Blue solutions will turn yellow with the addition of the Stop Solution.

Note: The same pipette tip can be used repeatedly for this step.

Measuring the Color

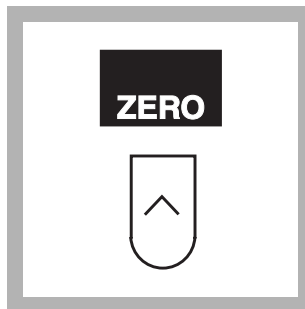


16. Label and fill a Zeroing Cuvette with deionized water. Wipe the outside of all the cuvettes with a tissue to remove water, smudges, and fingerprints.



17. Place the filled zeroing cuvette into the cell holder with the arrow pointing left.

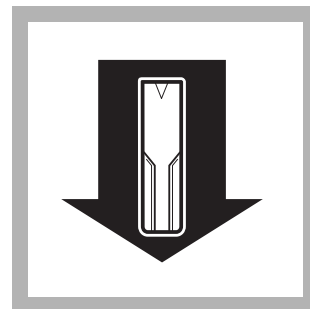
Orient the arrow in the same direction for all cuvettes.



18. Press the soft key under **ZERO**.

The display will show:

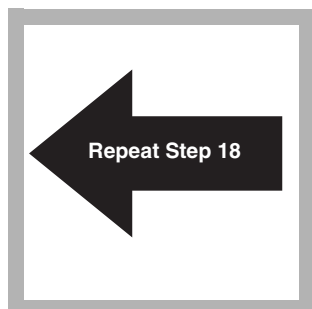
0.000 Abs



19. Place the first calibrator into the cell holder. Read the results.

The display will give an absorbance reading. Record the results for each calibrator and sample.

Note: See the *Instrument Manual* for more information on taking a reading.



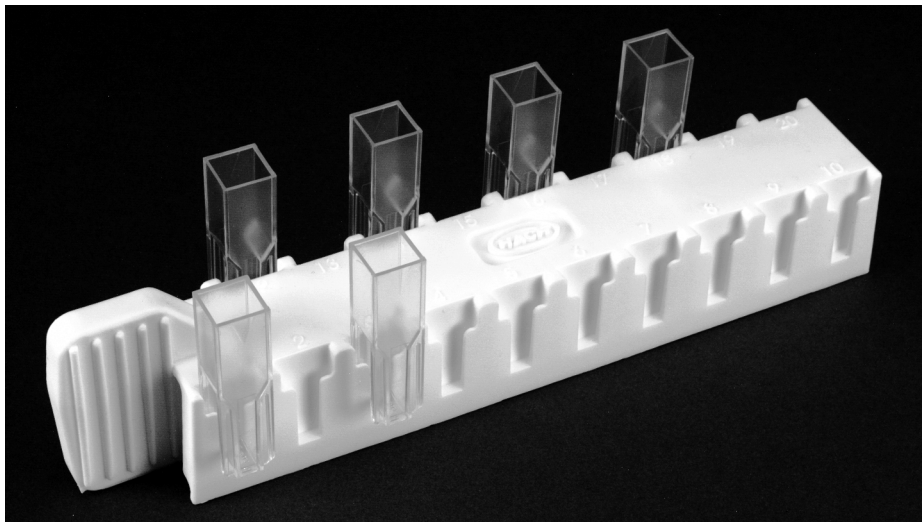
20. Repeat *step 19* for all remaining calibrators and samples.

See *Interpreting and Reporting Results* for help with interpretation of results.

Using the 1-cm MicroCuvette Rack

This rack (see *Figure 1*) has been designed specifically to aid in achieving precise and accurate results when using the immunoassay technique to analyze several samples at the same time.

Figure 1 **The 1-cm MicroCuvette Rack**



Loading the Rack — The cuvette rack is designed so that it may be inverted with the cuvettes in place. Identify each cuvette with a sample or calibrator number and place all the cuvettes in the rack before beginning the procedure. Fit the cuvettes snugly into the rack, but do not force them or they may be difficult to remove and their contents may spill. The cuvettes should remain in place when the rack is inverted and tapped lightly.

Mixing — Set the rack on a hard, flat surface that is at least twice the length of the rack. Hold the rack by one end and vigorously slide it back and forth along its long axis for 30 seconds. The rack should move through a distance equal to its own length in each direction.

Interpreting and Reporting Results

There is an inverse relationship between the concentration of Metolachlor and the reading. In other words, the higher the reading, the lower the concentration of Metolachlor.

If the sample reading is...	the sample Metolachlor Concentration is...
...less than calibrator reading	...greater than the calibrator concentration
...greater than calibrator reading	...less than the calibrator concentration

Example

Readings:

0.5 ppm Metolachlor Calibrator: **0.475 ABS**

2.0 ppm Metolachlor Calibrator: **0.245 ABS**

Sample #1: **0.140 ABS**

Sample #2: **0.300 ABS**

Sample #3: **0.550 ABS**

Interpretation

Sample #1 — Sample reading is less than the readings for both calibrators. Therefore the sample concentration of Metolachlor is greater than both 0.5 ppb and 2.0 ppb Metolachlor.

Sample #2 — Sample reading is between the readings for the 0.5 ppb and 2.0 ppb Metolachlor calibrators. Therefore the sample concentration of Metolachlor is between 0.5 ppb and 2.0 ppb.

Sample #3 — Sample reading is greater than the readings for both calibrators. Therefore the sample concentration of Metolachlor is less than both 2.0 ppb and 0.5 ppb.

Storing and Handling Reagents

- Wear protective gloves and eyewear.
- When storing reagent sets for extended periods of time, keep them out of direct sunlight. Store reagents at a temperature of 4 °C when not in use.
- Keep the foil pouch containing the Metolachlor Antibody Cuvettes sealed when not in use.
- If Stop Solution comes in contact with eyes, wash thoroughly for 15 minutes with cold water and seek immediate medical help.

Sensitivity

The Metolachlor immunoassay cannot differentiate between the various triazines and metabolites, but it detects their presence in differing degrees.

Table 1 Required Concentrations for Selected Chemicals

Compound	Concentration to give a positive result at 0.5 ppb Metolachlor	Concentration to give a positive result at 2.0 ppb Metolachlor
Acetochlor	74 ppm	398 ppm
Butachlor	84 ppb	550 ppb
2 Chloro-2'6'-Diethylacetaniline	8 ppm	60 ppm
2,6-Diethylaniline	61 ppm	313 ppm
Propachlor	60 ppb	295 ppb

The following compounds are not detectable at 10,000 ppb.

Atrazine	Carbofuran	Carbendazim
Aldicarb	2,4-D	
Diazoton	Chlorpyrifos	

Sample Collection and Storage

Collect samples in a clean glass bottle. Do not pre-rinse the bottle with the sample. If the sample cannot be analyzed immediately, store the sample at 4 °C. Samples may be kept for as long as 14 days. Warm the samples to room temperature before analysis.

Summary of Method

Hach immunoassay tests use antigen/antibody reactions to test for specific organic compounds in water and soil. Metolachlor-specific antibodies, attached to the walls of plastic cuvettes, selectively bind and remove Metolachlor from complex sample matrices. A prepared sample and a reagent containing enzyme-conjugate molecules (analyte molecules attached to molecules of an enzyme) are added to the Antibody Cuvettes. During incubation, enzyme-conjugate molecules and Metolachlor compete for binding sites on the antibodies. Samples with higher levels of analyte will have more antibody sites occupied by Metolachlor and fewer antibody sites occupied by the enzyme-conjugate molecules.

After incubation, the sample and unbound enzyme conjugate are washed from the cuvette and a color-development reagent is added. The enzyme in the conjugate catalyzes the development of color. Therefore, there is an inverse relationship between color intensity and the amount of Metolachlor in the sample. The resulting color is then compared with a calibrator to determine whether the Metolachlor concentration in the sample is greater or less than the threshold levels.

Required Reagents

Description	Unit	Cat. No.
Reagent Set, Metolachlor*	20 cuvettes.....	28135-00

Required Apparatus

Caps, flip spout.....	2/pkg.....	25818-02
Cell Adapter, 1-cm MicroCell.....	each.....	48588-00
Marker, laboratory	each.....	20920-00
Rack, for 1-cm Micro Cuvettes	each.....	48799-00
Wipes, disposable.....	box.....	20970-00
TenSette®, Pipet, 0.1–1.0 mL.....	each.....	19700-01
Tips, for pipettor 19700-01	1000/pkg.....	21856-28

* Immunoassay components are manufactured for Hach Company by Beacon Analytical Systems, Inc.



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Method 8036

Mercaptoacetic Acid Method*

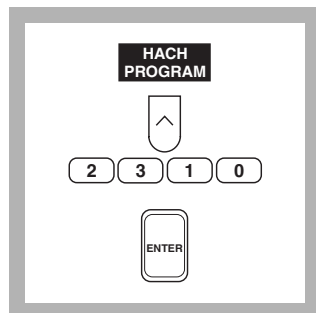
Powder Pillows or AccuVac® Ampuls

HR (0 to 50.0 mg/L)

Scope and Application: For water and wastewater. The estimated detection limit for program numbers 2310 and 2320 is 0.1 mg/L Mo⁶⁺.

* Adapted from *Analytical Chemistry*, 25 (9) 1363 (1953)

Using Powder Pillows



1. Press the soft key under **HACH PROGRAM**.

Select the stored program number for high range molybdenum (Mo) by pressing **2310** with the numeric keys.

Press: **ENTER**

Note: Collect samples in glass or plastic bottles. Samples must be analyzed immediately.

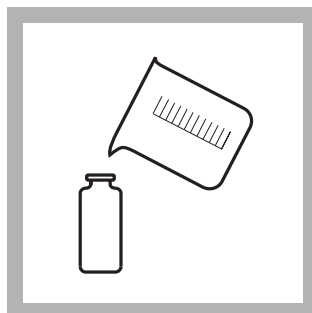
Note: The Flow Cell and Sipper Modules can be used with this procedure. Use a 25-mL sample and reagents with the Flow Cell Module.



2. The display will show: **HACH PROGRAM: 2310 Molybdenum, HR**

The wavelength (λ), **420 nm**, is automatically selected.

Note: For best results, determine a reagent blank for each new lot of reagent as follows. Prepare a reagent blank by repeating steps 3 through 11, using deionized water as the sample. Zero the instrument on deionized water by pressing the soft key under **ZERO**. Insert the reagent blank and the blank value will be displayed. Correct for the reagent blank by pressing the soft keys under **OPTIONS, (MORE)**, and then **BLANK:OFF**. Enter the reagent blank value and press **ENTER**. Repeat for each new lot of reagent.



3. Fill a sample cell with 10 mL of sample.

Note: For proof of accuracy, use a 10.0-mg/L Molybdenum Standard Solution (listed under **OPTIONAL REAGENTS AND STANDARDS**) in place of the sample.

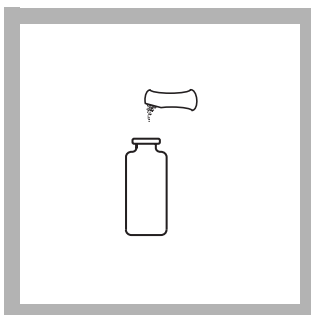
Note: Filter turbid samples using the labware listed under **OPTIONAL EQUIPMENT AND SUPPLIES**.



4. Add the contents of one MolyVer 1 Reagent Powder Pillow. Swirl to mix.

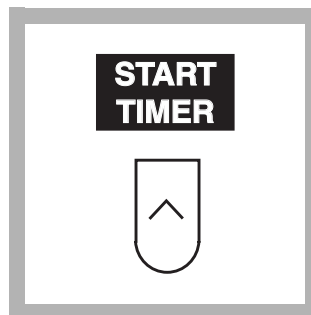


5. Add the contents of one MolyVer 2 Reagent Powder Pillow. Swirl to mix.



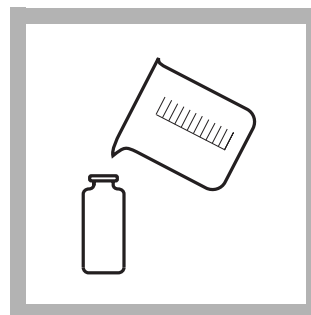
6. Add the contents of one MolyVer 3 Reagent Powder Pillow. Swirl to mix. This is the prepared sample.

Note: Molybdenum will cause a yellow color to form.

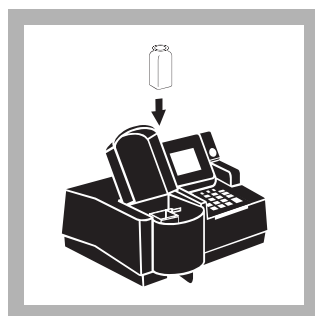


7. Press the soft key under **START TIMER**.

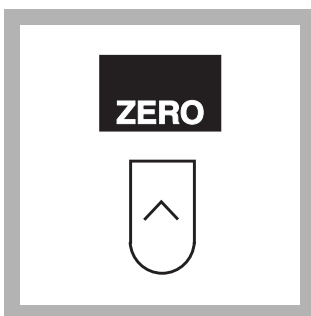
A 5-minute reaction period will begin.



8. When the timer beeps, fill a second sample cell with 10 mL of the original sample (the blank).



9. Insert the blank into the cell holder. Close the light shield.



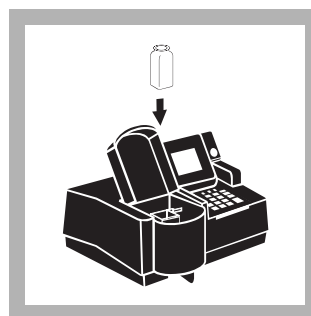
10. Press the soft key under **ZERO**.

The display will show:

0.0 mg/L Mo⁶⁺

Note: If you are using a reagent blank correction, the display will show the correction.

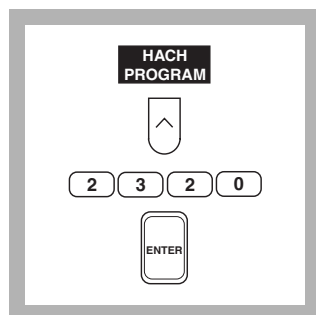
Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



11. Place the prepared sample into the cell holder. Close the light shield. The results in mg/L molybdenum as Mo⁶⁺ (or chosen units) will be displayed.

Note: Results can also be expressed as molybdate (MoO₄²⁻) or sodium molybdate (Na₂MoO₄). Press the soft key under **OPTIONS**, then **FORM**: to scroll through the available options. Press **ENTER** to return to the read screen.

Using AccuVac Ampuls



1. Press the soft key under **HACH PROGRAM**.

Select the stored program number for the high range molybdenum (Mo) AccuVac method by pressing **2320** with the numeric keys.

Press: **ENTER**

Note: Collect samples in glass or plastic bottles. Samples must be analyzed immediately.



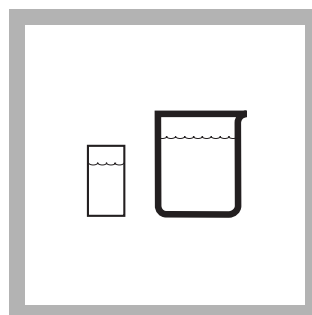
2. The display will show:
HACH PROGRAM: 2320 Molybdenum, HR AV

The wavelength (λ), **420 nm**, is automatically selected.

Note: For best results, determine a reagent blank for each new lot of reagent as follows. Prepare a reagent blank by repeating steps 3 through 10, using deionized water as the sample. Zero the instrument on deionized water by pressing the soft key under **ZERO**. Insert the reagent blank and the blank value will be displayed. Correct for the reagent blank by pressing the soft keys under **OPTIONS, (MORE)**, and then **BLANK:OFF**. Enter the reagent blank value and press **ENTER**. Repeat for each new lot of reagent.



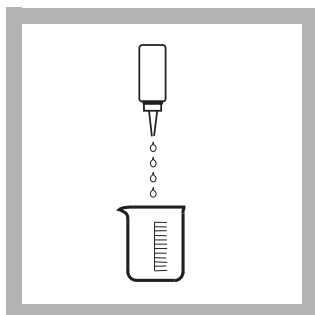
3. Insert the AccuVac Ampul Adapter into the sample cell module by sliding it under the thumb screw and into the alignment grooves. Fasten with the thumb screw.



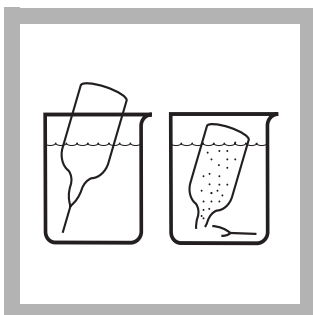
4. Fill a zeroing vial with at least 10 mL of sample (the blank). Collect 40 mL of sample in a 50-mL beaker.

Note: For proof of accuracy, use a 10.0-mg/L Molybdenum Standard Solution (listed under **OPTIONAL REAGENTS AND STANDARDS**) in place of the sample.

Note: Filter turbid samples using the labware listed under **OPTIONAL EQUIPMENT AND SUPPLIES**.



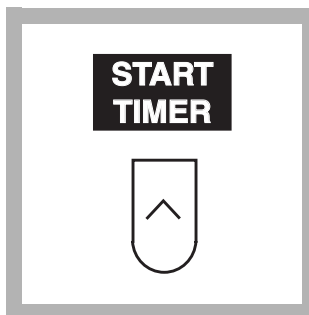
5. Add 4 drops of 0.4 M CDTA standard solution to the sample in the beaker and swirl to mix.



6. Fill a MolyVer 6 Reagent AccuVac Ampul with the treated sample and invert the ampule several times to mix (the prepared sample).

Note: Molybdenum will cause a yellow color to form.

Note: Undissolved reagent will not affect the results.

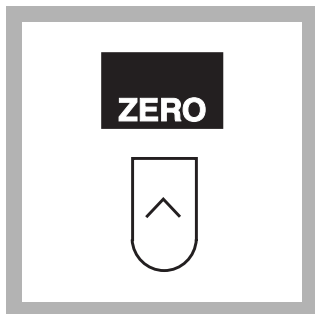


7. Press the soft key under **START TIMER**.

A 5-minute reaction period will begin.



8. When the timer beeps, place the blank into the cell holder. Close the light shield.



9. Press the soft key under **ZERO**.

The display will show:

0.0 mg/L Mo⁶⁺

Note: If you are using a reagent blank correction, the display will show the correction.

Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



10. Place the prepared sample into the cell holder. Close the light shield. The results in mg/L molybdenum as Mo⁶⁺ (or chosen units) will be displayed.

Note: Results can also be expressed as molybdate (MoO₄²⁻) or sodium molybdate (Na₂MoO₄). Press the soft key under **OPTIONS**, then **FORM**: to scroll through the available options. Press **ENTER** to return to the read screen.

Interferences

Interfering Substance	Interference Levels and Treatments
Aluminum	Greater than 50 mg/L
Chromium	Greater than 1000 mg/L
Copper	Samples containing 10 mg/L copper or more will exhibit an increasing positive interference upon standing. Read these samples as soon as possible after the five minute reaction period is complete.
Iron	Greater than 50 mg/L
Nickel	Greater than 50 mg/L
Nitrite	Interference from up to 2000 mg/L as NO ₂ ⁻ can be eliminated by adding one Sulfamic Acid Powder Pillow to the sample.
Highly buffered samples or extreme sample pH	May exceed the buffering capacity of the reagents and require sample pretreatment; see Section 1.3.1 <i>pH Interference</i> .

Sample Collection, Storage and Preservation

Collect samples in clean plastic or glass bottles. Sample must be analyzed immediately.

Accuracy Check

Standard Additions Method

- a. Leave the unspiked sample in the sample compartment. Verify that the units displayed are in mg/L Mo⁶⁺. Select standard additions mode by pressing the soft keys under **OPTIONS, (MORE)** and then **STD ADD**.
- b. Press **ENTER** to accept the default sample volume (mL), 30.
- c. Press **ENTER** to accept the default standard concentration (mg/L), 1000.
- d. Press the soft key under **ENTRY DONE**.
- e. Open a bottle of Molybdenum Standard Solution, 1000-mg/L Mo⁶⁺.
- f. Use the TenSette Pipet to add 0.2 mL, 0.4 mL and 0.6 mL of standard, respectively to three 30-mL samples and mix each thoroughly (for AccuVac ampuls, use 50-mL beakers).
- g. Analyze each standard addition sample as described above. Accept the standard additions reading by pressing the soft key under **READ** each time. Each addition should reflect approximately 100% recovery.
- h. After completing the sequence, the display will show the extrapolated concentration value and the “best-fit” line through the standard additions data points, accounting for matrix interferences.
- i. See Section 1.4.1 *Standard Additions* for more information.

Standard Solution Method

Use a Molybdenum Standard Solution, 10.0 mg/L Mo⁶⁺, listed under **OPTIONAL REAGENTS AND STANDARDS**, and perform the molybdenum procedure as described above.

To adjust the calibration curve using the reading obtained with the 10.0-mg/L standard solution, press the soft keys under **OPTIONS, (MORE)** then **STD: OFF**. Press **ENTER** to accept the default concentration, the value of which will depend on the selected units. If an alternate concentration is used, enter the actual concentration and press **ENTER** to return to the read screen. See Section 1.5.5 *Adjusting the Standard Curve* for more information.

Method Performance

Precision

Standard: 10.0 mg/L Mo⁶⁺

Program	95% Confidence Limits
2310	9.9–10.1 mg/L Mo ⁶⁺
2320	9.9–10.1 mg/L Mo ⁶⁺

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
2310	0.1 mg/L Mo ⁶⁺
2320	0.1 mg/L Mo ⁶⁺

For more information on derivation and use of Hach's estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, Appendix B, see Section 1.5.1.

Sensitivity

Program Number: 2310

Portion of Curve	ΔAbs	ΔConcentration
Entire Range	0.010	0.23 mg/L

Program Number: 2320

Portion of Curve	ΔAbs	ΔConcentration
0.010 Abs	0.010	0.24 mg/L
25.0 mg/L	0.010	0.25 mg/L
45.0 mg/L	0.010	0.25 mg/L

See Section 1.5.3 *Sensitivity Explained* for more information.

Calibration Standard Preparation

To perform a molybdenum calibration using the mercaptoacetic acid method, prepare a 500-mg/L Mo stock solution by pipetting 50.00 mL of a 1000-mg/L Molybdenum Standard Solution (Cat. No. 14186-42) into a 100-mL volumetric flask using Class A glassware. Dilute to the mark with deionized water and mix thoroughly. Use this 500-mg/L Mo stock standard solution to prepare the working standards.

Prepare calibration standards containing 5.0, 10.0, 20.0, 30.0, 40.0, and 50.0 mg/L Mo as follows:

- a. Into six different 100-mL volumetric Class A flasks, pipet 1.00, 2.00, 4.00, 6.00, 8.00 and 10.00 mL of the 500-mg/L Mo stock solution using Class A glassware.
- b. Dilute to the mark with deionized water. Mix thoroughly.
- c. Using the mercaptoacetic acid method and the calibration procedure described in the *User-Entered Programs* section of the *DR/4000 Spectrophotometer Instrument Manual*, generate a calibration curve from the standards prepared above.

Summary of Method

MolyVer 1 and 2 Reagents are added to buffer and condition the sample. MolyVer 3 provides the mercaptoacetic acid which reacts with molybdate molybdenum to form a yellow color proportional to the molybdenum concentration.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to Section 1.

Pollution Prevention and Waste Management

For information on pollution prevention and waste management, refer to Section 1.

REQUIRED REAGENTS AND STANDARDS (Using Powder Pillows)

	Cat. No.
Molybdenum Reagent Set, for 10-mL samples (100 tests).....	26041-00
Includes (1) 26042-99, (1) 26043-99, and (1) 26044-99	

Description	Quantity Required per test	Unit	Cat. No.
MolyVer 1 Molybdenum Reagent Powder Pillows	1 pillow	100/pkg	26042-99
MolyVer 2 Molybdenum Reagent Powder Pillows	1 pillow	100/pkg	26043-99
MolyVer 3 Molybdenum Reagent Powder Pillows	1 pillow	100/pkg	26044-99

REQUIRED REAGENTS AND STANDARDS (Using AccuVac Ampuls)

CDTA Solution, 0.4 M	4 drops ... 15 mL SCDB.....	26154-36
MolyVer 6 Reagent AccuVac Ampuls	1 ampul	25220-25

REQUIRED EQUIPMENT AND SUPPLIES (Using Powder Pillows)

DR/4000 1-Inch Cell Adapter	1	each.....	48190-00
-----------------------------------	---------	-----------	----------

REQUIRED EQUIPMENT AND SUPPLIES (Using AccuVac Ampuls)

Beaker, 50-mL	1	each.....	500-41
DR/4000 AccuVac Ampul Adapter.....	1	each.....	48187-00

MOLYBDENUM, Molybdate, continued

OPTIONAL REAGENTS AND STANDARDS

Description	Quantity Required per test	Unit	Cat. No.
Molybdenum Standard Solution, 10-mg/L as Mo ⁶⁺		100 mL.....	14187-42
Molybdenum Standard Solution, 1000-mg/L as Mo ⁶⁺		100 mL.....	14186-42
Molybdenum Standard Solution, 10-mL Voluette Ampule, 250-mg/L as Mo ⁶⁺		16/pkg.....	25574-10
Molybdenum Standard Solution, 2-mL Voluette Ampule, 75-mg/L Mo ⁶⁺		20/pkg.....	25575-20
Molybdenum Standard Solution, 2-mL Voluette Ampule, 250-mg/L Mo ⁶⁺		20/pkg.....	25574-20
Molybdenum Standard Solution, 10-mL Voluette Ampule, 500-mg/L Mo ⁶⁺		16/pkg.....	14265-10
Sulfamic Acid Powder Pillows.....		100/pkg.....	1055-99
Water, deionized.....		4 liters.....	272-56

OPTIONAL EQUIPMENT AND SUPPLIES

Ampule Breaker Kit	each.....	21968-00
DR/4000 Carousel Module Kit	each.....	48070-02
DR/4000 Flow Cell Module Kit, 1-inch.....	each.....	48070-04
DR/4000 Sipper Module Kit, 1-inch	each.....	48090-03
Filter Paper, folded, 12.5-cm.....	100/pkg.....	1894-57
Flask, volumetric, Class A, 100-mL	each.....	14574-42
Flask, volumetric, Class A, 100-mL	pkg/6.....	14574-72
Funnel, poly, 65 mm.....	each.....	1083-67
Pipet, TenSette, 0.1 to 1.0 mL	each.....	19700-01
Pipet Tips, for 19700-01 TenSette Pipet	50/pkg.....	21856-96
Pipet, TenSette, 1.0 to 10.0 mL	each.....	19700-10
Pipet Tips, for 19700-10 TenSette Pipet	50/pkg.....	21997-96
Pipet, volumetric, Class A, 1.00-mL	each.....	14515-35
Pipet, volumetric, Class A, 2.00-mL	each.....	14515-36
Pipet, volumetric, Class A, 4.00-mL	each.....	14515-04
Pipet, volumetric, Class A, 6.00-mL	each.....	14515-06
Pipet, volumetric, Class A, 8.00-mL	each.....	14515-08
Pipet, volumetric, Class A, 10.00-mL	each.....	14515-38
Pipet, volumetric, Class A, 100.0-mL	each.....	14515-42



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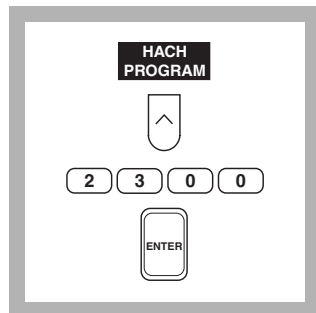
Method 8169

Ternary Complex Method

Powder Pillows

LR (0 to 3.00 mg/L)

Scope and Application: For boiler and cooling tower waters.
The estimated detection for program number 2300 is 0.03 mg/L Mo^{6+} .



1. Press the soft key under **HACH PROGRAM**.

Select the stored program for low range molybdate molybdenum by pressing **2300** with the numeric keys.

Press: **ENTER**

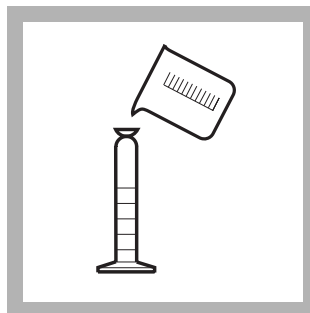
Note: Analyze samples immediately after collection.

Note: The Flow Cell and Sipper Modules can be used with this procedure.



2. The display will show:
HACH PROGRAM: 2300 Molybdenum, LR

The wavelength (λ), **610 nm**, is automatically selected.

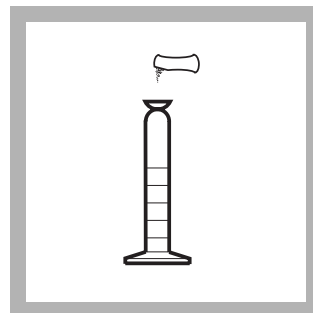


3. Fill a 25-mL graduated mixing cylinder with 20 mL of the sample to be tested.

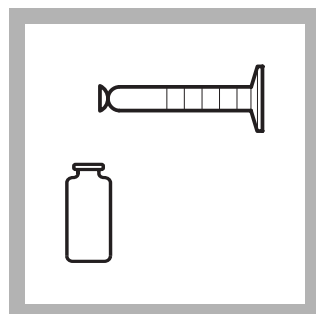
Note: For proof of accuracy, use a 2.0-mg/L molybdenum standard solution (preparation given in the Accuracy Check section) in place of the sample.

Note: Filter turbid samples using the labware listed under **OPTIONAL EQUIPMENT AND SUPPLIES**.

Note: For samples with extreme pH, see the Interferences section.



4. Add the contents of one Molybdenum 1 Reagent Powder Pillow to the graduated cylinder. Stopper, then shake the graduated cylinder to dissolve the reagents. This is the prepared sample.



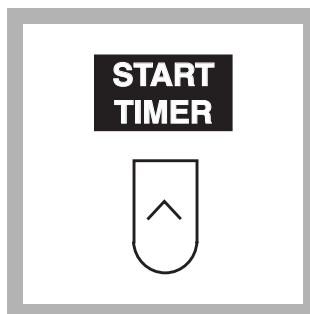
5. Pour 10 mL of the prepared sample into one sample cell of a matched pair.

Note: The remaining sample in the graduated cylinder is used as the blank in steps 8 and 9.

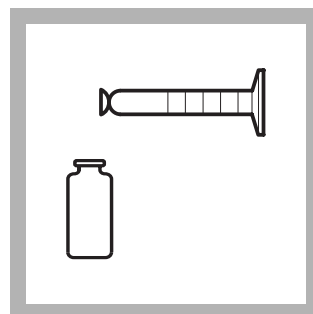


6. Add 0.5 mL of Molybdenum 2 Reagent to the sample cell. Swirl to mix. This is the developed sample.

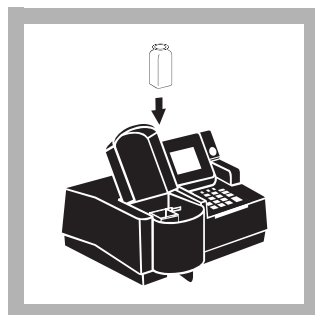
Note: Molybdenum will cause a green color to form.



7. Press the soft key under **START TIMER**. A 2-minute reaction period will begin.



8. When the timer beeps, fill a second sample cell with 10 mL of prepared sample with the remaining sample in the graduate cylinder. This is the blank.



9. Insert the blank into the cell holder. Close the light shield.

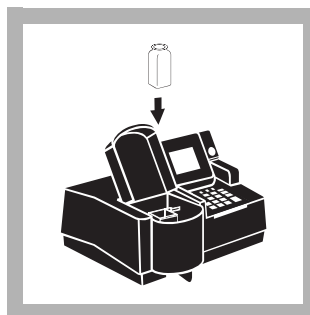


10. Press the soft key under **ZERO**.

The display will show:

0.000 mg/L Mo⁶⁺

Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



11. Place the developed sample into the cell holder. Close the light shield. The results in mg/L molybdenum (or chosen units) will be displayed.

Note: Results may be expressed as molybdate (MoO_4^{2-}) or as sodium molybdate (Na_2MoO_4). Press the soft keys under **OPTIONS** and then **FORM** to scroll through the available options. Press **ENTER** to return to the read screen.

Interferences

Interferences studies were conducted by preparing a molybdenum standard solution (2 mg/L Mo⁶⁺) as well as a solution of the potential interfering ion. When the standard solution concentration changed by $\pm 5\%$ with a given ion concentration, the ion was considered an interference. The tables below list the details of these studies.

Table 1 Interfering Substances That Cause a Negative Interference

Interfering Substance	Interference Levels and Treatments
Alum	Greater than 7 mg/L
Aluminum	Greater than 2 mg/L
AMP (Phosphonate)	Greater than 15 mg/L
Bicarbonate	Greater than 5,650 mg/L
Bisulfate	Greater than 3,300 mg/L
Borate	Greater than 5,250 mg/L
Chloride	Greater than 1,400 mg/L
Chromium	Greater than 4.5 mg/L
Copper	Greater than 98 mg/L
Diethanoldithiocarbamate	Greater than 6,500 mg/L
EDTA	Greater than 1,500 mg/L
Ethylene Glycol	Greater than 2% (by volume)
Iron	Greater than 200 mg/L
Lignin Sulfonate	Greater than 105 mg/L
Nitrite	Greater than 350* mg/L

Table 1 Interfering Substances That Cause a Negative Interference (Continued)

Interfering Substance	Interference Levels and Treatments
Orthophosphate	Greater than 4,500 mg/L
Phosphonohydroxy-Acetic Acid	Greater than 32 mg/L
Phosphonate HEDP	The presence of the phosphonate HEDP at concentrations up to 30 mg/L will increase the apparent molybdenum concentration reading by approximately 10% (positive interference). Multiply the value obtained in step 11 by 0.9 to obtain the actual Mo6+ concentration.
Sulfite	Greater than 6,500 mg/L

* Read the molybdenum concentration immediately after the beep of the 2-minute reaction period.

Table 2 Interfering Substances That Cause a Positive Interference

Interfering Substance	Interference Level and Treatment
Benzotriazole	Greater than 210 mg/L
Carbonate	Greater than 1,325 mg/L
Morpholine	Greater than 6 mg/L
Phosphonate HEDP	Positive interference of about 10% up to 30 mg/L. As the concentration increases above 30 mg/L, a decrease in the molybdenum concentration reading occurs (negative interference).
Silica	Greater than 600 mg/L

The following substances did not interfere at the level listed:

Substance	Highest Concentration Tested
Bisulfite	9,600 mg/L
Calcium	720 mg/L
Chlorine	7.5 mg/L
Magnesium	8,000 mg/L
Manganese	1,600 mg/L
Nickel	250 mg/L
PBTC (phosphonate)	500 mg/L
Sulfate	12,800 mg/L
Zinc	400 mg/L

Highly buffered samples or extreme sample pH may exceed the buffering capacity of the reagent and require sample pretreatment. Adjust the sample pH to between 3 and 5 by using a pH meter or pH paper and adding, dropwise, an appropriate amount of acid or base such as 1.0 N Sulfuric Acid Standard Solution, or 1.0 N Sodium Hydroxide Standard Solution. If significant volumes of acid or base are used, a volume correction should be made by dividing the total volume (sample + acid + base) by the original volume and multiplying the test result by this factor.

After a number of samples have been analyzed, the sample cells may exhibit a build-up of a slight blue color. Rinse the cells with 1:1 Hydrochloric Acid Solution to eliminate this build-up.

Sample Collection, Storage and Preservation

Collect samples in glass or plastic bottles. Analyze samples immediately.

Accuracy Check

Standard Additions Method

- a. Leave the unspiked sample in the sample compartment. Verify that the units displayed are in mg/L. Select standard additions mode by pressing the soft keys under **OPTIONS, (MORE)** and then **STD ADD**.
- b. Press **ENTER** to accept the default sample volume (mL), 50.
- c. Press **ENTER** to accept the default standard concentration (mg/L), 250.
- d. Press the soft key under **ENTRY DONE**.
- e. Snap the neck off a Molybdenum Voluette Ampule Standard, 250-mg/L Mo⁶⁺.
- f. Use the TenSette Pipet to add 0.1, 0.2 mL and 0.3 mL of standard, respectively to three 50-mL samples and mix each thoroughly.
- g. Analyze 20 mL of each standard addition sample as described above. Accept the standard additions reading by pressing the soft key under **READ** each time. Each addition should reflect approximately 100% recovery.
- h. After completing the sequence, the display will show the extrapolated concentration value and the “best-fit” line through the standard additions data points, accounting for matrix interferences.
- i. See Section 1.4.1 *Standard Additions* for more information.

Standard Solutions Method

Using Class A glassware, prepare a 2.00-mg/L molybdenum standard solution by pipetting 10.00 mL of Molybdenum Standard Solution, 10.00-mg/L, into a 50-mL volumetric flask. Dilute to the mark with deionized water. Prepare this solution daily. Perform the molybdenum procedure as described above.

To adjust the calibration curve using the reading obtained with the 2.00-mg/L Mo standard solution, press the soft keys under **OPTIONS, MORE** then **STD: OFF**. Press **ENTER** to accept the displayed concentration, the value of which depends on the selected units. If an alternate concentration is used, enter the actual concentration and press **ENTER** to return to the read screen. See Section 1.5.5 *Adjusting the Standard Curve* for more information.

Method Performance

Precision

Standard: 2.00 mg/L Mo⁶⁺

Program	95% Confidence Limits
2300	1.90–2.03 mg/L Mo ⁶⁺

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
2300	0.03 mg/L Mo ⁶⁺

For more information on derivation and use of Hach's estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, appendix B, see Section 1.5.1.

Sensitivity

Program Number: 2300

Portion of Curve	ΔAbs	ΔConcentration
0.010 Abs	0.010	0.023 mg/L
1.50 mg/L	0.010	0.021 mg/L
2.70 mg/L	0.010	0.026 mg/L

See Section 1.5.3 *Sensitivity Explained* for more information.

Calibration Standard Preparation

To perform a molybdenum calibration using the ternary complex method, prepare a 40.0-mg/L Mo stock solution by pipetting 4.00 mL of a 1000-mg/L Molybdenum Standard Solution into a Class A 100-mL volumetric flask using Class A glassware. Dilute to the mark with deionized water and mix thoroughly.

Prepare calibration standards containing 0.40, 0.80, 1.20, 1.60, 2.00, 2.40, and 2.80 mg/L Mo as follows:

- a. Into seven different 100-mL volumetric flasks, pipet 1.00, 2.00, 3.00, 4.00, 5.00, 6.00 and 7.00 mL of the 40-mg/L Mo stock solution using Class A glassware.
- b. Dilute to the mark with deionized water. Stopper and mix thoroughly.
- c. Using the ternary complex method and the calibration procedure described in the *User-Entered Programs* section of the *DR/4000 Spectrophotometer Instrument Manual*, generate a calibration curve from the standards prepared above.

Summary of Method

The ternary complex method for molybdenum determination is a method in which molybdate molybdenum reacts with an indicator and sensitizing agent to give a stable blue complex.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to Section 1.

MOLYBDENUM, Molybdate, continued

Pollution Prevention and Waste Management

For information on pollution prevention and waste management, refer to Section I.

REQUIRED REAGENTS AND STANDARDS

	Cat. No.
Molybdenum Reagent Set for 20 mL sample (100 tests).....	24494-00
Includes (1) 23524-49, (1) 23525-12	

Description	Quantity Required per test	Unit	Cat. No.
Molybdenum 1 Reagent (Low Range) Molybdate Powder Pillows	1 pillow	100/pkg	23524-49
Molybdenum 2 Reagent Solution	0.5 mL	50 mL MDB	23525-12

REQUIRED EQUIPMENT AND SUPPLIES

Cylinder, graduated mixing, 25-mL	1	each	1896-40
DR/4000 1-Inch Cell Adapter	1	each	48190-00

OPTIONAL REAGENTS AND STANDARDS

Molybdenum Standard Solution, 10-mg/L as Mo ⁶⁺	100 mL	14187-42
Molybdenum Standard Solution, 1000-mg/L as Mo ⁶⁺	100 mL	14186-42
Molybdenum Standard Solution, 10-mL Voluette ampule, 250-mg/L Mo ⁶⁺	16/pkg	25574-10
Molybdenum Standard Solution, 2-mL Voluette ampule, 75-mg/L Mo ⁶⁺	20/pkg	25575-20
Molybdenum Standard Solution, 2-mL Voluette ampule, 250-mg/L Mo ⁶⁺	20/pkg	25574-20
Molybdenum Standard Solution, 10-mL Voluette ampule, 500-mg/L Mo ⁶⁺	16/pkg	14265-10
Hydrochloric Acid Solution, 1:1, 6.0 N	500 mL	884-49
Water, deionized	4 liters	272-56

OPTIONAL EQUIPMENT AND SUPPLIES

Ampule Breaker Kit	each	21968-00
DR/4000 Carousel Module Kit	each	48070-02
DR/4000 Flow Cell Module Kit, 1-inch	each	48070-04
DR/4000 Sipper Module Kit, 1-inch	each	48090-03
Filter Paper, folded, 12.5-cm	100/pkg	1894-57
Flask, volumetric, 50-mL, Class A	each	14547-41
Flask, volumetric, 100-mL, Class A	each	14547-42
Flask, volumetric, 100-mL, Class A	6/pkg	14547-72
Funnel, analytical, poly, 65-mm	each	1083-67
pH Meter, <i>sensio</i> TM 1, portable	each	51700-00
Pipet, TenSette, 0.1 to 1.0 mL	each	19700-01
Pipet Tips, for 19700-01 TenSette Pipet	50/pkg	21856-96
Pipet, volumetric, Class A, 1.00-mL	each	14515-35
Pipet, volumetric, Class A, 2.00-mL	each	14515-36
Pipet, volumetric, Class A, 3.00-mL	each	14515-03
Pipet, volumetric, Class A, 4.00-mL	each	14515-04
Pipet, volumetric, Class A, 5.00-mL	each	14515-37
Pipet, volumetric, Class A, 6.00-mL	each	14515-06
Pipet, volumetric, Class A, 7.00-mL	each	14515-07
Pipet, volumetric, Class A, 10.00-mL	each	14515-38
Pipet Filler	each	12189-00



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Method 8150

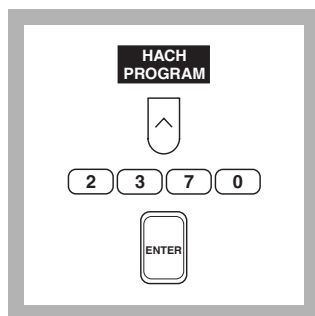
1-(2 Pyridylazo)-2-Naphthol (PAN)* Method

Powder Pillows

(0 to 1.000 mg/L)

Scope and Application: For water and wastewater; digestion is required for determining total nickel.
See SECTION 2 for digestion procedure. The estimated detection limit for program number 2370 is 0.005 mg/L Ni.

* Adapted from Watanabe, H., *Talanta*, 21 295 (1974)



1. Press the soft key under **HACH PROGRAM**.

Select the stored program for nickel (Ni) by pressing **2370** with the numeric keys.
Press: **ENTER**

Note: If sample cannot be analyzed immediately, see Sample Collection, Storage and Preservation following these steps. Adjust the pH of preserved samples before analysis.

Note: The Flow Cell and Sipper Modules can be used if rinsed well with deionized water between the blank and prepared sample.



2. The display will show:
HACH PROGRAM: 2370 Nickel, PAN

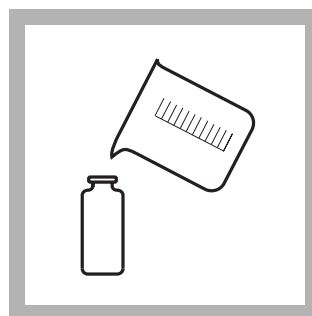
The wavelength (λ), **560 nm**, is automatically selected.



3. Fill a glass-stoppered cell to the 25-mL mark with sample (the prepared sample).

Note: If sample is less than 10 °C (50 °F), warm to room temperature before analysis.

Note: For proof of accuracy, use a 0.5-mg/L nickel standard solution (preparation given in the Accuracy Check section) in place of the sample.

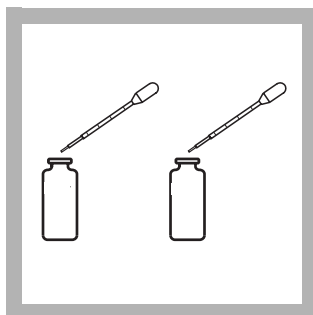


4. Fill another glass-stoppered cell to the 25-mL mark with deionized water (the blank).



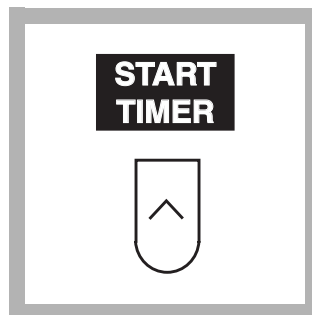
5. Add the contents of one Phthalate-Phosphate Reagent Powder Pillow to each cell. Stopper. Immediately shake to dissolve.

Note: If sample contains iron (Fe^{3+}) it is important that all powder be dissolved completely before continuing with Step 6.



6. Add 1.0 mL of 0.3% PAN Indicator Solution to each cell. Stopper. Invert several times to mix.

Note: Use the plastic dropper provided.



7. Press the soft key under **START TIMER**.

A 15-minute reaction period will begin.

Note: During color development, the sample solution color may vary from yellowish-orange to dark red depending on the chemical make-up of the sample. The deionized water blank should be yellow.



8. When the timer beeps add the contents of one EDTA Reagent Powder Pillow to each cell. Stopper. Shake to dissolve.



9. Place the blank into the cell holder. Close the light shield.



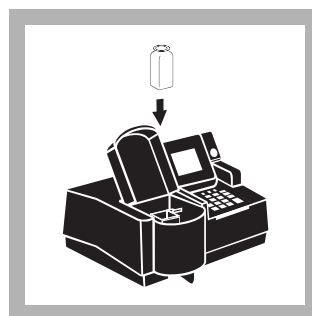
10. Press the soft key under **ZERO**.

The display will show:

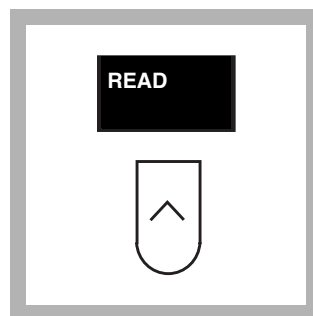
0.000 mg/L Ni

Note: The instrument will zero at 560 nm and 620 nm.

Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



11. Place the sample into the cell holder. Close the light shield.



12. Press the soft key under **READ**.

The instrument will read the sample at 560 nm and 620 nm. When finished, the result in mg/L nickel (or chosen units) will be displayed.

Note: Determination of cobalt concentration may be made with the same prepared sample by using **HACH METHOD** program number 1600.

Interferences

The following may interfere when present in concentrations exceeding those listed below:

Table 1 Interfering Substances and Suggested Treatments

Interfering Substance	Interference Levels and Treatments
Al ³⁺	32 mg/L
Ca ²⁺	1000 mg/L as (CaCO ₃)
Cd ²⁺	20 mg/L
Cl ⁻	8000 mg/L
Chelating agents	Interfere at all levels. Use either the Digesdahl or vigorous digestion to eliminate this interference (see <i>SECTION 2</i>).
Cr ³⁺	20 mg/L
Cr ⁶⁺	40 mg/L
Cu ²⁺	15 mg/L
F ⁻	20 mg/L
Fe ³⁺	10 mg/L
Fe ²⁺	Interferes directly and must not be present.
K ⁺	500 mg/L
Mg ²⁺	400 mg/L
Mn ²⁺	25 mg/L
Mo ⁶⁺	60 mg/L
Na ⁺	5000 mg/L
Pb ²⁺	20 mg/L
Zn ²⁺	30 mg/L
Highly buffered samples or extreme sample pH	May exceed the buffering capacity of the reagents and require sample pretreatment; see Section 1.3.1 <i>pH Interference</i> .

Sample Collection, Storage and Preservation

Collect samples in acid-washed plastic bottles. Adjust the sample pH to 2 or less with nitric acid (about 5 mL per liter). Preserved samples can be stored up to six months at room temperature. Before analysis, adjust the sample pH to between 3 and 8 with 5.0 N Sodium Hydroxide Standard Solution. Do not exceed pH 8 as this may cause some loss of nickel as a precipitate. Correct test results for volume additions, see Section 1.2.2 *Correcting for Volume Additions*.

Accuracy Check

Standard Additions Method

- Leave the unspiked sample in the sample compartment. Verify that the units displayed are in mg/L. Select standard additions mode by pressing the soft keys under **OPTIONS, (MORE)** and then **STD ADD**.
- Press **ENTER** to accept the default sample volume (mL), 25.
- Press **ENTER** to accept the default standard concentration (mg/L), 50.
Note: Alternative standard concentrations and additions volumes can also be used.
- Press the soft key under **ENTRY DONE**.

- e. Snap the neck off a Nickel Voluette Ampule Standard, 50-mg/L Ni.
- f. Use the TenSette Pipet to add 0.1 mL, 0.2 mL and 0.3 mL of standard, respectively to three 25-mL samples and mix each thoroughly.
- g. Analyze each standard addition sample as described in the procedure. Accept the standard additions reading by pressing the soft key under **READ** each time. Each addition should reflect approximately 100% recovery.
- h. After completing the sequence, the display will show the extrapolated concentration value and the “best-fit” line through the standard additions data points, accounting for matrix interferences.
- i. See Section 1.4.1 *Standard Additions* for more information.

Standard Solution Method

Prepare a 5.00-mg/L Nickel stock solution by pipetting 5.00 mL of Nickel Standard Solution, 1000-mg/L as Ni, into a 1000-mL volumetric flask. Dilute to the mark with deionized water. Prepare this solution daily. Prepare a 0.5-mg/L Ni working solution by pipetting 10.0 mL of the 5.00-mg/L nickel stock solution into a 100-mL volumetric flask. Dilute to the mark with deionized water. Prepare this solution daily. Perform the nickel procedure as described above.

To adjust the calibration curve using the reading obtained with the 0.5-mg/L working solution, press the soft keys under **OPTIONS, MORE** then **STD: OFF**. Press **ENTER** to accept the displayed concentration, the value of which depends on the selected units. If an alternate concentration is used, enter the actual concentration and press **ENTER** to return to the read screen. See Section 1.5.5 *Adjusting the Standard Curve* for more information.

Method Performance

Precision

Standard: 0.500 mg/L Ni²⁺

Program	95% Confidence Limits
2370	0.498–0.502 mg/L Ni ²⁺

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
2370	0.005 mg/L Ni ²⁺

For more information on derivation and use of Hach’s estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, appendix B, see Section 1.5.1.

Sensitivity

Program Number: 2370

Portion of Curve	Δ Abs	Δ Concentration
Entire Range	0.010	0.006 Ni ²⁺

See Section 1.5.3 *Sensitivity Explained* for more information.

Calibration Standard Preparation

To perform a nickel calibration using the PAN method, prepare a 5.0-mg/L nickel stock solution by pipetting 5.0 mL of a 1000-mg/L Nickel Standard Solution (Cat. No. 23383-42) into a 1000-mL volumetric flask using Class A glassware. Dilute to the mark with deionized water and mix thoroughly.

Prepare calibration standards containing 0.150, 0.300, 0.450, 0.600, 0.750, 0.900 and 1.000 mg/L Ni as follows:

- Into seven different Class A 100-mL volumetric flasks, pipet 3, 6, 9, 12, 15, 18, and 20 mL of the 5.0 mg/L Ni stock solution using Class A glassware.
- Dilute to the mark with deionized water. Mix thoroughly.
- Using the PAN method and the calibration procedure described in *Creating and Running User Programs* in the *DR/4000 Instrument Manual*, generate a calibration curve from the standards prepared above.

Summary of Method

After buffering the sample and masking any Fe³⁺ with pyrophosphate, the nickel is reacted with 1-(2-Pyridylazo)-2-Naphthol indicator. The indicator forms complexes with most metals present. After color development, EDTA is added to destroy all metal-PAN complexes except nickel and cobalt. The DR/4000 automatically adjusts for cobalt interference by measuring the absorbance of the sample at both 560 nm and 620 nm. This method is unique because both nickel and cobalt can be determined on the same sample.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to Section 1.

Pollution Prevention and Waste Management

For information on pollution prevention and waste management, refer to Section 1.

REQUIRED REAGENTS AND STANDARDS

Nickel Reagent Set (100 Tests*)	22426-00
Includes: (2) 7005-99, (4) 21501-66, (2) 21502-32	

Description	Quantity Required		Unit	Cat. No.
	per test			
EDTA Reagent Powder Pillows	2 pillows	100/pkg		7005-99
Phthalate-Phosphate Reagent Powder Pillows	2 pillows	50/pkg		21501-66
PAN Indicator Solution, 0.3%	2 mL	100 mL MDB		21502-32
Water, deionized	25 mL	4 liters		272-56

REQUIRED EQUIPMENT AND SUPPLIES

Clippers, for opening powder pillows	1	each	968-00
DR/4000 1-Inch Cell Adapter	1	each	48190-00
Sample Cells, matched pair, 1-inch, glass, with stopper	2	pair	26126-02

OPTIONAL REAGENTS AND STANDARDS

Nickel Standard Solution, 1000-mg/L Ni	100 mL	14176-42
Nickel Standard Solution, 2-mL Voluette Ampule, 50-mg/L Ni	20/pkg	25576-20
Nickel Standard Solution, 10-mL Voluette Ampule, 300-mg/L Ni	16/pkg	14266-10
Nitric Acid, ACS	500 mL	152-49
Nitric Acid Solution, 1:1	500 mL	2540-49
Sodium Hydroxide Standard Solution, 5.0 N	100 mL MDB	2450-32

OPTIONAL EQUIPMENT AND SUPPLIES

DR/4000 Carousel Module Kit	each	48070-02
DR/4000 Flow Cell Module Kit, 1-inch	each	48070-04
DR/4000 Flow Cell Module Kit, 1-cm	each	48070-05
DR/4000 Sipper Module Kit, 1-inch	each	48090-03
Flask, volumetric, Class A, 100-mL	each	14574-42
Flask, volumetric, Class A, 1000-mL	each	14574-53
pH Paper, pH 1.0 to 11.0	5 rolls/pkg	391-33
pH Meter, <i>sensio</i> TM 1, portable	each	51700-00
Pipet, serological, 1-mL	each	532-35
Pipet, serological, 5-mL	each	532-37
Pipet, TenSette, 0.1 to 1.0 mL	each	19700-01
Pipet Tips, for 19700-01 TenSette Pipet	50/pkg	21856-96
Pipet, volumetric, Class A, 3.00-mL	each	14515-03
Pipet, volumetric, Class A, 5.00-mL	each	14515-37
Pipet, volumetric, Class A, 6.00-mL	each	14515-06
Pipet, volumetric, Class A, 9.00-mL	each	14515-09
Pipet, volumetric, Class A, 10.00-mL	each	14515-38
Pipet, volumetric, Class A, 15.00-mL	each	14515-39
Pipet, volumetric, Class A, 20.00-mL	each	14515-20
Pipet Filler, safety bulb	each	14651-00

* 100 Tests equals 50 sample and 50 blanks. Contact Hach for larger sizes.



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✓ Method 8037

Heptoxime Method*

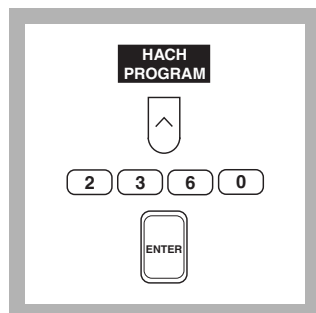
Powder Pillows

(0 to 1.80 mg/L Ni)

Scope and Application: For water, wastewater and seawater; USEPA accepted for reporting wastewater analyses (digestion required)**. See Section 1 for digestion procedure. The estimated detection limit for program number 2360 is 0.01 mg/L.

* Adapted from *Chimie Analytique*, 36 43 (1954)

** Procedure is equivalent to Standard Method 3500-Ni D for wastewater.



1. Press the soft key under **HACH PROGRAM**.

Select the stored program for nickel (Ni) by pressing **2360** with the numeric keys.

Press: **ENTER**

Note: If sample cannot be analyzed immediately, see Sample Collection, Storage and Preservation following these steps. Adjust the pH of preserved samples before analysis.

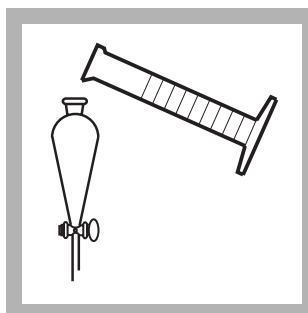
Note: The Flow Cell and Sipper Modules cannot be used with this procedure.



2. The display will show: **HACH PROGRAM: 2360 Nickel, Heptoxime**

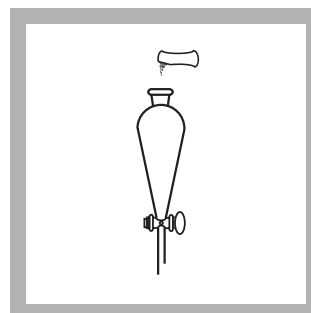
The wavelength (λ), **430 nm**, is automatically selected.

Note: For best results, determine a reagent blank for each new lot of reagent as follows. Prepare a reagent blank by repeating steps 3 through 14, using deionized water as the sample. Zero the instrument on deionized water by pressing the soft key under **ZERO**. Insert the reagent blank and the blank value will be displayed. Correct for the reagent blank by pressing the soft keys under **OPTIONS, (MORE)**, and then **BLANK:OFF**. Enter the reagent blank value and press **ENTER**. Repeat for each new lot of reagent.

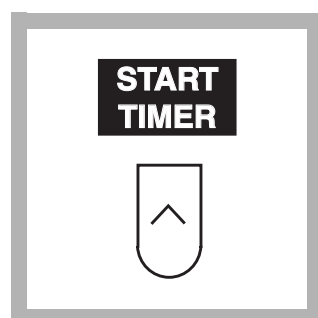


3. Measure 300 mL of sample in a 500-mL graduated cylinder. Pour into a 500-mL separatory funnel.

Note: For proof of accuracy, use a 1.0 mg/L nickel standard solution (preparation given in the Accuracy Check section) in place of the sample.

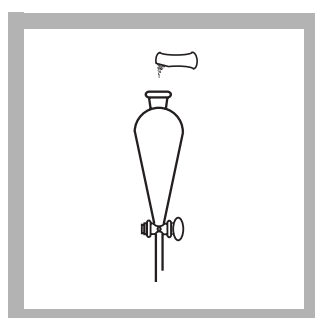


4. Add the contents of one Nickel 1 Reagent Powder Pillow to the funnel. Stopper. Shake to mix.

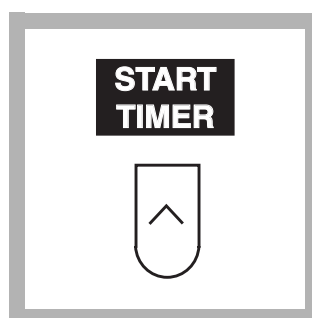


5. Press the soft key under **START TIMER**.

A 5-minute reaction period will begin.

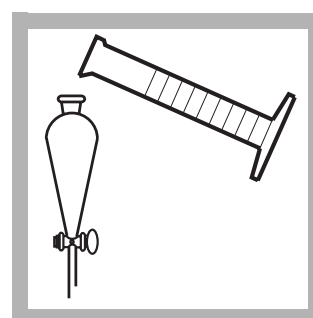


6. When the timer beeps, add the contents of one Nickel 2 Reagent Powder Pillow to the funnel. Stopper. Shake to mix.

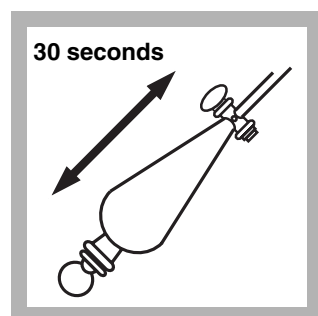


7. Press the soft key under **START TIMER**.

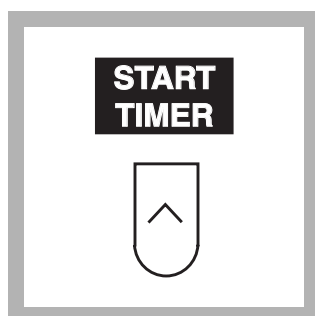
A second 5-minute reaction period will begin.



8. When the timer beeps, add 10 mL of chloroform. Stopper. Shake gently. Invert. With tip pointed up and away from people, open the stopcock to vent.

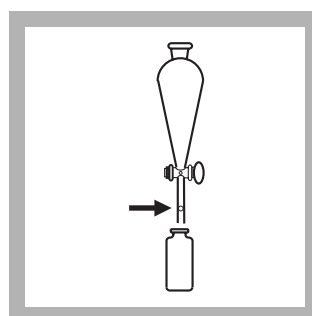


9. Close stopcock. Shake for 30 seconds.



10. Press the soft key under **START TIMER**.

A third 5-minute reaction period will begin. Shake the funnel several times over the five minute period.



11. When the timer beeps wait for the layers to separate. Insert a pea-sized cotton plug into the delivery tube of the funnel. Drain the chloroform layer (bottom layer) into a sample cell (the prepared sample). Stopper.

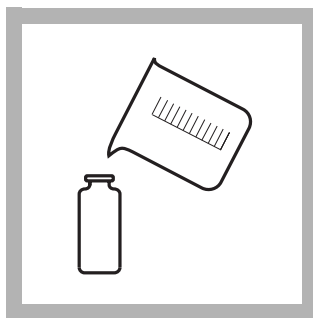


12. Repeat steps 8 to 11 two additional times with 10-mL portions of chloroform.

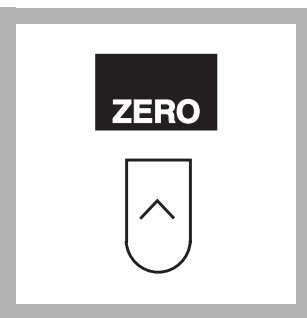
Note: The 5-minute reaction period is not necessary. Shake with chloroform, wait for layers to separate, then continue.

Note: The final volume of extract will be about 25 mL due to the slight solubility of chloroform in water.

Note: Swirl sample cell to mix extracts.



13. Fill a second cell (the blank) with 25 mL of chloroform. Stopper. Place the blank into the cell holder. Close the light shield.

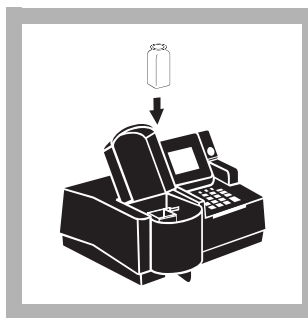


14. Press the soft key under **ZERO**.

The display will show:

0.00 mg/L Ni

Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



15. Place the prepared sample into the cell holder. Close the light shield. The result in mg/L nickel (or chosen units) will be displayed.

Interferences

Cobalt, copper and iron interferences can be overcome by adding additional Nickel 1 Reagent Powder Pillows in Step 4. The tolerance limits of these interferences are shown in the following table:

Table 1 Tolerance Limits vs. Number of Nickel 1 Reagent Powder Pillows

Pillows of Nickel 1 Reagent	Tolerance Limit (mg/L):		
	Cobalt	Copper	Iron
1	1	10	20
2	7	16	65
3	13	22	110
4	18	28	155
5	25	35	200

A preliminary acid digestion is required to determine any suspended or precipitated nickel and to eliminate interference by organic matter. To eliminate this interference or to determine total recoverable nickel perform the USEPA approved digestion in Section 2.

Sample Collection, Storage and Preservation

Collect samples in acid-washed plastic bottles. Adjust the sample pH to 2 or less with nitric acid (about 5 mL per liter). Preserved samples can be stored up to six months at room temperature. Before analysis, adjust the sample pH to between 3 and 8 with 5.0 N Sodium Hydroxide Standard Solution. Do not exceed pH 8 as this may cause some loss of nickel as a precipitate. Correct the test results for volume additions; see Section 1.2.2 *Correcting for Volume Additions*.

Accuracy Check

Standard Solutions Method

Prepare a 10.0-mg/L nickel working standard solution by pipetting 10.0 mL of a Nickel Standard Solution, 1000-mg/L, into a 1000-mL volumetric flask. Dilute to the mark with deionized water. Prepare this solution daily. Prepare a 1.0-mg/L nickel standard solution by diluting 50.0 mL of the 10-mg/L working standard solution to 500 mL in a volumetric flask. Perform the heptoxime procedure as described above.

To adjust the calibration curve using the reading obtained with the 1.0-mg/L standard solution, press the soft keys under **OPTIONS, MORE** then **STD: OFF**. Press **ENTER** to accept the displayed concentration, the value of which depends on the selected units. If an alternate concentration is used, enter the actual concentration and press **ENTER** to return to the read screen. See Section 1.5.5 *Adjusting the Standard Curve* for more information.

Or, use the TenSette Pipet to add 1.0 mL of a Nickel Voluette Ampule Standard Solution, 300-mg/L Ni, into a 500-mL volumetric flask and dilute to volume with deionized water. This is a 0.60-mg/L nickel standard solution.

Method Performance

Precision

Standard: 1.00 mg/L Ni²⁺

Program	95% Confidence Limits
2360	0.99–1.01 mg/L Ni ²⁺

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
2360	0.01 mg/L Ni ²⁺

For more information on derivation and use of Hach's estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, appendix B, see Section 1.5.1.

Sensitivity

Program Number: 2360

Portion of Curve	ΔAbs	ΔConcentration
Entire Range	0.010	0.02 mg/L

See Section 1.5.3 *Sensitivity Explained* for more information.

Calibration Standard Preparation

To perform a nickel calibration using the heptoxime method, prepare a 10-mg/L Ni stock solution by pipetting 10 mL of a 1000-mg/L Nickel Standard Solution (Cat. No. 23383-42) into a 1000-mL volumetric flask using Class A glassware. Dilute to the mark with deionized water and mix thoroughly.

Prepare calibration standards containing 0.10, 0.30, 0.60, 0.90, 1.20, 1.50 and 1.80 mg/L Ni as follows:

- a. Into seven different Class A 100-mL volumetric flasks, pipet 1, 3, 6, 9, 12, 15 and 18 mL of the 10-mg/L Ni stock solution using class A glassware.
- b. Dilute to the mark with deionized water. Mix thoroughly.
- c. Using the heptoxime method and the calibration procedure described in the *User-Entered Programs* section of the *DR/4000 Spectrophotometer Instrument Manual*, generate a calibration curve from the standards prepared above.

Summary of Method

Nickel ion reacts with heptoxime to form a yellow-colored complex which is then extracted into chloroform to concentrate the color and enable a more sensitive determination. Chelating agents are added to the sample to overcome the interferences caused by cobalt, copper and iron.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to Section 1.

Pollution Prevention and Waste Management

Chloroform (D022) solutions are regulated as hazardous waste by the Federal RCRA. Do not pour these materials down the drain. Water saturated with chloroform, chloroform solutions, and the cotton plug used in the delivery tube of the separatory funnel should be collected for disposal with laboratory solvent wastes. See Section 1 for more information on proper disposal of these materials.

REQUIRED REAGENTS AND STANDARDS

Nickel Reagent Set (50 Tests)	22435-00
Includes: (3) 14458-49, (2) 2123-68, (2) 2124-68	

Description	Quantity Required		Cat. No.
	per test	Unit	
Chloroform, ACS	55 mL	500 mL	14458-49
Nickel 1 Reagent Powder Pillows	1 pillow	25/pkg	2123-68
Nickel 2 Reagent Powder Pillows	1 pillow	25/pkg	2124-68

REQUIRED EQUIPMENT AND SUPPLIES

Clippers, for opening powder pillows	1	each	968-00
Cotton balls, absorbent	1	100/pkg	2572-01
Cylinder, graduated, 10-mL	1	each	508-38
Cylinder, graduated, 500-mL	1	each	508-49
DR/4000 1-Inch Cell Adapter	1	each	48190-00
Funnel, separatory, 500-mL	1	each	520-49
Ring, support, 4-inch	1	each	580-01
Sample Cells, matched pair, 1-inch, glass, with stoppers	2	pair	26126-02
Stand, support, 5" X 8" base	1	each	563-00
Stopper, hollow, Size 1	2	6/pkg	14480-00

OPTIONAL REAGENTS AND STANDARDS

Nickel Standard Solution, 1000-mg/L Ni	100 mL	14176-42
Nickel Standard Solution, 10-mL Voluette ampule, 300-mg/L Ni	16/pkg	14266-10
Nitric Acid, ACS	500 mL	152-49
Nitric Acid Solution, 1:1	500 mL	2540-49
Sodium Hydroxide Solution, 5.0 N	1 liter	2450-53
Water, deionized	4 liters	272-56

OPTIONAL EQUIPMENT AND SUPPLIES

DR/4000 Carousel Module Kit	each	48090-02
Flask, Erlenmeyer, 500-mL	each	505-49
Flask, volumetric, Class A, 500-mL	each	14574-49
Flask, volumetric, Class A, 1000-mL	each	14574-53
pH Paper, pH 1.0 to 11.0	5 rolls/pkg	391-33
Pipet, serological, 1-mL	each	532-35
Pipet, serological, 5-mL	each	532-37
Pipet, TenSette, 0.1 to 1.0 mL	each	19700-01
Pipet Tips, for 19700-01 TenSette Pipet	50/pkg	21856-96
Pipet, volumetric, Class A, 1.0-mL	each	14515-35
Pipet, volumetric, Class A, 3.0-mL	each	14515-03
Pipet, volumetric, Class A, 6.0-mL	each	14515-06
Pipet, volumetric, Class A, 9.0-mL	each	14515-09
Pipet, volumetric, Class A, 10.00-mL	each	14515-38
Pipet, volumetric, Class A, 15.00-mL	each	14515-39
Pipet, volumetric, Class A, 50.00-mL	each	14515-41
Pipet Filler, safety bulb	each	14651-00



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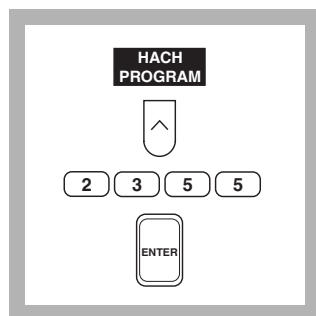


Dimethylglyoxime Method

UniCell™ Vials

(0 to 6.00 mg/L)

Scope and Application: For water, wastewater, raw water, and process control; Metal Prep Set (HCT 200) digestion is required for determining total nickel. The estimated detection limit for program number 2355 is 0.10 mg/L Ni.



1. Press the soft key under **HACH PROGRAM**.

Select the stored program for nickel (HCT 167 A), by pressing **2355** with the numeric keys.

Press: **ENTER**

Note: If the sample cannot be analyzed immediately, see Sample Collection, Storage and Preservation following these steps. Adjust the pH of preserved samples before analysis.

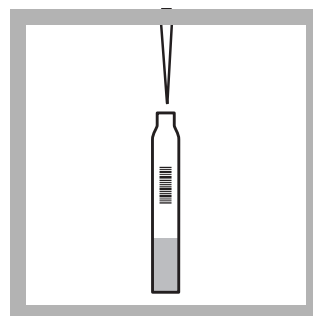


2. The display will show: **HACH PROGRAM: 2355 Nickel, HCT 167**

The wavelength (λ), **463 nm**, is automatically selected.

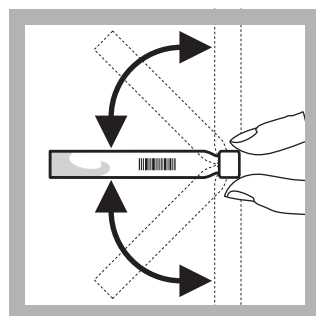


3. Insert the Test Tube Adapter into the sample cell module by sliding it under the thumb screw and into the alignment grooves. Fasten with the thumb screw.

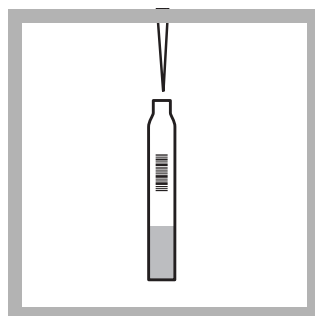


4. Pipet 4.0 mL of sample into the sample vial.

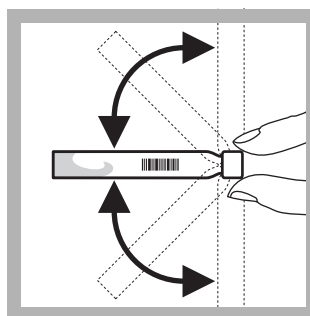
Note: For proof of accuracy, use a 2.00-mg/L nickel standard solution (preparation given in the Accuracy Check section) in place of the sample.



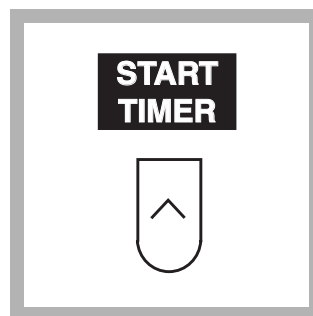
5. Cap and invert the sample vial until the solid in the vial is completely dissolved.



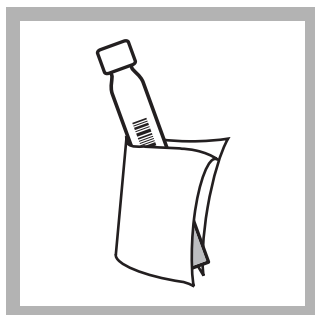
6. Pipet 0.4 mL of Dimethylglyoxime Solution A (HCT 167) into the sample vial.



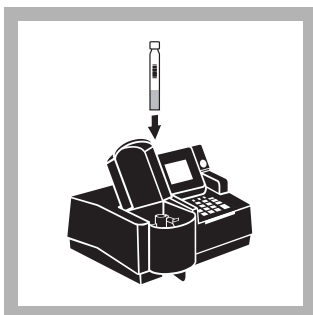
7. Cap and invert the vial several times to mix.



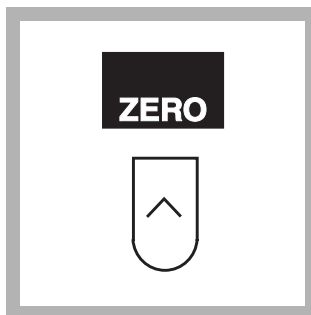
8. Press the soft key under **START TIMER**. A 3-minute reaction period will begin.



9. Wipe the outside of the sample and zero vials with a damp towel followed by a dry one to remove fingerprints and other marks.



10. Place the zero vial (white cap) into the cell holder. Close the light shield.

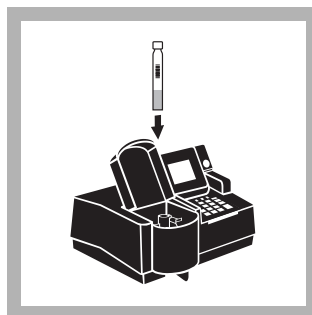


11. Press the soft key under **ZERO**.

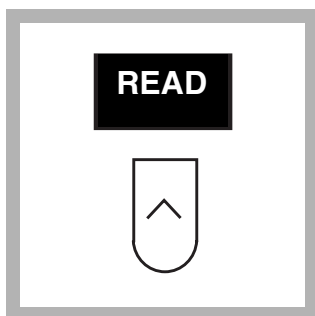
The display will show:

0.00 mg/L free Ni

Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



12. When the timer beeps, place the sample vial into the cell holder. Close the light shield.



13. Press the soft key under **READ**.

The result in mg/L free nickel (or chosen units) will be displayed.

Interferences

The ions listed in the table have been individually checked up to the given concentrations. Cumulative effects and the influence of other ions have not been determined.

Ion	No interference up to:
Cl ⁻	1000 mg/L
NH ₄ ⁺ , Ca ²⁺ , PO ₄ ³⁻ , CO ₃ ²⁻	500 mg/L
Cr ⁶⁺ , Zn ²⁺ , F ⁻ , NO ₂ ⁻	50 mg/L
Al ³⁺ , Cr ³⁺ , Cd ²⁺ , Co ²⁺ , Sn ²⁺ , Pb ²⁺	10 mg/L
Fe ²⁺ , Fe ³⁺ , Mn ²⁺ , Cu ²⁺ , Mg ²⁺ , Hg ²⁺	5 mg/L
Ag ⁺	1 mg/L

Total nickel, undissolved nickel, and complexed nickel can only be determined after digesting with the Metal Prep Set HCT 200. (Total nickel measuring range is 0.12 – 7.20 mg/L.)

Sample Collection, Storage and Preservation

Collect samples in acid-cleaned or plastic containers. No acid addition is necessary if analyzing the samples immediately. To preserve samples, adjust the pH to 2 or less with concentrated nitric acid (about 2 mL per liter). Preserved samples may be stored up to six months at room temperature.

Adjust the pH to between 3 and 8 with 5.0 N Sodium Hydroxide Standard Solution before analysis. Do not exceed pH 8 or nickel may precipitate.

Accuracy Check

Standard Additions Method

- a. Leave the unspiked sample in the sample compartment. Verify that the units displayed are in mg/L. Select standard additions mode by pressing the soft keys under **OPTIONS, (MORE)** and then **STD ADD**.
- b. Press **ENTER** to accept the default sample volume (mL), 100.
- c. Press **ENTER** to accept the default standard concentration (mg/L), 1000.

Note: Alternative standard concentrations and additions volumes can also be used.

- d. Press the soft key under **ENTRY DONE**.
- e. Use a pipet to add 0.1 mL, 0.2 mL and 0.3 mL of 1000-mg/L Ni standard, respectively, to three 100-mL samples and mix each thoroughly.
- f. Analyze each standard addition sample as described in the procedure. Accept the standard additions reading by pressing the soft key under **READ** each time. Each addition should reflect approximately 100% recovery.
- g. After completing the sequence, the display will show the extrapolated concentration value and the “best-fit” line through the standard additions data points, accounting for matrix interferences.
- h. See Section 1.4.1 *Standard Additions* for more information.

Standard Solution Method

Prepare a 2.00-mg/L Nickel stock solution by pipetting 0.2 mL of Nickel Standard Solution, 1000-mg/L as Ni, into a 100-mL volumetric flask. Dilute to the mark with deionized water. Prepare this solution daily.

To adjust the calibration curve using the reading obtained with the 2.00-mg/L working solution, press the soft keys under **OPTIONS, (MORE)** then **STD: OFF**. Press **ENTER** to accept the displayed concentration, the value of which depends on the selected units. If an alternate concentration is used, enter the actual concentration and press **ENTER** to return to the read screen. See Section 1.5.5 *Adjusting the Standard Curve* for more information.

Method Performance

Precision

Standard: 2.00 mg/L Free Ni

Program	95% Confidence Limits
2355	1.76–2.24 mg/L Free Ni

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
2355	0.10 mg/L Free Ni

For more information on derivation and use of Hach's estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, appendix B, see Section 1.5.1.

Sensitivity

Program Number: 2355

Portion of Curve	Δ Abs	Δ Concentration
Entire Range	0.010	0.038 mg/L

See Section 1.5.3 *Sensitivity Explained* for more information.

Summary of Method

In the presence of an oxidizing agent, nickel ions react with dimethylglyoxime in an alkaline solution to form an orange-brown colored complex.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the Material Safety Data Sheet for information specific to the reagents used.

Pollution Prevention and Waste Management

For information on pollution prevention and waste management, refer to Section 1.

REQUIRED EQUIPMENT AND SUPPLIES

Description	Unit	Cat. No.
Nickel - Ni, UniCell™ HCT 167	23/pkg	HCT 167

OPTIONAL REAGENTS AND STANDARDS

Metal-Prep-Set, UniCell™ HCT 200	50 digestions	HCT 200
Nickel Standard, 1000-mg/L as Ni	100 mL	14176-42
Sodium Hydroxide, 5 N	1L	2450-53
Nitric Acid Solution, 1:1	500 mL	2540-49

OPTIONAL APPARATUS

DRB100 Digital Reactor Block	each	DRB 100
Graduated cylinder, mixing, 100-mL	each	20886-42
Flask, volumetric 100-mL	each	14574-42
Pipettor, (Jencons) 1–5 mL	each	27951-00
Replacement tips for 27951-00	pkg/100	27952-00
Pipettor, (Jencons) 100–1000 µL	each	27949-00
Replacement tips for 27949-00	pkg/400	27950-00
pH Paper	pkg/100	26013-00



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FAX: (970) 669-2932



Method 8192

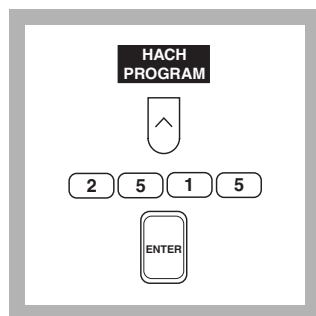
Cadmium Reduction Method

Powder Pillows

LR (0 to 0.50 mg/L NO_3^- -N)

Scope and Application: For water, wastewater and seawater.

The estimated detection limit for program number 2515 is 0.01 mg/L NO_3^- -N.



- 1.** Press the soft key under **HACH PROGRAM**.

Select the stored program number for nitrate, low range, by pressing **2515** with the numeric keys.

Press: **ENTER**

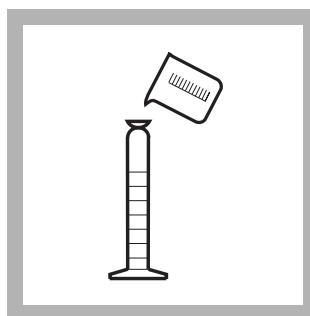
Note: If samples cannot be analyzed immediately, see *Sample Collection, Storage and Preservation* following these steps. Adjust the pH of preserved samples before analysis.



- 2.** The display will show:
HACH PROGRAM: 2515 N, Nitrate LR

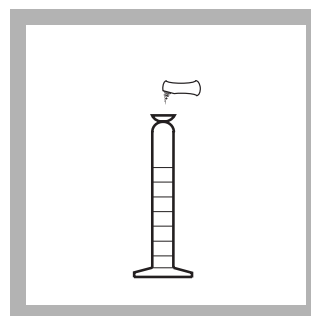
The wavelength (λ), **507 nm**, is automatically selected.

Note: A reagent blank must be determined on each new lot of reagent as follows. Prepare a reagent blank by repeating Steps 3 through 14, using deionized water as the sample. Zero the instrument on deionized water by pressing the soft key under **ZERO**. Insert the reagent blank and the blank value will be displayed. Correct for the reagent blank by pressing the soft keys under **OPTIONS, (MORE)**, and then **BLANK:OFF**. Enter the reagent blank value and press **ENTER**. Repeat for each new lot of reagent.



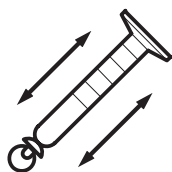
- 3.** Fill a 25-mL graduated mixing cylinder with 15 mL of sample.

Note: For proof of accuracy, use a 0.2 mg/L nitrate nitrogen standard solution (see the *Accuracy Check* section) in place of the sample.



- 4.** Add the contents of one NitraVer 6 Reagent Powder Pillow to the cylinder. Stopper.

3 minutes



5. Press the soft key under **START TIMER**.
Shake the cylinder vigorously for 3 minutes.

**START
TIMER**



6. When the timer beeps, press the soft key under **START TIMER**.

A 2-minute reaction period will begin.

Note: A deposit of unoxidized metal will remain after the NitraVer 6 dissolves. The deposit will not affect results.

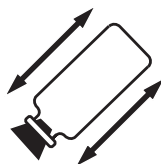


7. When the timer beeps, carefully pour 10 mL of the sample into a clean sample cell. Take care not to transfer any cadmium particles.



8. Add the contents of one NitraVer 3 Nitrite Reagent Powder Pillow to the sample cell (the prepared sample). Stopper.

30 seconds



9. Press the soft key under **START TIMER**.
Shake the sample cell gently for 30 seconds.

Note: A pink color will develop if nitrate is present.

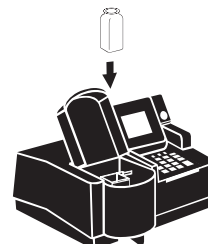
**START
TIMER**



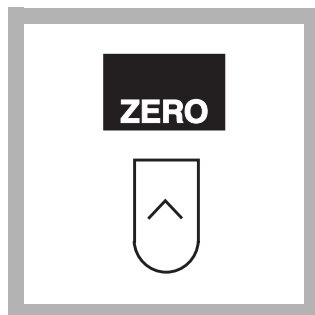
10. Press the soft key under **START TIMER**.
A 15-minute reaction period will begin.



11. When the timer beeps, fill a second sample cell with 10 mL of original sample (the blank).



12. Place the blank into the cell holder and close the light shield.



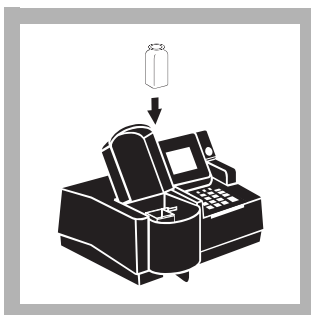
13. Press the soft key under **ZERO**.

The display will show:

0.00 mg/L NO₃⁻-N

Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.

Note: If you have entered a reagent blank correction, the display will show the correction.



14. Place the prepared sample into the cell holder and close the light shield. The result in mg/L nitrate nitrogen will be displayed.

Note: The results can be expressed as mg/L nitrate (NO₃⁻). Press the soft keys under **OPTIONS**, and then **FORM** to scroll through the available options.

Note: Rinse the sample cell and mixing cylinder immediately after use to remove all cadmium particles. Retain the spent sample for proper hazardous waste disposal for cadmium.

Interferences

Table 1 Interfering Substances and Suggested Treatments

Interfering Substance	Interference Level and Treatment
Calcium	100 mg/L
Chloride	Chloride concentrations above 100 mg/L will cause low results. The test may be used at high chloride concentrations (seawater) but a calibration must be done using standards spiked to the same chloride concentration.
Ferric iron	All levels
Nitrite	All levels: This method measures both the nitrate and nitrite in the sample. If nitrite is present, the nitrite nitrogen test (Program #2610) should be done on the sample. Pretreat the nitrate nitrogen sample with the following pretreatment. Then subtract the amount of nitrite found from the results of the LR nitrate nitrogen test. <ol style="list-style-type: none"> 1. Add 30-g/L Bromine Water dropwise to the sample in Step 3 until a yellow color remains. Mix after each drop. 2. Add one drop of 30-g/L Phenol Solution to destroy the color. 3. Proceed with the LR Nitrate procedure.
pH	Highly buffered samples or extreme sample pH may exceed the buffering capacity of the reagents and require sample pretreatment.
Strong oxidizing and reducing substances	Interfere at all levels

Sample Collection, Storage and Preservation

Most reliable results are obtained when samples are analyzed as soon as possible after collection. If prompt analysis is impossible, store samples in clean plastic or glass bottles for up to 48 hours at 4 °C. To preserve samples for longer periods, add 2 mL of concentrated sulfuric acid (H₂SO₄) per liter and store at 4 °C.

Before analysis, warm the sample to room temperature and adjust the pH to 7 with 5.0 N Sodium Hydroxide Standard Solution. Do not use mercury compounds as preservatives. Correct the test result for volume additions by dividing the total volume (acid + base + sample) by the original sample volume and multiplying the test result by this factor.

Accuracy Check

Standard Solution Method

To test accuracy, use a 0.20-mg/L NO₃⁻-N standard in place of the sample and perform the procedure as described. Prepare this standard by diluting 2.00 mL of a 10-mg/L Nitrate Nitrogen Standard Solution to 100.0 mL with deionized water.

To adjust the calibration curve using the reading obtained with the 0.20-mg/L nitrate nitrogen standard, press the soft keys under **OPTIONS, (MORE)** then **STD: OFF**. Press **ENTER** to accept the default concentration, 0.20-mg/L NO₃⁻-N. If an alternate concentration is used, enter the actual concentration and press **ENTER** to return to the read screen. See Section 1.5.5 *Adjusting the Standard Curve* for more information.

Standard Additions Method

- a. Leave the unspiked sample in the sample compartment. Verify that the units displayed are in mg/L. Select standard additions mode by pressing the soft keys under **OPTIONS, (MORE)** and then **STD ADD**.
- b. Press **ENTER** to accept the default sample volume (mL), 15. (This is the volume to which standard addition aliquots are added.)
- c. Press **ENTER** to accept the default standard concentration (mg/L), 12.0.
- d. Press the soft key under **ENTRY DONE**.
- e. Fill three 25-mL graduated mixing cylinders with 15 mL of sample.
- f. Snap the neck off a Nitrate Nitrogen PourRite Ampule Standard, 12.0-mg/L NO₃⁻-N.
- g. Use the TenSette Pipet to add 0.1, 0.2 and 0.3 mL of standard, respectively to the three mixing cylinders. Stopper each and mix thoroughly.
- h. Analyze each standard addition sample as described above. Accept the standard additions reading by pressing the soft key under **READ** each time. Each additions reading should reflect approximately 100% recovery.
- i. After completing the sequence, the display will show the extrapolated concentration value and the “best-fit” line through the standard additions data points, accounting for matrix interferences.
- j. See Section 1.4.1 *Standard Additions* for more information.

Method Performance

Precision

Standard: 0.30 mg/L NO₃⁻-N₂

Program	95% Confidence Limits
2515	0.29–0.31 mg/L NO ₃ ⁻ -N

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
2515	0.01 mg/L NO ₃ ⁻ -N

For more information on derivation and use of Hach's estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, appendix B, see Section 1.5.1.

Sensitivity

Program Number: 2515

Portion of Curve:	ΔAbs	ΔConcentration
0.004 mg/L NO ₃ ⁻ -N	0.010	0.0041 mg/L NO ₃ ⁻ -N
0.25 mg/L NO ₃ ⁻ -N	0.010	0.0034 mg/L NO ₃ ⁻ -N
0.45 mg/L NO ₃ ⁻ -N	0.010	0.0027 mg/L NO ₃ ⁻ -N

See Section 1.5.3 *Sensitivity Explained* for more information.

Calibration Standard Preparation

To perform a nitrate calibration using the Low Range Cadmium Reduction method, prepare calibration standards containing 0.10, 0.30, and 0.50 mg/L NO₃⁻-N as follows:

- a. Into three different 100-mL class A volumetric flasks, pipet 1.00, 3.00, and 5.00 mL of a 10.0-mg/L Nitrate Nitrogen Standard Solution using Class A glassware.
- b. Dilute to the mark with deionized water. Mix thoroughly.
- c. Using the Low Range Cadmium Reduction method and the calibration procedure described in the *User-Entered Programs* section of the *DR/4000 Spectrophotometer Instrument Manual*, generate a calibration curve from the standards prepared above.

Summary of Method

Cadmium metal reduces nitrates in the sample to nitrite. The nitrite ion reacts in an acidic medium with sulfanilic acid to form an intermediate diazonium salt. The salt couples with chromotropic acid to form a pink-colored product.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to Section 1.

Pollution Prevention and Waste Management

Prepared samples will contain cadmium and must be disposed of according to Federal, State and local hazardous waste regulations. For information on pollution prevention and waste management, refer to Section 1.

REQUIRED REAGENTS AND STANDARDS

Low Range Nitrate Reagent Set (100 tests)24298-00
Includes: (1) 21072-49, (1) 21071-69

Description	Quantity Required		Unit	Cat. No.
	per test			
NitraVer 6 Nitrate Reagent Powder Pillows	1 pillow	100/pkg		21072-49
NitriVer 3 Nitrite Reagent Powder Pillows	1 pillow	100/pkg		21071-69

REQUIRED APPARATUS

Cylinder, graduated, mixing, 25-mL1each.....20886-40
DR/400- 1-inch Cell Adapter1each.....48190-00
Sample Cell, matched pair, 1-inch, glass, with stoppers2pair.....26126-02

OPTIONAL REAGENTS AND STANDARDS

Bromine Water, 30-g/L.....29 mL*.....2211-20
Nitrate Nitrogen Standard Solution, 10.0-mg/L NO₃⁻-N500 mL.....307-49
Nitrate Nitrogen Standard Solution,
10-mL PourRite Ampule, 12-mg/L NO₃⁻-N.....16/pkg.....14333-10
Phenol Solution, 30-g/L25 mL*.....2112-20
Sodium Hydroxide Standard Solution, 5.0 N.....1 liter*.....2450-53
Sulfuric Acid, ACS, concentrated500 mL*.....979-49
Water, deionized4 liters.....272-56

OPTIONAL APPARATUS

Ampule Breaker, PourRiteeach.....24846-00
Brush, test tubeeach.....690-00
Dropper, for 29-mL bottleeach.....2258-00
DR/4000 Carousel Module Kiteach.....48070-02
Flask, volumetric, 100-mL, Class Aeach.....14574-42
pH Paper, pH 1.0 to 11.05/pkg.....391-33
Pipet, serological, 2-mLeach.....532-36
Pipet, TenSette, 0.1 to 1.0 mLeach.....19700-01
Pipet Tips, for 19700-01 TenSette Pipet50/pkg.....21856-96
Pipet, volumetric, Class A, 1.00-mLeach.....14515-35
Pipet, volumetric, Class A, 3.00-mLeach.....14515-03
Pipet, volumetric, Class A, 5.00-mLeach.....14515-37
Pipet Filler, safety bulb.....each.....14651-00

* Larger sizes available



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Method 8171

Cadmium Reduction Method

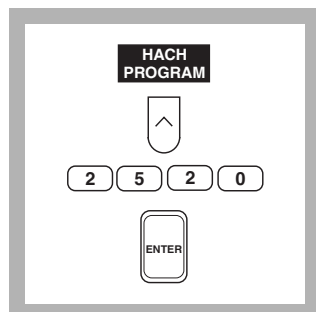
Powder Pillows or AccuVac® Ampuls

MR (0 to 5.0 mg/L NO₃⁻-N)

Scope and Application: For water, wastewater and seawater.

The estimated detection limit for program numbers 2520 and 2525 are 0.1 and 0.1 mg/L NO₃⁻-N, respectively.

Using Powder Pillows



- 1.** Press the soft key under **HACH PROGRAM**.

Select the stored program number for mid range nitrate by pressing **2520** with the numeric keys.

Press: **ENTER**

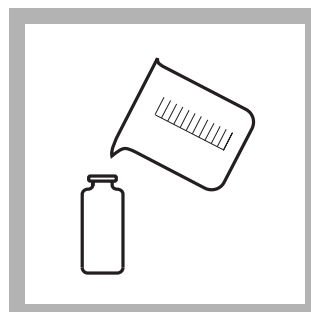
Note: If samples cannot be analyzed immediately, see *Sample Collection, Storage and Preservation* following these steps. Adjust the pH of preserved samples before analysis.



- 2.** The display will show:
HACH PROGRAM: 2520 Nitrate MR

The wavelength (λ), **400 nm**, is automatically selected.

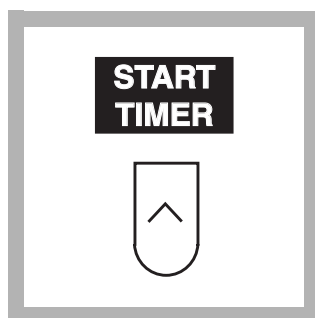
Note: For best results, determine a reagent blank for each new lot of reagent as follows. Prepare a reagent blank by repeating steps 3 through 9, using deionized water as the sample. Zero the instrument on deionized water by pressing the soft key under **ZERO**. Insert the reagent blank and the blank value will be displayed. Correct for the reagent blank by pressing the soft keys under **OPTIONS, (MORE)**, and then **BLANK:OFF**. Enter the reagent blank value and press **ENTER**. Repeat for each new lot of reagent.



- 3.** Fill a sample cell with 10 mL of sample.



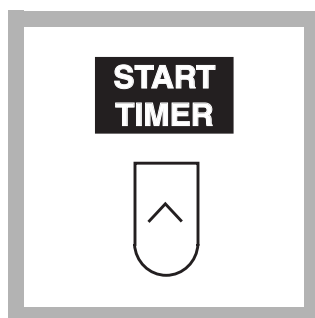
- 4.** Add the contents of one NitraVer 5 Nitrate Reagent Powder Pillow (the prepared sample). Stopper.



5. Press the soft key under **START TIMER**.
Shake the cell vigorously until the timer beeps in one minute.

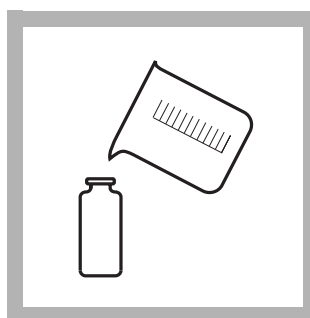
Note: A deposit of unoxidized metal will remain after the NitraVer 5 dissolves. The deposit will not affect results.

Note: Shaking time and technique influence color development. For most accurate results, make successive tests on a 1.00 mg/L Nitrate Nitrogen Standard solution (listed under **OPTIONAL REAGENTS AND STANDARDS**). Adjust shaking times to obtain the correct result.

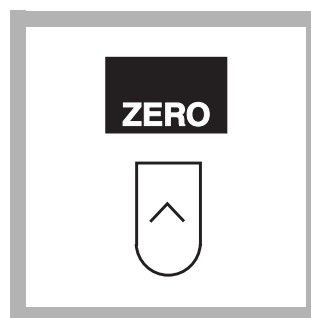


6. When the timer beeps, press the soft key under **START TIMER**.
A 5-minute reaction period will begin.

Note: An amber color will develop if nitrate nitrogen is present.



7. When the timer beeps, fill a second sample cell with 10 mL of sample (the blank). Place the blank into the cell holder.

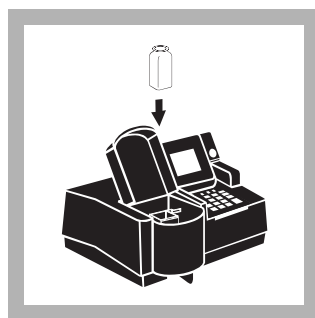


8. Press the soft key under **ZERO**.
The display will show:

0.0 mg/L NO₃⁻-N

Note: If you have entered a reagent blank correction, the display will show the correction.

Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



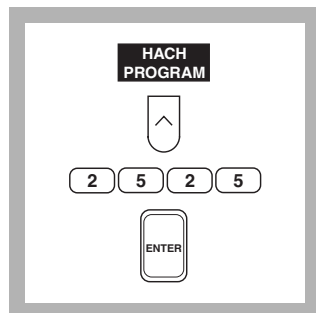
9. Place the prepared sample into the cell holder. Close the light shield. The result in mg/L nitrate nitrogen (NO₃⁻-N) will be displayed.

Note: Measure sample within two minutes after timer beeps.

Note: The result can be expressed as mg/L nitrate (NO₃⁻). Press the soft keys under **OPTIONS** and then **FORM** to scroll through the available options.

Note: Rinse the sample cell immediately after use to remove all cadmium particles. Retain the spent sample for proper hazardous waste disposal for cadmium.

Using AccuVac Ampuls



1. Press the soft key under **HACH PROGRAM**.

Select the stored program number for mid range nitrate by pressing **2525** with the numeric keys.

Press: **ENTER**

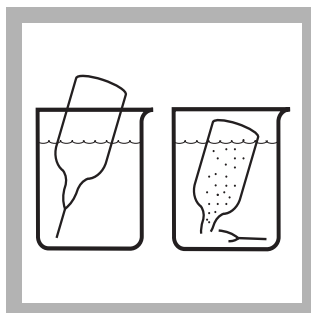
Note: If samples cannot be analyzed immediately, see *Sample Collection, Storage and Preservation* following these steps. Adjust the pH of preserved samples before analysis.



2. The display will show:
HACH PROGRAM: 2525 N, Nitrate MR AV

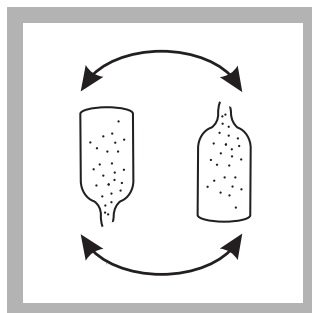
The wavelength (λ), **400 nm**, is automatically selected.

Note: For best results, determine a reagent blank for each new lot of reagent as follows. Prepare a reagent blank by repeating steps 3 through 10, using deionized water as the sample. Zero the instrument on deionized water by pressing the soft key under **ZERO**. Insert the reagent blank and the blank value will be displayed. Correct for the reagent blank by pressing the soft keys under **OPTIONS, (MORE)**, and then **BLANK:OFF**. Enter the reagent blank value and press **ENTER**. Repeat for each new lot of reagent.



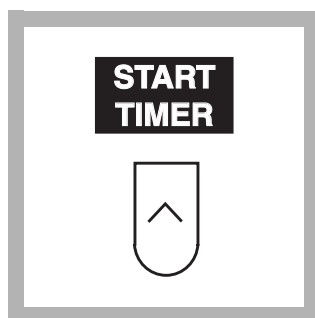
3. Collect at least 40 mL of sample in a 50-mL beaker. Fill a NitraVer 5 Nitrate AccuVac Ampul with sample. Place a stopper over the tip of the ampul.

Note: Keep the tip immersed while the ampul fills.



4. Press the soft key under **START TIMER**. A one-minute reaction period will begin. Invert the ampul repeatedly until the timer beeps. Wipe off any liquid or fingerprints.

Note: Inversion rate can influence color development. For most accurate results, make successive tests on a 1.00 mg/L Nitrate Nitrogen Standard solution (listed under **OPTIONAL REAGENTS AND STANDARDS**.) Adjust inversion rate to obtain the correct result.

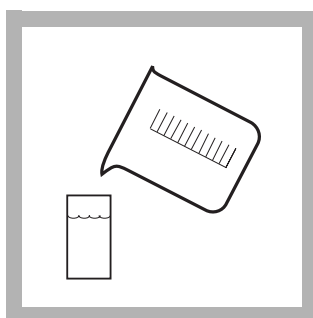


5. When the timer beeps, press the soft key under **START TIMER**.

A 5-minute reaction period will begin.

Note: A deposit of unoxidized metal will remain after the NitraVer 5 dissolves. The deposit will not affect results.

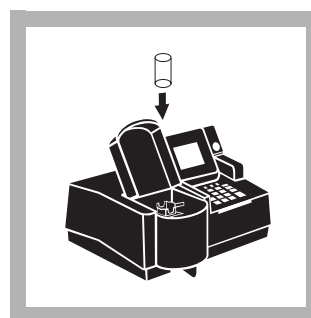
Note: An amber color will develop if nitrate nitrogen is present.



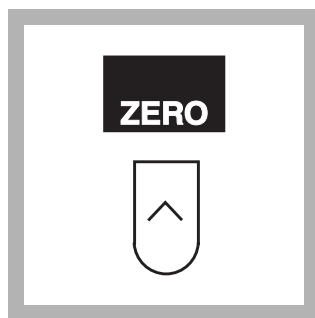
6. When the timer beeps, fill a zeroing vial (the blank) with at least 10 mL of sample.



7. Insert the AccuVac Ampul Adapter into the sample cell module by sliding it under the thumb screw and into the alignment grooves. Fasten with the thumb screw.



8. Place the blank into the cell holder. Close the light shield.



9. Press the soft key under **ZERO**.

The display will show:

0.0 mg/L NO_3^- -N

Note: If you have entered a reagent blank correction, the display will show the correction.

Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



10. Place the AccuVac ampul into the cell holder. Close the light shield. Results in mg/L nitrate expressed as nitrogen (NO_3^- -N) will be displayed.

Note: Measure sample within two minutes after timer beeps.

Note: The results can be expressed as mg/L nitrate (NO_3^-). Press the soft keys under **OPTIONS**, then **FORM**: to scroll through the available options.

Interferences

Table 1 Interfering Substances and Suggested Treatments

Interfering Substance	Interference Level and Treatment
Chloride	Chloride concentrations above 100 mg/L will cause low results. The test may be used at high chloride concentrations (seawater) but a calibration must be done using standards spiked to the same chloride concentration.
Ferric iron	All levels
Nitrite	All levels Compensate for nitrite interference as follows: a) Add 30-g/L Bromine Water dropwise to the sample in Step 3 until a yellow color remains. b) Add one drop of 30-g/L Phenol Solution to destroy the color. c) Proceed with Step 4. Report the results as total nitrate and nitrite.
pH	Highly buffered samples or extreme sample pH may exceed the buffering capacity of the reagents and require sample pretreatment.
Strong oxidizing and reducing substances	Interfere at all levels

Sample Collection, Storage and Preservation

Most reliable results are obtained when samples are analyzed as soon as possible after collection. If prompt analysis is impossible, store samples in clean plastic or glass bottles for up to 24 hours at 4 °C. To preserve samples for longer periods, add 2 mL of concentrated sulfuric acid (H₂SO₄) per liter and store at 4 °C.

Before analysis, warm the sample to room temperature and adjust the pH to 7 with 5.0 N Sodium Hydroxide Standard Solution. Do not use mercury compounds as preservatives. Correct the test result for volume additions by dividing the total volume (acid + base + sample) by the original sample volume and multiplying the test result by this factor.

Accuracy Check

Standard Solution Method

To test accuracy, use a 1.00 mg/L Nitrate Nitrogen Standard Solution in place of the sample and perform the procedure as described.

To adjust the calibration curve using the reading obtained with the 1.00 mg/L Nitrate Nitrogen Standard Solution, press the soft keys under **OPTIONS, (MORE)** then **STD: OFF**. Press **ENTER** to accept the default concentration, 1.00 mg/L NO₃⁻-N. If an alternate concentration is used, enter the actual concentration and press **ENTER** to return to the read screen. See Section 1.5.5 *Adjusting the Standard Curve* for more information.

Standard Additions Method

- Leave the unspiked sample in the sample compartment. Verify that the units displayed are in mg/L. Select standard additions mode by pressing the soft keys under **OPTIONS, (MORE)** and then **STD ADD**.
- Press **ENTER** to accept the default sample volume (mL), 25. (This is the volume to which standard addition aliquots are added.)
- Press **ENTER** to accept the default standard concentration (mg/L), 100.

- d. Press the soft key under **ENTRY DONE**.
- e. Fill three 25-mL graduated mixing cylinders with 25 mL of sample.
- f. Snap the neck off a Nitrate Nitrogen Voluette Ampule Standard, 100 mg/L NO_3^- -N.
- g. Use the TenSette Pipet to add 0.1, 0.2 and 0.3 mL of standard, respectively, to the three mixing cylinders. Stopper each and mix thoroughly.
- h. For analysis with AccuVac Ampuls, transfer solutions to dry, clean 50-mL beakers to facilitate filling of the ampules. For analysis with powder pillows, transfer only 10 mL of solution to the 10-mL sample cells.
- i. Analyze each standard addition sample as described above. Accept the standard additions readings by pressing the soft key under **READ** each time. Each addition should reflect approximately 100% recovery.
- j. After completing the sequence, the display will show the extrapolated concentration value and the “best-fit” line through the standard additions data points, accounting for matrix interferences.
- k. See Section 1.4.1 *Standard Additions* for more information.

Method Performance

Precision

Standard: 3.0 mg/L NO_3^- -N

Program	95% Confidence Limits
2520	2.9–3.1 mg/L NO_3^- -N
2525	2.9–3.1 mg/L NO_3^- -N

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
2520	0.1 mg/L NO_3^- -N
2525	0.1 mg/L NO_3^- -N

For more information on derivation and use of Hach’s estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, appendix B, see Section 1.5.1.

Sensitivity

Program Number: 2520

Portion of Curve	Δ Abs	Δ Concentration
Entire Range	0.010	0.04 mg/L NO ₃ ⁻ -N

Program Number: 2525

Portion of Curve	Δ Abs	Δ Concentration
Entire Range	0.010	0.04 mg/L NO ₃ ⁻ -N

See Section 1.5.3 *Sensitivity Explained* for more information.

Calibration Standard Preparation

To perform a nitrate calibration using the NitraVer 5 method, prepare calibration standards containing 1, 3, and 5 mg/L NO₃⁻-N as follows:

- Into three different 100-mL Class A volumetric flasks, pipet 1.00, 3.00, and 5.00 mL of a 100-mg/L Nitrate Nitrogen Standard Solution using Class A glassware.
- Dilute to the mark with deionized water. Mix thoroughly.
- Using the NitraVer 5 Powder Pillow or AccuVac method and the calibration procedure described in the *User-Entered Programs* section of the *DR/4000 Spectrophotometer Instrument Manual*, generate a calibration curve from the standards prepared above.

Summary of Method

Cadmium metal reduces nitrates in the sample to nitrite. The nitrite ion reacts in an acidic medium with sulfanilic acid to form an intermediate diazonium salt. The salt couples with gentisic acid to form an amber colored solution.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to Section 1.

Pollution Prevention and Waste Management

Prepared samples will contain cadmium and must be disposed of according to Federal, State and local hazardous waste regulations. For information on pollution prevention and waste management, refer to Section 1.

NITRATE, continued

REQUIRED REAGENTS AND STANDARDS (Using Powder Pillows)

Description	Quantity Required per test	Unit	Cat. No.
NitraVer 5 Nitrate Reagent Powder Pillows (for 10-mL sample) .. 1 pillow	1	100/pkg	21061-69

REQUIRED REAGENTS AND STANDARDS (Using AccuVac Ampuls)

NitraVer 5 Nitrate Reagent AccuVac Ampul	1 ampul	25/pkg	25110-25
--	---------	--------	----------

REQUIRED EQUIPMENT AND SUPPLIES (Using Powder Pillows)

DR/4000 1-Inch Cell Adapter	1	each	48190-00
Sample Cells, matched pair, 1-inch, glass, with stoppers	2	pair	26126-02

REQUIRED EQUIPMENT AND SUPPLIES (Using AccuVac Ampuls)

Beaker, 50-mL	1	each	500-41
DR/4000 AccuVac Ampul Adapter	1	each	48187-00
Sample Cell, 10-mL with cap (zeroing vial)	1	each	21228-00
Stopper, for 18-mm tube	1	6/pkg	1731-06

OPTIONAL REAGENTS AND STANDARDS

Bromine Water, 30-g/L	29 mL*	2211-20
Nitrate Nitrogen Standard Solution, 1.00-mg/L NO ₃ ⁻ -N	500 mL	2046-49
Nitrate Nitrogen Standard Solution, 100-mg/L NO ₃ ⁻ -N	500 mL	1947-49
Nitrate Nitrogen Standard Solution, 2-mL PourRite Ampule, 100-mg/L NO ₃ ⁻ -N ...	20/pkg	1947-20
Phenol Solution, 30-g/L	29 mL*	2112-20
Sodium Hydroxide Standard Solution, 5.0 N	1 liter	2450-53
Sulfuric Acid, ACS, concentrated	500 mL*	979-49
Water, deionized	4 liters	272-56

OPTIONAL EQUIPMENT AND SUPPLIES

AccuVac Snapper	each	24052-00
Cylinder, graduated, mixing, 25-mL	each	20886-40
Dropper, for 29-mL bottle	each	2258-00
DR/4000 Carousel Module Kit	each	48070-02
Flask, volumetric, 100-mL, Class A	each	14574-42
pH Paper, pH 1.0 to 11.0	5/pkg	391-33
Pipet, serological, 2-mL	each	532-36
Pipet, TenSette, 0.1 to 1.0 mL	each	19700-01
Pipet Tips for 19700-01 TenSette Pipet	50/pkg	21856-96
Pipet, volumetric, Class A, 1.00-mL	each	14515-35
Pipet, volumetric, Class A, 3.00-mL	each	14515-03
Pipet, volumetric, Class A, 5.00-mL	each	14515-37
Pipet Filler, safety bulb	each	14651-00
PourRite Ampule Breaker	each	24846-00

* Larger sizes available



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Telephone: (970) 669-3050
FAX: (970) 669-2932



Method 8039

Cadmium Reduction Method

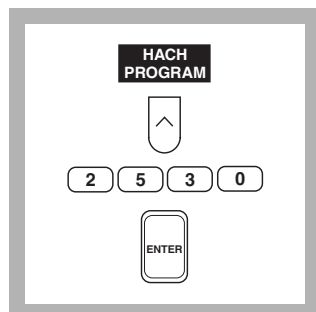
Powder Pillows or AccuVac® Ampuls

HR (0 to 30.0 mg/L NO₃⁻-N)

Scope and Application: For water, wastewater and seawater.

The estimated detection limits for program numbers 2530 and 2535 are 0.5 and 0.3 mg/L NO₃⁻-N, respectively.

Using Powder Pillows



1. Press the soft key under **HACH PROGRAM**.

Select the stored program number for high range nitrate by pressing **2530** with the numeric keys.

Press: **ENTER**

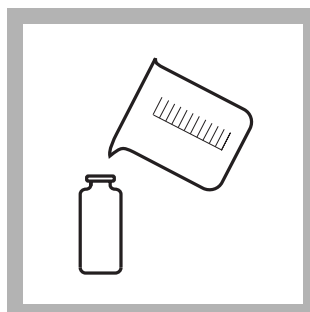
Note: If samples cannot be analyzed immediately, see *Sample Collection, Storage and Preservation* following these steps. Adjust the pH of preserved samples before analysis.



2. The display will show:
**HACH PROGRAM: 2530
N, Nitrate HR**

The wavelength (λ), **500 nm**, is automatically selected.

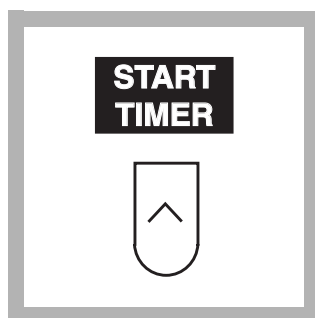
Note: For best results, determine a reagent blank for each new lot of reagent as follows. Prepare a reagent blank by repeating steps 3 through 9, using deionized water as the sample. Zero the instrument on deionized water by pressing the soft key under **ZERO**. Insert the reagent blank and the blank value will be displayed. Correct for the reagent blank by pressing the soft keys under **OPTIONS, (MORE)**, and then **BLANK:OFF**. Enter the reagent blank value and press **ENTER**. Repeat for each new lot of reagent.



3. Fill a sample cell with 10 mL of sample.



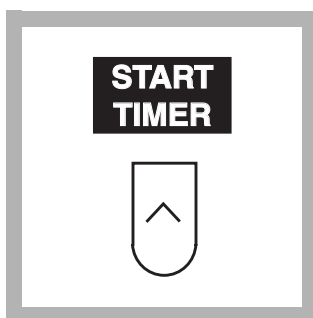
4. Add the contents of one NitraVer 5 Nitrate Reagent Powder Pillow (the prepared sample). Stopper.



5. Press the soft key under **START TIMER**.
Shake the cell vigorously until the timer beeps in one minute.

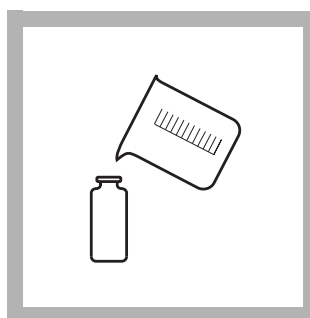
Note: A deposit of unoxidized metal will remain after the NitraVer 5 dissolves. The deposit will not affect results.

Note: Shaking time and technique influence color development. For most accurate results, make successive tests on a 10-mg/L Nitrate Nitrogen Standard Solution listed under **OPTIONAL REAGENTS AND STANDARDS**. Adjust shaking times to obtain the correct result.

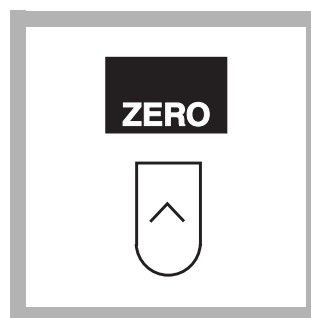


6. When the timer beeps, press the soft key under **START TIMER**.
A 5-minute reaction period will begin.

Note: An amber color will develop if nitrate nitrogen is present.



7. When the timer beeps, fill a second sample cell with 10 mL of sample (the blank). Place the blank into the cell holder.

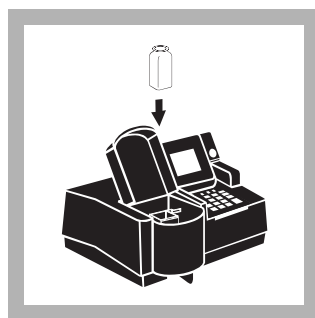


8. Press the soft key under **ZERO**.
The display will show:

0.0 mg/L NO₃⁻-N

Note: If you are using a reagent blank correction, the display will show the correction.

Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



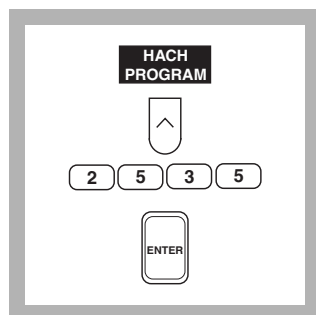
9. Place the prepared sample into the cell holder. Close the light shield. The result in mg/L nitrate nitrogen (NO₃⁻-N) will be displayed.

Note: Measure sample within one minute after timer beeps.

Note: The result can be expressed as mg/L nitrate (NO₃⁻). Press the soft keys under **OPTIONS** and then **FORM** to scroll through the available options.

Note: Rinse the sample cell immediately after use to remove all cadmium particles. Retain the spent sample for proper hazardous waste disposal for cadmium.

Using AccuVac Ampuls



1. Press the soft key under **HACH PROGRAM**.

Select the stored program number for high range nitrate by pressing **2535** with the numeric keys.

Press: **ENTER**

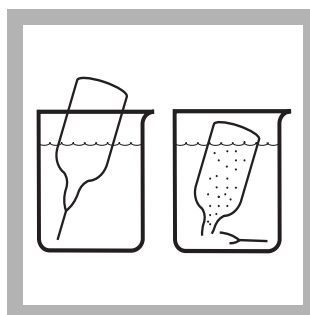
Note: If samples cannot be analyzed immediately, see *Sample Collection, Storage and Preservation* following these steps. Adjust the pH of preserved samples before analysis.



2. The display will show:
HACH PROGRAM: 2535 N, Nitrate HR AV

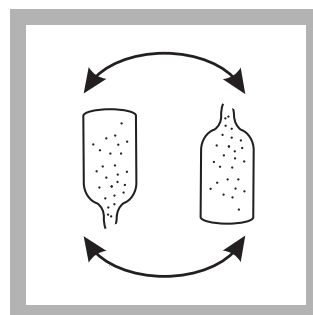
The wavelength (λ), **500 nm**, is automatically selected.

Note: For best results, determine a reagent blank for each new lot of reagent as follows. Prepare a reagent blank by repeating steps 3 through 10, using deionized water as the sample. Zero the instrument on deionized water by pressing the soft key under **ZERO**. Insert the reagent blank and the blank value will be displayed. Correct for the reagent blank by pressing the soft keys under **OPTIONS, (MORE)**, and then **BLANK:OFF**. Enter the reagent blank value and press **ENTER**. Repeat for each new lot of reagent.



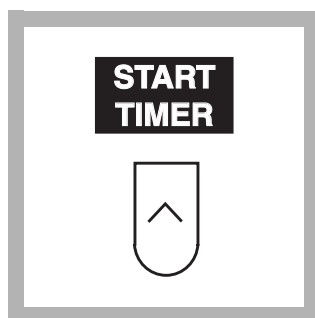
3. Collect at least 40 mL of sample in a 50-mL beaker. Fill a NitraVer 5 Nitrate AccuVac Ampul with sample. Place a stopper over the tip of the ampul.

Note: Keep the tip immersed while the ampul fills.



4. Press the soft key under **START TIMER**. Invert the ampul repeatedly until the timer beeps in one minute. Wipe off any liquid or fingerprints.

Note: Inversion rate can influence color development. For most accurate results, make successive tests on a 10-mg/L Nitrate Nitrogen Standard Solution listed under **OPTIONAL REAGENTS AND STANDARDS**. Adjust inversion rate to obtain the correct result.

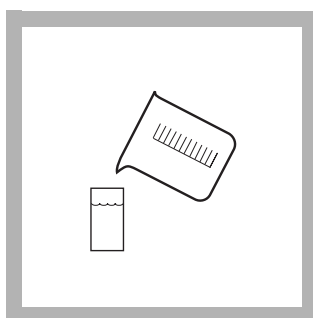


5. When the timer beeps, press the soft key under **START TIMER**.

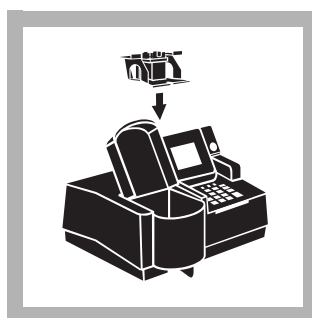
A 5-minute reaction period will begin.

Note: A deposit of unoxidized metal will remain after the NitraVer 5 dissolves. The deposit will not affect results.

Note: An amber color will develop if nitrate nitrogen is present.



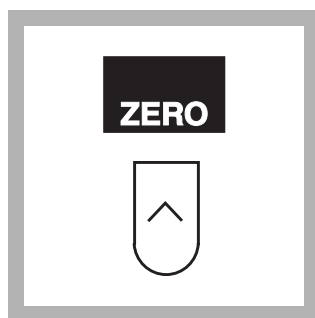
6. When the timer beeps, fill a zeroing vial (the blank) with at least 10 mL of sample.



7. Insert the AccuVac Ampul Adapter into the sample cell module by sliding it under the thumb screw and into the alignment grooves. Fasten with the thumb screw.



8. Place the blank into the cell holder. Close the light shield.



9. Press the soft key under **ZERO**.

The display will show:

0.0 mg/LNO₃⁻-N

Note: If you have entered a reagent blank correction, the display will show the correction.

Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



10. Place the AccuVac Ampul into the cell holder. Close the light shield. Results in mg/L nitrate expressed as nitrogen (NO₃⁻-N) will be displayed.

Note: Measure sample within 1 minute after timer beeps.

Note: The results can be expressed as mg/L nitrate (NO₃⁻). Press the soft keys under **OPTIONS**, then **FORM**: to scroll through the available options.

Interferences

Table 1 Interfering Substances and Suggested Treatments

Interfering Substance	Interference Level and Treatment
Chloride	Chloride concentrations above 100 mg/L will cause low results. The test may be used at high chloride concentrations (seawater) but a calibration must be done using standards spiked to the same chloride concentration.
Ferric iron	All levels
Nitrite	All levels Compensate for nitrite interference as follows: <ol style="list-style-type: none"> 1. Add 30-g/L Bromine Water dropwise to the sample in Step 3 until a yellow color remains. 2. Add one drop of 30-g/L Phenol Solution to destroy the color. 3. Proceed with Step 4. Report the results as total nitrate and nitrite.
pH	Highly buffered samples or extreme sample pH may exceed the buffering capacity of the reagents and require sample pretreatment.
Strong oxidizing and reducing substances	Interfere at all levels

Sample Collection, Storage and Preservation

Most reliable results are obtained when samples are analyzed as soon as possible after collection. If prompt analysis is impossible, store samples in clean plastic or glass bottles for up to 24 hours at 4 °C. To preserve samples for longer periods, add 2 mL of concentrated sulfuric acid (H₂SO₄) per liter and store at 4 °C.

Before analysis, warm the sample to room temperature and adjust the pH to 7 with 5.0 N Sodium Hydroxide Standard Solution. Do not use mercury compounds as preservatives. Correct the test result for volume additions by dividing the total volume (acid + base + sample) by the original sample volume and multiplying the test result by this factor.

Accuracy Check

Standard Solution Method

To test accuracy, use a 10.0 mg/L Nitrate Nitrogen Standard Solution in place of the sample and perform the procedure as described.

To adjust the calibration curve using the reading obtained with the 10.0 mg/L Nitrate Nitrogen Standard Solution, press the soft keys under **OPTIONS, (MORE)** then **STD: OFF**. Press **ENTER** to accept the default concentration, 10.0-mg/L NO₃⁻-N. If an alternate concentration is used, enter the actual concentration and press **ENTER** to return to the read screen. See Section 1.5.5 *Adjusting the Standard Curve* for more information.

Standard Additions Method

- Leave the unspiked sample in the sample compartment. Verify that the units displayed are in mg/L. Select standard additions mode by pressing the soft keys under **OPTIONS, (MORE)** and then **STD ADD**.
- Press **ENTER** to accept the default sample volume (mL), 25. (This is the volume to which standard addition aliquots are added.)
- Press **ENTER** to accept the default standard concentration (mg/L), 500.

- d. Press the soft key under **ENTRY DONE**.
- e. Fill three 25-mL graduated mixing cylinders with 25 mL of sample.
- f. Snap the neck off a Nitrate Nitrogen PourRite Ampule Standard, 500-mg/L NO_3^- -N.
- g. Use the TenSette Pipet to add 0.1, 0.2 and 0.3 mL of standard, respectively, to the three mixing cylinders. Stopper each and mix thoroughly.
- h. For analysis with AccuVac Ampuls, transfer solutions to dry, clean 50-mL beakers to facilitate filling of the ampules. For analysis with powder pillows, transfer only 10 mL of solution to the 10-mL sample cells.
- i. Analyze each standard addition sample as described above. Accept the standard additions readings by pressing the soft key under **READ** each time. Each addition should reflect approximately 100% recovery.
- j. After completing the sequence, the display will show the extrapolated concentration value and the “best-fit” line through the standard additions data points, accounting for matrix interferences.
- k. See Section 1.4.1 *Standard Additions* for more information.

Method Performance

Precision

Standard: 20.0 mg/L NO_3^- -N₂

Program	95% Confidence Limits
2530	19.5–20.5 mg/L NO_3^- -N
2535	19.6–20.4 mg/L NO_3^- -N

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
2530	0.5 mg/L NO_3^- -N
2535	0.3 mg/L NO_3^- -N

For more information on derivation and use of Hach’s estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, Appendix B, see Section 1.5.1.

Sensitivity

Program Number: 2530

Portion of Curve	ΔAbs	$\Delta\text{Concentration}$
0.4 mg/L NO_3^- -N	0.010	0.35 mg/L NO_3^- -N
15.0 mg/L NO_3^- -N	0.010	0.59 mg/L NO_3^- -N
27.0 mg/L NO_3^- -N	0.010	0.72 mg/L NO_3^- -N

Program Number: 2535

Portion of Curve	ΔAbs	$\Delta\text{Concentration}$
0.3 mg/L NO_3^- -N	0.010	0.35 mg/L NO_3^- -N
15.0 mg/L NO_3^- -N	0.010	0.56 mg/L NO_3^- -N
27.0 mg/L NO_3^- -N	0.010	0.68 mg/L NO_3^- -N

See Section 1.5.3 *Sensitivity Explained* for more information.

Calibration Standard Preparation

To perform a nitrate calibration using the NitraVer 5 method, prepare calibration standards containing 4, 14, and 30 mg/L NO_3^- -N as follows:

- Into three different 500-mL Class A volumetric flasks, pipet 2.00, 7.00, and 15.00 mL of a 1000-mg/L Nitrate Nitrogen Standard Solution using Class A glassware.
- Dilute to the mark with deionized water. Mix thoroughly.
- Using the NitraVer 5 Powder Pillow or AccuVac method and the calibration procedure described in the *User-Entered Programs* section of the *DR/4000 Spectrophotometer Instrument Manual*, generate a calibration curve from the standards prepared above.

Summary of Method

Cadmium metal reduces nitrates in the sample to nitrite. The nitrite ion reacts in an acidic medium with sulfanilic acid to form an intermediate diazonium salt. The salt couples with gentisic acid to form an amber colored solution.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to Section 1.

Pollution Prevention and Waste Management

Prepared samples will contain cadmium and must be disposed of according to Federal, State and local hazardous waste regulations. For information on pollution prevention and waste management, refer to Section 1.

NITRATE, continued

REQUIRED REAGENTS AND STANDARDS (Using Powder Pillows)

Description	Quantity Required per test	Unit	Cat. No.
NitraVer 5 Nitrate Reagent Powder Pillows for 10 mL sample	1 pillow	100/pkg	21061-69

REQUIRED REAGENTS AND STANDARDS (Using AccuVac Ampuls)

NitraVer 5 Nitrate Reagent AccuVac Ampul	1 ampul	25/pkg	25110-25
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REQUIRED EQUIPMENT AND SUPPLIES (Using Powder Pillows)

DR/4000 1-Inch Cell Adapter	1	each	48190-00
Sample Cells, matched pair, 1-inch, glass, with stoppers	2	pair	26126-02

REQUIRED EQUIPMENT AND SUPPLIES (Using AccuVac Ampuls)

Beaker, 50-mL	1	each	500-41
DR/4000 AccuVac Ampul Adapter	1	each	48187-00
Sample Cell, 10-mL with cap (zeroing vial)	1	each	21228-00
Stopper	1	6/pkg	1731-06

OPTIONAL REAGENTS AND STANDARDS

Bromine Water, 30-g/L	29 mL*	2211-20
Nitrate Nitrogen Standard Solution, 10.0-mg/L NO ₃ ⁻ -N	500 mL	307-49
Nitrate Nitrogen Standard Solution, 1000-mg/L NO ₃ ⁻ -N	500 mL	12792-49
Nitrate Nitrogen Standard Solution, PourRite Ampule, 500-mg/L NO ₃ ⁻ -N, 2 mL	20/pkg	14260-20
Phenol Solution, 30-g/L	29 mL*	2112-20
Sodium Hydroxide Standard Solution, 5.0 N	1 liter	2450-53
Sulfuric Acid, ACS	500 mL*	979-49
Water, deionized	4 liters	272-56

OPTIONAL EQUIPMENT AND SUPPLIES

AccuVac Snapper	each	24052-00
Cylinder, graduated, mixing, 25 mL	each	20886-40
Dropper, for 29-mL bottle	each	2258-00
DR/4000 Carousel Module Kit	each	48070-02
Flask, volumetric, 500-mL, Class A	each	14574-49
Pipet, serological, 2 mL	each	532-36
Pipet, TenSette, 0.1 to 1.0 mL	each	19700-01
Pipet Tips for 19700-01 TenSette Pipet	50/pkg	21856-96
Pipet, volumetric, Class A, 2.00-mL	each	14515-36
Pipet, volumetric, Class A, 7.00-mL	each	14515-07
Pipet, volumetric, Class A, 15.00-mL	each	14515-39
Pipet Filler, safety bulb	each	14651-00
pH Paper, pH 1.0 to 11.0	5/pkg	391-33
PourRite Ampule Breaker	each	24846-00

* Larger sizes available



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Method 10020

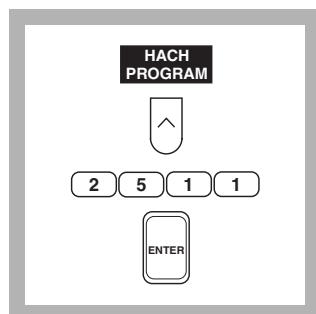
Chromotropic Acid Method

Test 'N Tube™ Vials

HR (0 to 30.0 mg/L NO₃⁻-N)

Scope and Application: For water and wastewater.

The estimated detection limit for program number 2511 is 0.2 mg/L NO₃⁻-N.



1. Press the soft key under **HACH PROGRAM**.

Select the stored program number for Nitrate, Test 'N Tube method, by pressing **2511** with the numeric keys.

Press: **ENTER**

Note: If samples cannot be analyzed immediately, see Sample Collection, Preservation and Storage following these steps.

Note: The Flow Cell and Sipper Modules cannot be used with this method.



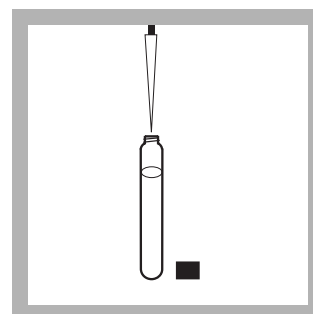
2. The display will show:

**HACH PROGRAM: 2511
N, Nitrate HR TNT**

The wavelength (λ), **410 nm**, is automatically selected.



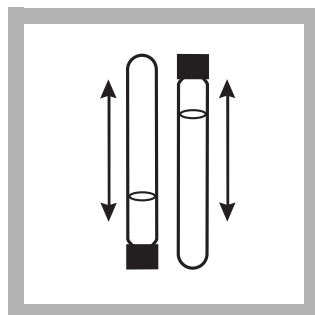
3. Insert the Test Tube Adapter into the sample cell module by sliding it under the thumb screw and into the alignment grooves. Fasten with the thumb screw.



4. Remove the cap from a Nitrate Pretreatment Solution Test 'N Tube vial and add 1.00 mL of sample (this will be the sample blank).

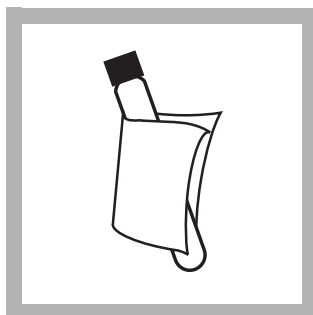
Note: For proof of accuracy, use a 15-mg/L Nitrate Nitrogen Standard Solution (listed under **OPTIONAL REAGENTS AND STANDARDS**) in place of the sample.

Note: Run a reagent blank for this test. Use nitrate-free water in place of the sample. Subtract this result from all test results run with these lots of reagents. Determine a new reagent blank when the reagent lots change.



5. Cap the tube and invert it 10 times to mix.

Note: This test is technique-sensitive. If these instructions are not followed, low results may occur. Hold the tube in a vertical position with the cap pointing up. Invert the vial so the cap now points down. Wait for all of the solution to flow to the cap end. Pause. Return the vial to the original position. Wait for all the solution to flow to the vial bottom. This process equals one inversion. Do this 10 times.

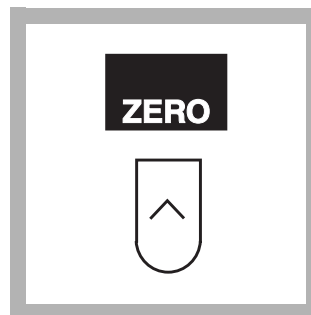


6. Clean the outside of the vial with a towel.

Note: Wiping with a damp towel, followed by a dry one, will remove fingerprints or other marks.



7. Place the sample blank into the cell holder and close the light shield.

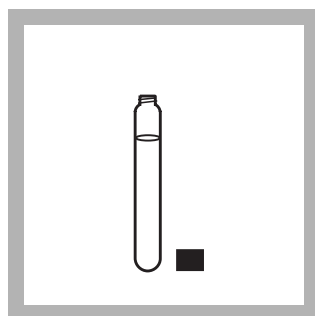


8. Press the soft key under **ZERO**.

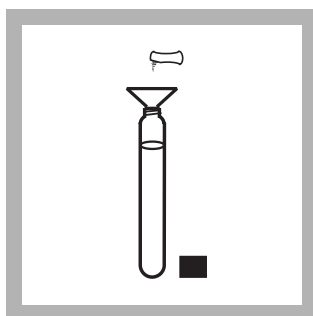
The display will show:

0.0 mg/L NO₃⁻-N

Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



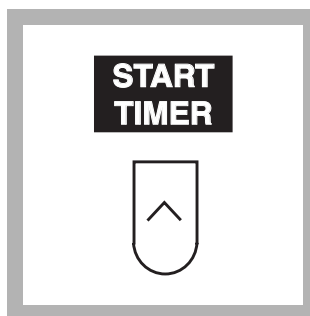
9. Remove the vial from the instrument. Remove the cap from the vial.



10. Using a funnel, add the contents of one NitraVer X Reagent B Powder Pillow to the vial. Cap and invert 10 times to mix. (This will be the prepared sample.)

Note: See Step 5 for inversion instructions.

Note: Some solid matter will not dissolve.



11. Press the soft key under **START TIMER**.

A 5-minute reaction period will begin. Do not invert the vial again.

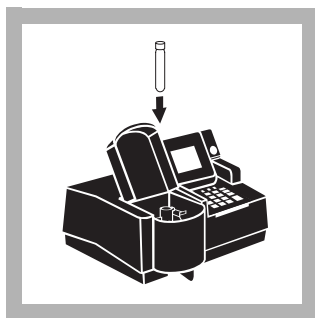
Note: A yellow color will develop if nitrate nitrogen is present.

Note: Complete steps 12-13 within 5 minutes after the timer beeps.



12. When the timer beeps, clean the outside of the vial with a towel.

Note: Wiping with a damp towel, followed by a dry one, will remove fingerprints or other marks.



13. Place the prepared sample into the cell holder and close the light shield. The result in mg/L nitrate nitrogen (or chosen units) will be displayed.

Note: Measure the sample within 5 minutes after the timer beeps.

Note: The results can be expressed as nitrate (NO_3^-) or NO_3^- -N. Press the soft keys under **METHOD OPTIONS**, then **FORM**: to scroll through the available options.

Interferences

Table 1 Interfering Substances and Suggested Treatments

Interfering Substance	Interference Level
Barium	A negative interference at concentrations greater than 1 mg/L
Chloride	Does not interfere below 1000 mg/L
Hardness	Does not interfere
Nitrite	A positive interference at concentrations greater than 12 mg/L. Remove nitrite interference up to 100 mg/L by adding 400 mg of urea (one full 0.5 g Hach measuring spoon) to 10 mL of sample. Swirl to dissolve. Proceed with the nitrate test as usual.

Sample Collection, Preservation and Storage

Collect samples in clean plastic or glass bottles. Store at 4 °C (39 °F) or lower if the sample is to be analyzed within 24 to 48 hours. Warm to room temperature before running the test. For longer storage periods (up to 14 days), adjust sample pH to 2 or less with concentrated sulfuric acid, ACS (about 2 mL per liter). Sample refrigeration is still required.

Before testing the stored sample, warm to room temperature and neutralize with 5.0 N Sodium Hydroxide Standard Solution.

Do not use mercury compounds as preservatives.

Correct the test result for volume additions; see Section 1.2.2 *Correcting for Volume Additions*.

Accuracy Check

Standard Additions Method

- a. Leave the unspiked sample in the sample compartment. Verify that the units displayed are in mg/L. Select standard additions mode by pressing the soft keys under **OPTIONS, (MORE)** and then **STD ADD**.
- b. Press **ENTER** to accept the default sample volume (mL), 25.0.
- c. Press **ENTER** to accept the default standard concentration (mg/L), 500.0.
- d. Press the soft key under **ENTRY DONE**.
- e. Snap the neck off a High Range Nitrate Nitrogen Voluette Ampule Standard, 500-mg/L NO₃⁻-N.
- f. Use the TenSette Pipet to add 0.1, 0.2, and 0.3 mL of standard, respectively, to three 25-mL samples and mix each thoroughly.
- g. Analyze each standard addition sample as described above. Accept the standard additions reading by pressing the soft key under **READ** each time. Each addition should reflect approximately 100% recovery.
- h. After completing the sequence, the display will show the extrapolated concentration value and the “best-fit” line through the standard additions data points, accounting for matrix interferences.
- i. See Section 1.4.1 *Standard Additions* for more information.

Standard Solution Method

Use a 15.0-mg/L Nitrate Nitrogen Standard Solution listed under *OPTIONAL REAGENTS AND STANDARDS* to check test accuracy. Or, this can be prepared by pipetting 3.00 mL of solution from a High Range Nitrate Nitrogen 10-mL Voluette Ampule Standard Solution, 500-mg/L NO₃⁻-N, into a 100-mL Class A volumetric flask. Dilute to the mark with deionized water.

Method Performance

Precision

Standard: 15.0 mg/L NO₃⁻-N

Program	95% Confidence Limits
2511	14.8–15.2 mg/L NO ₃ ⁻ -N

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
2511	0.2 mg/L NO ₃ ⁻ -N

For more information on derivation and use of Hach’s estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, Appendix B, see Section 1.5.1.

Sensitivity

Program Number: 2511

Portion of Curve	Δ Abs	Δ Concentration
Entire Range	0.010	0.22 mg/L

See Section 1.5.3 *Sensitivity Explained* for more information.

Calibration Standard Preparation

To perform a nitrate calibration using the Test 'N Tube Chromotropic Acid method, prepare calibration standards containing 4, 14, and 30 mg/L NO_3^- -N as follows:

- a. Into three different 500-mL Class A volumetric flasks, pipet 2.00, 7.00, and 15.00 mL of a 1000-mg/L Nitrate Nitrogen Standard Solution using Class A glassware.
- b. Dilute to the mark with deionized water. Mix thoroughly.
- c. Using the Test 'N Tube Chromotropic Acid method and the calibration procedure described in the *User-Entered Programs* section of the *DR/4000 Spectrophotometer Instrument Manual*, generate a calibration curve from the standards prepared above.

Summary of Method

Nitrate in the sample reacts with chromotropic acid under strongly acidic conditions to yield a yellow product with a maximum absorbance at 410 nm.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to Section 1.

Pollution Prevention and Waste Management

For information on pollution prevention and waste management, refer to Section 1.

NITRATE, continued

REQUIRED REAGENTS AND STANDARDS

	Cat. No.
Test 'N Tube NitraVer X Nitrate Reagent Set (50 tests)	26053-45
Includes: (1) 26055-46, (1) 272-42, (50) Nitrate Pretreatment Solution Vials*	

Description	Quantity Required		Unit	Cat. No.
	Per Test			
Nitrate Pretreatment Solution Vials.....	1		50/pkg	*
NitraVer X Reagent B Powder Pillows	1		50/pkg	26055-46

REQUIRED EQUIPMENT AND SUPPLIES

DR/4000 Test Tube Adapter.....	1	each	48189-00
Funnel, micro, poly	1	each	25843-35
Pipet, TenSette, 0.1 to 1.0 mL	1	each	19700-01
Pipet Tips, for 19700-01 TenSette Pipet	varies	50/pkg	21856-96
Test Tube Rack, cooling	1-3	each	18641-00

OPTIONAL REAGENTS AND STANDARDS

Nitrate Nitrogen Standard Solution, 15-mg/L N	100 mL	MDB	24151-32
Nitrate Nitrogen Standard Solution, 1000-mg/L N	500 mL		12792-49
Nitrate Nitrogen Standard Solution, Voluette Ampule, 500-mg/L N	16/pkg		14260-10
Sodium Hydroxide Standard Solution, 5.0 N	50 mL		2450-26
Sulfuric Acid, concentrated, ACS	500 mL		979-49
Urea, ACS	100 g		11237-26
Water, deionized	4 liters		272-56

OPTIONAL EQUIPMENT AND SUPPLIES

Ampule Breaker Kit	each	21968-00
Flask, volumetric, Class A, 100-mL	each	14574-42
Flask, volumetric, Class A, 500-mL	each	14574-49
pH Paper, pH 1.0 to 11.0	5 rolls/pkg	391-33
Pipet, serological, 2-mL	each	532-36
Pipet, volumetric, Class A, 2.00-mL	each	14515-36
Pipet, volumetric, Class A, 3.00-mL	each	14515-03
Pipet, volumetric, Class A, 7.00-mL	each	14515-07
Pipet, volumetric, Class A, 15.00-mL	each	14515-39
Pipet Filler	each	12189-00
Spoon, measuring, 0.5-g	each	907-00

* Items not sold separately.



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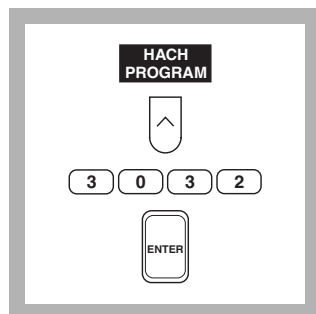


UniCell™ Vials

(0.0 to 13.5 mg/L NO_3^- -N)

Scope and Application: For water and wastewater process control.

The estimated detection limit for program number 3032 is 0.2 mg/L NO_3^- -N.



1. Press the soft key under **HACH PROGRAM**.

Select the stored program number for UniCell Nitrate, by pressing **3032** with the numeric keys.

Press: **ENTER**

Note: If samples cannot be analyzed immediately, see Sample Collection, Preservation, and Storage following these steps.

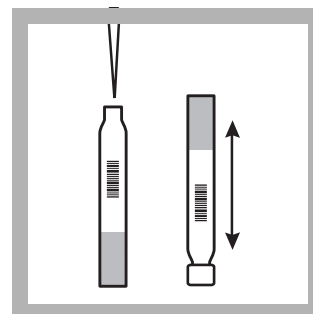


2. The display will show:
HACH PROGRAM: 3032 Nitrate, HCT 106

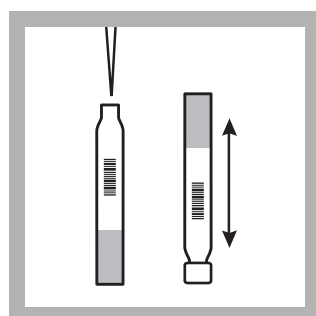
The wavelength (λ), **370 nm**, is automatically selected.



3. Insert the Test Tube Adapter into the sample cell module by sliding it under the thumb screw and into the alignment grooves. Fasten with the thumb screw.

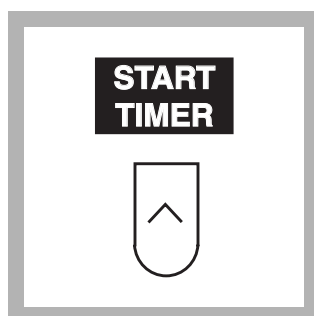


4. Remove the cap from a sample vial and add 0.2 mL of dimethylphenol solution (HCT 106 A). Cap and invert to mix.



5. Immediately remove the cap and pipet 1.0 mL of sample into the vial. Cap and invert to mix.

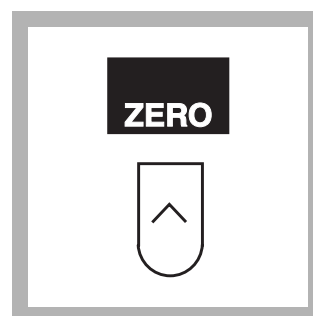
Note: For proof of accuracy, use a 6-mg/L Nitrate Nitrogen Standard Solution in place of the sample. See Accuracy Check, Standard Solution Method.



6. Press the soft key under **START TIMER**. A 15-minute reaction period will begin.



7. Wipe the zero vial. Place the provided zero vial (white cap) into the cell holder and close the light shield.

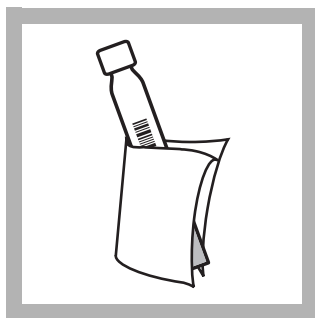


8. Press the soft key under **ZERO**.

The display will show:

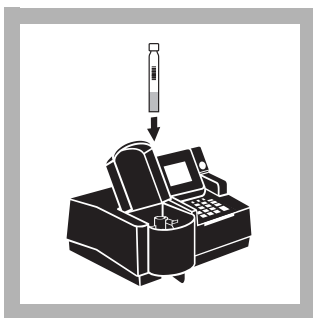
0.0 mg/L NO_3

Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



9. When the timer beeps, clean the outside of the sample vial with a towel.

Note: Wiping with a damp towel, followed by a dry one, will remove fingerprints or other marks.



10. Place the prepared sample into the cell holder and close the light shield. The result in mg/L nitrate (NO_3^-) will be displayed.

Note: The results can also be expressed as NO_3^- -N. Press the soft keys under **METHOD OPTIONS**, then **FORM**, to scroll through the available options.

Interferences

The ions listed in the following table have been individually tested up to the given concentrations. Cumulative effects and the influence of other ions have not been evaluated.

Table 1 Substances That Do Not Interfere

Ion	No interference up to:
K^+ , Na^+ , Cl^-	500 mg/L
COD	200 mg/L
Ag^+	100 mg/L
Pb^{2+} , Zn^{2+} , Ni^{2+} , Fe^{3+} , Cd^{2+} , Sn^{2+} , Ca^{2+} , Cu^{2+}	50 mg/L
Co^{2+} , Fe^{2+}	10 mg/L
Cr^{6+}	5 mg/L
NO_2^-	2 mg/L

Table 2 Substances That Interfere

Substance	Interferes at levels greater than:
COD	200 mg/L (high bias results)
Nitrite	2 mg/L (high bias results)
Chloride	500 mg/L (low bias results)

Removal of Interferences

Nitrite concentrations of more than 2 mg/L can be removed by adding 30 mg of sulfamic acid per 10 mL of sample. High Chloride (more than 500 mg/L) can be precipitated out as silver chloride by adding 1 g of silver oxide per 10 mL of sample. The addition of 5 drops of EDTA to the sample prevents turbidity caused by high calcium concentrations (above 50 mg/L).

Sample Collection, Preservation, and Storage

Analyze samples within 3 hours after collection for best results. For longer storage periods, add 1 mL of concentrated sulfuric acid per liter of sample and store at 4 °C.

Before testing the stored sample, warm to room temperature and adjust the pH to 3–10 with 5.0 N Sodium Hydroxide Standard Solution.

Do not use mercury compounds as preservatives.

Correct for volume additions by dividing the total final volume (acid + base + sample) by the initial sample volume and multiplying the test result by this factor. See Section 1.2.2 *Correcting for Volume Additions* for more information.

Accuracy Check

Standard Additions Method

- a. Leave the unspiked sample in the sample compartment. Verify that the units displayed are in mg/L. Select standard additions mode by pressing the soft keys under **OPTIONS, (MORE)** and then **STD ADD**.
- b. Press **ENTER** to accept the default sample volume (mL), 100.
- c. Use the numeric keys to enter the standard concentration, 4427-mg/L NO₃.

Note: This is equivalent to 1000 mg/L NO₃⁻-N.

- d. Press the soft key under **ENTRY DONE**.
- e. Use a pipet to add 0.2, 0.4, and 0.6 mL of 1000-mg/L NO₃⁻-N (4427-mg/L NO₃) standard, respectively, to three 100-mL mixing cylinders filled with 100 mL of sample. Mix each sample thoroughly.
- f. Analyze each standard addition sample as described above. Accept the standard additions reading by pressing the soft key under **READ** each time. Each addition should reflect approximately 100% recovery.
- g. After completing the sequence, the display will show the extrapolated concentration value and the “best-fit” line through the standard additions data points, accounting for matrix interferences.
- h. See Section 1.4.1 *Standard Additions* for more information.

Standard Solution Method

Prepare a 6-mg/L NO₃⁻-N standard solution by pipetting 6 mL of 1000-mg/L NO₃⁻-N into a 1000-mL volumetric flask. Dilute to the mark with deionized water. Stopper and invert to mix. Prepare this solution daily. Perform the nitrate procedure as described.

Method Performance

Precision

Standard: 6.0 mg/L NO₃⁻-N

Program	95% Confidence Limits
3032	5.4–6.6 mg/L NO ₃ ⁻ -N

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
3032	0.2 mg/L NO ₃ ⁻ -N

For more information on derivation and use of Hach's estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by 40 CFR part 136, Appendix B, see Section 1.5.1.

Sensitivity

Program Number: 3032

Portion of Curve	ΔAbs	ΔConcentration
Entire Range	0.010	0.1 mg/L NO ₃ ⁻ -N

See Section 1.5.3 *Sensitivity Explained* for more information.

Summary of Method

Nitrate ions react with 2,6-dimethylphenol in a solution of sulfuric and phosphoric acids to form 4-nitro-2,6-dimethylphenol.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to Section 1.

Sample vial — contains 60% sulfuric acid and 33% phosphoric acid.

Dimethylphenol Solution A —(HCT 106 A) contains 2 propanol.

Pollution Prevention and Waste Management

For information on pollution prevention and waste management, refer to Section 1.

REQUIRED REAGENTS AND STANDARDS

	Unit	Cat. No.
Nitrate UniCell™ HCT 106	23/pkg	HCT 106

OPTIONAL REAGENTS AND STANDARDS

Description	Quantity Required Per Test	Unit	Cat. No.
Nitrate Nitrogen Standard Solution, 1000-mg/L NO ₃ ⁻ -N	500	mL	12792-49
Sulfuric Acid, concentrated, ACS	500	mL	979-49
Sodium Hydroxide Standard Solution, 5.0 N	50	mL SCDB	2450-26
EDTA Solution	50	mL SCDB	22419-26

OPTIONAL EQUIPMENT AND SUPPLIES

Cylinder, mixing, 100-mL	3	each	20886-42
Flask, volumetric, Class A, 1000-mL	1	each	14574-53
Pipettor, Jencon, 100–1000 µL	1	each	27949-00
Replacement tips for 27949-00	400	/pkg	27950-00
Pipettor, Jencon, 1–5 mL	1	each	27951-00
Replacement tips for 27951-00	100	/pkg	27952-00



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✓ Method 8507

Diazotization Method

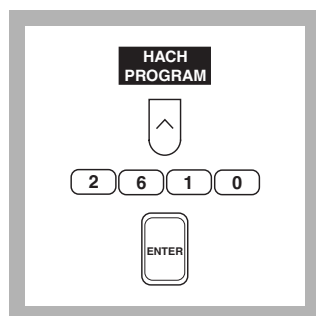
Powder Pillows or AccuVac® Ampuls

LR (0 to 0.300 mg/L NO₂⁻-N)

Scope and Application: For water, wastewater and seawater; USEPA Approved* for wastewater analysis. The estimated detection limit for program numbers 2610 and 2620 are 0.0008 and 0.004 mg/L NO₂⁻-N, respectively.

* Federal Register, 44(85), 25505 (May 1, 1979)

Using Powder Pillows



- 1.** Press the soft key under **HACH PROGRAM**.

Select the stored program number for low range nitrite by pressing **2610** with the numeric keys.

Press: **ENTER**

Note: If samples cannot be analyzed immediately, see *Sample Collection, Storage and Preservation* following these steps.

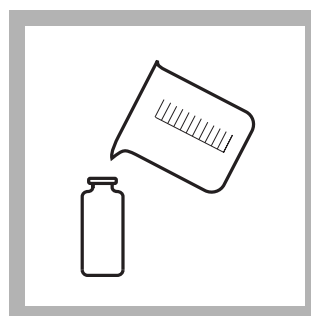
Note: The Flow Cell and Sipper Modules can be used with this procedure. Use 25-mL samples and reagents with the Flow Cell Module.



- 2.** The display will show:
**HACH PROGRAM: 2610
Nitrite, LR**

The wavelength (λ), **507 nm**, is automatically selected.

Note: For best results, determine a reagent blank for each new lot of reagent as follows. Prepare a reagent blank by repeating steps 3 through 8, using deionized water as the sample. Zero the instrument on deionized water by pressing the soft key under **ZERO**. Insert the reagent blank and the blank value will be displayed. Correct for the reagent blank by pressing the soft keys under **OPTIONS, (MORE)**, and then **BLANK:OFF**. Enter the reagent blank value and press **ENTER**. Repeat for each new lot of reagent.

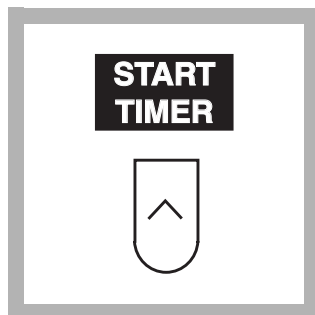


- 3.** Fill a sample cell with 10 mL of sample.



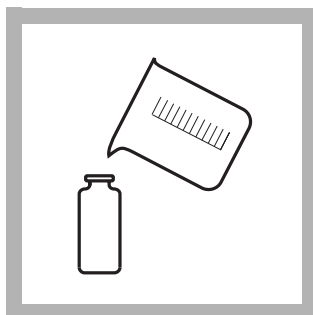
- 4.** Add the contents of one NitraVer 3 Nitrate Reagent Powder Pillow (the prepared sample). Stopper. Shake to dissolve.

Note: A pink color will develop if nitrite is present.

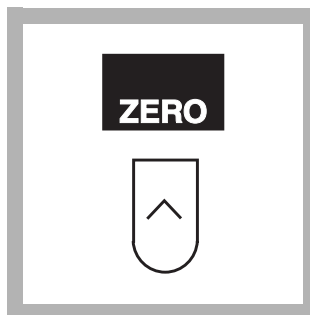


5. Press the soft key under **START TIMER**.

A 20-minute reaction period will begin.



6. When the timer beeps, fill a second sample cell with 10 mL of sample (the blank). Place the blank into the cell holder.



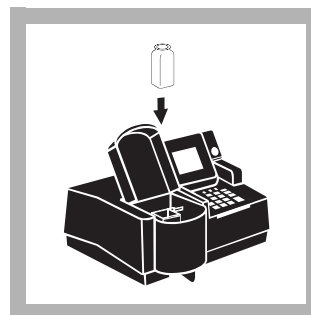
7. Press the soft key under **ZERO**.

The display will show:

0.0000 mg/L NO₂⁻-N

Note: If you are using a reagent blank correction, the display will show the correction.

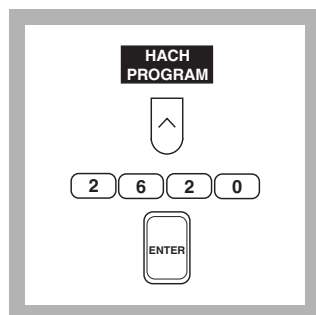
Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



8. Remove the stopper. Place the prepared sample into the cell holder. Close the light shield. Result in mg/L nitrite nitrogen (NO₂⁻-N) will be displayed.

Note: The result can be expressed as mg/L nitrite (NO₂⁻). Press the soft keys under **OPTIONS** and then **FORM** to scroll through the available options.

Using AccuVac Ampuls



1. Press the soft key under **HACH PROGRAM**.

Select the stored program number for low range nitrite by pressing **2620** with the numeric keys.

Press: **ENTER**

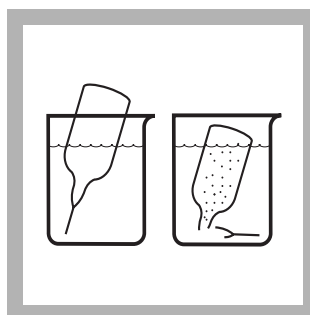
Note: If samples cannot be analyzed immediately, see *Sample Collection, Storage and Preservation* following these steps.



2. The display will show: **HACH PROGRAM: 2620 Nitrate, LR AV**

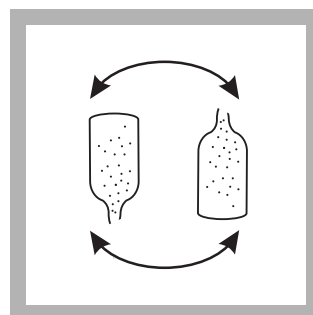
The wavelength (λ), **507 nm**, is automatically selected.

Note: For best results, determine a reagent blank for each new lot of reagent as follows. Prepare a reagent blank by repeating steps 3 through 10, using deionized water as the sample. Zero the instrument on deionized water by pressing the soft key under **ZERO**. Insert the reagent blank and the blank value will be displayed. Correct for the reagent blank by pressing the soft keys under **OPTIONS, (MORE)**, and then **BLANK:OFF**. Enter the reagent blank value and press **ENTER**. Repeat for each new lot of reagent.



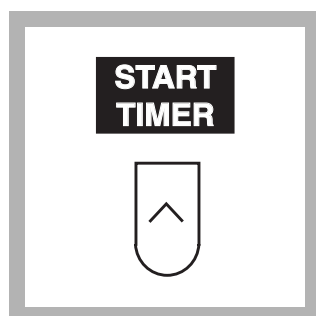
3. Collect at least 40 mL of sample in a 50-mL beaker. Fill a NitraVer 3 Nitrate AccuVac Ampul with sample.

Note: Keep the tip immersed while the ampul fills.



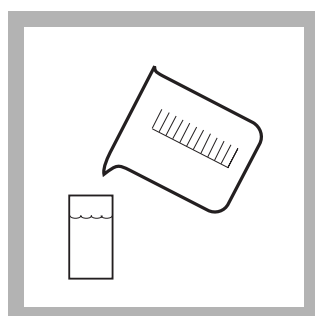
4. Invert the ampul several times to mix. Wipe off any liquid or fingerprints.

Note: A pink color will develop if nitrite is present.



5. Press the soft key under **START TIMER**.

A 20-minute reaction period will begin.



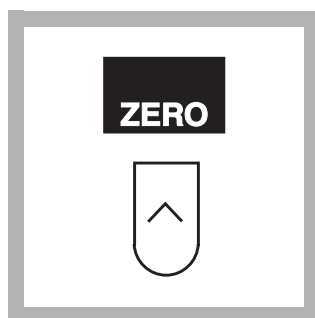
6. When the timer beeps, fill a zeroing vial (the blank) with at least 10 mL of sample.



7. Insert the AccuVac Ampul Adapter into the sample cell module by sliding it under the thumb screw and into the alignment grooves. Fasten with the thumb screw.



8. Place the blank into the cell holder. Close the light shield.



9. Press the soft key under **ZERO**.

The display will show:

0.0000 mg/L NO₂⁻-N

Note: If you are using a reagent blank correction, the display will show the correction.

Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



10. Place the AccuVac Ampul into the cell holder. Close the light shield. Results in mg/L nitrate expressed as nitrogen (NO₂⁻-N) will be displayed.

Note: The results can be expressed as mg/L nitrate (NO₂⁻). Press the soft keys under **METHOD OPTIONS**, then **FORM**: to scroll through the available options.

Interferences

Table 1 Interfering Substances and Suggested Treatments

Interfering Substance	Interference Levels
Antimonious ions	Interfere by causing precipitation
Auric ions	Interfere by causing precipitation
Bismuth ions	Interfere by causing precipitation
Chloroplatinate ions	Interfere by causing precipitation
Cupric ions	Cause low results
Ferric ions	Interfere by causing precipitation
Ferrous ions	Cause low results
Lead ions	Interfere by causing precipitation
Mercurous ions	Interfere by causing precipitation
Metavanadate ions	Interfere by causing precipitation
Nitrate	Very high levels of nitrate (>100 mg/L nitrate as N) appear to undergo a slight amount of reduction to nitrite, either spontaneously or during the course of the test. A small amount of nitrite will be found at these levels.
Silver ions	Interfere by causing precipitation
Strong oxidizing and reducing substances	Interfere at all levels

Sample Collection, Storage and Preservation

Collect samples in clean plastic or glass bottles. Store at 4 °C (30 °F) or lower if the sample is to be analyzed within 24 to 48 hours. Warm to room temperature before running the test.

Accuracy Check

Standard Solution Method

Preparing nitrite standards is difficult. A standard should be prepared by a trained chemist. Hach recommends using the standard preparation instructions in *Standard Methods for the Examination of Water and Wastewater*, Method 4500-NO₂⁻ B (p. 4–86 of 18th edition) Prepare a 0.150-mg/L standard.

Method Performance

Precision

Standard: 0.1500 mg/L NO₂⁻-N

Program	95% Confidence Limits
2610	0.1494–0.1506 mg/L NO ₂ ⁻ -N
2620	0.1496–0.1504 mg/L NO ₂ ⁻ -N

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
2610	0.0008 mg/L NO ₂ ⁻ -N
2620	0.0043 mg/L NO ₂ ⁻ -N

For more information on derivation and use of Hach's estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, appendix B, see Section 1.5.1.

Sensitivity

Program Number: 2610

Portion of Curve	ΔAbs	ΔConcentration
Entire Range	0.010	0.00187 mg/L

Program Number: 2620

Portion of Curve	ΔAbs	ΔConcentration
Entire Range	0.010	0.00203 mg/L

See Section 1.5.3 *Sensitivity Explained* for more information.

Calibration Standard Preparation

Preparing nitrite standards is difficult. Calibration should be performed by a trained chemist. Hach recommends using the standard preparation instructions in *Standard Methods for the Examination of Water and Wastewater*, Method 4500-NO₂⁻ B (p. 4–86 of 18th edition).

Using the standards prepared above and the analysis procedure, generate a calibration curve.

Summary Of Method

Nitrite in the sample reacts with sulfanilic acid to form an intermediate diazonium salt. This couples with chromotropic acid to produce a pink colored complex directly proportional to the amount of nitrite present.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to Section 1.

Pollution Prevention and Waste Management

For information on pollution prevention and waste management, refer to Section 1.

REQUIRED REAGENTS AND STANDARDS (Using Powder Pillows)

Description	Quantity Required		Unit	Cat. No.
	Per Test			
NitriVer 3 Nitrite Reagent Powder Pillows	1 pillow	100/pkg		21071-69

REQUIRED REAGENTS AND STANDARDS (Using AccuVac Ampuls)

NitriVer 3 Nitrite Reagent AccuVac Ampul	1 ampul	25/pkg	25120-25
--	---------------	--------------	----------

REQUIRED EQUIPMENT AND SUPPLIES (Using Powder Pillows)

DR/4000 1-Inch Cell Adapter	1	each	48190-00
Sample Cells, matched pair, 1-inch, glass, with stoppers	2	pair	26126-02

REQUIRED EQUIPMENT AND SUPPLIES (Using AccuVac Ampuls)

Beaker, 50-mL	1	each	500-41
DR/4000 AccuVac Ampul Adapter	1	each	48187-00
Sample Cell, with cap (zeroing vial)	1	each	21228-00

OPTIONAL REAGENTS AND STANDARDS

Sodium Nitrite, ACS	454 g	2452-01
Water, deionized	4 liters	272-56

OPTIONAL EQUIPMENT AND SUPPLIES

Balance, analytical, 110 VAC	each	26103-00
Balance, analytical, 220 VAC	each	26103-02
DR/4000 Carousel Module Kit	each	48070-02
DR/4000 Flow Cell Module Kit, 1-inch	each	48070-04
DR/4000 Flow Cell Module Kit, 1-cm	each	48070-05
DR/4000 Sipper Module Kit, 1-inch	each	48090-03
Flask, volumetric, 1000-mL, Class B	each	547-53
Pipet, serological, 10-mL	each	532-38
Pipet, TenSette, 0.1 to 1.0 mL	each	19700-01
Pipet Tips for 19700-01 TenSette Pipet	50/pkg	21856-96
Pipet, volumetric, Class A, 1.00-mL	each	14515-35
Pipet Filler, safety bulb	each	14651-00



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FAX: (970) 669-2932



Method 10019

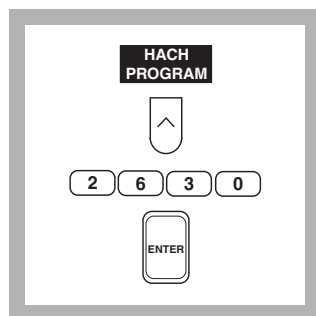
Diazotization Method

Test 'N Tube™ Vials

LR (0 to 0.500 mg/L NO₂⁻-N)

Scope and Application: For water, wastewater and seawater.

The estimated detection limit for program number 2630 is 0.0013 mg/L NO₂⁻-N.



- 1.** Press the soft key under **HACH PROGRAM**.

Select the stored program for the Test 'N Tube Nitrite method by pressing **2630** with the numeric keys.

Press: **ENTER**

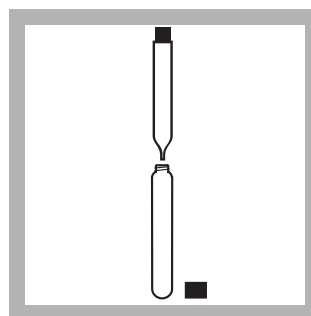
Note: If sample cannot be analyzed immediately, see *Sample Collection, Storage and Preservation* following these steps.



- 2.** The display will show: **HACH PROGRAM: 2630 Nitrite, TNT**

The wavelength (λ), **507 nm**, is automatically selected.

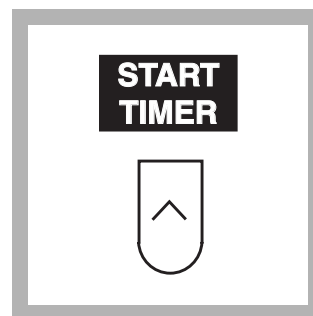
Note: For best results, determine a reagent blank for each new lot of reagent as follows. Prepare a reagent blank by repeating Steps 3 through 10, using deionized water as the sample. Zero the instrument on deionized water by pressing the soft key under **ZERO**. Insert the reagent blank and the blank value will be displayed. Correct for the reagent blank by pressing the soft keys under **OPTIONS, (MORE)**, and then **BLANK:OFF**. Enter the reagent blank value and press **ENTER**. Repeat for each new lot of reagent.



- 3.** Fill a Test 'N Tube NitriVer 3 Nitrite vial with 5 mL of sample. Cap and shake to dissolve powder. This is the prepared sample.

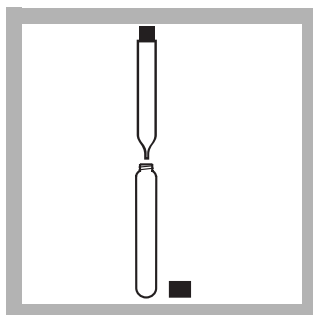
Note: For proof of accuracy, use a 0.250-mg/L nitrite nitrogen standard solution (preparation given in the *Accuracy Check* section) in place of the sample.

Note: A pink color will develop if nitrite nitrogen is present.



- 4.** Press the soft key under **START TIMER**.

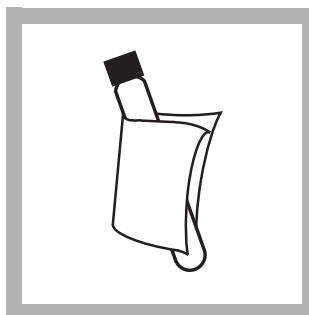
A 20-minute reaction period will begin.



5. When the timer beeps, fill an empty Test 'N' Tube vial with 5 mL of sample (the blank).



6. Insert the Test 'N' Tube Adapter into the sample cell module by sliding it under the thumb screw and into the alignment grooves. Fasten with the thumb screw.

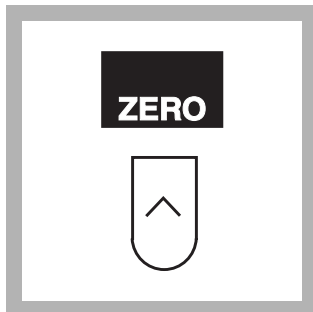


7. Wipe the outside of the vials with a towel.

Note: Wiping with a damp towel, followed by a dry one, removes fingerprints and other marks.



8. Place the blank into the cell holder.



9. Press the soft key under **ZERO**.

The display will show:

0.0000 mg/L NO₂⁻-N

Note: If you are using a reagent blank correction, the display will show the correction.

Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



10. Place the prepared sample into the cell holder. The result in mg/L nitrite expressed as nitrogen will be displayed.

Note: The results can be expressed as NO₂⁻. Press the soft keys under **METHOD OPTIONS**, then **FORM**: to scroll through the available options. Press **ENTER** to return to the read screen.

Interferences

Interfering Substance	Interference Levels and Treatments
Antimonious ions	Interfere by causing precipitation
Auric ions	Interfere by causing precipitation
Bismuth ions	Interfere by causing precipitation
Chloroplatinate ions	Interfere by causing precipitation
Cupric ions	Cause low results
Ferric ions	Interfere by causing precipitation
Ferrous ions	Cause low results
Lead ions	Interfere by causing precipitation
Mercurous ions	Interfere by causing precipitation
Metavanadate ions	Interfere by causing precipitation
Nitrate	Very high levels of nitrate (>100 mg/L nitrate as N) appear to undergo a slight amount of reduction to nitrite, either spontaneously or during the course of the test. A small amount of nitrite will be found at these levels.
Silver ions	Interfere by causing precipitation
Strong oxidizing and reducing substances	Interfere at all levels

Sample Collection, Storage and Preservation

Collect samples in clean plastic or glass bottles.

Store at 4 °C (30 °F) or lower if the sample is to be analyzed within 24 to 48 hours. Warm to room temperature before running the test.

Accuracy Check

Standard Solution Method

Preparing nitrite standards is difficult. A standard should be prepared by a trained chemist. Hach recommends using the standard preparation instructions in *Standard Methods for the Examination of Water and Wastewater*, 18th ed., under the headings “Stock nitrite solution:,” “Intermediate nitrite solution:,” and “Standard nitrite solution:.” These can be found on pp. 4–86. Prepare a 0.250-mg/L standard. Perform the nitrite test on the standard solution.

Method Performance

Precision

Standard: 0.2500 mg/L NO₂⁻-N

Program	95% Confidence Limits
2630	0.2493–0.2507 mg/L NO ₂ ⁻ -N

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
2630	0.0013 mg/L NO ₂ ⁻ -N

For more information on derivation and use of Hach's estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, appendix B, see Section 1.5.1.

Sensitivity

Program Number: 2630

Program	ΔAbs	ΔConcentration
Entire Range	0.010	0.0035 mg/L

See Section 1.5.3 *Sensitivity Explained* for more information.

Calibration Standard Preparation

Preparing nitrite standards is difficult. Calibration should be performed by a trained chemist. Hach recommends using the standard preparation instructions in *Standard Methods for the Examination of Water and Wastewater*, 18th ed., under the headings “Stock nitrite solution;” “Intermediate nitrite solution;” and “Standard nitrite solution”. These can be found on pp. 4–86.

Using the standards prepared above and the analysis procedure, generate a calibration curve.

Summary of Method

Nitrite in the sample reacts with sulfanilic acid to form an intermediate diazonium salt. This couples with chromotropic acid to produce a pink colored complex directly proportional to the amount of nitrite present.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to Section 1.

Pollution Prevention and Waste Management

For information on pollution prevention and waste management, refer to Section 1.

REQUIRED REAGENTS AND STANDARDS

NitriVer 3 Low range Nitrite Test 'N Tube Vial Set (50 tests).....26083-45
Includes: (50) NitriVer 3 Nitrite Vials*, (1) 22758-06, (1) 22411-06, (1) 272-42

REQUIRED EQUIPMENT AND SUPPLIES

Description	Quantity Required per test	Unit	Cat. No.
DR/4000 Test Tube Adapter.....	1	each.....	48189-00
NitriVer 3 Nitrite Vials	1	50/pkg.....	*
Pipet, TenSette, 1.0 to 10.0 mL	1	each.....	19700-10
Pipet Tips, for 19700-10 TenSette Pipet	varies.....	50/pkg.....	21997-96
Test 'N Tube Vials.....	1	50/pkg.....	22758-00
Test 'N Tube Vial caps	1	6/pkg.....	22411-06

OPTIONAL REAGENTS AND STANDARDS

Sodium Nitrite, ACS 454 g.....2452-01
Water, deionized..... 4 liters.....272-56
Water, deionized..... 100 mL.....272-42

OPTIONAL EQUIPMENT AND SUPPLIES

Balance, analytical, 110 VAC..... each.....26103-00
Balance, analytical, 220 VAC..... each.....26103-02
Flask, volumetric, 1000-mL each.....547-53
Test Tube Rack..... each..... 18641-00

* Items not sold separately



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Method 8153

Ferrous Sulfate Method*

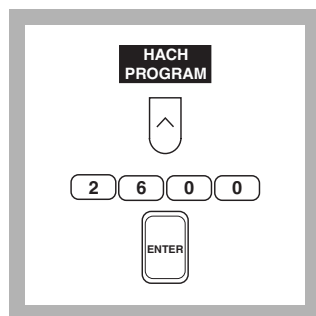
Powder Pillows

HR (0 to 250 mg/L NO₂⁻)

Scope and Application: For water and wastewater.

The estimated detection limit for program number 2600 is 1 mg/L NO₂⁻.

* Adapted from McAlpine, R. and Soule, B., *Qualitative Chemical Analysis*, New York, 476, 575 (1933)



- 1.** Press the soft key under **HACH PROGRAM**.

Select the stored program number for high range nitrite by pressing **2600** with the numeric keys.

Press: **ENTER**

Note: If samples cannot be analyzed immediately, see *Sample Collection, Storage and Preservation* following these steps.

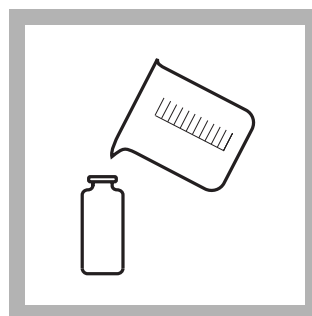
Note: The Flow Cell and Sipper Modules cannot be used with this procedure.



- 2.** The display will show:
HACH PROGRAM: 2600 Nitrite, HR

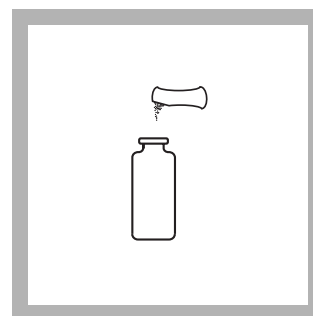
The wavelength (λ), **585 nm**, is automatically selected.

Note: For best results, determine a reagent blank for each new lot of reagent as follows. Prepare a reagent blank by repeating steps 3 through 9, using deionized water as the sample. Zero the instrument on deionized water by pressing the soft key under **ZERO**. Insert the reagent blank and the blank value will be displayed. Correct for the reagent blank by pressing the soft keys under **OPTIONS, (MORE)**, and then **BLANK:OFF**. Enter the reagent blank value and press **ENTER**. Repeat for each new lot of reagent.



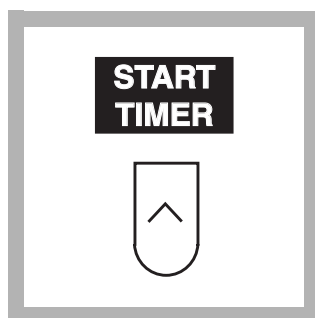
- 3.** Fill a sample cell with 10 mL of sample.

Note: For proof of accuracy, use a 200-mg/L nitrite solution in place of the sample. See the *Accuracy Check* section for preparation.

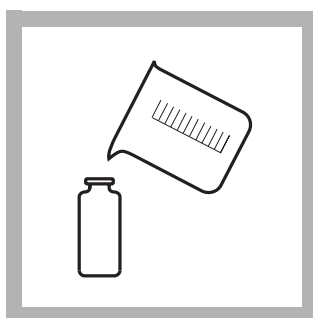


- 4.** Add the contents of one NitriVer 2 Nitrite Reagent Powder Pillow, stopper and shake to dissolve (the prepared sample).

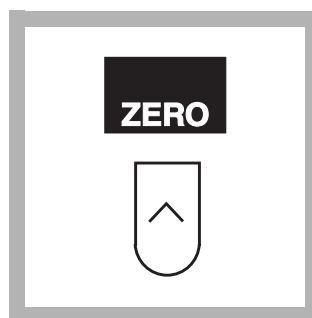
Note: A greenish-brown color will develop if nitrite is present.



5. Press the soft key under **START TIMER**. A 10-minute reaction period will begin. **It is critical to leave the sample undisturbed on a flat surface** for the reaction period or low results may occur.



6. Fill another sample cell with 10 mL of sample (the blank). Place it into the cell holder.

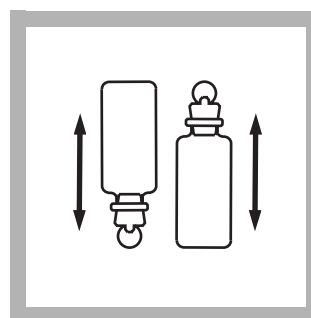


7. Press the soft key under **ZERO**. The display will show:

0 mg/L NO₂⁻

Note: If you are using a reagent blank correction, the display will show the correction.

Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



8. Gently invert the prepared sample twice. Remove the stopper.

Note: Avoid excessive mixing or low results may occur.



9. Place the prepared sample into the cell holder. Close the light shield. Results in mg/L NO₂⁻ (or chosen units) will be displayed.

Note: The results can be expressed as nitrite nitrogen (NO₂⁻-N) or as sodium nitrite (NaNO₂). Press the soft keys under **METHOD OPTIONS** and then **FORM**: to scroll through the available options.

Interferences

This test does not measure nitrates nor is it applicable to glycol-based samples. Dilute glycol-based samples and follow the Low Range Nitrite procedure.

Sample Collection, Storage and Preservation

Collect samples in clean plastic or glass bottles.

The following storage instructions are necessary only when prompt analysis is impossible. Store at 4 °C (39 °F) or lower if the sample is to be analyzed within 24 to 48 hours. Warm to room temperature before running the test.

Accuracy Check

Standard Solution Method

Preparing nitrite standards is difficult. A standard should be prepared by a trained chemist. Hach recommends using the standard preparation instructions in *Standard Methods for the Examination of Water and Wastewater*. Prepare a 200 mg/L standard.

Method Performance

Precision

Standard: 200 mg/L NO₂⁻

Program	95% Confidence Limits
2600	199–201 mg/L NO ₂ ⁻

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
2600	1 mg/L NO ₂ ⁻

For more information on derivation and use of Hach's estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, appendix B, see Section 1.5.1.

Sensitivity

Program Number: 2600

Portion of Curve	ΔAbs	ΔConcentration
Entire Range	0.010	1.4 mg/L

See Section 1.5.3 *Sensitivity Explained* for more information.

Calibration Standard Preparation

Preparing nitrite standards is difficult. Calibration should be performed by a trained chemist. Hach recommends using the standard preparation instructions in *Standard Methods for the Examination of Water and Wastewater*, 18th ed. under the headings “Stock nitrite solution:,” “Intermediate nitrite solution:,” and “Standard nitrite solution:.” These can be found on pp. 4–86.

Using the standards prepared above and the analysis procedure, generate a calibration curve.

Summary of Method

The method uses ferrous sulfate in an acidic medium to reduce nitrite to nitrous oxide. Ferrous ions combine with the nitrous oxide to form a greenish-brown complex in direct proportion to the nitrite present.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to Section 1.

Pollution Prevention and Waste Management

For information on pollution prevention and waste management, refer to Section 1.

REQUIRED REAGENTS AND STANDARDS

Description	Quantity Required		Cat. No.
	Per Test	Unit	
NitriVer 2 Nitrite Reagent Powder Pillows	1 pillow	100/pkg	21075-69

REQUIRED EQUIPMENT AND SUPPLIES

DR/4000 1-Inch Cell Adapter	1	each	48190-00
Sample Cells, matched pair, 1-inch, glass, with stoppers	2	pair	26126-02

OPTIONAL REAGENTS AND STANDARDS

Sodium Nitrite, ACS	454 g	2452-01
Water, deionized	4 liters	272-56

OPTIONAL EQUIPMENT AND SUPPLIES

Balance, analytical, 110 VAC	each	26103-00
Balance, analytical, 220 VAC	each	26103-02
DR/4000 Carousel Module Kit	each	48070-02
Flask, volumetric, 1000-mL	each	547-53
Pipet, serological, 10-mL	each	532-38
Pipet Filler, safety bulb	each	14651-00



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Diazotization Method*

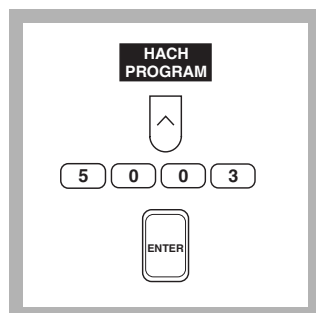
(0 to 0.600 mg/L NO_2^- -N)

UniCell™ Vials

Scope and Application: For water, and wastewater.

The estimated detection limit for program number 5003 is 0.020 mg/L NO_2^- -N.

* Reagent sets for this method are only available in Europe.



1. Press the soft key under **HACH PROGRAM**.

Select the stored program for the UniCell Nitrite method by pressing **5003** with the numeric keys.

Press: **ENTER**

Note: If sample cannot be analyzed immediately, see *Sample Collection, Storage and Preservation* following these steps.

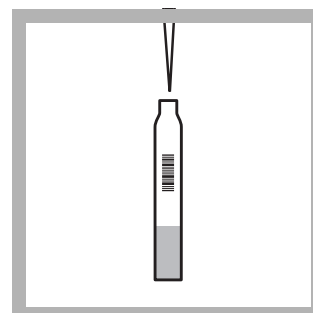


2. The display will show:
**HACH PROGRAM: 5003
Nitrite, HCT 116**

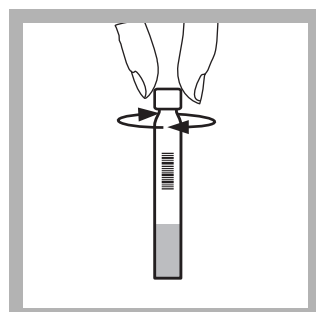
The wavelength (λ), **515 nm**, is automatically selected.



3. Insert the Test Tube Adapter into the sample cell module by sliding it under the thumb screw and into the alignment grooves. Fasten with the thumb screw.

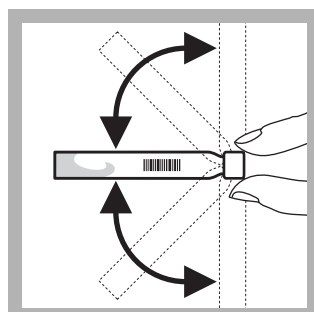


4. Pipet 4.0 mL of sample into a sample vial.

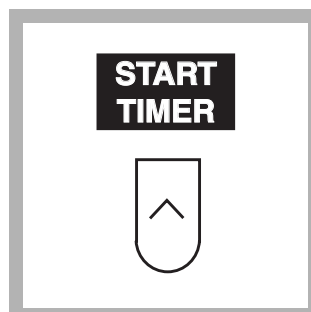


5. Immediately screw a **green** UniCap A (HCT 116A) onto the sample vial.

Note: Close the UniCap bottle immediately after use.



6. Invert the sample vial repeatedly until the reagent in the cap is completely dissolved.



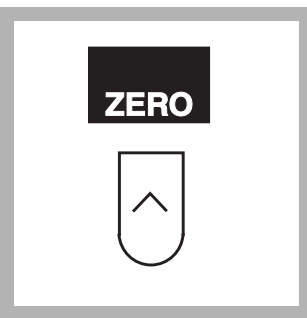
7. Press the soft key under Start Timer.
A 10-minute reaction period will begin.
After the reaction period, invert the sample again.



8. Wipe the outside of the zero vial (**white** cap) and the sample vial with a damp towel, followed by a dry one, to remove fingerprints and other marks.



9. Place the zero vial into the cell holder.



10. Press the soft key under **ZERO**.

The display will show:

0.000 mg/L NO₂⁻-N

Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



11. Place the sample vial into the cell holder. The result in mg/L nitrite expressed as nitrogen will be displayed.

Note: The results can be expressed as NO₂⁻. Press the soft keys under **METHOD OPTIONS**, then **FORM**: to scroll through the available options. Press **ENTER** to return to the read screen.

Interferences

The ions listed in the table have been individually checked up to the given concentrations. Cumulative effects and the influence of other ions have not been evaluated.

Ion	No interference above:
Cl ⁻ , SO ₄ ²⁻	2000 mg/L
K ⁺ , NO ₃ ⁻ , Ca ²⁺	1000 mg/L
NH ₄ ⁺ , PO ₄ ³⁻	500 mg/L
Mg ²⁺	100 mg/L
Cr ³⁺ , Hg ²⁺	50 mg/L
Co ²⁺ , Zn ²⁺ , Cd ²⁺	25 mg/L
Fe ³⁺ , Ni ²⁺	12 mg/L
Ag ⁺ , Fe ²⁺	10 mg/L
Sn ²⁺	5 mg/L

Chromium (VI) ions interfere. Copper (II) ions interfere even at concentrations below 1 mg/L.

Sample Collection, Storage and Preservation

If prompt analysis is impossible, store sample for 24 to 48 hours at 4 °C (39 °F) or lower. Warm sample to room temperature before testing. Do not use acid preservation.

Accuracy Check

Standard Solution Method

Prepare a 0.25-mg/L NO_2^- -N standard solution by pipetting 1.00 mL of 250-mg/L NO_2^- -N standard into a 1000-mL volumetric flask. Dilute to the mark with deionized water. Stopper and invert to mix. Prepare this solution daily. Perform the nitrite procedure as described.

Method Performance

Precision

Standard: 0.25 mg/L NO_2^- -N

Program	95% Confidence Limits
5003	0.180–0.320 mg/L NO_2^- -N

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
5003	0.020 mg/L NO_2^- -N

For more information on derivation and use of Hach's estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, appendix B, see Section 1.5.1.

Sensitivity

Program Number: 5003

Program	ΔAbs	$\Delta\text{Concentration}$
Entire Range	0.010	0.004 mg/L

See Section 1.5.3 *Sensitivity Explained* for more information.

Summary of Method

Nitrites react with primary aromatic amines in an acidic solution to form diazonium salts. These combine with aromatic compounds that contain an amino group or a hydroxyl group to form intensely colored azo dyes.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used.

UniCap (HCT 116 A) contains a 1-naphthylamine sulphononic acid salt.

Pollution Prevention and Waste Management

For information on pollution prevention and waste management, refer to Section 1.

NITRITE, continued

REQUIRED EQUIPMENT AND SUPPLIES

Description	Unit	Cat. No.
Nitrite, (NO ₂ -N) UniCell™ HCT 116*	23/pkg	HCT 116

OPTIONAL REAGENTS AND STANDARDS

Nitrite Standard, 250-mg/L	500 mL	23402-49
----------------------------	--------	----------

OPTIONAL APPARATUS

Graduated cylinder, mixing, 100-mL	each	20886-42
Flask, volumetric, 100-mL	each	14574-42
Flask, volumetric, 50-mL	each	14574-41
Flask, volumetric, 1000-mL	each	14574-53

* Available in Europe only



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WORLD HEADQUARTERS
Telephone: (970) 669-3050
FAX: (970) 669-2932



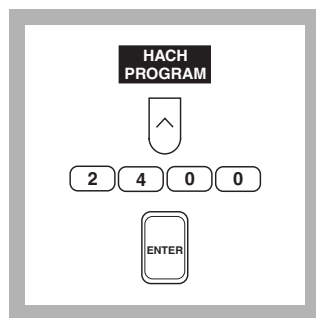
✓ Method 8038

Nessler Method*

(0 to 2,500 mg/L NH₃-N)

Scope and Application: For water, wastewater, seawater; distillation is required for wastewater and seawater; USEPA accepted for wastewater analyses (distillation is required). See Distillation following this procedure. The estimated detection limit for program number 2400 is 0.017 mg/L.

* Adapted from Standard Methods for the Examination of Water and Wastewater 4500-NH₃ B & C.



1. Press the soft key under **HACH PROGRAM**.

Select the stored program for low range ammonia nitrogen (NH₃-N) by pressing **2400** with the numeric keys.

Press: **ENTER**

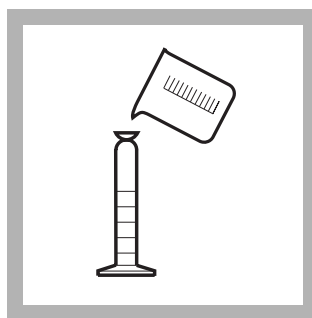
Note: If samples cannot be analyzed immediately, see Sample collection, Storage and Preservation following these steps. Adjust the pH of preserved samples before analysis.

Note: The Flow Cell and Sipper Modules can be used with this procedure. Periodically clean the cells by pouring a few sodium thiosulfate pentahydrate crystals into the cell funnel. Flush it through the funnel and cell with enough deionized water to dissolve. Rinse out the crystals.



2. The display will show: **HACH PROGRAM: 2400 N, Ammonia Nessler**

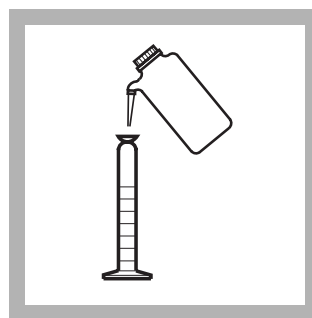
The wavelength (λ), **425 nm**, is automatically selected.



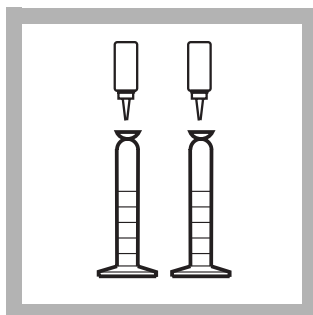
3. Fill a 25-mL mixing graduated cylinder (the prepared sample) to the 25-mL mark with sample.

Note: For proof of accuracy, use a 1.0-mg/L Ammonia Nitrogen Standard Solution (listed under OPTIONAL REAGENTS AND STANDARDS) in place of the sample.

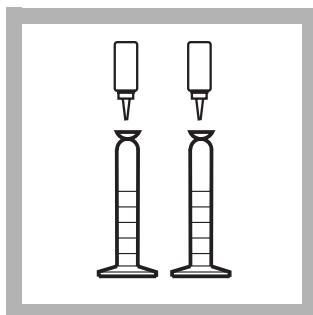
Note: For non-preserved samples with extreme pH, see the Interferences section.



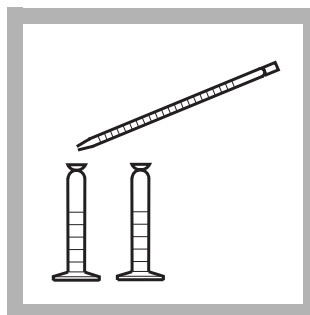
4. Fill another 25-mL mixing graduated cylinder (the blank) with deionized water.



5. Add three drops of Mineral Stabilizer to each cylinder. Stopper. Invert several times to mix.



6. Add three drops of Polyvinyl Alcohol Dispersing Agent to each cylinder by holding the dropping bottle vertically. Invert several times to mix.

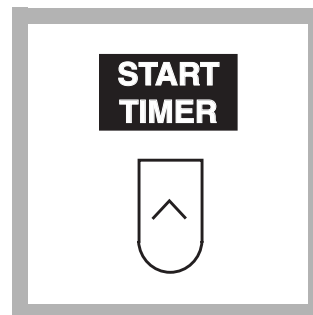


7. Pipet 1.0 mL of Nessler Reagent into each cylinder. Stopper. Invert several times to mix.

Note: Nessler Reagent is toxic and corrosive. Pipet carefully and use a pipet filler.

Note: A yellow color will develop if ammonia is present. (The reagent will cause a faint yellow color in the blank.)

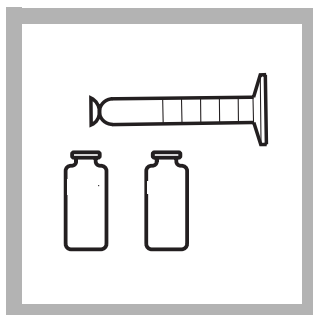
Note: Do not wait more than 15 minutes after reagent addition (Step 7) before performing Step 12.



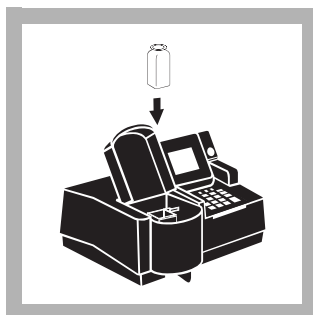
8. Press the soft key under **START TIMER**.

A one-minute reaction period will begin.

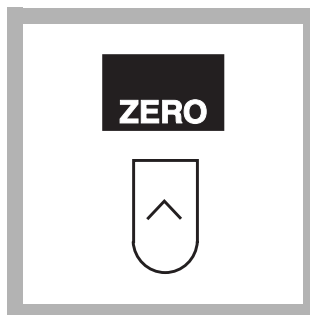
Note: Continue with Step 9 while timer is running.



9. Pour each solution into a sample cell.



10. When the timer beeps, place the blank into the cell holder. Close the light shield.

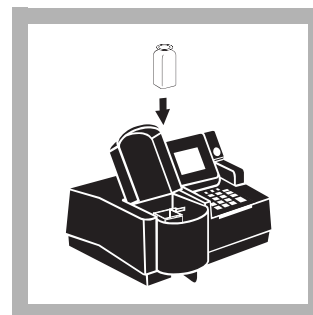


11. Press the soft key under **ZERO**.

The display will show:

0.000 mg/L N NH₃

Note: For alternate concentration units press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



12. Place the prepared sample into the cell holder. Close the light shield. Results in mg/L ammonia expressed as nitrogen (NH₃-N) (or chosen units) will be displayed.

Note: The results may be expressed as mg/L ammonia (NH₃) or mg/L ammonium (NH₄⁺). Press the soft keys under **OPTIONS**, then **FORM**: to scroll through the available options. Press **ENTER** to return to the read screen.

Interferences

Interfering Substance	Interference Levels and Treatments
Chlorine	Remove residual chlorine by adding 2 drops of sodium arsenite for each mg/L Cl from a 250 mL sample. Sodium thiosulfate can be used in place of sodium arsenite. See <i>Sample collection, Storage and Preservation</i> below.
Hardness	A solution containing a mixture of 500 mg/L CaCO ₃ and 500 mg/L Mg as CaCO ₃ does not interfere. If the hardness concentration exceeds these concentrations, add extra Mineral Stabilizer.
Iron	Interferes at all levels by causing turbidity with Nessler Reagent.
Seawater	May be analyzed by adding of 1.0 mL (27 drops) of Mineral Stabilizer to the sample before analysis. This complexes the high magnesium concentrations found in sea water, but the sensitivity of the test is reduced by 30 percent due to the high chloride concentration. For best results, perform a calibration, using standards spiked to the equivalent chloride concentration, or distill the sample as described below.
Sulfide	Interferes at all levels by causing turbidity with Nessler Reagent.
Glycine, various aliphatic and aromatic amines, organic chloramines, acetone, aldehydes and alcohols	May cause greenish or other off colors or turbidity. Distill the sample if these compounds are present.

Sample collection, Storage and Preservation

Collect samples in clean glass or plastic bottles. If chlorine is present, add one drop of 0.1 N Sodium Thiosulfate for each 0.3 mg/L Cl₂ in a 1-liter sample. Preserve the sample by reducing the pH to 2 or less with sulfuric acid (at least 2 mL). Store at 4 °C (39 °F) or less. Preserved samples may be stored up to 28 days. Warm samples to room temperature and neutralize with 5 N Sodium Hydroxide before analysis. Correct the test result for volume additions; see Section 1.2.2 *Correcting for Volume Additions*.

Accuracy Check

Standard Additions Method

- a. Leave the unspiked sample in the sample compartment. Verify that the units displayed are in mg/L. Select standard additions mode by pressing the soft keys under **OPTIONS, (MORE)** and then **STD ADD**.
- b. Press **ENTER** to accept the default sample volume (mL), 25.
- c. Press **ENTER** to accept the default standard concentration (mg/L), 50.
- d. Press the soft key under **ENTRY DONE**.
- e. Snap the neck off a Nitrogen Ammonia Voluette Ampule Standard, 50-mg/L NH₃-N.
- f. Use the TenSette Pipet to add 0.1 mL, 0.2 mL and 0.3 mL of standard, respectively to three 25-mL samples and mix each thoroughly.
- g. Analyze each standard addition sample as described above. Accept the standard additions reading by pressing the soft key under **READ** each time. Each addition should reflect approximately 100% recovery.
- h. After completing the sequence, the display will show the extrapolated concentration value and the “best-fit” line through the standard additions data points, accounting for matrix interferences.

- i. See Section 1.4.1 *Standard Additions* for more information.

Standard Solutions Method

To check accuracy, use a 1.0-mg/L Nitrogen Ammonia Standard Solution listed under *OPTIONAL REAGENTS AND STANDARDS*. Or, prepare a 1.0-mg/L ammonia nitrogen standard solution by pipetting 1.00 mL of Nitrogen Ammonia Voluette Ampule Standard, 50-mg/L, into a 50-mL volumetric flask. Dilute to the mark with deionized water. Prepare this solution daily. Perform the Nessler procedure as described above.

To adjust the calibration curve using the reading obtained with the 1.0-mg/L Nitrogen Ammonia Standard Solution, press the soft keys under **OPTIONS, MORE** then **STD: OFF**. Press **ENTER** to accept the displayed concentration, the value of which depends on the selected units. If an alternate concentration is used, enter the actual concentration and press **ENTER** to return to the read screen. See Section 1.5.5 *Adjusting the Standard Curve* for more information.

Method Performance

Precision

Standard: 1.00 mg/L NH₃-N

Program	95% Confidence Limits
2400	0.99–1.01 mg/L NH ₃ -N

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
2400	0.017 mg/L NH ₃ -N

For more information on derivation and use of Hach's estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, Appendix B, see Section 1.5.1.

Sensitivity

Program Number: 2400

Portion of Curve	ΔAbs	ΔConcentration
Entire Range	0.010	0.01717 mg/L

See Section 1.5.3 *Sensitivity Explained* for more information.

Distillation

- a. Measure 250 mL of sample into a 250-mL graduated cylinder and pour into a 400-mL beaker. Destroy chlorine, if necessary, by adding 2 drops of Sodium Arsenite Solution per mg/L Cl_2 .
- b. Add 25 mL of Borate Buffer Solution and mix. Adjust the pH to about 9.5 with 1 N Sodium Hydroxide solution. Use a pH meter.
- c. Set up the general purpose distillation apparatus as shown in the *Hach Distillation Apparatus Manual*. Pour the solution into the distillation flask. Add a stir bar.
- d. Use a graduated cylinder to measure 25 mL of deionized water into a 250-mL erlenmeyer flask. Add the contents of one Boric Acid Powder Pillow. Mix thoroughly. Place the flask under the still drip tube. Elevate so the end of the tube is immersed in the solution.
- e. Turn on the heater power switch. Set the stir control to 5 and the heat control to 10. Turn on the water and adjust to maintain a constant flow through the condenser.
- f. Turn off the heater after collecting 150 mL of distillate. Immediately remove the collection flask to avoid sucking solution into the still. Measure the distillate to ensure 150 mL was collected (total volume = 175 mL).
- g. Adjust the pH of the distillate to about 7 with 1 N Sodium Hydroxide. Use a pH meter.
- h. Pour the distillate into a 250-mL volumetric flask; rinse the erlenmeyer with deionized water. Add the rinsings to the volumetric. Dilute to the mark. Stopper. Mix thoroughly. Analyze as described above.

Calibration Standard Preparation

To perform an ammonia calibration using the Nessler method, prepare standards containing 0.5, 1.0 and 2.0 mg/L ammonia-nitrogen as follows:

- a. Into three different 100-mL volumetric flasks pipet 0.5, 1.0 and 2.0 mL of the 100-mg/L Nitrogen Ammonia Standard Solution (Cat No. 24065-49) using Class A glassware.
- b. Dilute to the mark with deionized ammonia-free water. Mix thoroughly.
- c. Using the Nessler method and the calibration procedure described in the *User-Entered Programs* section of the *DR/4000 Spectrophotometer Instrument Manual*, generate a calibration curve from the standard prepared above.

NITROGEN, Ammonia, continued

Summary of Method

The Mineral Stabilizer complexes hardness in the sample. The Polyvinyl Alcohol Dispersing Agent aids the color formation in the reaction of Nessler Reagent with ammonium ions. A yellow color is formed proportional to the ammonia concentration.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to Section 1.

Pollution Prevention and Waste Management

Nessler Reagent contains mercuric iodide. Both the sample and the blank will contain mercury (D009) at a concentration regulated as a hazardous waste by the Federal RCRA. Do not pour these solutions down the drain. See Section 1 for further information on proper disposal of these materials.

REQUIRED REAGENTS AND STANDARDS

Ammonia Nitrogen Reagent Set24582-00
Includes: (1) 21194-49, (1) 23766-26, (1) 23765-26

Description	Quantity Required		Cat. No.
	Per Test	Unit	
Nessler Reagent.....	2 mL	500 mL	21194-49
Mineral Stabilizer.....	6 drops	50 mL* SCDB	23766-26
Polyvinyl Alcohol Dispersing Agent	6 drops	50 mL SCDB	23765-26
Water, deionized	25 mL	4 liters	272-56

REQUIRED EQUIPMENT AND SUPPLIES

Cylinder, graduated, mixing, 25-mL	2	each	21190-40
DR/4000 1-Inch Cell Adapter	1	each	48190-00
Pipet, serological, 1-mL	2	each	532-35
Pipet Filler, safety bulb.....	1	each	14651-00

OPTIONAL REAGENTS AND STANDARDS

Borate Buffer Solution	1000 mL	14709-53
Boric Acid Powder Pillows	50/pkg	14817-66
Nitrogen, Ammonia Standard Solution, 1-mg/L NH ₃ -N	500 mL	1891-49
Nitrogen, Ammonia Standard Solution, 100-mg/L NH ₃ -N	500 mL	24065-49
Nitrogen, Ammonia Standard Solution, 0-mL Voluette Ampule, 50-mg/L NH ₃ -N	16/pkg	14791-10
Sodium Arsenite Solution, 5.0-g/L	100 mL	MDB..... 1047-32
Sodium Hydroxide Standard Solution, 5.0 N.....	100 mL	* MDB..... 2450-32
Sodium Hydroxide Standard Solution, 1.0 N.....	100 mL	* MDB..... 1045-32
Sodium Thiosulfate Standard Solution, 0.1 N.....	100 mL	* MDB..... 323-32
Sulfuric Acid, ACS, concentrated	500 mL	*..... 979-49

* Contact Hach for larger sizes.

OPTIONAL EQUIPMENT AND SUPPLIES

Description	Unit	Cat. No.
Ampule Breaker Kit	each.....	21968-00
Beaker, 400-mL	each.....	500-48
Cylinder, graduated, 25-mL	each.....	508-40
Cylinder, graduated, 250-mL	each.....	508-46
Distillation Apparatus, general purpose accessories	each.....	22653-00
Distillation heater and support apparatus set, 115 VAC.....	each.....	22744-00
Distillation heater and support apparatus set, 230 VAC.....	each.....	22744-02
Dropper, plastic, 0.5- and 1.0-mL marks	10/pkg.....	21247-10
DR/4000 Carousel Module Kit	each.....	48070-02
DR/4000 Flow-Thru Cell Module.....	each.....	48090-04
DR/4000 Sipper Cell Module, 1-inch	each.....	48090-03
Flask, Erlenmeyer, 250-mL.....	each.....	505-46
Flask, volumetric, 50-mL	each.....	547-41
Flask, volumetric, 250-mL	each.....	547-46
pH Meter, <i>sens^{ion}</i> TM 1, portable	each.....	51700-00
Pipet, serological, 2-mL	each.....	532-36
Pipet, TenSette, 0.1 to 1.0 mL	each.....	19700-01
Pipet Tips, for 19700-01 TenSette Pipet	50/pkg.....	21856-96
Pipet, volumetric, Class A, 1-mL.....	each.....	14515-35
Thermometer, -10 to 110 °C.....	each.....	1877-01



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Method 8155

Salicylate Method*

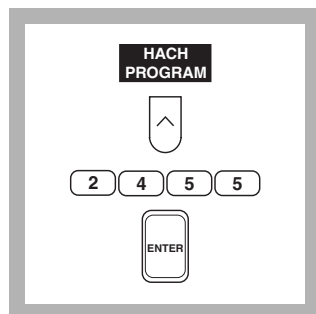
Powder Pillows

(0 to 0.80 mg/L $\text{NH}_3\text{-N}$)

Scope and Application: For water, wastewater, and seawater.

The estimated detection limit for program number 2455 is 0.09 mg/L $\text{NH}_3\text{-N}$.

* Adapted from *Clin. Chim. Acta.*, 14, 403 (1966)



1. Press the soft key under **HACH PROGRAM**.

Select the stored program number for ammonia nitrogen ($\text{NH}_3\text{-N}$) by pressing **2455** with the numeric keys.

Press: **ENTER**

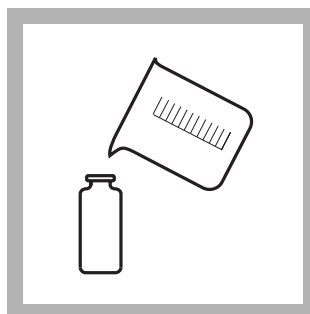
Note: If samples cannot be analyzed immediately, see *Sample Collection, Storage and Preservation* following these steps.

Note: The Flow Cell and Sipper Modules can be used with this procedure.



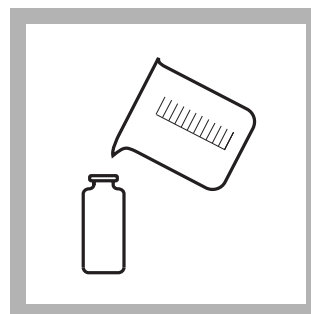
2. The display will show:
HACH PROGRAM: 2455 N, Ammonia Salic.

The wavelength (λ), **655 nm**, is automatically selected.

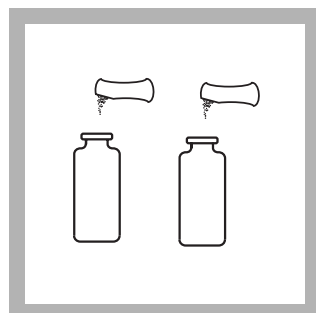


3. Fill a glass-stoppered sample cell to the 25-mL mark with sample.

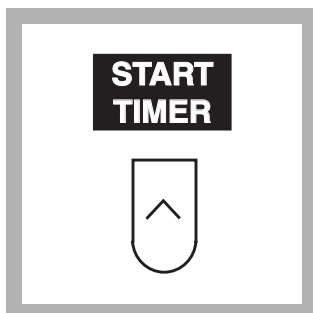
Note: For proof of accuracy, a 0.60-mg/L $\text{NH}_3\text{-N}$ solution (preparation given in the *Accuracy Check* section) can be used in place of the sample.



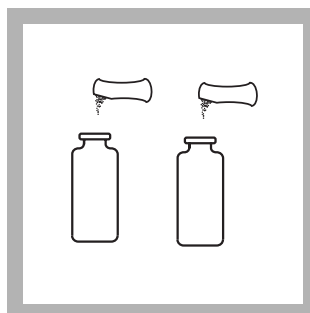
4. Fill another glass-stoppered sample cell to the 25-mL mark with deionized water (the blank).



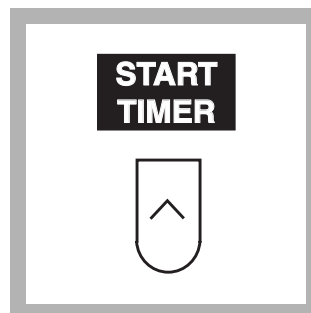
5. Add the contents of one Ammonia Salicylate Powder Pillow to each cell. Stopper and shake to dissolve the powder.



6. Press the soft key under **START TIMER**. A 3-minute reaction period will begin.



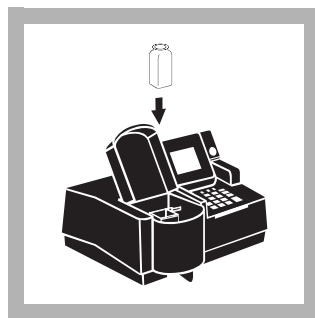
7. When the timer beeps, add the contents of one Ammonia Cyanurate Reagent Powder Pillow to each cell. Stopper and shake to dissolve the reagent.



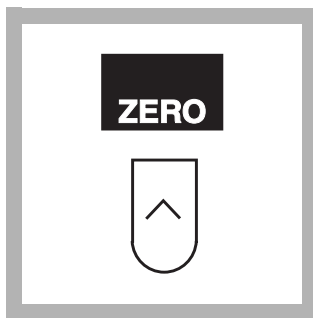
8. Press the soft key under **START TIMER**. A 15-minute reaction period will begin.

Note: A green color will develop if ammonia nitrogen is present.

NITROGEN, Ammonia, continued



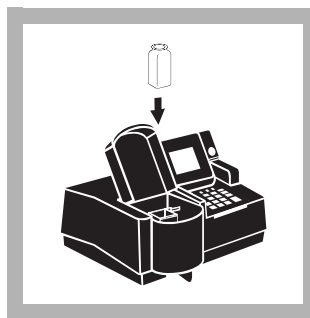
9. When the timer beeps, place the blank into the cell holder. Close the light shield.



10. Press the soft key under **ZERO**.

The display will show:

0.00 mg/L NH₃-N



11. Place the prepared sample into the cell holder. Close the light shield. Result in mg/L ammonia as nitrogen (NH₃-N) (or chosen units) will be displayed.

Note: Results may be expressed as mg/L ammonia (NH₃) or mg/L ammonium (NH₄⁺). Press the soft keys under **OPTIONS**, then **FORM**: to scroll through the available options. Press **ENTER** to return to the read screen.

Interferences

Table 1 Interfering Substances and Suggested Treatments

Interfering Substance	Interference Levels
Calcium	Greater than 1000 mg/L as CaCO ₃
Iron	All levels. Correct for iron interference as follows: <ol style="list-style-type: none">1. Determine the amount of iron present in the sample by following one of the Iron, Total, procedures.2. Add the same iron concentration to the ammonia-free water in Step 3. The interference will be successfully blanked out.
Magnesium	Greater than 6000 mg/L as CaCO ₃
Nitrate	Greater than 100 mg/L as NO ₃ ⁻ -N
Nitrite	Greater than 12 mg/L as NO ₂ ⁻ -N
Phosphate	Greater than 100 mg/L as PO ₄ ³⁻ -P
Sulfate	Greater than 300 mg/L as SO ₄ ²⁻
Sulfide	Sulfide will intensify the color. Eliminate sulfide interference as follows: <ol style="list-style-type: none">1. Measure about 350 mL of sample in a 500-mL erlenmeyer flask.2. Add the contents of one Sulfide Inhibitor Reagent Powder Pillow. Swirl to mix.3. Filter the sample through a folded filter paper.4. Use the filtered solution in Step 3.
Other Substances	Less common interferences such as hydrazine and glycine will cause intensified colors in the prepared sample. Turbidity and color will give erroneous high values. Samples with severe interferences require distillation. Hach recommends the distillation procedure using the Hach General Purpose Distillation Set. See <i>OPTIONAL REAGENTS AND STANDARDS</i> .

Sample Collection, Storage and Preservation

Collect samples in clean plastic or glass bottles. Most reliable results are obtained when samples are analyzed as soon as possible after collection.

If chlorine is known to be present, the sample must be treated immediately with sodium thiosulfate. Add one drop of Sodium Thiosulfate Standard Solution, 0.1 N, for each 0.3 mg of chlorine present in a one-liter sample.

For longer storage, adjust the pH to 2 or less with concentrated sulfuric acid (about 2 mL per liter). Store samples at 4 °C or less. Samples preserved in this manner can be stored up to 28 days. Just before testing the stored sample, warm to room temperature and neutralize with 5.0 N Sodium Hydroxide Standard Solution. Correct the test result for volume additions; see Section 1.2.2 *Correcting for Volume Additions*.

Accuracy Check

Standard Additions Method

- a. Leave the unspiked sample in the sample compartment. Verify that the units displayed are in mg/L. Select standard additions mode by pressing the soft keys under **OPTIONS, (MORE)** and then **STD ADD**.
- b. Press **ENTER** to accept the default sample volume (mL), 25.
- c. Press **ENTER** to accept the default standard concentration (mg/L), 10.
- d. Press the soft key under **ENTRY DONE**.
- e. Open an Ammonia Nitrogen Standard Solution, 10-mg/L as $\text{NH}_3\text{-N}$.
- f. Use the TenSette Pipet to add 0.2 mL, 0.4 mL and 0.6 mL of standard, respectively to three 25-mL samples and mix each thoroughly.
- g. Analyze each standard addition sample as described above. Accept the standard addition reading by pressing the soft key under **READ** each time. Each addition should reflect approximately 100% recovery.
- h. After completing the sequence, the display will show the extrapolated concentration value and the “best-fit” line through the standard additions data points, accounting for matrix interferences.
- i. See Section 1.4.1 *Standard Additions* for more information.

Standard Solution Method

Prepare a 0.60-mg/L ammonia nitrogen standard by diluting 6.00 mL of the Ammonia Nitrogen Standard Solution, 10-mg/L, to 100 mL with deionized water. Or, using the TenSette Pipet, prepare a 0.60-mg/L ammonia nitrogen standard by diluting 1.2 mL of a Ammonia Nitrogen Volumetric Standard Solution, 50-mg/L as $\text{NH}_3\text{-N}$, to 100 mL with deionized water.

To adjust the calibration curve using the reading obtained with the 0.60-mg/L standard solution, press the soft keys under **OPTIONS, MORE** then **STD: OFF**. Press **ENTER** to accept the displayed concentration, the value of which depends on the selected units. If an alternate concentration is used, enter the actual concentration and press **ENTER** to return to the read screen. See Section 1.5.5 *Adjusting the Standard Curve* for more information.

Method Performance

Precision

Standard: 0.60 mg/L NH₃-N

Program	95% Confidence Limits
2455	0.54–0.66 mg/L NH ₃ -N

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
2455	0.09 mg/L NH ₃ -N

For more information on derivation and use of Hach's estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, appendix B, see Section 1.5.1.

Sensitivity

Program Number: 2455

Portion of Curve	ΔAbs	ΔConcentration
Entire Range	0.010	0.004 mg/L

See Section 1.5.3 *Sensitivity Explained* for more information.

Calibration Standard Preparation

To perform an nitrogen calibration using the salicylate method, prepare a 10 mg/L ammonia nitrogen stock solution by pipetting 10 mL of a 100-mg/L Nitrogen Ammonia Standard Solution (Cat. No. 24065-49) into a 100-mL volumetric flask using Class A glassware. Dilute to the mark with deionized water and mix thoroughly. Or use the 10 mg/L Nitrogen Ammonia Standard Solution from Hach (Cat. No. 153-49).

Prepare calibration standards containing 0.20, 0.50, and 0.80 mg/L ammonia nitrogen as follows:

- Into three different 100-mL volumetric flasks, pipet 2.00, 5.00, and 8.00 mL of the 10-mg/L Nitrogen Ammonia Standard Solution using Class A glassware.
- Dilute to the mark with deionized water. Mix thoroughly.
- Using the salicylate method and the calibration procedure described in the *User-Entered Programs* section of the *DR/4000 Spectrophotometer Instrument Manual*, generate a calibration curve from the standards prepared above.

Summary of Method

Ammonia compounds combine with chlorine to form monochloramine. Monochloramine reacts with salicylate to form 5-aminosalicylate. The 5-aminosalicylate is oxidized in the presence of a sodium nitroprusside catalyst to form a blue-colored compound. The blue color is masked by the yellow color from the excess reagent present to give a final green-colored solution.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to Section 1.

Pollution Prevention and Waste Management

For information on pollution prevention and waste management, refer to Section 1.

REQUIRED REAGENTS AND STANDARDS

Description	Cat. No
Ammonia Nitrogen Reagent Set, (100 tests).....	22437-00
Includes: (4) 23955-66, (4) 23953-66	

Description	Quantity Required		Cat. No.
	Per Test	Unit	
Ammonia Cyanurate Reagent Powder Pillows	2	25/pkg	23955-66
Ammonia Salicylate Reagent Powder Pillows	2	25/pkg	23953-66

REQUIRED EQUIPMENT AND SUPPLIES

Description	Quantity Required		Cat. No.
	Per Test	Unit	
Clippers, for opening powder pillows	1	each	968-00
DR/4000 1-Inch Cell Adapter	1	each	48190-00
Sample Cells, matched pair, 1 inch, w/ stopper	2	pair	26126-02

OPTIONAL REAGENTS AND STANDARDS

Ammonia Nitrogen Standard Solution,			
10-mg/L as NH ₃ -N	500 mL		153-49
Ammonia Nitrogen Standard Solution,			
10-mL Voluette Ampule, 50-mg/L as NH ₃ -N	16/pkg		14791-10
Ammonia Nitrogen Standard Solution,			
100-mg/L as NH ₃ -N	500 mL		24065-49
Sodium Hydroxide Standard Solution, 1.00 N.....	100 mL MDB		1045-32
Sodium Hydroxide Standard Solution, 5.0 N.....	59 mL DB		2450-26
Sodium Thiosulfate Standard Solution, 0.1 N.....	100 mL MDB		323-32
Sulfide Inhibitor Reagent Powder Pillows	100/pkg		2418-99
Sulfuric Acid, concentrated, ACS	500 mL		979-49
Sulfuric Acid Standard Solution, 1.000 N.....	100 mL MDB		1270-32
Water, deionized	4 liters		272-56

NITROGEN, Ammonia, continued

OPTIONAL EQUIPMENT AND SUPPLIES

Cylinder, graduated, polypropylene, 500-mL	each.....	1081-49
Distillation Heater and Support Apparatus, 115 VAC.....	each.....	22744-00
Distillation Heater and Support Apparatus, 230 VAC.....	each.....	22744-02
Distillation Apparatus Set, general purpose	each.....	22653-00
DR/4000 Carousel Module Kit	each.....	48070-02
DR/4000 Flow Cell Module Kit, 1-inch	each.....	48070-04
DR/4000 Flow Cell Module Kit, 1-cm.....	each.....	48070-05
DR/4000 Sipper Module Kit, 1-inch	each.....	48090-03
Filter Paper, folded, 12.5-cm.....	100.....	1894-57
Flask, Erlenmeyer, polypropylene, 500-mL.....	each.....	1082-49
Flask, volumetric, Class A, 100-mL	each.....	14574-42
Funnel, poly, 65-mm	each.....	1083-67
pH Meter, <i>sensio</i> TM 1 , portable	each.....	517000-00
Pipet Filler, safety bulb.....	each.....	14651-00
Pipet, TenSette, 0.1 to 1.0 mL	each.....	19700-01
Pipet Tips, for 19700-01 TenSette Pipet	50/pkg.....	21856-96
Pipet, volumetric, Class A, 2.00-mL	each.....	14515-36
Pipet, volumetric, Class A, 5.00-mL	each.....	14515-37
Pipet, volumetric, Class A, 6.00-mL	each.....	14515-06
Pipet, volumetric, Class A, 8.00-mL	each.....	14515-08
Thermometer, -10 to 110 °C.....	each.....	1877-01



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FAX: (970) 669-2932



Method 10031

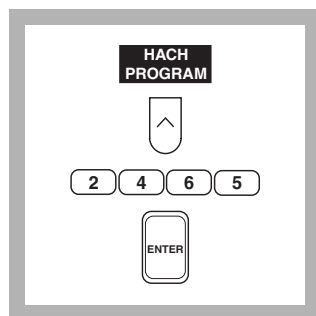
Salicylate Method

Test 'N Tube™ Vials

HR (0 to 50.0 mg/L $\text{NH}_3\text{-N}$)

Scope and Application: For water, wastewater, and seawater.

The estimated detection limit for program number 2465 is 0.6 mg/L $\text{NH}_3\text{-N}$.



- 1.** Press the soft key under **HACH PROGRAM**.

Select the stored program number for the High Range Nitrogen Ammonia Test 'N Tube method by pressing **2465** with the numeric keys.

Press: **ENTER**

Note: If samples cannot be analyzed immediately, see Sample Collection, Storage and Preservation following these steps. Adjust the pH of preserved samples before analysis.

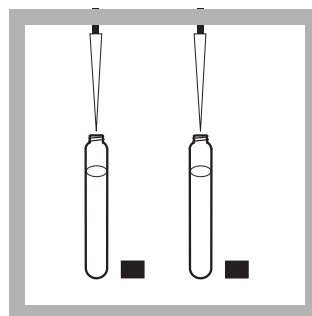
Note: The Flow Cell and Sipper Modules cannot be used with this method.



- 2.** The display will show: **HACH PROGRAM: 2465 N, Ammonia HR TNT**

The wavelength (λ), **655 nm**, is automatically selected.

Note: For proof of accuracy, use a 10-mg/L Nitrogen Ammonia standard in place of the sample.

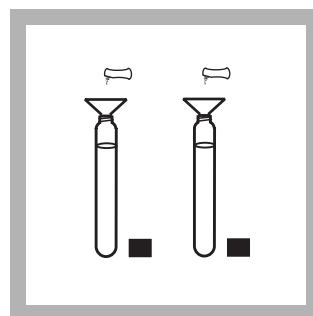


- 3.** Remove the caps from two AmVer Diluent Reagent High Range vials. Add 0.1 mL of ammonia-free water to the other vial (the blank).

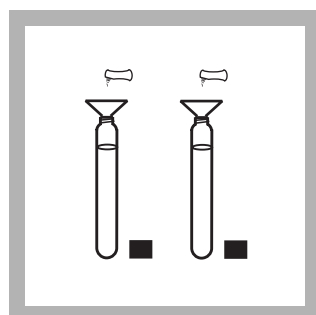
Add 0.1 mL of sample to one vial (the sample).

Note: For non-preserved samples with extreme pH, see the Interferences section.

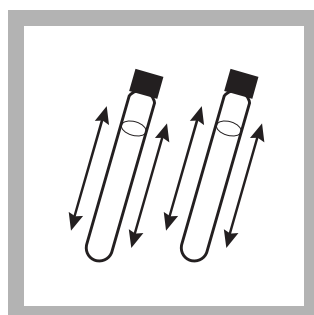
Note: Small sample sizes may not be representative of the entire sample. Mix the sample well before testing or repeat the test, sampling from different portions of the sample.



- 4.** Add the contents of one Ammonia Salicylate Reagent Powder Pillow (for 5-mL sample) to each vial.

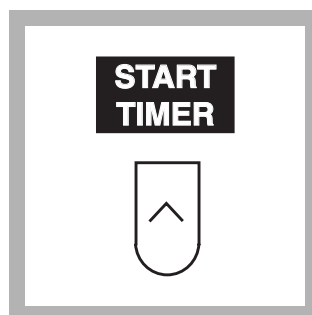


- 5.** Add the contents of one Ammonia Cyanurate Reagent Powder Pillow (for 5-mL sample) to each vial.



- 6.** Cap the vials tightly and shake thoroughly to dissolve the powder.

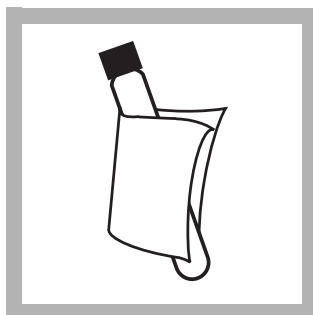
Note: A green color will develop if ammonia is present.



- 7.** Press the soft key under **START TIMER**. A 20-minute reaction period will begin.

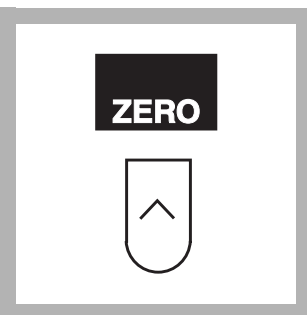


- 8.** Insert the Test Tube Adapter into the sample cell module by sliding it under the thumb screw and into the alignment grooves. Fasten with the thumb screw.



9. When the timer beeps, clean the outside of the vial with a towel, and place the blank into the cell holder. Close the light shield.

Note: Wiping with a damp cloth followed by a dry one removes fingerprints and other marks.

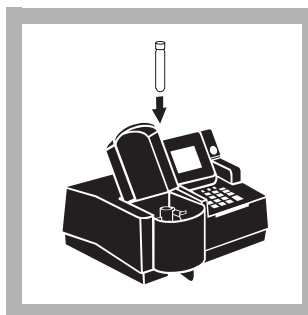


10. Press the soft key under **ZERO**.

The display will show:

0.0 mg/L NH₃-N

Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



11. Place the prepared sample into the cell holder and close the light shield. The result in mg/L ammonia nitrogen (or chosen units) will be displayed.

Note: The results can be expressed as ammonia (NH₃). Press the soft keys under **METHOD OPTIONS**, then **FORM**: to scroll through the available options. Press **ENTER** to return to the read screen.

Interferences

In some lab environments, airborne cross contamination of the blank is possible. Complete preparation of the blank before opening or handling any samples or standards to avoid transfer of ammonia. If sample or standard containers have already been open, move to a separate area of the lab to prepare the blank.

Substance	Concentration and Suggested Treatments
Acidic or basic samples	Adjust to approximately pH 7. Use 1 N Sodium Hydroxide Standard Solution for acidic samples and 1 N Hydrochloric Acid Standard Solution for basic samples.
Calcium	50,000 mg/L as CaCO ₃
Glycine, hydrazine	Will cause intensified colors in the prepared sample.
Magnesium	300,000 mg/L as CaCO ₃
Iron	Eliminate iron interference as follows: <ol style="list-style-type: none">1. Determine the amount of iron present in the sample using one of the total iron procedures.2. Add the same iron concentration to the deionized water in step 4.3. The interference will then be successfully blanked out.
Nitrite	600 mg/L as NO ₂ ⁻ -N
Nitrate	5,000 mg/L as NO ₃ ⁻ -N
Orthophosphate	5,000 mg/L as PO ₄ ³⁻ -P
Sulfate	6,000 mg/L as SO ₄ ²⁻

Substance	Concentration and Suggested Treatments
Sulfide	Sulfide will intensify the color. Eliminate sulfide interference as follows: <ol style="list-style-type: none"> 1. Measure about 350 mL of sample in a 500 mL erlenmeyer flask. 2. Add the contents of one Sulfide Inhibitor Reagent Powder Pillow. Swirl to mix. 3. Filter the sample through folded filter paper. Use the solution in step 4.
Turbidity and color	Give erroneous high values. Samples with severe interferences require distillation. Hach recommends the distillation procedure using the Hach General Purpose Distillation Set.

Sample Collection, Storage and Preservation

Collect samples in clean plastic or glass bottles. Best results are obtained with immediate analysis. If chlorine is known to be present, add one drop of 0.1 N Sodium Thiosulfate for each 0.3 mg/L Cl_2 in a one liter sample. Preserve the sample by reducing the pH to 2 or less with hydrochloric acid (at least 2 mL). Store at 4 °C (39 °F) or less. Preserved samples may be stored up to 28 days. Warm samples to room temperature. Neutralize with 5.0 N Sodium Hydroxide before analysis. Correct the test result for volume additions.

Accuracy Check

Standard Additions Method

- a. Leave the unspiked sample in the sample compartment. Verify that the units displayed are in mg/L. Select standard additions mode by pressing the soft keys under **OPTIONS, (MORE)** and then **STD ADD**.
- b. Press **ENTER** to accept the default sample volume (mL), 25.0.
- c. Press **ENTER** to accept the default standard concentration (mg/L $\text{NH}_3\text{-N}$), 150.0.
- d. Press the soft key under **ENTRY DONE**.
- e. Snap the neck off a Nitrogen, Ammonia Voluette Ampule Standard, 150-mg/L $\text{NH}_3\text{-N}$.
- f. Use the TenSette Pipet to add 0.2, 0.4 mL and 0.6 mL of standard, respectively to three 25-mL samples and mix each thoroughly.
- g. Analyze each standard addition sample as described above. Accept the standard additions reading by pressing the soft key under **READ** each time. Each addition should reflect approximately 100% recovery.
- h. After completing the sequence, the display will show the extrapolated concentration value and the “best-fit” line through the standard additions data points, accounting for matrix interferences.
- i. See Section 1.4.1 *Standard Additions* for more information.

Standard Solution Method

To check accuracy, use the 10-mg/L Nitrogen, Ammonia Standard Solution or a 50-mg/L Nitrogen, Ammonia Voluette Ampule Standard listed under **OPTIONAL REAGENTS AND STANDARDS**.

Alternatively, prepare a 40.0-mg/L ammonia nitrogen standard solution by pipetting 4.00 mL of 100-mg/L Ammonia Nitrogen standard into a 100-mL, Class A volumetric flask. Dilute to the mark with deionized water.

To adjust the calibration curve using the reading obtained with the 40.0 mg/L nitrogen ammonia standard solution, press the soft keys under **OPTIONS, MORE**, then **STD: OFF**. Press **ENTER** to accept the displayed concentration, the value of which depends on the selected units. If an alternate concentration is used, enter the actual concentration and press **ENTER** to return to the read screen. See *Section 1.5.5 Adjusting the Standard Curve* for more information.

Method Performance

Precision

Standard: 10.0 mg/L NH₃-N

Program	95% Confidence Limits
2465	9.6–10.4 mg/L NH ₃ -N

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
2465	0.6 mg/L NH ₃ -N

For more information on derivation and use of Hach's estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, appendix B, see Section 1.5.1.

Sensitivity

Program Number: 2465

Portion of Curve	ΔAbs	ΔConcentration
Entire Range	0.010	0.38 mg/L

See Section 1.5.3 *Sensitivity Explained* for more information.

Calibration Standard Preparation

To perform an ammonia calibration using the Test 'N Tube HR Salicylate method, prepare calibration standards containing 10.0, 30.0, and 50.0 mg/L NH₃-N as follows:

- Into three different 50-mL Class A volumetric flasks, pipet 5.00, 15.00, and 25.00 mL of a 100-mg/L Nitrogen Ammonia Standard Solution (Cat. No. 24065-49) using Class A glassware.
- Dilute to the mark with deionized water. Mix thoroughly.
- Using the Test 'N Tube HR salicylate method and the calibration procedure described in the *User-Entered Programs* section of the *DR/4000 Spectrophotometer Instrument Manual*, generate a calibration curve from the standards prepared above.

Summary of Method

Ammonia compounds combine with chlorine to form monochloramine. Monochloramine reacts with salicylate to form 5-aminosalicylate. The 5-aminosalicylate is oxidized in the presence of a sodium nitroprusside catalyst to form a blue colored compound. The blue color is masked by the yellow color from the excess reagent present to give a green-colored solution.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to Section 1.

Pollution Prevention and Waste Management

The ammonia salicylate reagent contains sodium nitroferricyanide. Cyanide solutions are regulated as hazardous wastes by the Federal RCRA. Collect cyanide solutions for disposal as reactive (D001) waste. Be sure cyanide solutions are stored in a caustic solution with pH >11 to prevent release of hydrogen cyanide gas. See Section 1 for further information in proper disposal of these materials.

REQUIRED REAGENTS AND STANDARDS

	Cat. No.
High Range Test 'N Tube AmVer Nitrogen Ammonia Reagent Set (50 tests).....	26069-45
Includes: (50) HR TNT AmVer Diluent Vials*, (1) 23952-66, (1) 23954-66, (1) 272-42	

Description	Quantity Required		Cat. No.
	per test	Unit	
AmVer Reagent HR TNT Vials.....	2 vials	50/pkg	*
Ammonia Salicylate Reagent Powder Pillows	2 pillows	50/pkg	23952-66
Ammonia Cyanurate Reagent Powder Pillows	2 pillows	50/pkg	23954-66

REQUIRED EQUIPMENT AND SUPPLIES

DR/4000 Test Tube Adapter.....	1	each.....	48189-00
Funnel, micro (for adding reagent)	1	each.....	25843-35
Test Tube Rack	1	each.....	18641-00
Pipet, TenSette, 0.1 to 1.0 mL	1	each.....	19700-01
Pipet Tips, for TenSette Pipet 19700-01	varies.....	50/pkg	21856-96

OPTIONAL REAGENTS AND STANDARDS

Hydrochloric Acid, ACS	500 mL.....	134-49
Nitrogen Ammonia Standard Solution, 10-mg/L NH ₃ -N.....	500 mL.....	153-49
Nitrogen Ammonia Standard Solution, 100-mg/L NH ₃ -N.....	500 mL.....	24065-49
Nitrogen Ammonia Standard Solution, 150-mg/L NH ₃ -N, 10-mL Voluette Ampules.....	16/pkg	21284-10
Sodium Hydroxide Standard Solution, 5.0 N.....	50 mL	2450-26
Sodium Thiosulfate Standard Solution, 0.1 N.....	100 mL.....	323-32
Sulfide Inhibitor Powder Pillows	100/pkg	2418-99
Water, deionized	4 L.....	272-56

* Item not sold separately

NITROGEN, Ammonia, continued

OPTIONAL EQUIPMENT AND SUPPLIES

Description	Unit	Cat. No.
Distillation Apparatus Set, general purpose	each.....	22653-00
Distillation Heater & Support Apparatus Set, 115 VAC	each.....	22744-00
Distillation Heater & Support Apparatus Set, 230 VAC	each.....	22744-02
Filter Paper, folded, 12.5-cm dia.	100/pkg.....	692-57
Flask, Erlenmeyer, 500-mL	each.....	505-49
Flask, volumetric, Class A, 100-mL	each.....	14574-42
Funnel, analytical, plastic, for filtering	each.....	1083-67
pH Paper, 1.0 to 11.0 pH units	5 rolls/pkg.....	391-33
Pipet, serological, 2.00-mL	each.....	532-36
Pipet, volumetric, Class A, 4.00-mL	each.....	14515-04
Pipet, volumetric, Class A, 5.00-mL	each.....	14515-37
Pipet, volumetric, Class A, 10.00-mL	each.....	14515-38
Pipet, volumetric, Class A, 15.00-mL	each.....	14515-39
Pipet, volumetric, Class A, 25.00-mL	each.....	14515-40
Pipet Filler	each.....	12189-00
Pipet, TenSette, 1.0 to 10.0 mL	each.....	19700-10
Pipet Tips, for TenSette Pipet 19700-10	50/pkg.....	21997-96
Thermometer, pocket, -10 to 110 °C	each.....	1877-01



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Method 10023

Salicylate Method*

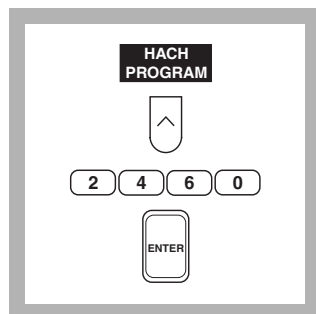
Test 'N Tube™ Vials

LR (0 to 2.500 mg/L $\text{NH}_3\text{-N}$)

Scope and Application: For water, wastewater and seawater.

The estimated detection limit for program number 2460 is 0.031 mg/L $\text{NH}_3\text{-N}$.

* Adapted from *Clin. Chim. Acta*, 14, 403 (1966).



1. Press the soft key under **HACH PROGRAM**.

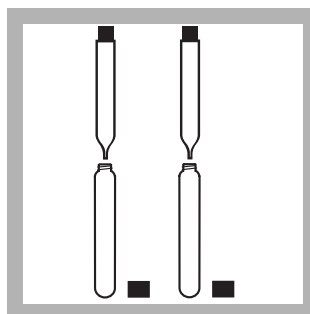
Select the stored program number for Low Range Nitrogen-Ammonia, Test 'N Tube method, by pressing **2460** with the numeric keys.

Press: **ENTER**

Note: If samples cannot be analyzed immediately, see *Sample Collection, Storage and Preservation* following these steps. Adjust the pH of preserved samples before analysis.

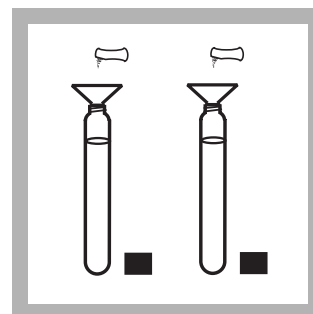


2. The display will show: **HACH PROGRAM: 2460 N, Ammonia LR TNT**
The wavelength (λ), **655nm**, is automatically selected.

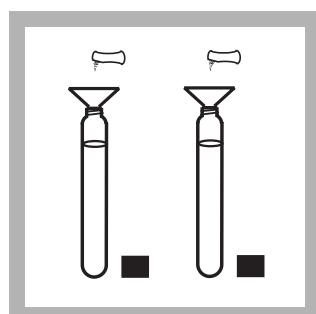


3. Remove the caps from two AmVer Diluent Reagent Test 'N Tubes for Low Range Ammonia Nitrogen. Add 2.0 mL of sample to one vial (the sample). Add 2.0 mL of ammonia-free water to the other vial (the blank).

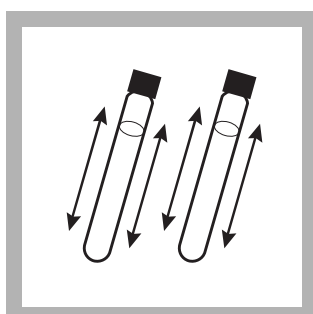
Note: For non-preserved samples with extreme pH, see the *Interferences* section.



4. Using a funnel, add the contents of one Ammonia Salicylate Reagent Powder Pillow for 5 mL sample to each vial.

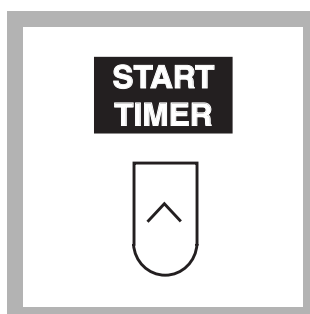


5. Add the contents of one Ammonia Cyanurate Reagent Powder Pillow (for 5-mL sample) to each vial.



6. Cap the vials tightly and shake thoroughly to dissolve the powder.

Note: A green color will develop if ammonia is present.



7. Press the soft key under **START TIMER**. A 20-minute reaction period will begin.

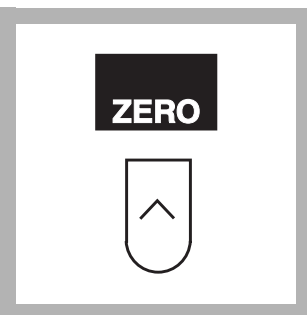


8. Insert the Test Tube Adapter into the sample cell module by sliding it under the thumb screw and into the alignment grooves. Fasten with the thumb screw.



9. When the timer beeps, clean the outside of the vial with a towel, and place the blank into the cell holder. Close the light shield.

Note: Wiping with a damp cloth followed by a dry one removes fingerprints and other marks.



10. Press the soft key under **ZERO**.

The display will show:

0.000 mg/L NH₃-N

Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



11. Place the prepared sample into the cell holder and close the light shield. The result in mg/L ammonia nitrogen (or chosen units) will be displayed.

Note: The results can be expressed as NH₃-N ammonia (NH₃). Press the soft keys under **OPTIONS**, then **FORM**: to scroll through the available options.

Interferences

Interfering Substance	Interference Levels and Treatments
Calcium	2,500 mg/L as CaCO ₃
Iron	<ol style="list-style-type: none"> Determine the amount of iron present in the sample following one of the Iron, Total, procedures. Add the same iron concentration to the ammonia-free water in Step 4. The interference will then be successfully blanked out.
Magnesium	15,000 mg/L as CaCO ₃
Nitrite	30 mg/L as NO ₂ -N
Nitrate	250 mg/L as NO ₃ -N
Orthophosphate	250 mg/L as PO ₄ ³⁻ -P
pH	Acidic or basic samples should be adjusted to approximately pH 7. Use 1 N Sodium Hydroxide Standard Solution for acidic samples and 1 N Hydrochloric Acid Standard Solution for basic samples.
Sulfate	300 mg/L as SO ₄ ²⁻
Sulfide	<ol style="list-style-type: none"> Measure about 350 mL of sample in a 500 mL Erlenmeyer flask. Add the contents of one Sulfide Inhibitor Reagent Powder Pillow. Swirl to mix. Filter the sample through a folded filter paper. Use the filtered solution in Step 3.
Other	Less common interferences such as hydrazine and glycine will cause intensified colors in the prepared sample. Turbidity and color will give erroneous high values. Samples with severe interferences require distillation. Hach recommends the distillation procedure using the Hach General Purpose Distillation Set. See OPTIONAL REAGENTS AND STANDARDS at the end of this procedure.

Sample Collection, Storage and Preservation

Collect samples in clean plastic or glass bottles. Best results are obtained with immediate analysis. If chlorine is known to be present, add one drop of 0.1 N sodium thiosulfate for each 0.3 mg/L Cl_2 in a one liter sample. Preserve the sample by reducing the pH to 2 or less with hydrochloric acid (at least 2 mL). Store at 4 °C (39 °F) or less. Preserved samples may be stored up to 28 days. Before analysis, warm samples to room temperature and neutralize with 5.0 N Sodium Hydroxide. Correct the test result for volume additions, see Section 1.2.2 *Correcting for Volume Additions*.

Accuracy Check

Standard Additions Method

- a. Leave the unspiked sample in the sample compartment. Verify that the units displayed are in mg/L. Select standard additions mode by pressing the soft keys under **OPTIONS, (MORE)** and then **STD ADD**.
- b. Press **ENTER** to accept the default sample volume (mL), 25.0.
- c. Press **ENTER** to accept the default standard concentration (mg/L), 50.000.
- d. Press the soft key under **ENTRY DONE**.
- e. Snap the neck off an Ammonia Nitrogen Ampule Standard, 50-mg/L as $\text{NH}_3\text{-N}$.
- f. Use the TenSette Pipet to add 0.1 mL, 0.2 mL and 0.3 mL of standard to three 25-mL samples. Mix thoroughly.
- g. Analyze each spiked sample as described above. Accept the standard additions reading by pressing the soft key under **READ** each time. Each addition should reflect approximately 100% recovery.
- h. After completing the sequence, the display will show the extrapolated concentration value and the “best-fit” line through the standard additions data points, accounting for matrix interferences.
- i. See Section 1.4.1 *Standard Additions* for more information.

Standard Solution Method

To check accuracy, use the Nitrogen Ammonia Standard Solution, 1.0-mg/L listed under **OPTIONAL REAGENTS AND STANDARDS**. Or, dilute 1 mL of 50-mg/L Nitrogen Ammonia Standard Solution to 50 mL with deionized water in a 50-mL volumetric flask.

Method Performance

Precision

Standard: 1.000 mg/L $\text{NH}_3\text{-N}$

Program	95% Confidence Limits
2460	0.985–1.014 mg/L $\text{NH}_3\text{-N}$

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
2460	0.031 mg/L NH ₃ -N

For more information on derivation and use of Hach's estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, appendix B, see Section 1.5.1.

Sensitivity

Program Number: 2460

Portion of Curve	Δ Abs	Δ Concentration
Entire Range	0.010	0.01604 mg/L

See Section 1.5.3 *Sensitivity Explained* for more information.

Calibration Standard Preparation

To perform an ammonia calibration using the Test 'N Tube LR salicylate method, prepare calibration standards containing 0.5, 1.0, 1.5, 2.0, and 2.5 mg/L NH₃-N as follows:

- Into five different 1000-mL Class A volumetric flasks, pipet 5, 10, 15, 20, and 25 mL of a 100-mg/L Ammonia Nitrogen Standard (Cat. No 24065-49) using Class A glassware.
- Dilute to the mark with deionized water. Mix thoroughly.
- Using the salicylate method and the calibration described in the *User-Entered Programs* section of the *DR/4000 Spectrophotometer Instrument Manual*, generate a calibration curve from the standards prepared above.

Summary of Method

Ammonia compounds combine with chlorine to form monochloramine. Monochloramine reacts with salicylate to form 5-aminosalicylate. The 5-aminosalicylate is oxidized in the presence of a sodium nitroprusside catalyst to form a blue colored compound. The blue color is masked by the yellow color from the excess reagent present to give a final green colored solution.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to Section 1.

Pollution Prevention and Waste Management

The ammonia salicylate reagent contains sodium nitroferricyanide. Cyanide solutions are regulated as hazardous wastes by the Federal RCRA. Collect cyanide solutions for disposal as reactive (D001) waste. Be sure cyanide solutions are stored in a caustic solution with pH >11 to prevent release of hydrogen cyanide gas. See Section 1 for further information in proper disposal of these materials.

REQUIRED REAGENTS AND STANDARDS

	Cat. No.
Low Range Test 'N Tube Nitrogen-Ammonia AmVer Reagent Set (25 tests)	26045-45
Includes:(50) AmVer Diluent LR Vials*, (1) 23952-66, (1) 23954-66, (1) 272-42	

Description	Quantity Required per test	Unit	Cat. No.
AmVer Diluent Reagent Test 'N Tube, Low Range.....	2 vials	50/pkg	*
Ammonia Cyanurate Reagent Powder Pillows, (5-mL sample) ...	2 pillows	50/pkg	23954-66
Ammonia Salicylate Reagent Powder Pillows, (5-mL sample)	2 pillows	50/pkg	23952-66

REQUIRED EQUIPMENT AND SUPPLIES

DR/4000 Test Tube Adapter.....	1	each.....	48189-00
Funnel, micro, poly	1	each.....	25843-35
Pipet, TenSette, 0–10 mL.....	1	each.....	19700-10
Pipet Tips, for TenSette Pipet 19700-10	varies.....	50/pkg	21997-96
Test Tube Rack	1–3	each.....	18641-00

OPTIONAL REAGENTS AND STANDARDS

Nitrogen Ammonia Standard Solution, 1.0-mg/L NH ₃ -N	500 mL.....	1891-49
Nitrogen Ammonia Standard Solution, 100-mg/L NH ₃ -N	500 mL.....	24065-49
Nitrogen Ammonia Standard Solution, 2-mL PourRite Ampule, 50-mg/L NH ₃ -N..	20/pkg.....	14791-20
Nitrogen Ammonia Standard Solution, 10-mL PourRite Ampule, 50-mg/L NH ₃ -N	16/pkg.....	14791-10
Hydrochloric Acid, ACS	500 mL.....	134-49
Sodium Hydroxide Standard Solution, 5.0 N.....	50 mL SCDB.....	2450-26
Sodium Thiosulfate Standard Solution, 0.1 N.....	100 mL MDB.....	323-32
Description	Unit	Cat. No.
Sulfide Inhibitor Reagent Powder Pillows	100/pkg	2418-99
Water, deionized	4 L.....	272-56

OPTIONAL EQUIPMENT AND SUPPLIES

Ampule Breaker Kit, Voluette	each.....	21968-00
Ampule Breaker Kit, PourRite	each.....	24846-00
Distillation Apparatus Set	each.....	22653-00
Distillation Heater & Support Apparatus Set, 120 VAC	each.....	22744-00
Distillation Heater & Support Apparatus Set, 230 VAC	each.....	22744-02
Filter Paper, folded	100/box.....	1894-57
Flask, Erlenmeyer, 500-mL.....	each.....	505-49
Flask, volumetric, Class A, 50.0-mL	each.....	14574-41
Flask, volumetric, Class A, 1000-mL	each.....	14574-53
Funnel, analytical (for filtration).....	each.....	1083-68
pH Indicator Paper, pH 1.0 to 11.0.....	5 rolls/pkg	391-33
Pipet, TenSette, 0.1–1.0 mL	each.....	19700-01
Pipet Tips, for TenSette Pipet 19700-01	50/pkg	21856-96
Pipet, volumetric, Class A, 5.00-mL	each.....	14515-37
Pipet, volumetric, Class A, 10.00-mL	each.....	14515-38
Pipet, volumetric, Class A, 15.00-mL	each.....	14515-39
Pipet, volumetric, Class A, 20.00-mL	each.....	14515-20
Pipet, volumetric, Class A, 25.00-mL	each.....	14515-40
Pipet Filler	each.....	12189-00
Thermometer, -20 to 100 °C.....	each.....	566-01
Thermometer, -10 to 260 °C.....	each.....	20959-26

* Not sold separately.



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FAX: (970) 669-2932



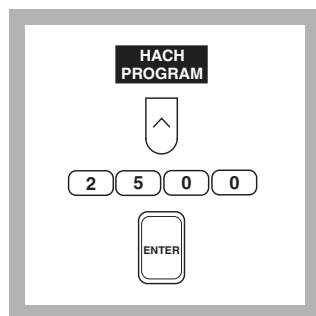
Method 10049

UV Direct Reading Method*

(0.0 to 10.2 mg/L NO_3^- -N)

Scope and Application: For uncontaminated natural and potable water supplies containing low concentrations of organic matter. The estimated detection limit for program number 2500 is 0.2 mg/L NO_3^- -N.

* Adapted from *Standard Methods for the Examination of Water and Wastewater*, 18th ed., part 4500, pages 4–87.



1. Press the soft key under **HACH PROGRAM**.

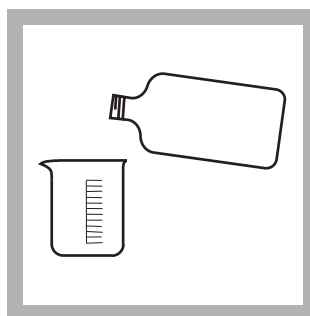
Select the stored program number for Nitrate, UV direct reading method, by pressing **2500** with the numeric keys.

Press: **ENTER**



2. The display will show:
HACH PROGRAM: 2500 N, Nitrate

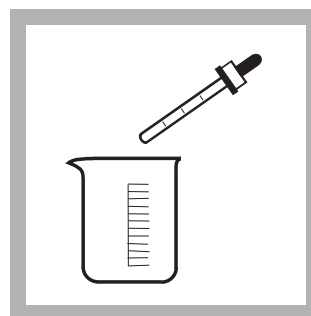
The wavelength (λ), **220 nm**, is automatically selected.



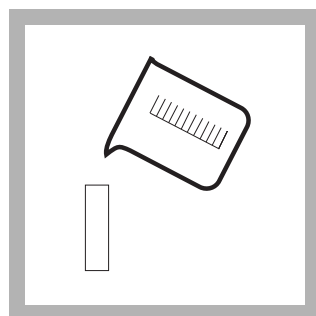
3. Collect 50 mL of clear sample in a 100-mL beaker.

Note: Turbid samples must be filtered prior to analysis.

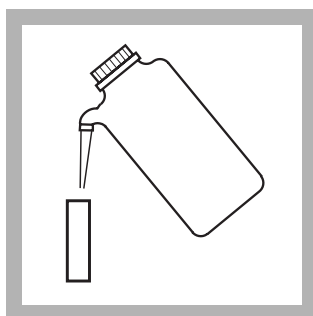
Note: For proof of accuracy, use a 10.0 -g/L Nitrate Nitrogen Standard Solution in place of sample.



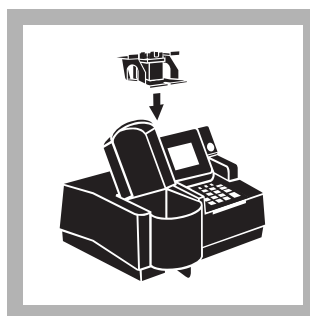
4. Add 1 mL of 1.0 N Hydrochloric Acid Standard Solution to the beaker and swirl to mix.



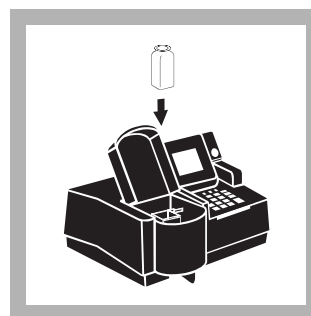
5. Fill a 1-cm quartz sample cell with sample. Discard the excess.



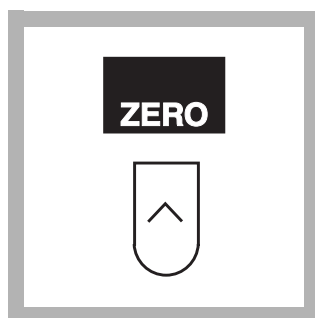
6. Fill a 1-cm quartz sample cell (the blank) with deionized water.



7. Insert the 1-cm Cell Adapter into the sample cell compartment.



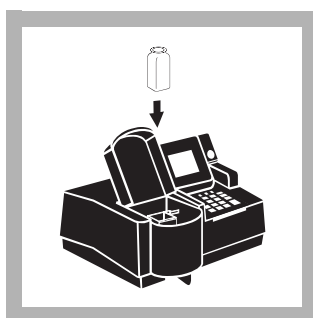
8. Place the blank into the cell holder and close the light shield.



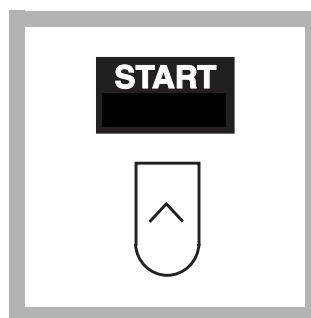
9. Press the soft key under **ZERO**.

The instrument will zero at 220 nm and at 275 nm.

Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



10. When prompted, place the sample in the cell holder and close the light shield.



11. Press the soft key under **START**.

The instrument will read the sample at 220 and 275 nm. When finished, the display will show the sample nitrate nitrogen concentration.

Note: The results can be expressed as nitrate (NO_3^-). Press the soft keys under **OPTIONS**, then **FORM**: to scroll through the available options. Press **ENTER** to return to the read screen.

Interferences

Interfering Substance	Interference Levels and Treatments
Chlorate	May interfere
Cr^{6+}	All levels
Dissolved organic matter	All levels
NO_2^-	All levels
Surfactants	All levels
Suspended particulate matter	Remove using filtration.

Sample Collection, Preservation and Storage

Most reliable results are obtained when samples are analyzed as soon as possible after collection. If prompt analysis is impossible, store samples in clean plastic or glass bottles for up to 24 hours at 4 °C. To preserve samples for longer periods, add 2 mL of concentrated sulfuric acid (H_2SO_4) per liter and store at 4 °C.

Accuracy Check

Standard Solution Method

To test accuracy, use a 10-mg/L Nitrate Nitrogen Standard Solution (44.3-mg/L as NO_3^-) in place of the sample and perform the procedure as described.

To adjust the calibration curve using the reading obtained with the 10.0 mg/L Nitrate Nitrogen Standard Solution, press the soft keys under **OPTIONS, (MORE)** then **STD: OFF**. Press **ENTER** to accept the default concentration, 10.0 mg/L NO_3^- -N. If an alternate concentration is used, enter the actual concentration and press **ENTER** to return to the read screen. See Section 1.5.5 *Adjusting the Standard Curve* for more information.

Method Performance

Precision

Standard: 10.0 mg/L NO_3^- -N

Program	95% Confidence Limits
2500	9.9–10.1 mg/L NO_3^- -N

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
2500	0.2 mg/L NO_3^- -N

For more information on derivation and use of Hach's estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, Appendix B, see Section 1.5.1.

Sensitivity

Program Number: 2470

Portion of Curve:	ΔAbs	$\Delta\text{Concentration}$
0.010 Abs	0.010	0.04 mg/L NO_3^- -N
5.1 mg/L NO_3^- -N	0.010	0.04 mg/L NO_3^- -N
9.2 mg/L NO_3^- -N	0.010	0.06 mg/L NO_3^- -N

See Section 1.5.3 *Sensitivity Explained* for more information.

Summary of Method

The UV nitrate direct screening method offers rapid determination of nitrate. Because both nitrate and organic constituents absorb at 220 nm and nitrate does not absorb at 275 nm, the second reading at 275 nm is used to correct for the absorbance attributed to organic matter. Although this method is useful for monitoring nitrate, it is not recommended for samples containing high concentrations of organics. Adding hydrochloric acid prevents interference from hydroxide or carbonate concentrations up to 1000 mg/L CaCO_3 .

NITROGEN, Nitrate, continued

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult *Material Safety Data Sheets (MSDS)* for information specific to the standard used. For additional information, refer to Section I.

Pollution Prevention and Waste Management

For information on pollution prevention and waste management, refer to Section I.

REQUIRED REAGENTS AND STANDARDS

Description	Quantity Required		Cat. No.
	Per Test	Unit	
Hydrochloric Acid Standard Solution, 1.0 M	1 mL	1 liter.....	23213-53
Water, deionized	10 mL	4 liters.....	272-56

REQUIRED EQUIPMENT AND SUPPLIES

Beaker, 100-mL	1	each.....	500-42
DR/4000 1-cm Cell Adapter	1	each.....	48584-00
Nitrate Nitrogen Standard Solution, 10-mg/L.....	varies.....	500 mL.....	307-49
Sample cells, 1-cm, quartz	2	each.....	26244-10

OPTIONAL REAGENTS AND STANDARDS

Sulfuric Acid, ACS, concentrated	500 mL.....	979-49
--	-------------	--------

OPTIONAL EQUIPMENT AND SUPPLIES

Aspirator, Nalgene vacuum pump	each.....	2131-00
Filter Holder, 47-mm,.....	each.....	13529-00
Filter, membrane, 47-mm, 0.45 microns	100/pkg.....	13530-00
Flask, filtering, 500-mL	each.....	546-49
Pipet, serological, 2-mL	each.....	532-36
Stopper, No. 7, one-hole.....	6/pkg.....	2119-07
Tubing, rubber latex	12 ft.....	560-19



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Method 10071

Persulfate Digestion Method

Test 'N Tube™ Vials

(0.0 to 25.0 mg/L N)

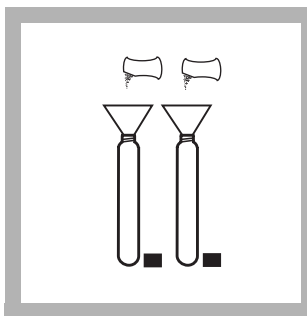
Scope and Application: For water and wastewater; digestion is required for determining total nitrogen.
Digestion procedure included in method.



1. Turn on the COD Reactor. Heat to 103–106 °C (best temperature is 105 °C). Place the plastic shield in front of the reactor.

Note: Ensure safety devices are in place to protect the analyst from splattering due to leakage.

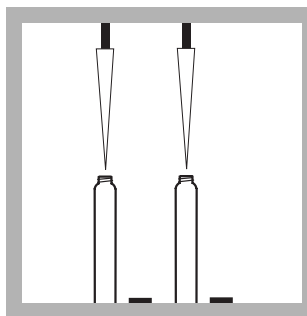
Note: For proof of accuracy, run a 20-mg/L $\text{NH}_3\text{-N}$ standard through digestion and analysis.



2. Using a funnel, add the contents of one Total Nitrogen Persulfate Reagent Powder Pillow to each of two Total Nitrogen Hydroxide Reagent vials.

Note: Wipe reagent from the lid and the tube threads.

Note: One reagent blank is sufficient for each set of samples.



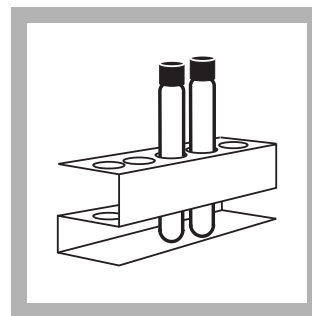
3. Add 2 mL of sample to a vial. This is the prepared sample.

Add 2 mL of the deionized water included in the kit to a second vial. This is the reagent blank.

Cap both vials, shake vigorously to mix (more than 30 seconds), and place the vials in the COD Reactor. Heat for 30 minutes.

Note: Use only water that is free of all nitrogen-containing species as a substitute for the deionized water provided.

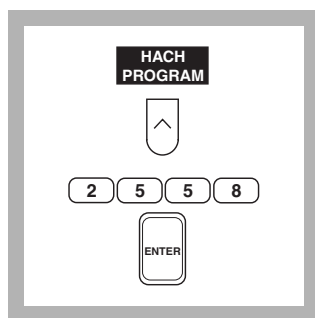
Note: The persulfate reagent may not dissolve completely after shaking. This will not affect accuracy.



4. Using finger cots or gloves, remove the hot vials from the reactor and allow to cool to room temperature.

Note: It is important to remove the vials from the COD Reactor after exactly 30 minutes.

NITROGEN, Total, continued



5. Press the soft key under **HACH PROGRAM**.

Select the stored program for Test 'N Tube Total Nitrogen by pressing **2558** with the numeric keys.

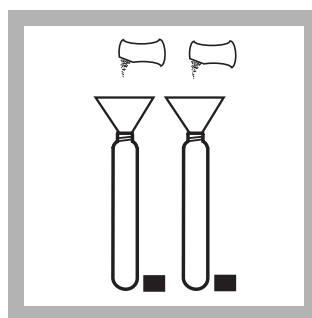
Press: **ENTER**



6. The display will show:

**HACH PROGRAM:2558
N, Total, TNT**

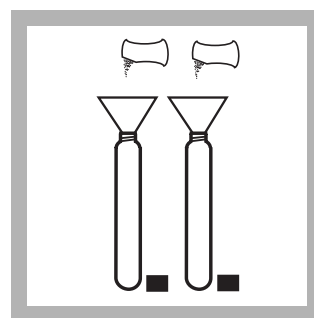
The wavelength (λ), **410 nm**, is automatically selected.



7. Remove the caps from the digested vials and add the contents of one TN Reagent A Powder Pillow to each vial. Cap tubes and shake for 15 seconds.

Press the soft key under **START TIMER** after shaking.

A 3-minute reaction period will begin.

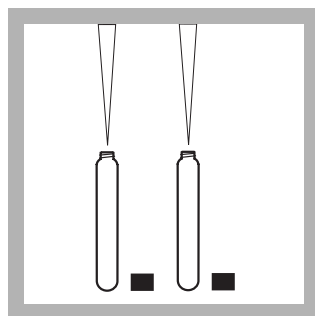


8. After the timer beeps, remove the caps from the vials and add one TN Reagent B Powder Pillow to each vial. Cap the tubes and shake for 15 seconds.

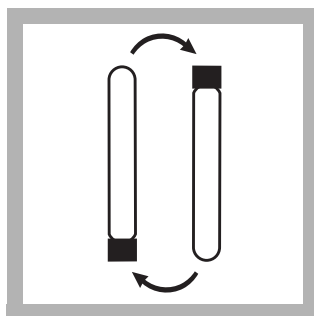
Press the soft key under **START TIMER** after shaking.

A 2-minute reaction period will begin.

Note: The reagent will not completely dissolve. The solution will begin to turn yellow.

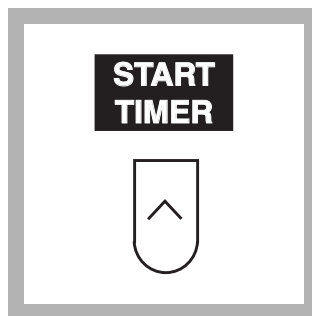


9. After the timer beeps, remove the caps from two TN Reagent C Vials and add 2 mL of digested, treated sample to one vial. Add 2 mL of the digested, treated reagent blank to the second TN Reagent C Vial.



10. Cap and invert 10 times to mix. Use slow, deliberate inversions for complete recovery. The tubes will be warm.

Note: Hold the tube vertical with the cap up. Invert the vial and wait for all of the solution to flow to the cap end. Return the vial to the upright position and wait for all of the solution to flow to the vial bottom. This is one inversion (10 inversions = 30 seconds).



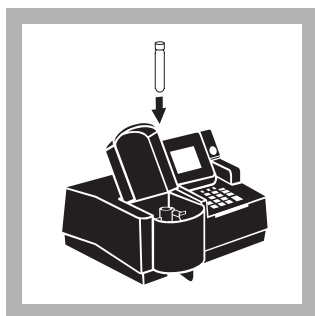
11. Press the soft key under **START TIMER**.

A 5-minute reaction period will begin.

Note: The yellow color will intensify.



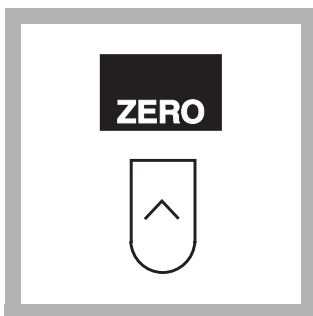
12. Insert the Test 'N Tube Adapter into the sample cell module by sliding it under the thumb screw and into the alignment grooves. Fasten with the thumb screw.



13. When the timer beeps, clean the outside of the TN Reagent C Vial containing the reagent blank. Place the vial in the adapter and close the light shield.

Note: Wiping with a damp towel followed by a dry one will remove fingerprints or other marks.

Note: The reagent blank is stable when stored in the dark; see Blanks For Colorimetric Measurement following these steps.



14. Press the soft key under **ZERO**.

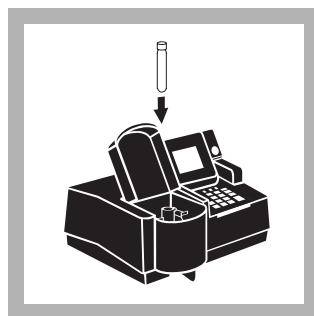
The display will show:

0.0 mg/L N

Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



15. Wipe the TN Reagent C Vial containing the sample.



16. Place the prepared sample into the cell holder and close the light shield. The result in mg/L total nitrogen will be displayed.

Note: Multiple samples may be read after zeroing on one reagent blank.

Note: Results may be expressed as N, NH_3 or NO_3^- . Press the soft keys under **OPTIONS** and then **FORM** to scroll through the available options. Press **ENTER** to return to the read screen.

Note: If the test overranges, repeat the digestion and measurement with diluted sample. The digestion must be repeated for accurate results.

Interferences

The substances in the following table have been tested and found **not** to interfere up to the indicated levels (in mg/L):

Substance	Maximum Level Tested (mg/L)
Barium	2.6
Calcium	300
Chromium (3^+)	0.5
Iron	2
Lead	6.6 ppb
Magnesium	500
Organic Carbon	150
pH	13 pH units
Phosphorus	100
Silica	150
Silver	0.9
Tin	1.5

NITROGEN, Total, continued

Interfering substances that resulted in a concentration change of $\pm 10\%$:

Substance	Level and Effect
Bromide	>60ppm; positive interference
Chloride	>1000 ppm; positive interference

Hach chemists tested this chemistry on standard nitrogen solutions prepared from the following compounds and obtained 95% recovery:

- Ammonium chloride
- Ammonium sulfate
- Ammonium acetate
- Urea
- Glycine

Ammonium chloride or nicotinic-PTSA spikes in domestic influent, effluent and the ASTM standard specification for substitute wastewater (D 5905-96) also resulted in $\geq 95\%$ recovery.

The large amounts of nitrogen-free organic compounds in some samples may decrease digestion efficiency by consuming some of the persulfate present. Samples known to contain high levels of organics should be diluted and re-run to verify digestion efficiency.

Sample Collection, Storage and Preservation

Collect samples in clean plastic or glass bottles. Best results are obtained with immediate analysis.

Preserve the sample by reducing the pH to 2 or less with concentrated sulfuric acid (at least 2 mL). Store at 4 °C (39 °F) or less. Preserved samples may be stored up to 28 days. Warm samples to room temperature and neutralize with 5 N sodium hydroxide before analysis. Correct the test result for volume additions; see Section 1.2.2 *Correcting for Volume Additions*.

Method Performance

Precision

Hach analysis of two independent nutrient standards shows the lowest average percent recovery at 95% with a standard deviation of $\pm 2\%$.

Precision

Standard: 15 mg/L $\text{NH}_3\text{-N}$

Program	95% Confidence Limits
2558	14.2–15.8 mg/L N

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
2558	2 mg/L N

For more information on derivation and use of Hach's estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by 40 CFR part 136, appendix B, see Section 1.5.1.

Sensitivity

Program Number: 2558

Program	ΔAbs	ΔConcentration
Entire Range	0.010	0.52 mg/L N

See Section 1.5.3 *Sensitivity Explained* for more information.

Accuracy Check

This method generally yields 95–100% recovery on organic nitrogen standards. For proof of accuracy Hach offers a set of 3 Primary Standards for Kjeldahl Nitrogen.

1. Prepare one or more of the following 3 solutions. Each preparation is for an equivalent 25-mg/L N standard. Use the deionized water included in the kit or water that is free of all organic and nitrogen-containing species.
 - a. Weigh 0.3379 g of Ammonium p-Toluenesulfonate (PTSA). Dissolve in a 1000-mL volumetric flask with deionized water. Add deionized water to the 1000-mL mark.
 - b. Weigh 0.4416 g of Glycine p-Toluenesulfonate. Dissolve in a 1000-mL volumetric flask with deionized water. Add deionized water to the 1000-mL mark.
 - c. Weigh 0.5274 g of Nicotinic p-Toluenesulfonate. Dissolve in a 1000-mL volumetric flask with deionized water. Add deionized water to the 1000-mL mark.
2. Analyze each of these solutions using the test procedure above. Calculate the percent recovery for each using this formula:

$$\% \text{ recovery} = \frac{\text{measured concentration}}{25} \times 100$$

The percent recovery should be:

Compound	Lowest Expected % Recovery
Ammonia-PTSA	95%
Glycine-PTSA	95%
Nicotinic-PTSA	95%

Hach analysts have found Ammonia-PTSA to be the most difficult to digest.

Other compounds may yield different percent recoveries.

Standard Solution Method

For proof of accuracy, substitute 2 mL of a 20-mg/L ammonia nitrogen standard solution for the sample in the procedure*. A single analyst should obtain less than 5% variation on replicates.

To adjust the calibration curve using the reading obtained with the 20-mg/L N standard solution, press the soft keys under **OPTIONS, (MORE)** then **STD: (OFF)**. Press **ENTER** to accept the default concentration. If an alternate concentration is used, enter the actual concentration and press **ENTER** to return to the “read” screen. See Section 1.5.5 *Adjusting the Standard Curve*, for more information.

Standard Additions Method

- a. Fill three 25-mL graduated mixing cylinders with 25 mL of sample.
- b. Snap the neck off an Ammonia Nitrogen Voluette® Ampule Standard Solution, 160-mg/L as $\text{NH}_3\text{-N}$
- c. Use the TenSette® Pipet to add 0.3, 0.6, and 0.9 mL of standard, respectively, to the three mixing cylinders.
- d. Stopper each cylinder and mix thoroughly.
- e. Add 2 mL of each prepared solution, respectively, to three Total Nitrogen Hydroxide Reagent Vials.
- f. Analyze each standard addition sample as described in the procedure. The nitrogen concentration should increase by approximately 1.9, 3.8, and 5.6 mg/L N, respectively.
- g. If these increases do not occur, see Section 1.4.1 *Standard Additions* for troubleshooting information.

Blanks For Colorimetric Measurement

The reagent blank may be used up to seven days for measurements using the same lots of reagents. Store it in the dark at room temperature (18–25 °C). If a small amount of white floc appears prior to the end of one week, discard the reagent blank and prepare a new one.

Calibration Standard Preparation

To perform a total nitrogen calibration using the Test ‘N Tube Persulfate Digestion method, prepare calibration standards containing 5.00, 10.00, 15.00, and 20 mg/L nitrogen ($\text{NH}_3\text{-N}$) as follows:

- a. Into three different 100-mL Class A volumetric flasks, pipet 5.00, 10.00, 15.00, and 20 mL of a 100-mg/L Ammonia Nitrogen Standard Solution using Class A glassware.
- b. Using the Basic Persulfate Digestion method and the calibration procedure described in the *User-Entered Programs* section of the *DR/4000*

* To prepare a 20-mg/L ammonia nitrogen standard, use a 20-mL Class A pipet to transfer 20 mL of a 100-mg/L Ammonia Nitrogen Standard Solution (see *OPTIONAL REAGENTS AND STANDARDS*) to a 100-mL Class A volumetric flask. Dilute to the line with deionized water.

Spectrophotometer Instrument Manual, generate a calibration curve from the standards prepared above.

Summary of Method

An alkaline persulfate digestion converts all forms of nitrogen to nitrate. Sodium metabisulfite is added after the digestion to eliminate halogen oxide interferences. Nitrate then reacts with chromotropic acid under strongly acidic conditions to form a yellow complex with an absorbance maximum at 410 nm.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to Section 1.

Pollution Prevention and Waste Management

For information on pollution prevention and waste management, refer to Section 1.

REQUIRED REAGENTS AND STANDARDS

Test 'N Tube Total Nitrogen Reagent Set (50 sets of vials).....26722-45
Includes: (50) Total Nitrogen Hydroxide Reagent Vials*, (50) TN Reagent C Vials*, (1) 26718-46, (1) 26719-46, (1) 26720-46, (1) 272-42

Description	Quantity Required		Cat. No.
	Per Test	Unit	
Total Nitrogen Hydroxide Reagent Vials, 0.1 N	2 vials	50/pkg	*
Total Nitrogen Persulfate Reagent Powder Pillows	2 pillows	50/pkg	26718-46
TN Reagent A Powder Pillows	2 pillows	50/pkg	26719-46
TN Reagent B Powder Pillows	2 pillows	50/pkg	26720-49
TN Reagent C Vials.....	2 vials	50/pkg	*
Water, deionized	4 mL	100 mL.....	272-42f

REQUIRED EQUIPMENT AND SUPPLIES

COD Reactor, 115/230 VAC, North American Plug.....	1	each.....	45600-00
COD Reactor, 230 VAC, European Plug.....	1	each.....	45600-02
DR/4000 Test Tube Adapter.....	1	each.....	48189-00
Funnel, micro	1	each.....	25843-35
Safety Shield, laboratory bench, 38 x 40 cm.....	1	each.....	23810-00
Test Tube Cooling Rack	1-3	each.....	18641-00

OPTIONAL REAGENTS AND STANDARDS

Description	Unit	Cat. No.
Ammonia Nitrogen Standard Solution as N, 100-mg/L.....	500 mL.....	24065-49
Ammonia Nitrogen Standard Solution, 10-mg/L NH ₃ -N.....	500 mL.....	153-49
Ammonia Nitrogen Standard Solution, 100-mg/L NH ₃ -N.....	500 mL.....	24065-49
Ammonia Nitrogen Standard Solution, 10-mL Voluette Ampule, 160-mg/L NH ₃ -N	16/pkg	21091-10
Primary Standard Set, for Kjeldahl Nitrogen	set of 3	22778-00
Sodium Hydroxide Standard Solution, 5.0 N.....	59 mL SCDB.....	2450-26
Sulfuric Acid, ACS, concentrated	500 mL.....	979-49
Water, organic-free	500 mL.....	26415-49

* These items are not sold separately. Please reorder the complete set (Cat. No. 26722-45).

NITROGEN, Total, continued

OPTIONAL EQUIPMENT AND SUPPLIES

Balance, analytical, 110 VAC.....	each.....	26103-00
Balance, analytical, 220 VAC.....	each.....	26103-02
Cylinder, mixing, graduated, 25-mL (3 required)	each.....	20886-40
Flask, volumetric, Class A, 100-mL	each.....	14574-42
Flask, volumetric, Class A, 1000-mL (3 required)	each.....	14574-53
pH Paper, pH 1.0 to 11.0.....	5 rolls/pkg.....	391-33
Pipet, volumetric, Class A, 20-mL.....	each.....	14515-20
Pipet, TenSette, 1.0- to 10.0-mL.....	each.....	19700-10
Pipet Tips, for 19700-10 TenSette Pipet	50/pkg.....	21997-96
Pipet, TenSette, 0.1- to 1.0-mL	each.....	19700-01
Pipet Tips, for 19700-01 TenSette Pipet	50/pkg.....	21856-96



FOR TECHNICAL ASSISTANCE, PRICE INFORMATION AND ORDERING:

In the U.S.A. – Call toll-free 800-227-4224

Outside the U.S.A. – Contact the HACH office or distributor serving you.

On the Worldwide Web – www.hach.com; E-mail – techhelp@hach.com

HACH COMPANY

WORLD HEADQUARTERS

Telephone: (970) 669-3050

FAX: (970) 669-2932



✓ Method 10072

Persulfate Digestion Method

Test 'N Tube™ Vials

HR (10 to 150 mg/L N)

Scope and Application: For water and wastewater.

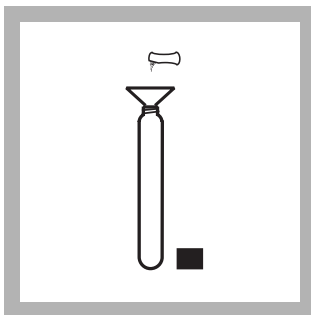
Digestion is required for determining total nitrogen. The digestion procedure is included in the method



1. Turn on the COD Reactor. Heat to 103-106 °C (optimal temperature is 105 °C). Place the plastic shield in front of the reactor.

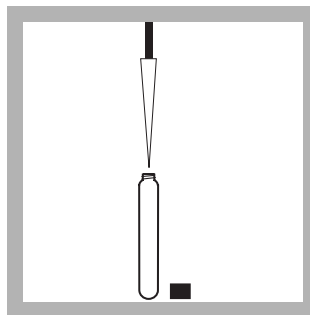
Note: Ensure safety devices are in place to protect the analyst should splattering and leakage occur.

Note: For proof of accuracy, run a 125 mg/L $\text{NH}_3\text{-N}$ standard through digestion and analysis.



2. Prepare a reagent blank: Using a funnel, add the contents of one Total Nitrogen Persulfate Reagent Powder Pillow to one HR Total Nitrogen Hydroxide Digestion Vial.

Note: Wipe off any reagent that gets on the lid or the tube threads.



3. Add 0.5 mL of the deionized water provided. Cap and shake vigorously for about 30 seconds.

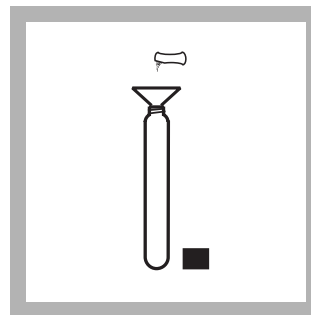
Process this reagent blank exactly the same as the sample, including digestion and color finish. Proceed to step 6.

Note: Alternate water must be free of all nitrogen-containing species.

Note: The persulfate reagent may not dissolve completely after shaking.

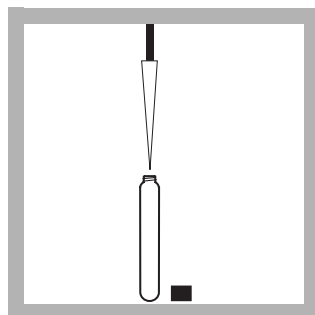
Note: One reagent blank is sufficient for each set of samples using the same lots of reagents.

Note: The reagent blank is stable for as long as seven days when stored in the dark; see Blanks for Colorimetric Measurement following this procedure.



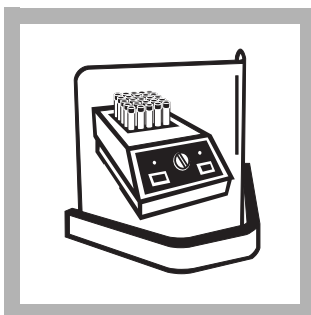
4. Prepare the sample: Using a funnel, add the contents of one Total Nitrogen Persulfate Reagent Powder Pillow to one HR Total Nitrogen Hydroxide Digestion Vial.

Note: Wipe off any reagent that gets on the lid or the tube threads.

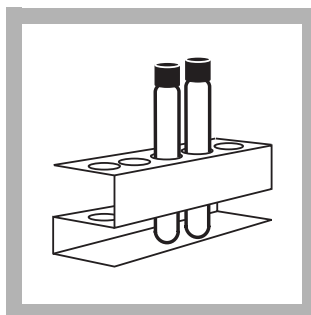


5. Add 0.5 mL of sample to the vial. Cap the vial, shake vigorously for about 30 seconds.

Note: The persulfate reagent may not dissolve completely after shaking.

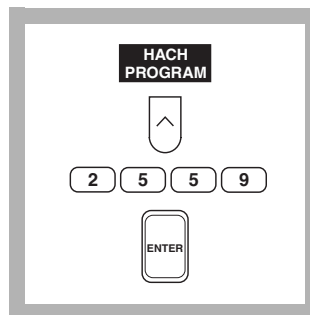


6. Place the vials in the COD Reactor. Heat for 30 minutes.



7. Using finger cots or gloves, remove the hot vials from the reactor and allow to cool to room temperature.

Note: It is very important to remove the vials from the COD Reactor after exactly 30 minutes.



8. Press the soft key under **HACH PROGRAM**.

Select the stored program for Test 'N Tube HR Total Nitrogen by pressing **2559** with the numeric keys.

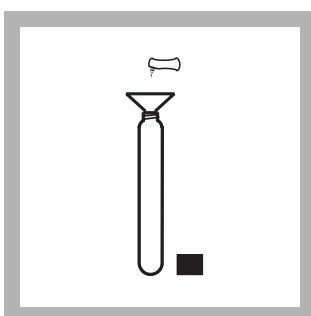
Press: **ENTER**

Note: A software update disc may be required to install this method.



9. The display will show:
HACH PROGRAM: 2559
N, Total, HR, TNT

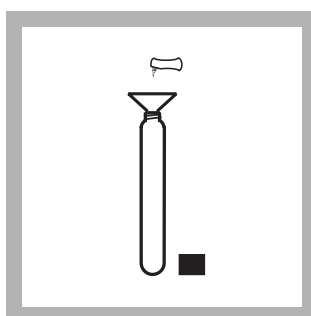
The wavelength (λ), **410 nm**, is automatically selected.



10. Add the contents of one Total Nitrogen Reagent A Powder Pillow to the vial containing the digested blank or sample. Cap the vial and shake for 15 seconds.

Press the soft key under **START TIMER** after shaking.

A 3-minute reaction period will begin.

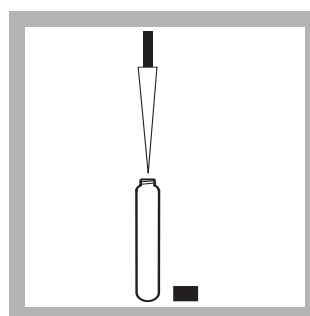


11. After the timer beeps, add one Total Nitrogen Reagent B Powder Pillow to the vial. Cap the vial and shake for 15 seconds.

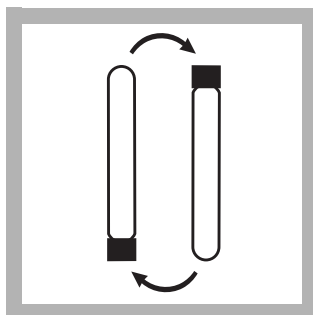
Press the soft key under **START TIMER** after shaking.

A 2-minute reaction period will begin.

Note: The reagent will not completely dissolve. The solution will begin to turn yellow.

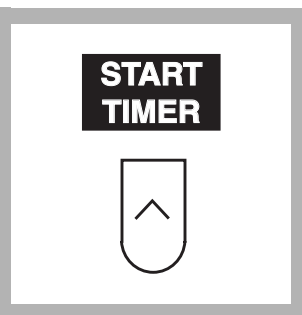


12. After the timer beeps, remove the cap from one Total Nitrogen Reagent C Vial. Add 2 mL of digested, treated sample (or reagent blank) to the vial. The vial will be warm.



13. Cap and invert slowly 10 times to mix.

Note: Proper mixing is important for complete recovery. Hold the vial vertical with the cap up. Invert the vial and wait for all of the solution to flow to the cap end. Pause. Return the vial to the upright position and wait for all of the solution to flow to the vial bottom. This is one inversion (10 inversions = 30 seconds).



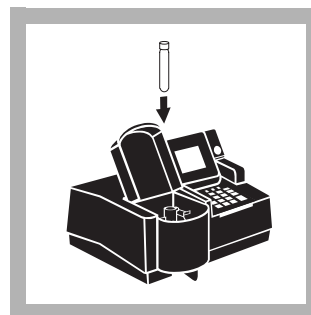
14. Press the soft key under **START TIMER**.

A 5-minute reaction period will begin. Do not invert the vial again.

Note: The yellow color will intensify.



15. Insert the Test 'N Tube Adapter into the sample cell module by sliding it under the thumb screw and into the alignment grooves. Fasten with the thumb screw.



16. When the timer beeps, clean the outside of the Total Nitrogen Reagent C Vial containing the reagent blank. Place the vial in the adapter with the Hach logo facing the front of the instrument and close the light shield.

Note: Wipe with a damp towel, followed by a dry one, to remove fingerprints or other marks.



17. Press the soft key under **ZERO**.

The display will show:

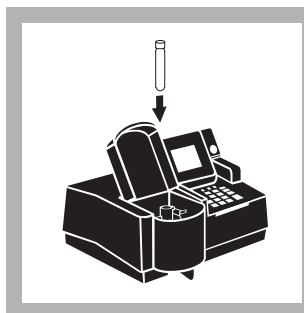
0 mg/L N

Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



18. Wipe the Total Nitrogen Reagent C vial containing the sample.

Note: Wipe with a damp towel, followed by a dry one, to remove fingerprints or other marks.



19. Place the vial into the cell holder with the Hach logo facing the front of the instrument and close the light shield. The result in mg/L total nitrogen will be displayed.

Note: Multiple samples may be read after zeroing on one reagent blank.

Note: Results may be expressed as N, NH_3 or NO_3^- . Press the soft keys under **OPTIONS** and then **FORM** to scroll through the available options. Press **ENTER** to return to the read screen.

Note: If the test overranges, repeat the digestion and measurement with diluted sample. The digestion must be repeated for accurate results.

NITROGEN, Total, continued

Interferences

The substances in the following table have been tested and found **not** to interfere up to the indicated levels (in mg/L):

Substance	Maximum Level Tested (mg/L)
Barium	10.4
Calcium	1200
Chromium (3+)	2
Iron	8
Lead	26.4 ppb
Magnesium	2000
Organic Carbon	600
pH	13 pH units
Phosphorus	400
Silica	600
Silver	3.6
Tin	6

Interfering substances that resulted in a concentration change of $\pm 10\%$:

Substance	Level and Effect
Bromide	> 240 ppm; positive interference
Chloride	≥ 3000 ppm; positive interference

The large amounts of nitrogen-free organic compounds in some samples may decrease digestion efficiency by consuming some of the persulfate reagent. Samples known to contain high levels of organics should be diluted and re-run to verify digestion efficiency.

Sample Collection, Storage and Preservation

Collect samples in clean plastic or glass bottles. Best results are obtained with immediate analysis.

Preserve the sample by reducing the pH to 2 or less with concentrated sulfuric acid (at least 2 mL/L). Store at 4 °C (39 °F) or less. Preserved samples may be stored up to 28 days. Warm samples to room temperature and neutralize with 5 N Sodium Hydroxide before analysis. Correct the test result for volume additions; see Section 1.2.2 *Correcting For Volume Additions*.

Accuracy Check

This method generally yields 95–100% recovery on organic nitrogen standards. For proof of accuracy Hach offers a set of three Primary Standards for Kjeldahl Nitrogen.

1. Prepare one or more of the following 3 solutions. Each preparation is for an equivalent 120-mg/L N standard. Use the deionized water included in the kit or water that is free of all organic and nitrogen-containing species.
 - a. Weigh 1.6208 g of Ammonium p-Toluenesulfonate (PTSA). Dissolve in a 1000-mL volumetric flask with deionized water. Add deionized water to the 1000-mL mark.

- b. Weigh 2.1179 g of Glycine p-Toluenesulfonate. Dissolve in a 1000-mL volumetric flask with deionized water. Add deionized water to the 1000-mL mark.
 - c. Weigh 2.5295 g of Nicotinic p-Toluenesulfonate. Dissolve in a 1000-mL volumetric flask with deionized water. Add deionized water to the 1000-mL mark.
2. Analyze each of these solutions using the test procedure above. Calculate the percent recovery for each using this formula:

$$\% \text{ recovery} = \frac{\text{measured concentration}}{120} \times 100$$

The percent recovery should be:

Compound	Lowest Expected % Recovery
Ammonia-PTSA	95%
Glycine-PTSA	95%
Nicotinic-PTSA	95%

Hach analysts have found Ammonia-PTSA to be the most difficult to digest. Other compounds may yield different percent recoveries.

Standard Solution Method

For proof of accuracy, substitute 0.5 mL of a 125-mg/L ammonia nitrogen standard solution for the sample in the procedure*.

To adjust the calibration curve using the reading obtained with a 120 mg/L N standard solution, press the soft keys under **OPTIONS, (MORE)**, then **STD: (OFF)**. Press **KEEP** to retain the default concentration. If an alternate concentration is used, enter the actual concentration and press **ENTER** to return to the read screen. See *Adjusting the Standard Curve* in the DR/4000 Procedures Manual for more information.

Standard Additions Method

- a. Fill three 25-mL graduated mixing cylinders with 25 mL of sample.
- b. Open an Ammonia Nitrogen Standard Solution, 1000-mg/L as $\text{NH}_3\text{-N}$.
- c. Use the TenSette® Pipet to add 0.1, 0.2 and 0.3 mL of standard, respectively, to the three mixing cylinders.
- d. Stopper each cylinder and mix thoroughly.
- e. Add 0.5 mL of each prepared solution, respectively, to three HR Total Nitrogen Hydroxide Digestion vials.
- f. Analyze each standard addition sample as described in the procedure. The nitrogen concentration should increase by approximately 4 mg/L N for each 0.1 mL of standard added.
- g. If these increases do not occur, see *Standard Additions* in *Section 1* of the Procedure Manual for troubleshooting information.

* To prepare a 125-mg/L ammonia nitrogen standard, use a 25-mL Class A pipet to transfer 25.00 mL of a 1000-mg/L Ammonia Nitrogen Standard Solution (see *OPTIONAL REAGENTS AND STANDARDS*) to a 200-mL Class A volumetric flask. Dilute to the line with organic-free water.

Blanks for Colorimetric Measurement

The reagent blank may be used repeatedly for measurements using the same lots of reagents. Store the reagent blank in the dark at room temperature (18–25 °C) for a maximum of seven days. If a small amount of white floc appears prior to the end of one week, discard the reagent blank and prepare a new one.

Calibration Standard Preparation

To perform a HR total nitrogen calibration using the Test 'N Tube Persulfate Digestion method, prepare calibration standards containing 20.00, 50.00, 80.00 and 125 mg/L nitrogen (NH₃-N) as follows:

- Using Class A glassware, pipet 5.00 mL of a 1000-mg/L Ammonia Nitrogen Standard Solution into a 250-mL Class A volumetric flasks.
- Using Class A glassware, pipet 25.00 mL of a 1000-mg/L Ammonia Nitrogen Standard Solution into a 500-mL Class A volumetric flask.
- Using Class A glassware, pipet 8.00 mL of a 1000-mg/L Ammonia Nitrogen Standard Solution into a 100-mL Class A volumetric flask.
- Using Class A glassware, pipet 25.00 mL of a 1000-mg/L Ammonia Nitrogen standard solution into a 200-mL class A volumetric flask.
- Using the Basic Persulfate Digestion method and the calibration procedure described in the *User-Entered Programs* section of the *DR/4000 Spectrophotometer Instrument Manual*, generate a calibration curve from the standards prepared above.

Method Performance

Precision:

Standard: 125 mg/L NH₃-N

Program	95% Confidence Limits
2559	122.5–127.5 mg/L N

For more information on determining precision data and method detection limits refer to *Section 1.5*.

Estimated Detection Limit:

Program	EDL
2559	7 mg/L N

For more information on derivation and use of Hach's estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40CFR, part 136, Appendix B, see *Section 1.5.1*.

Sensitivity:

Program Number: 2559

Program	ΔAbs	ΔConcentration
Entire Range	0.010	2 mg/L

See *Section 1.5.3 Sensitivity Explained* for more information.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used.

Summary of Method

An alkaline persulfate digestion converts all forms of nitrogen to nitrate. Sodium metabisulfite is added after the digestion to eliminate halogen oxide interferences. Nitrate then reacts with chromotropic acid under strongly acidic conditions to form a yellow complex with an absorbance maximum at 410 nm.

REQUIRED REAGENTS

Test 'N Tube HR Total Nitrogen Reagent Set (50 vials).....27141-00
Includes: (1) 26718-46, (1) 26719-46, (1) 26720-46, (1) 272-42, (50) Hydroxide digestion vials,*
(50) Acid Solution vials*, (1) 272-42

Description	Quantity Required		Cat. No.
	Per Test	Unit	
HR Total Nitrogen Hydroxide Digestion Vials	1 vial	50/pkg	*
Total Nitrogen Persulfate Reagent Powder Pillows	1 pillow	50/pkg	26718-46
Total Nitrogen Reagent A, Bisulfite Powder Pillows.....	1 pillow	50/pkg	26719-46
Total Nitrogen Reagent B, Indicator Powder Pillows	1 pillow	50/pkg	26720-46
Total Nitrogen Reagent C Vials, Acid Solution	1 vial	50/pkg	*
Water, deionized	1 mL	100 mL.....	272-42

REQUIRED EQUIPMENT AND SUPPLIES

COD Reactor, 115/230 VAC, North American Plug.....	1	each.....	45600-00
COD Reactor, 230 VAC, European Plug	1	each.....	45600-02
DR/4000 Test Tube Adapter.....	1	each.....	48189-00
Funnel, micro	1	each.....	25843-35
Pipet, TenSette, 0.1- to 1.0-mL	1	each.....	19700-01
Pipet Tips, for 19700-01 TenSette Pipet	2	50/pkg.....	21856-96
Safety Shield	1	each.....	23810-00
Test Tube Cooling Rack	1-3	each.....	18641-00

* These items are not sold separately. Please reorder the complete set (Cat. No. 27141-00).

NITROGEN, Total, continued

OPTIONAL REAGENTS AND STANDARDS

Description	Unit	Cat. No.
Ammonia Standard Solution as N, 1000-mg/L	500 mL	23541-53
Primary Standard Set, for Kjeldahl Nitrogen	set of 3	22778-00
Sodium Hydroxide Standard Solution, 5.0 N	59 mL SCDB	2450-26
Sulfuric Acid, ACS, concentrated	500 mL	979-49
Water, organic-free	500 mL	26415-49

OPTIONAL EQUIPMENT AND SUPPLIES

Balance, analytical, 115 VAC	each	26103-00
Balance, analytical, 230 VAC	each	26103-02
Cylinder, mixing, graduated, 25-mL	each	20886-40
Flask, volumetric, Class A, 100-mL	each	14574-42
Flask, volumetric, Class A, 200-mL	each	14574-45
Flask, volumetric, Class A, 250 mL	each	14574-46
Flask, volumetric, Class A, 500-mL	each	14574-49
Flask, volumetric, Class A, 1000-mL	each	14574-53
pH Paper, pH 1.0 to 11.0	5 rolls/pkg	391-33
Pipet, volumetric, Class A, 5-mL	each	14515-37
Pipet, volumetric, Class A, 8-mL	each	14515-08
Pipet, volumetric, Class A, 20-mL	each	14515-20
Pipet, volumetric, Class A, 25-mL	each	14515-40



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HACH COMPANY
WORLD HEADQUARTERS
Telephone: (970) 669-3050
FAX: (970) 669-2932



DR/4000 PROCEDURE

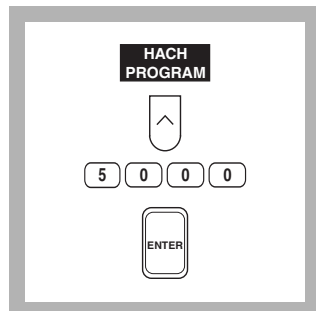
NITROGEN, Total

Persulfate Digestion Method

UniCell™ Vials

(0 to 40.0 mg/L N or TN_b)

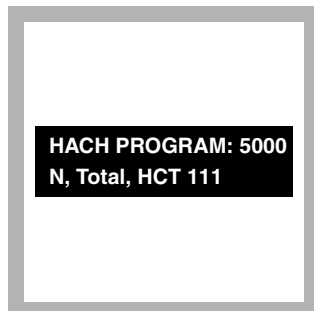
Scope and Application: For water and wastewater.



1. Press the soft key under **HACH PROGRAM**.

Select the stored program for UniCell Total Nitrogen by pressing **5000** with the numeric keys.

Press: **ENTER**.

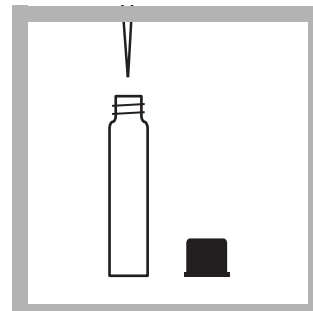


2. The display will show: **HACH PROGRAM: 5000 N, Total, HCT 111.**

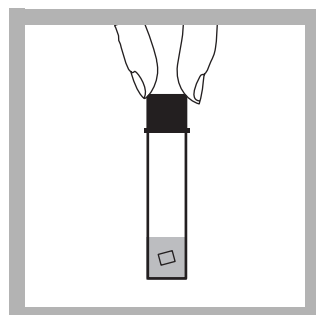
The wavelength (λ), **370 nm**, is automatically selected.



3. Insert the Test Tube Adapter into the sample cell module by sliding it under the thumb screw and into the alignment grooves. Fasten with the thumb screw.

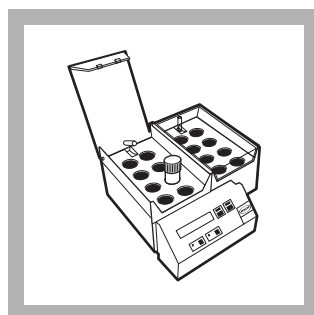


4. Pipet 0.5 mL of sample into a reaction tube (**red cap**). Immediately add 2.0 mL of Sodium Hydroxide Solution A (HCT 111 A).

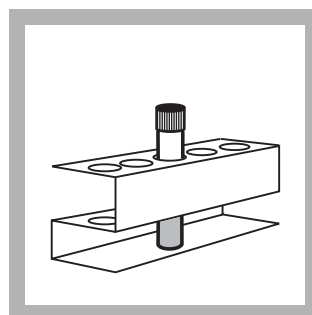


5. Immediately add one Oxidant Tablet B (HCT 111 B) and cap the reaction tube.

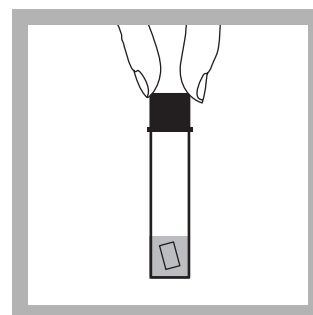
Do not invert.



6. Heat the reaction tube in the reactor block at 100 °C for 60 minutes.

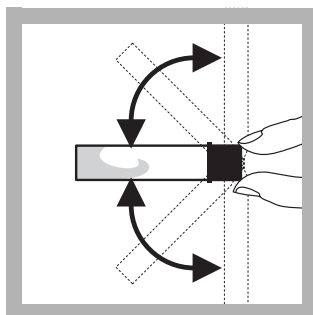


7. After the heating period, remove the tube from the reactor block and place it in a cooling rack.

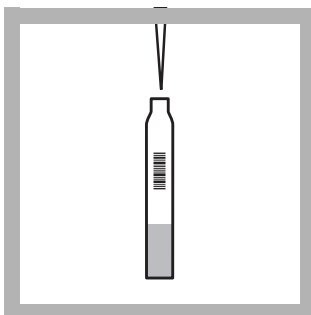


8. When the reaction tube has cooled to room temperature, add a colorless MicroCap C (HCT 111 C) and cap the tube.

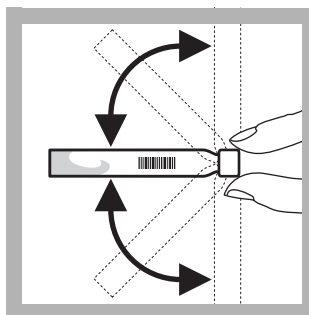
NITROGEN, Total, continued



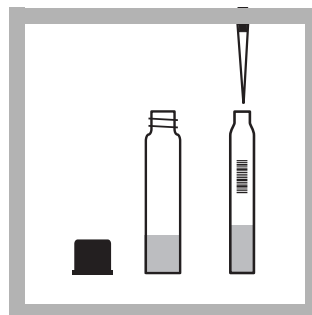
9. Invert the tube several times until the solid material in the cap is fully dissolved.



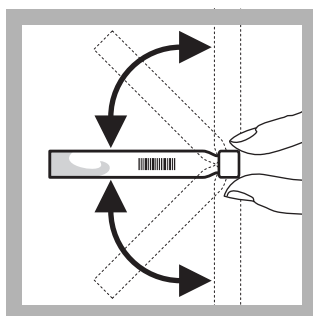
10. Pipet 0.2 mL of Dimethylphenol Solution D (HCT 111 D) into a sample vial (**blue cap**).



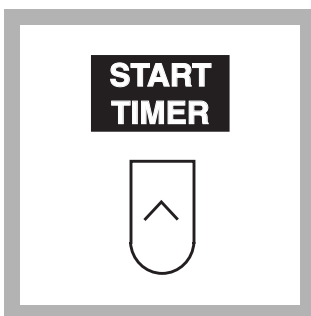
11. Cap the vial and invert three times to mix.



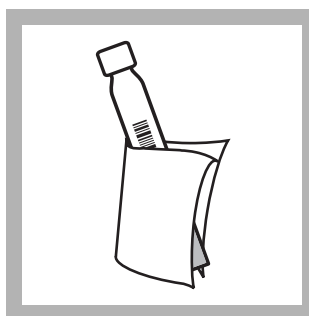
12. Immediately pipet 1.0 mL of digested sample from the reaction tube into the sample vial.



13. Cap the sample vial and invert it several times until the reagents are mixed and appear uniform.



14. Press the soft key under **START TIMER**. A 15-minute reaction period will begin.



15. Wipe the outside of the zero vial (**white cap**) and the sample vial with a damp towel followed with a dry one to remove fingerprints and other marks.



16. Place the zero vial into the cell holder. Close the light shield.

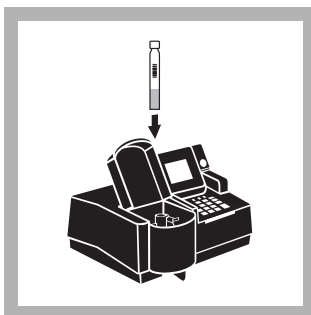


17. Press the soft key under **ZERO**.

The display will show:

0.00 mg/L N

Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



18. Place the sample vial into the cell holder. Close the light shield. Results in mg/L total nitrogen will be displayed.

Note: Results may be expressed as N or TN_b. Press the soft key under **OPTIONS** and then **FORM**: to scroll through the available options. Press **ENTER** to return to the read screen.

Interferences

The ions listed in the table have been tested up to the given concentrations. Cumulative effects and the influence of other ions have not been evaluated. There is no interference from:

Ion	No interference up to:
COD	1000 mg/L
Cl ⁻	2000 mg/L

Sample Collection, Preservation, and Storage

Collect samples in clean glass or plastic containers. Best results are obtained when samples are analyzed within 3 hours after collection. Store in a cool place. To preserve samples for longer periods, add 1mL of concentrated sulfuric acid per liter and store at 4 °C (39 °F).

Before analysis, adjust the pH to between 3 and 9 with 5.0 N Sodium Hydroxide standard solution.

Accuracy Check

Standard Additions Method

- Select standard additions mode by pressing the soft keys under **OPTIONS**, **(MORE)** and then **STD ADD**.
- Press **ENTER** to accept the default sample volume (mL), 50.
- Press **ENTER** to accept the default standard concentration (mg/L), 1000.
- Press the soft key under **ENTRY DONE**.
- Measure 50 mL of sample into each of three graduated cylinders.
- Use a pipet to add 0.20, 0.40 and 0.60 mL of 50-mg/L standard, respectively, to three 50-mL samples and mix each thoroughly.
- Stopper each cylinder and mix thoroughly.
- Analyze each standard addition sample as described above. Accept the standard additions reading by pressing the soft key under **READ** each time. The total nitrogen concentration should increase by approximately 4.0 mg/L N for each 0.2 mL of standard solution added.
- After completing the sequence, the display will show the extrapolated concentration value and the “best-fit” line through the standard additions data points, accounting for matrix interferences.

See Section 1.4.1 *Standard Additions* for more information.

Standard Solution Method

Prepare a 20.0-mg/L NO₃-N standard solution by pipetting 2.00 mL 1000-mg/L NO₃-N standard into a 100-mL volumetric flask. Dilute to the mark with deionized water. Stopper and invert to mix. Prepare this solution daily. Perform the total nitrogen procedure as described above.

To adjust the calibration curve using the reading obtained with the 20.0-mg/L standard solution, press the soft keys under **OPTIONS, (MORE)** then **STD: OFF**. Press **ENTER** to accept the displayed concentration, the value of which depends on the selected units. If an alternate concentration is used, enter the actual concentration and press **ENTER** to return to the read screen. See Section 1.5.5 *Adjusting the Standard Curve* for more information.

Method Performance

Precision

Standard: 20.0 mg/L -N

Program	95% Confidence Limits
5000	15.2–24.8 mg/L N

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
5000	5.0 mg/L N

For more information on derivation and use of Hach's estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, Appendix B, see Section 1.5.1.

Sensitivity

Program Number: 5000

Portion of Curve	ΔAbs	ΔConcentration
Entire Range	0.010	0.57 mg/L

See Section 1.5.3 *Sensitivity Explained* for more information.

Summary of Method

Bound nitrogen, both inorganically and organically bound, is oxidized to nitrate by digestion with peroxodisulphate. The nitrate ions react with 2,6-dimethylphenol in a solution of sulphuric and phosphoric acids to form a nitrophenol. Results are expressed as mg/L N or mg/L TN_b (Total Bound Nitrogen), with the same numeric value.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to Section I.

Sample vial contains: 60% phosphoric acid, 33% phosphoric acid.
 Sodium hydroxide solution A (HCT 111 A) contains: Sodium hydroxide.
 Oxidant tablet B (HCT 111 B) contains: Potassium peroxodisulphate.
 Dimethylphenol solution D (HCT 111 D) contains: 2-propanol.

REQUIRED EQUIPMENT AND SUPPLIES

Description	Unit	Cat. No.
UniCell™ HCT 111	23/pkg	HCT 111
Reaction Tube.....	5 each.....	HCT 211
Digital Reactor Block.....	each.....	DRB 100
Test tube rack, cooling	each.....	18641-00

OPTIONAL REAGENTS AND STANDARDS

Nitrogen-Nitrate (NO ₃ -N) Standard, 1000-mg/L	500 mL.....	12792-49
Sulfuric Acid, ACS.....	500 mL.....	979-49
Sodium Hydroxide, 5 N	50 mL SCDB.....	2450-26

OPTIONAL APPARATUS

Graduated cylinder, mixing, 100-mL	each.....	20886-42
Flask, volumetric, 100-mL	each.....	14574-42
Pipettor, (Jencons) 1–5 mL	each.....	27951-00
Replacement tips for 27951-00	100/pkg.....	27952-00
Pipettor, (Jencons) 100–1000 µL.....	1 each.....	27949-00
Replacement tips for 27949-00	400/pkg.....	27950-00
pH Paper	100/pkg.....	26013-00



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FAX: (970) 669-2932



Method 10021 Requires Centrifuge

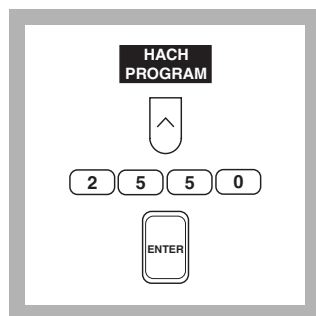
Titanium Trichloride Reduction Method

Test 'N Tube™ Vials

(0 to 25.0 mg/L N)

Scope and Application: For water, wastewater, and seawater.

The estimated detection limit for program number 2550 is 0.3 mg/L N.



1. Press the soft key under **HACH PROGRAM**.

Select the stored program number for total inorganic nitrogen, Test 'N Tube method, by pressing **2550** with the numeric keys.

Press: **ENTER**

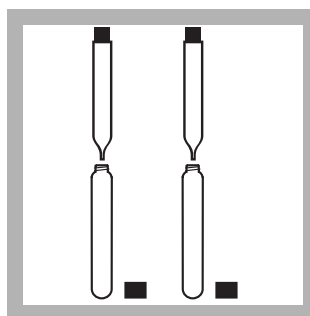
Note: If samples cannot be analyzed immediately, see *Sample Collection, Preservation and storage following these steps. Adjust the pH of preserved samples before analysis.*



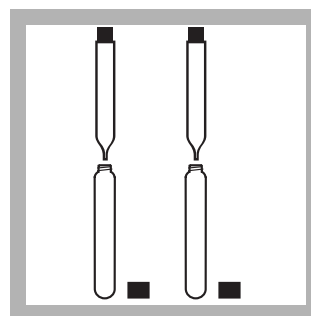
2. The display will show:

**HACH PROGRAM: 2550
N, Inorganic TNT**

The wavelength (λ), **655 nm**, is automatically selected.

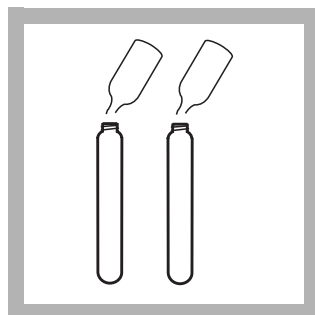


3. Pipet 1 mL of Total Inorganic Nitrogen Pretreatment Base Concentrate into each of 2 Total Inorganic Nitrogen Pretreatment Diluent Vials.



4. Pipet 1 mL of sample into 1 vial (the sample). Pipet 1 mL of deionized water into the other vial (the blank). Cap the vials and shake for 30 seconds to mix.

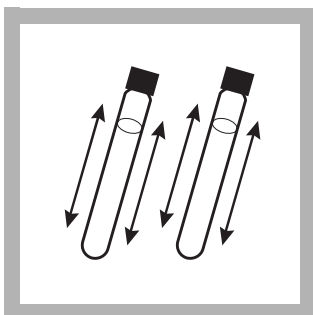
NITROGEN, Total Inorganic, continued



5. Snap the neck off two Total Inorganic Nitrogen Reductant ampules and pour the contents of one into the TIN Diluent Vial containing sample. Repeat for the second vial (the blank).

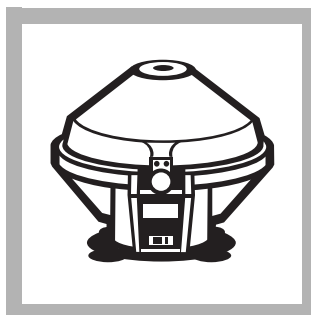
Note: For safety, wear gloves while breaking the ampules.

Note: A black precipitate will form immediately.



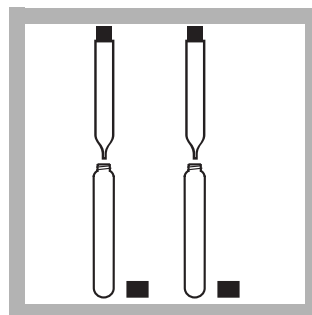
6. Cap the vials. Shake gently for 30 seconds to mix the reagents. Allow the vials to sit for at least one minute.

Note: The precipitate should remain black after shaking. Excessive shaking will result in a white precipitate and low results.



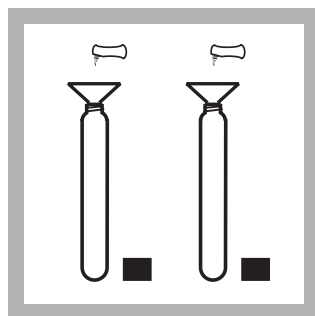
7. Centrifuge vials for 3 minutes or wait until solids settle to bottom of vial. Press the soft key under **START TIMER** to start a 3-minute period.

Note: The solids will settle without use of centrifuge, but can take up to 30 minutes.

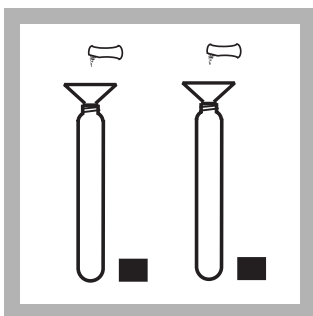


8. Remove the caps from two AmVer™ Diluent Reagent Test 'N Tubes for Low Range Ammonia Nitrogen. Using a pipet, add 2 mL of centrifuged sample to one vial. Add 2 mL of centrifuged blank to the other Test 'N Tube vial. Label the tubes appropriately.

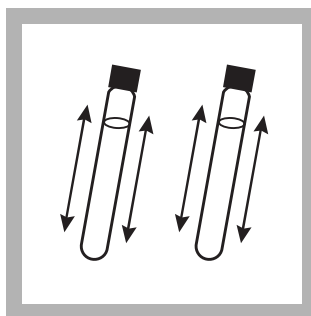
Note: Pipet carefully to avoid disturbing the sediment.



9. Using a funnel add the contents of one Ammonia Salicylate Reagent Powder Pillow (for 5-mL sample) to each vial.

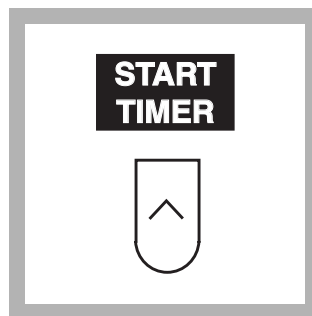


10. Using a funnel, add the contents of one Ammonia Cyanurate Reagent Powder Pillow (for 5-mL sample) to each vial.



11. Cap the vials tightly and shake thoroughly to dissolve the powder.

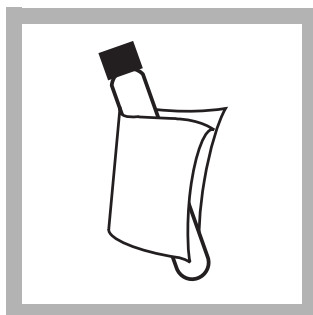
Note: A green color will develop if inorganic nitrogen is present.



12. Press the soft key under **START TIMER**. A 20-minute reaction period will begin.

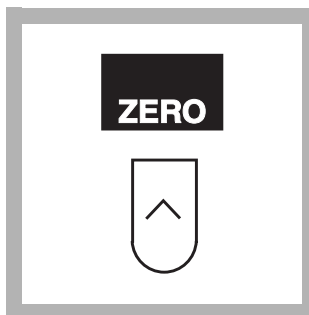


13. Insert the Test Tube Adapter into the sample cell module by sliding it under the thumb screw and into the alignment grooves. Fasten with the thumb screw.



14. When the timer beeps, clean the outside of the vials with a towel, and place the blank into the cell holder.

Note: Wiping with a damp cloth followed with a dry removes fingerprints and other marks.



15. Press the soft key under **ZERO**.

The display will show:

0.0 mg/L N

Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



16. Place the prepared sample into the cell holder and close the light shield. The result in mg/L total inorganic nitrogen as nitrogen (N) (or chosen units) will be displayed.

Note: The result can be expressed as $N-NH_3$ or NO_3^- . Press the soft keys under **OPTIONS**, then **FORM**: to scroll through the available options. Press **ENTER** to return to the read screen.

Interferences

The following ions may interfere when present in concentrations exceeding those listed below:

Species	Level	Effect
Calcium	1000 mg/L as $CaCO_3$	Positive
Manganese (IV)	3 mg/L	Negative
Magnesium	1000 mg/L as $CaCO_3$	Positive
Sulfide	3 mg/L	Negative
Sulfate	250 mg/L	Negative

The following do not interfere below the levels listed:

Species	Level
Al^{3+}	8 mg/L
Ba^{2+}	40 mg/L
Cu^{2+}	40 mg/L
Fe^{3+}	8 mg/L
Zn^{2+}	80 mg/L
F^-	40 mg/L
$PO_4^{3-}-P$	8 mg/L
SiO_2	80 mg/L
EDTA	80 mg/L

Sample Collection, Preservation and storage

Collect samples in clean plastic or glass bottles. Best results are obtained with immediate analysis. If chlorine is known to be present, add one drop of 0.1 N sodium thiosulfate for each 0.3 mg/L Cl_2 in a one-liter sample. Preserve the sample by reducing the pH to 2 or less with concentrated hydrochloric acid (at least 2 mL). Store at 4 °C (39 °F) or less. Preserved samples may be stored up to 28 days. Warm samples to room temperature. Neutralize with 5 N Sodium Hydroxide before analysis. Correct the test result for volume additions; see Section 1.2.2 *Correcting for Volume Additions*.

Accuracy Check

Standard Additions Method

- a. Leave the unspiked sample in the sample compartment. Verify that the units displayed are in mg/L. Select standard additions mode by pressing the soft keys under **OPTIONS, (MORE)** and then **STD ADD**.
- b. Press **ENTER** to accept the default sample volume (mL), 25.0.
- c. Press **ENTER** to accept the default standard concentration (mg/L), 500.0.
- d. Press the soft key under **ENTRY DONE**.
- e. Snap the neck off a fresh HR Nitrate Nitrogen PourRite Ampule Standard, 500-mg/L NO_3^- -N.
- f. Use the TenSette Pipet to add 0.1 mL, 0.2 mL and 0.3 mL of standard, respectively to three 25-mL samples and mix each thoroughly.
- g. Analyze each standard addition sample as described above. Accept the standard additions reading by pressing the soft key under **READ** each time. Each addition should reflect approximately 90-95% recovery.
- h. After completing the sequence, the display will show the extrapolated concentration value and the “best-fit” line through the standard additions data points, accounting for matrix interferences.
- i. See Section 1.4.1 *Standard Additions* for more information.

Standard Solution Method

To check accuracy, use a 10.0-mg/L Nitrate Nitrogen Standard Solution listed under *OPTIONAL REAGENTS AND STANDARDS*. Or, prepare this by diluting 1 mL of solution from a Nitrate Nitrogen Volute Ampule Standard, 500-mg/L NO_3^- -N, to 50 mL with deionized water. The result should be 9–10 mg/L N.

Method Performance**Precision/Accuracy**

The total inorganic nitrogen test is designed to provide an estimate of the total nitrite, nitrate, and ammonia nitrogen load present in a water or wastewater sample. This test is most applicable to the monitoring of samples taken from an industrial process stream or a wastewater treatment stream where it is important to track the inorganic nitrogen load as it passes through the treatment process. The test does exhibit different recoveries of each of the three nitrogen species, as summarized below. The test is not recommended for use when quantifying only one of the three species. In that case, specific procedures for each particular analyte would be more appropriate.

Ammonia Nitrogen

In a single laboratory, using a standard solution of 20.0-mg/L $\text{NH}_3\text{-N}$ and two representative lots of reagent with the instrument, a single operator obtained a mean recovery of 22.7 mg/L with a standard deviation of ± 0.88 mg/L N (replicate number = 7 per reagent lot).

Nitrate Nitrogen

In a single laboratory, using a standard solution of 20.0-mg/L $\text{NO}_3\text{-N}$ and two representative lots of reagent with the instrument, a single operator obtained a mean recovery of 20.2 mg/L with a standard deviation of ± 0.67 mg/L N (replicate number = 7 per reagent lot).

Nitrite Nitrogen

In a single laboratory, using a standard solution of 20.0-mg/L $\text{NO}_2\text{-N}$ and two representative lots of reagent with the instrument, a single operator obtained a mean recovery of 15.4 mg/L with a standard deviation of ± 0.74 mg/L N (replicate number = 7 per reagent lot).

Estimated Detection Limit

Program	EDL
2550	0.3 mg/L $\text{NO}_3\text{-N}$

For more information on derivation and use of Hach's estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 *CFR* part 136, Appendix B, see Section 1.5.1.

Sensitivity

Program Number: 2550

Portion of Curve	ΔAbs	$\Delta\text{Concentration}$
Entire Range	0.010	0.16 mg/L

See Section 1.5.3 *Sensitivity Explained* for more information.

NITROGEN, Total Inorganic, continued

Calibration Standard Preparation

To perform a total inorganic nitrogen calibration for the Test 'N Tube titanium trichloride reduction method, follow the steps under *Calibration Standard Preparation* in the LR Ammonia Test 'N Tube procedure. One change is required: You must enter the concentrations into the calibration table as 5, 10, 15, 20, and 25 mg/L TIN instead of the 0.5, 1.0, 1.5, 2.0, and 2.5 mg/L NH₃-N stated. This will account for a 10-fold dilution in the total inorganic nitrogen method.

Summary of Method

Titanium (III) ions reduce nitrate and nitrite to ammonia in a basic environment. After centrifugation to remove solids, the ammonia is combined with chlorine to form monochloramine. Monochloramine reacts with salicylate to form 5-aminosalicylate. The 5-aminosalicylate is oxidized in the presence of a sodium nitroprusside catalyst to form a blue colored compound. The blue color is masked by the yellow color from the excess reagent present to give a final green colored solution.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to Section 1.

Pollution Prevention and Waste Management

The ammonia salicylate reagent contains sodium nitroferricyanide. Cyanide solutions are regulated as hazardous wastes by the Federal RCRA. Collect cyanide solutions for disposal as reactive (D001) waste. Be sure cyanide solutions are stored in a caustic solution with pH >11 to prevent release of hydrogen cyanide gas. See Section 1 for further information in proper disposal of these materials.

REQUIRED REAGENTS AND STANDARDS

	Cat. No.
Total Inorganic Nitrogen Pretreatment Reagent Set (TiCl ₃ Reduction Method) (25 tests)	26049-45
Includes: (1) 2040-59, (1) 26051-50, *(50) TIN Pretreatment Diluent Vials	
Test 'N Tube AmVer Nitrogen-Ammonia Reagent Set (25 tests)	26045-45
Includes: (1) 23952-66, (1) 23954-66, (1) 272-42, *(50) AmVer Diluent LR Vials	

Description	Quantity Required		Cat. No.
	Per Test	Unit	
AmVer Diluent Reagent, Low Range Vials.....	2 vials	50/pkg	*
Ammonia Salicylate Reagent Powder Pillows	2 pillows	50/pkg	23952-66
Ammonia Cyanurate Reagent Powder Pillows	2 pillows	50/pkg	23954-66
Total Inorganic Nitrogen Pretreatment Diluent Vials.....	2 vials	50/pkg	*
Total Inorganic Nitrogen Reductant Ampule, 1 mL.....	2 ampules	50/pkg	26051-50
Total Inorganic Nitrogen Pretreatment Base Concentrate.....	2 mL	50 mL.....	2040-59

* These items are not sold separately. Please order the complete set (Cat. No. 26049-45 or 26045-45) as a replacement.

REQUIRED EQUIPMENT AND SUPPLIES

Centrifuge, 115 VAC, 6 x 15 mL	1	each.....	26765-00
Centrifuge, 230 VAC, 6 x 15 mL	1	each.....	26765-02
DR/4000 Test Tube Adapter.....	1	each.....	48189-00
Funnel, micro	1	each.....	25843-35
Pipet, TenSette, 0 - 10 mL.....	1	each.....	19700-10
Pipet Tips, for 19700-10 Tensette Pipet	varies.....	50/pkg.....	21997-96
Pipette, volumetric, Class A, 1.00-mL	1	each.....	14515-35
Test Tube Rack	1	each.....	18641-00

OPTIONAL REAGENTS AND STANDARDS

Ammonia Nitrogen Standard Solution, 100-mg/L $\text{NH}_3\text{-N}$	500 mL	24065-49
Hydrochloric Acid, ACS	500 mL.....	134-49
Nitrate Nitrogen Standard Solution, 10-mg/L $\text{NO}_3\text{-N}$	500 mL.....	307-49
Nitrate Nitrogen Standard Solution, 2-mL Ampule, 500-mg/L $\text{NO}_3\text{-N}$	20/pkg.....	14260-20
Sodium Hydroxide Standard Solution, 5.0 N.....	59 mL SCDB.....	2450-26
Sodium Thiosulfate Standard Solution, 0.1 N.....	100 mL MDB.....	323-32
Water, deionized	4 liters.....	272-56

OPTIONAL EQUIPMENT AND SUPPLIES

Flask, volumetric, Class A, 50-mL	each.....	14574-41
pH Indicator Paper, 1 to 11 pH.....	5 rolls/pkg.....	391-33
Pipet, volumetric, Class A, 5.00-mL	each.....	14515-37
Pipet, volumetric, Class A, 10.00-mL	each.....	14515-38
Pipet, volumetric, Class A, 15.00-mL	each.....	14515-39
Pipet, volumetric, Class A, 20.00-mL	each.....	14515-20
Pipet, volumetric, Class A, 25.00-mL	each.....	14515-40
Pipet Filler	each.....	12189-00
PourRite Ampule Breaker	each.....	24846-00



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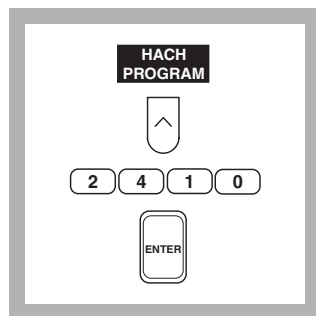
Method 8075

Nessler Method* (Digestion Required)

(0 to 150.0 mg/L)

Scope and Application: For water, wastewater and sludge; digestion is required for determining total kjeldahl nitrogen. The estimated detection limit for program number 2410 is 1.2 mg/L TKN.

* Adapted from Hach, et. al., *Journal of Association of Official Analytical Chemists*, 70 (5) 783-787 (1987); Hach, et. al., *Journal of Agricultural and Food Chemistry*, 33 (6) 1117-1123 (1985); *Standard Methods for the Examination of Water and Wastewater*



- 1.** Press the soft key under **HACH PROGRAM**.

Select the stored program for total kjeldahl nitrogen by pressing **2410** with the numeric keys.

Press: **ENTER**

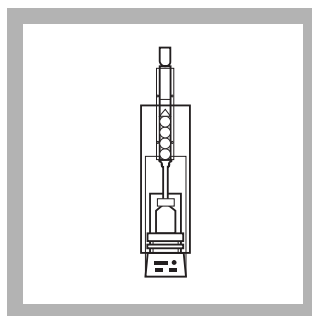
Note: The Flow Cell and Sipper Modules can be used with this procedure. If the Flow-Thru Cell is used, periodically clean the cell by pouring a few sodium thiosulfate pentahydrate crystals into the cell funnel. Flush it through the funnel and cell with enough deionized water to dissolve. Rinse out the crystals.



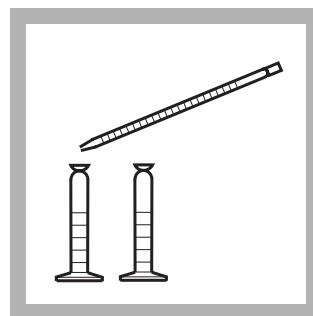
- 2.** The display will show:
HACH PROGRAM: 2410 Nitrogen, TKN

The wavelength (λ), **460 nm**, is automatically selected.

Note: For best results, determine a reagent blank for each new lot of reagent as follows. Prepare a reagent blank by repeating steps 3 through 15, using deionized water as the sample. Zero the instrument on deionized water by pressing the soft key under **ZERO**. Insert the reagent blank and the blank value will be displayed. Correct for the reagent blank by pressing the soft keys under **OPTIONS, (MORE)**, and then **BLANK:OFF**. Enter the reagent blank value and press **ENTER**. Repeat for each new lot of reagent.

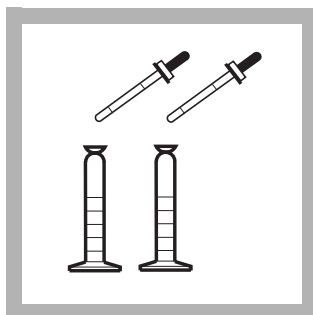


- 3.** Digest the sample amount as described in the *Digesdahl Digestion Apparatus Instruction Manual*. Digest an equal amount of deionized water as the blank.



- 4.** Select the appropriate analysis volume of the digested sample given in *Table 1* on page 3. Pipet the analysis volume from the sample and the blank into separate 25-mL mixing graduated cylinders.

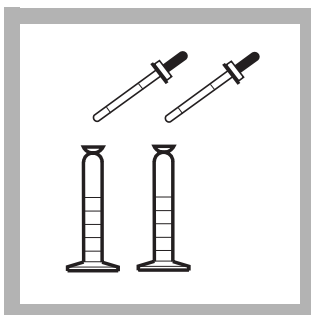
NITROGEN, Total Kjeldahl, continued



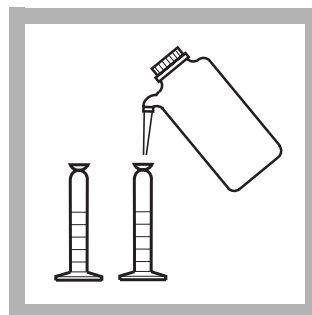
5. Add one drop of TKN Indicator to each cylinder. Add drops of 8.0 N KOH to each cylinder until the first flash of blue color appears. Stopper and invert the cylinder each addition.

Note: If aliquot is less than 1 mL, do not add KOH. Continue with Step 6.

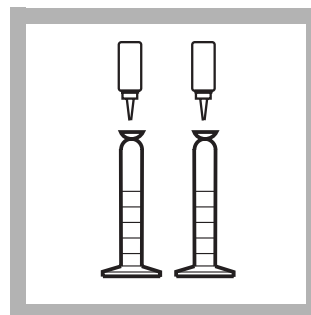
Note: Hold the dropping bottles upright while dispensing.



6. Add 1.0 N KOH to each cylinder, one drop at a time, mixing after each addition. Continue until the first permanent blue color appears.

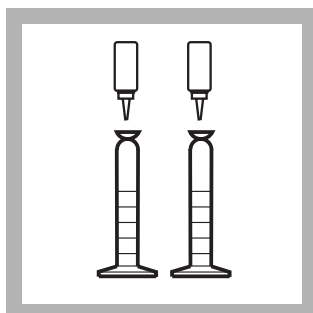


7. Fill both cylinders to the 20-mL mark with deionized water.



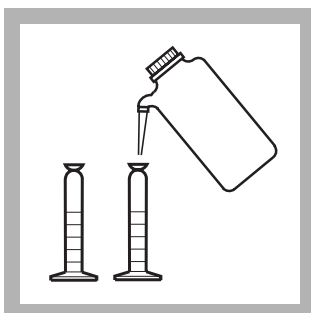
8. Add three drops of Mineral Stabilizer to each cylinder. Stopper. Invert several times to mix.

Note: Hold the dropping bottles upright while dispensing.

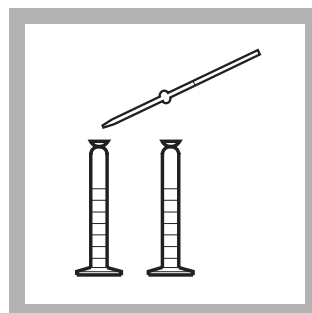


9. Add three drops of Polyvinyl Alcohol Dispersing Agent to each cylinder. Stopper. Invert several times to mix.

Note: Hold the dropping bottles upright while dispensing.

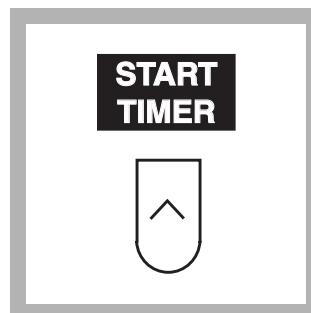


10. Fill both cylinders to the 25-mL mark with deionized water. Stopper. Invert several times to mix.



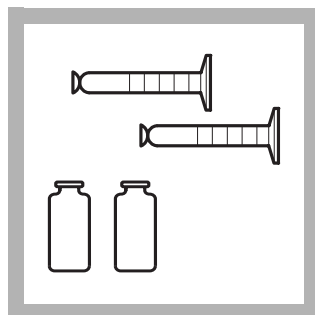
11. Pipet 1.00 mL of Nessler's Reagent to each cylinder. Stopper, invert repeatedly. The solution should not be hazy.

Any haze (turbidity) will cause inaccurate results.

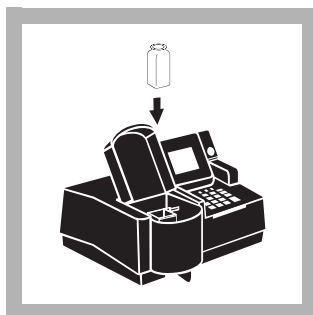


12. Press the soft key under **START TIMER**.

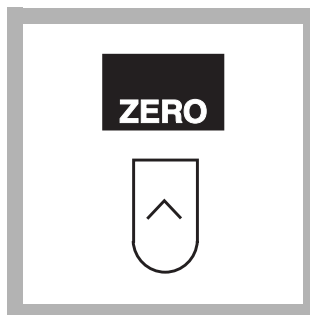
A 2-minute reaction period will begin.



13. When the timer beeps, pour the contents of each cylinder into separate 25-mL sample cells.



14. Place the blank into a cell holder. Close the light shield.



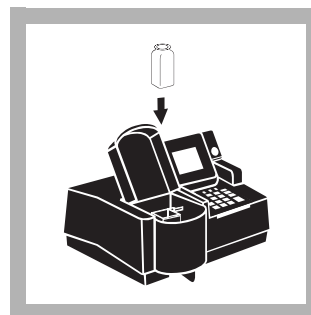
15. Press the soft key under **ZERO**.

The display will show:

0.0 mg/L TKN

Note: If you are using a reagent blank correction, the display will show the correction.

Note: For alternate concentration units press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



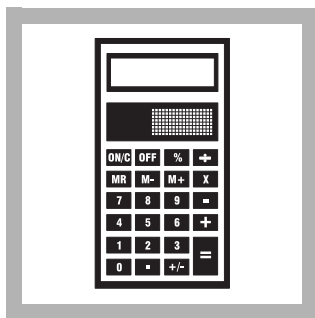
16. Place the prepared sample into the cell holder. Close the light shield. Results in mg/L total kjeldahl nitrogen as N (or chosen units) will be displayed.

Note: The readout is the actual concentration of total kjeldahl nitrogen when the sample amount is 25 mL and the analysis volume is 3 mL. If other volumes are used, the true concentration must be calculated using the formula in Step 17

Note: The results can be expressed as NH_3 , NH_4^+ or $\text{NH}_3\text{-N}$. Press the soft keys under **OPTIONS**, then **FORM**: to scroll through the available options. Press **ENTER** to return to the read screen.

Note: See Pollution Prevention and Waste Management following these steps about disposal of these reagents.

NITROGEN, Total Kjeldahl, continued



17. Calculate sample TKN as follows:

$$\text{ppm TKN} = \frac{75 \times A}{B \times C}$$

Where:

A = mg/L read from the display

B = g (or mL of water) sample taken for digest

C = mL analysis volume of digested sample.

Table 1 Digestion Table

AQUEOUS SAMPLES (Solutions of suspensions in water—less than 1% solids)	
Expected Nitrogen Concentration (mg/L)	Analysis Volume (mL)
0.5–28	10.0
2–112	5.0
11–560	2.00
45–2250	1.00
425–22500	0.500
DRY SAMPLES	
Expected Nitrogen Concentration (mg/L)	Analysis Volume (mL)
42–2200	10.0
106–5600	5.00
350–18000	2.00
1000–56000	1.00
4200–220000	0.50
OILS AND FATS	
Expected Nitrogen Concentration (mg/L)	Analysis Volume (mL)
85–4500	10.0
210–11000	5.00
2100–110000	1.00

Sample Collection, Storage and Preservation

Collect samples in clean glass or plastic containers. Adjust the pH to 2 or less with sulfuric acid (about 2 mL per liter) and cool to 4 °C. Preserved samples can be stored up to 28 days.

Accuracy Check

Kjeldahl Nitrogen Standard Method

This procedure checks digestion efficiency and indicates that amount of bound nitrogen that is freed during digestion. The methods and standards available to check digestion technique are found in the *Accuracy Check* section following the procedure in the *Digestion Apparatus Instruction Manual*. Using the digested Kjeldahl standard, perform the above TKN analysis on the colorimeter. The TKN value should come within about $\pm 3\%$ of the value of the prepared Kjeldahl standard.

Standard Solution Method (to check calibration accuracy only)

Add one drop of TKN Indicator to each of two 25-mL graduated mixing cylinders. Fill one cylinder to the 20-mL mark with deionized water. Fill the other cylinder to the 20-mL mark with a 1.0 mg/L $\text{NH}_3\text{-N}$ solution. Add 3 drops of Mineral Stabilizer to each cylinder. Invert several times to mix. Add 3 drops of Polyvinyl Alcohol Dispersing agent to each cylinder. Perform the TKN procedure as described in steps 10 to 16. This display should show 26–27 mg/L TKN.

Method Performance

Precision

Standard: 35.0 mg/L $\text{NH}_3\text{-N}$

Program	95% Confidence Limits
2410	34.3–35.7 mg/L $\text{NH}_3\text{-N}$

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
2410	1.2 mg/L $\text{NH}_3\text{-N}$

For more information on derivation and use of Hach's estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, appendix B, see Section 1.5.1.

Sensitivity

Program Number: 2410

Portion of Curve	ΔAbs	$\Delta\text{Concentration}$
Entire Range	0.010	1.02 mg/L

See Section 1.5.3 *Sensitivity Explained* for more information.

Calibration Standard Preparation

A new calibration may be performed for each lot of Nessler Reagent.

Prepare standards representing 28, 56, 84, 112 and 140 mg/L N as follows:

- a. Into five different 100-mL Class A volumetric flasks, pipet 7, 14, 21, 28, and 35 mL of a 100-mg/L Ammonia Nitrogen Standard Solution (Cat. No. 24065-49) using Class A glassware.
- b. Dilute to the mark with deionized water. Mix thoroughly. These standards are prepared as though the digestion were performed.
- c. Beginning at Step 4, use a 3-mL analysis volume and complete the procedure.
- d. This calibration can be stored as a **USER PROGRAM**. For more information, refer to the *User-Entered Programs* section of the *DR/4000 Spectrophotometer Instrument Manual*.

Summary of Method

The term “Total Kjeldahl Nitrogen” refers to the combination of ammonia and organic nitrogen. However, only the organic nitrogen compounds appearing as organically bound nitrogen in the trinegative state are determined in this test. Nitrogen in this form is converted into ammonium salts by the action of sulfuric acid and hydrogen peroxide. The ammonia is then analyzed by a modified Nessler method test.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to Section 1.

Pollution Prevention and Waste Management

Nessler reagent contains mercuric iodide. Both the sample and blank will contain mercury (D009) at concentrations regulated as a hazardous waste by the Federal RCRA. Do not pour these solutions down the drain. See Section 1 for more information on proper disposal of these materials.

REQUIRED REAGENTS AND STANDARDS

Kjeldahl Nitrogen Reagent Set.....	24953-00
Includes: (1) 21196-49, (1) 23766-26, (1) 21194-49, (1) 23765-26, (1) 23144-26, (1) 282-32, (1) 979-49, (1) 22519-26	

Description	Quantity Required		Cat. No.
	Per Test	Unit	
Hydrogen Peroxide, 50%	20 mL	490 mL	21196-49
Mineral Stabilizer.....	6 drops ... 50 mL SCDB.....		23766-26
Nesslers Reagent	2 mL	500 mL.....	21194-49
Polyvinyl Alcohol Dispersing Agent	6 drops ... 50 mL SCDB.....		23765-26
Potassium Hydroxide Standard Solution, 1.0 N.....	varies.....	50 mL SCDB.....	23144-26
Potassium Hydroxide Standard Solution, 8.0 N.....	varies.....	100 mL MDB.....	282-32
Sulfuric Acid, ACS, concentrated	6 mL	500 mL.....	979-49
TKN Indicator Solution.....	2 drops ... 50 mL SCDB.....		22519-26

REQUIRED EQUIPMENT AND SUPPLIES

Boiling Chips, silicon carbide	2-3	500 g	20557-34
Cots, finger	2	2/pkg	14647-02
DR/4000 1-Inch Cell Adapter	1	each.....	48190-00
Cylinder, graduated mixing, 25-mL	2	each.....	21190-40
Pipet, TenSette, 0.1 to 1.0 mL	1	each.....	19700-01
Pipet Tips, for 19700-01 TenSette Pipet	2	50/pkg	21856-96
Safety Shield, for Digesdahl.....	1	each.....	20974-00

Select one based on available voltage:

Digesdahl Digestion Apparatus, 115 VAC	1	each.....	23130-20
Digesdahl Digestion Apparatus, 230 VAC	1	each.....	23130-21

OPTIONAL REAGENTS AND STANDARDS

Nitrogen Standard Solution, 1 mg/L NH ₃ -N	500 mL.....	1891-49
Ammonia Nitrogen Standard Solution, 100 mg/L NH ₃ -N	500 mL.....	24065-49
Nitrogen Standard Solution, 10-mL Voluette ampule, 150 mg/L NH ₃ -N	16/pkg	21284-10
Potassium Hydroxide, 12.0 N	500 mL.....	230-49

OPTIONAL EQUIPMENT AND SUPPLIES

Ampule Breaker Kit	each.....	21968-00
Bottle, glass dispenser, 118-mL	each.....	591-00
Bottle, plastic, wash, 1000-mL.....	each.....	620-16
Cylinder, graduated, 50-mL	each.....	508-41
DR/4000 Carousel Module Kit	each.....	48070-02
DR/4000 Flow Cell Module Kit, 1-inch.....	each.....	48070-04
DR/4000 Flow Cell Module Kit, 1-cm.....	each.....	48070-05
DR/4000 Sipper Module Kit, 1-inch	each.....	48090-03
Mini-Grinder, 120 VAC.....	each.....	20991-00
Pipet Filler.....	each.....	12189-00
Pipet, volumetric, Class A, 0.50-mL	each.....	14515-34
Pipet, volumetric, Class A, 1.00-mL	each.....	14515-35
Pipet, volumetric, Class A, 2.00-mL	each.....	14515-36
Pipet, volumetric, Class A, 3.00-mL	each.....	14515-03
Pipet, volumetric, Class A, 4.00-mL	each.....	14515-04
Pipet, volumetric, Class A, 5.00-mL	each.....	14515-37
Pipet, volumetric, Class A, 10.00-mL	each.....	14515-38
Safety Glasses, clear.....	each.....	18421-00



FOR TECHNICAL ASSISTANCE, PRICE INFORMATION AND ORDERING:

In the U.S.A. – Call toll-free 800-227-4224

Outside the U.S.A. – Contact the HACH office or distributor serving you.

On the Worldwide Web – www.hach.com; E-mail – techhelp@hach.com

HACH COMPANY
WORLD HEADQUARTERS
Telephone: (970) 669-3050
FAX: (970) 669-2932



Method 10129

Direct Method*

LR (0.0 to 20.0 mg/L C)

Scope and Application: For water and wastewater

* Patent pending



1. Turn on the COD reactor. Heat to 103-105 °C. Place the plastic shield in front of the reactor.

Note: Ensure safety devices are in place to protect the analyst should leakage occur.

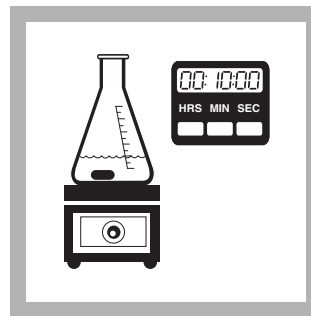


2. Use a graduated cylinder to add 10 mL of sample to a 50-mL erlenmeyer flask containing a stir bar.

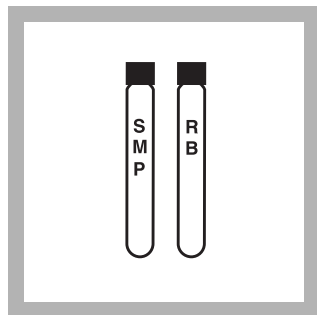


3. Add 0.4 mL of Buffer Solution, pH 2.0.

Note: Use pH paper to make sure the sample pH is 2.

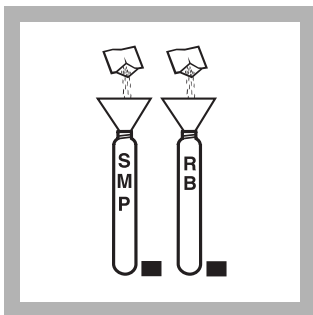


4. Place the flask on a stir plate and stir at a moderate speed for 10 minutes.

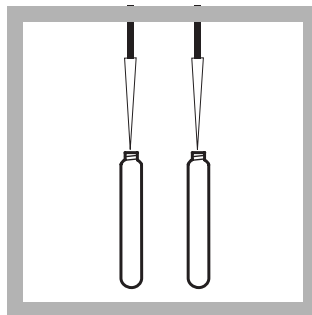


5. Label two Low Range Acid Digestion vials: **sample** and **reagent blank**.

Note: A reagent blank is required for each series of samples.



6. Using a funnel, add the contents of one TOC Persulfate Powder Pillow to each Acid Digestion vial (colorless liquid).



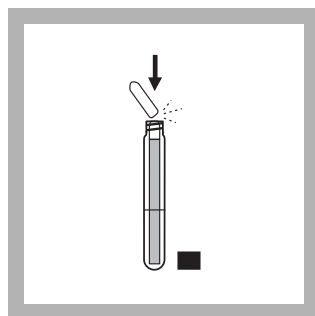
7. Use a TenSette Pipet to add 3.0 mL of **organic-free water** to the **reagent blank** vial and 3.0 mL of **prepared sample** to the **sample** vial. Do not cap; swirl gently to mix.

Note: The organic-free water must contain less than 0.05 mg/L carbon. See Reagent Blanks.



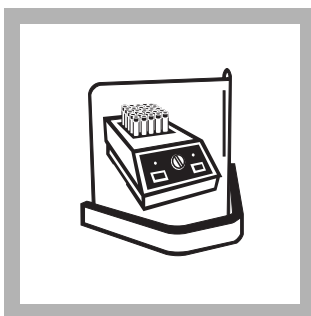
8. Rinse two blue Indicator Ampules with deionized water and wipe them with a soft, lint-free wipe.

Note: Do not touch the ampules on the sides after wiping. Pick them up by the top.

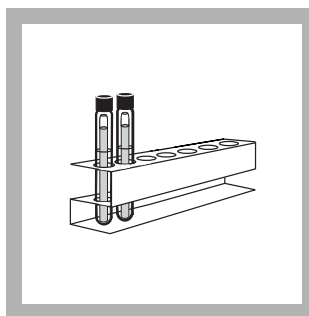


9. Lower one unopened ampule into each Acid Digestion vial. When the score mark on the ampule is level with the top of the Acid Digestion vial, snap the top off the ampule and allow it to drop into the Acid Digestion vial.

Note: Do not invert or tilt the vial after inserting the ampule to prevent the Indicator Reagent from mixing with the contents of the acid digestion vial.



10. Cap the vial assemblies tightly and place them in the COD reactor for 2 hours at 103-105 °C.

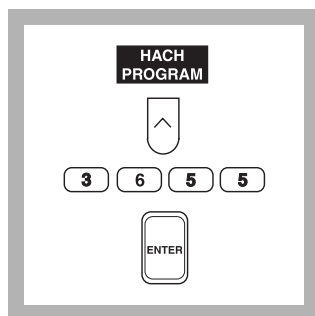


11. Carefully remove the vial assemblies from the reactor. Place them in a test tube rack.

Allow the vials to cool for **one hour** for accurate results.



12. Insert the Test 'N Tube™ adapter into the sample cell module by sliding it under the thumb screw and into the alignment grooves. Fasten with the thumb screw.



13. Press the soft key under **HACH PROGRAM**.

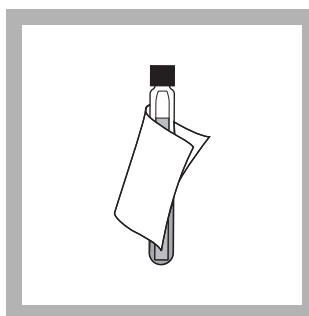
Select the stored program number for the Low Range TOC method by pressing **3655** with the numeric keys.

Press: **ENTER**



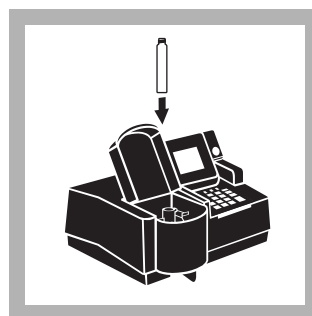
14. The display will show:
HACH PROGRAM: 3655 TOC, LR

The instrument automatically selects the multi-wavelength setting at 598 and 430 nm.

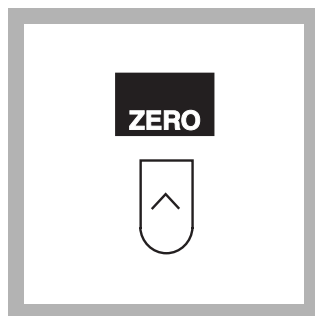


15. Wipe the reagent blank with a damp towel, followed by a dry one, to remove fingerprints or other marks.

Note: The liquid in the reagent blank vial should be dark blue.



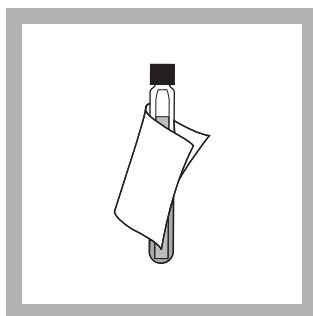
16. Place the **reagent blank** vial assembly in the adapter. Close the light shield.



17. Press the soft key under **ZERO**.

The display will show:

0.0 mg/L C



18. Wipe the sample vial assembly with a damp towel, followed by a dry one, to remove fingerprints or other marks.



19. Place the sample vial assembly in the adapter. Close the light shield. The result in mg/L C will be displayed.

Sampling and Storage

Collect samples in clean glass bottles. Rinse the sample bottle several times with the sample to be collected. Fill the bottle with minimum headspace before capping. Test samples as soon as possible. Acid preservation is not recommended. Homogenize samples containing solids to assure representative samples.

Reagent Blanks

Water used for the reagent blank must contain less than 0.05 mg/L carbon. If the organic-free water container is left open for extended periods, the water may absorb carbon dioxide (CO₂) from the atmosphere. To remove the dissolved CO₂ from the organic-free water, it is necessary to acid-sparge it (see steps 2–4 of the procedure).

Generally, water stored in plastic containers is not suitable for low-range TOC blanks. Water stored in plastic may leach organic compounds from the container walls. The leached organic compounds usually cannot be removed by acid sparging.

Accuracy Check

Standard Solutions Method

- a. Prepare a 1000 mg/L organic carbon stock standard by dissolving 2.1254 g dry primary standard Potassium Acid Phthalate in Organic-Free Reagent Water and dilute to 1000 mL. This stock standard is stable for about 1 month at room temperature.

Alternatively, open one ampule of TOC Standard Solution (Cat. No. 27915-05).

- b. Prepare a 10.0 mg/L C standard by transferring 10.00 mL of the stock standard to a 1000-mL Class A volumetric flask. Dilute to volume using Organic-Free Reagent Water. Stopper and mix thoroughly. Prepare this standard fresh daily.

ORGANIC CARBON, Total, continued

Standard Additions Method

- a. Prepare a 150 mg/L C standard by transferring 15.00 mL of 1000 mg/L C stock solution to a 100-mL Class A volumetric flask. Dilute to volume with organic-free water. Mix.
- b. Use the TenSette Pipet to add 0.1, 0.2, and 0.3 mL of the 150 mg/L C standard to each of three Acid Digestion vials.
- c. Add the contents of one TOC Persulfate powder pillow to each vial.
- d. Add 3.0 mL of sample to each vial. Swirl to mix.
- e. Proceed with the procedure starting at *step 8*.
- f. The mg/L C concentration should increase by 5.0 mg/L for each 0.1 mL increment.

Method Performance

Precision

at mg/L C	95% Confidence Limits
1	±1.3 mg/L
5	±1.0 mg/L
10	±0.8 mg/L
15	±0.7 mg/L
20	±0.7 mg/L

Estimated Detection Limit

Program	EDL
3655	0.3 mg/L C

For more information on derivation and use of Hach's estimated detection limit, see *Estimated Detection Limit*. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, Appendix B, see *Determining the Method Detection Limit (MDL)*.

Sensitivity

Program Number: 3655

Portion of Curve	Δ Abs	Δ Concentration
at 5.0 mg/L C	0.010	0.19 mg/L C
at 10.0 mg/L C	0.010	0.16 mg/L C
at 15.0 mg/L C	0.010	0.14 mg/L C

See *Sensitivity Explained* for more information.

Interferences

The following have been tested for interference and found not to interfere up to the indicated levels:

Table 1 Non-interfering Substances (Maximum Level Tested)

Substance	Maximum Level Tested
Aluminum	10 mg/L
Ammonia Nitrogen	1000 mg/L as N
ASTM Wastewater	No effect
Bromide	500 mg/L Br
Bromine	25 mg/L Br ₂
Calcium	2000 mg/L as CaCO ₃
Chloride	500 mg/L
Chlorine	10 mg/L Cl ₂
Chlorine Dioxide	6 mg/L ClO ₂
Copper	10 mg/L
Cyanide	10 mg/L CN
Iodide	50 mg/L
Iron (II)	10 mg/L
Iron (III)	10 mg/L
Magnesium	2000 mg/L as CaCO ₃
Manganese (VII)	1 mg/L
Monochloramine	14 mg/L NH ₂ Cl as Cl ₂
Nitrite	500 mg/L NO ₂ ⁻
Ozone	2 mg/L O ₃
Phosphate	3390 mg/L PO ₄ ²⁻
Silica	100 mg/L SiO ₂
Sulfate	5000 mg/L SO ₄ ²⁻
Sulfide	20 mg/L S ²⁻
Sulfite	50 mg/L SO ₃ ²⁻
Zinc	5 mg/L

If the sample contains greater than 600 mg/L CaCO₃ alkalinity, lower the sample pH to less than 7 before testing by adding sulfuric acid solution.

Most sample turbidity is either dissolved during the digestion stage or settled during the cooling period. Sample turbidities up to 50 NTU have been tested without interference.

Summary of Method

The total organic carbon (TOC) is determined by first sparging the sample under slightly acidic conditions to remove the inorganic carbon. In the outside vial, organic carbon in the sample is digested by persulfate and acid to form carbon dioxide. During digestion, the carbon dioxide diffuses into a pH indicator reagent in the inner ampule. The adsorption of carbon dioxide into the indicator forms carbonic acid. Carbonic acid changes the pH of the indicator solution which, in turn, changes the color. The amount of color change is related to the original amount of carbon present in the sample.

ORGANIC CARBON, Total, continued

REQUIRED REAGENTS

Total Organic Carbon Direct Method Low Range

Test 'N Tube Reagent Set.....50 vials.....27603-45

Includes:

Description	Quantity Required		Cat. No.
	Per Test	Unit	
Acid Digestion Solution Vials, Low Range TOC	1	50/pkg	*
Buffer Solution, Sulfate.....	0.4 mL.....	25 mL.....	452-33
Funnel, micro	1	each.....	25843-35
Indicator Ampules, Low Range TOC.....	1	10/pkg.....	*
TOC Persulfate Powder Pillows	1	50/pkg.....	*
Water, Organic-free**	3.0 mL.....	500 mL.....	26415-49

REQUIRED APPARATUS

COD Reactor, 115/230 V ac (U.S.A. and Canada)	1	each.....	45600-00
COD Reactor, 115/230 V ac (Europe)	1	each.....	45600-02
Cylinder, graduated, 10-mL	1	each.....	508-38
Flask, Erlenmeyer, 50-mL.....	1	each.....	505-41
Magnetic Stirrer.....	1	each.....	23436-00
Safety Shield, laboratory bench	1	each.....	50030-00
Test Tube Rack	1-3	each.....	18641-00
Pipet, TenSette®, 0.1 to 1.0 mL.....	1	each.....	19700-01
Pipet, TenSette®, 1.0 to 10.0 mL.....	1	each.....	19700-10
Pipet Tips, for 19700-01 TenSette® Pipet	2	50/pkg.....	21856-96
Pipet Tips, for 19700-10 TenSette® Pipet	2	50/pkg.....	25589-96
Stir Bar, Magnetic	1	each.....	45315-00
Wipes, Disposable, Kimwipes.....	1	280/pkg.....	20970-00

OPTIONAL REAGENTS

TOC Standard Solution (KHP Standard, 1000 mg/L C)	5/pkg.....	27915-05
Potassium Acid Phthalate.....	500 g.....	315-34
Sulfuric Acid Reagent Solution, 5.25 N.....	100 mL MDB.....	2449-32

OPTIONAL APPARATUS

Analytical Balance	each.....	26103-00
Flask, volumetric, 1000-mL	each.....	14574-53
Flask, volumetric, 100-mL	each.....	14574-42
Pipet, Class A, 10.00-mL	each.....	14515-38
Pipet, Class A, 15.00-mL	each.....	14515-39

* These items are not sold separately.

** This item must be purchased separately.



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Method 10173

Direct Method*

MR (15 to 150 mg/L C)

Scope and Application: For wastewater and industrial waters

* Patent pending



1. Turn on the COD reactor. Heat to 103–105 °C. Place the plastic shield in front of the reactor.

Note: Ensure safety devices are in place to protect the analyst should leakage occur.

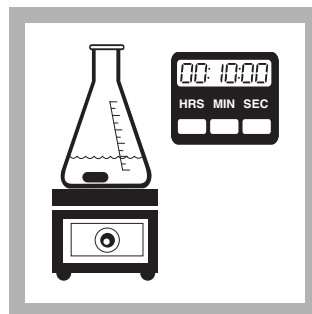


2. Use a graduated cylinder to add 10 mL of sample to a 50-mL erlenmeyer flask containing a stir bar.



3. Add 0.4 mL of Buffer Solution, pH 2.0.

Note: Use pH paper to make sure the sample pH is 2.

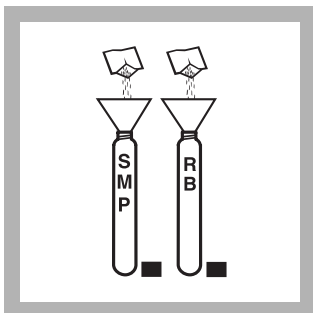


4. Place the flask on a stir plate and stir at a moderate speed for 10 minutes.

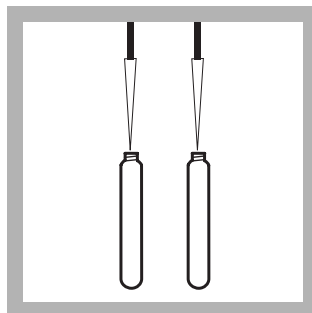


5. Label two Mid Range Acid Digestion vials: **sample** and **reagent blank**.

Note: A reagent blank is required for each series of samples.



6. Using a funnel, add the contents of one TOC Persulfate Powder Pillow to each Acid Digestion vial (colorless liquid).

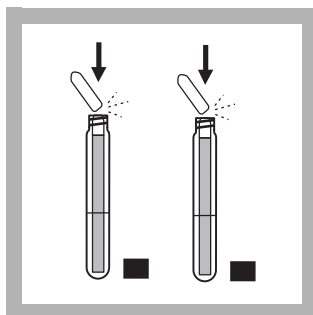


7. Use a TenSette Pipet to add 1.0 mL of **organic-free water** to the **reagent blank** vial and 1.0 mL of **prepared sample** to the **sample** vial. Do not cap; swirl gently to mix.



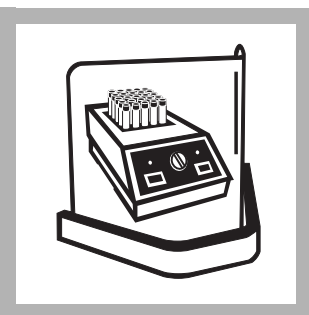
8. Rinse two blue Indicator Ampoules with deionized water and wipe them with a soft, lint-free wipe.

Note: Do not touch the ampoules on the sides after wiping. Pick them up by the top.

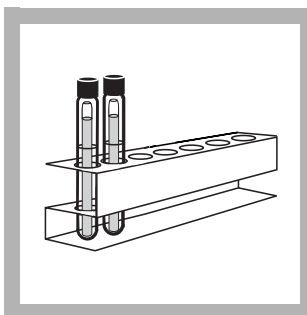


9. Lower one unopened ampule into each Acid Digestion vial. When the score mark on the ampule is level with the top of the Acid Digestion vial, snap the top off the ampule and allow it to drop into the Acid Digestion vial.

Note: Do not invert or tilt the vial after inserting the ampule to prevent the Indicator Reagent from mixing with the contents of the acid digestion vial.



10. Cap the vial assemblies tightly and place them in the COD reactor for 2 hours at 103–105 °C.

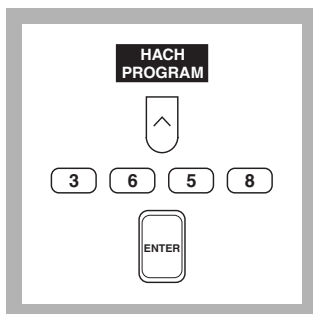


11. Carefully remove the vial assemblies from the reactor. Place them in a test tube rack.

Allow the vials to cool for **one hour** for accurate results.



12. Insert the Test 'N' Tube™ adapter into the sample cell module by sliding it under the thumb screw and into the alignment grooves. Fasten with the thumb screw.

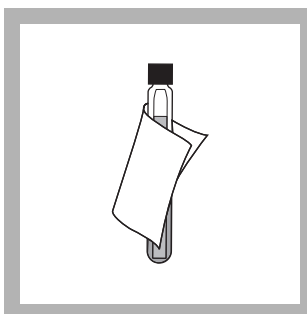


13. Press the soft key under **HACH PROGRAM**. Select the stored program number for the Mid Range TOC method by pressing **3658** with the numeric keys.

Press: **ENTER**

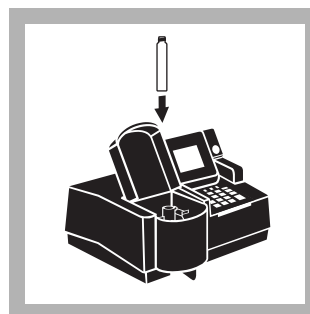


14. The display will show:
HACH PROGRAM: 3658 TOC, MR
The instrument automatically selects multi-wavelength settings at 598 and 430 nm.

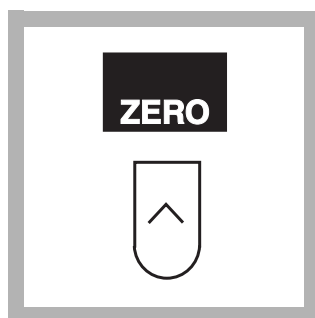


15. Wipe the reagent blank with a damp towel, followed by a dry one, to remove fingerprints or other marks.

Note: The liquid in the reagent blank vial should be dark blue.



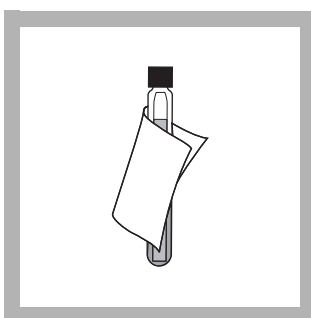
16. Place the **reagent blank** vial assembly in the adapter. Close the light shield.



17. Press the soft key under **ZERO**.

The display will show:

0 mg/L C



18. Wipe the sample vial assembly with a damp towel, followed by a dry one, to remove fingerprints or other marks.



19. Place the sample vial assembly in the adapter. Close the light shield. The result in mg/L C will be displayed.

Sampling and Storage

Collect samples in clean glass bottles. Rinse the sample bottle several times with the sample to be collected. Fill the bottle with minimum headspace before capping. Test samples as soon as possible. Acid preservation is not recommended. Homogenize samples containing solids to assure representative samples.

Accuracy Check

Standard Solutions Method

- a. Prepare a 1000 mg/L organic carbon stock standard by dissolving 2.1254 g dry primary standard Potassium Acid Phthalate in Organic-Free Reagent Water and dilute to 1000 mL. This stock standard is stable for about 1 month at room temperature.

Alternatively, open one ampule of TOC Standard Solution (Cat. No. 27915-05).

- b. Prepare a 100 mg/L C standard by transferring 5.00 mL of the stock standard to a 50-mL Class A volumetric flask. Dilute to volume using Organic-Free Reagent Water. Stopper and mix thoroughly. Prepare this standard fresh daily.

Standard Additions Method

- a. Prepare a 300 mg/L C standard by transferring 15.00 mL of 1000 mg/L C stock solution to a 50-mL Class A volumetric flask. Dilute to volume with organic-free water. Mix.
- b. Use the TenSette Pipet to add 0.1, 0.2, and 0.3 mL of the 300 mg/L C standard to each of three Acid Digestion vials.
- c. Add the contents of one TOC Persulfate powder pillow to each vial.
- d. Add 1.0 mL of sample to each vial. Swirl to mix.
- e. Proceed with the procedure starting at *step 8*.

ORGANIC CARBON, Total, continued

- f. The mg/L C concentration should increase by 30 mg/L for each 0.1 mL increment.

Method Performance

Precision

at mg/L C	95% Confidence Limits
15	±5 mg/L C
50	±4 mg/L
75	±6 mg/L
115	±5 mg/L
150	±6 mg/L

Estimated Detection Limit

To test TOC levels below 15 mg/L C, use Method Number 10129.

Sensitivity

Program Number: 3658

Portion of Curve	ΔAbs	ΔConcentration
At 20 mg/L C	0.010	1.6 mg/L
At 75 mg/L C	0.010	1.4 mg/L
At 125 mg/L C	0.010	1.1 mg/L

See *Sensitivity Explained* for more information.

Interferences

The following have been tested for interference and found not to interfere up to the indicated levels:

Table 1 Non-interfering Substances (Maximum Level Tested)

Substance	Maximum Level Tested
Aluminum	10 mg/L
Ammonia Nitrogen	1000 mg/L as N
ASTM Wastewater	No effect
Bromide	500 mg/L Br
Bromine	25 mg/L Br ₂
Calcium	2000 mg/L as CaCO ₃
Chloride	1500 mg/L
Chlorine	10 mg/L Cl ₂
Chlorine Dioxide	6 mg/L ClO ₂
Copper	10 mg/L
Cyanide	10 mg/L CN
Iodide	50 mg/L
Iron (II)	10 mg/L
Iron (III)	10 mg/L
Magnesium	2000 mg/L as CaCO ₃
Manganese (VII)	1 mg/L

Table 1 Non-interfering Substances (Maximum Level Tested) (Continued)

Monochloramine	14 mg/L NH ₂ Cl as Cl ₂
Nitrite	500 mg/L NO ₂ ⁻
Ozone	2 mg/L O ₃
Phosphate	3390 mg/L PO ₄ ³⁻
Silica	100 mg/L SiO ₂
Sulfate	5000 mg/L SO ₄ ²⁻
Sulfide	20 mg/L S ²⁻
Sulfite	50 mg/L SO ₃ ²⁻
Zinc	5 mg/L

If the sample contains greater than 1000 mg/L CaCO₃ alkalinity, lower the sample pH to less than 7 before testing by adding sulfuric acid solution.

Most sample turbidity is either dissolved during the digestion stage or settled during the cooling period. Sample turbidities up to 50 NTU have been tested without interference.

Summary of Method

The total organic carbon (TOC) is determined by first sparging the sample under slightly acidic conditions to remove the inorganic carbon. In the outside vial, organic carbon in the sample is digested by persulfate and acid to form carbon dioxide. During digestion, the carbon dioxide diffuses into a pH indicator reagent in the inner ampule. The adsorption of carbon dioxide into the indicator forms carbonic acid. Carbonic acid changes the pH of the indicator solution which, in turn, changes the color. The amount of color change is related to the original amount of carbon present in the sample.

REQUIRED REAGENTS

Total Organic Carbon Direct Method Mid Range

Test 'N Tube Reagent Set.....50 vials.....28159-45

Includes:

Description	Quantity Required		Cat. No.
	Per Test	Unit	
Acid Digestion Solution Vials, Mid Range TOC	1	50/pkg	*
Buffer Solution, Sulfate	0.4 mL	25 mL	452-33
Funnel, micro	1	each	25843-35
Indicator Ampules, Mid/High Range TOC	1	50/pkg	*
TOC Persulfate Powder Pillows	1	50/pkg	*
Water, Organic-free**	1.0 mL	500 mL	26415-49

REQUIRED APPARATUS

COD Reactor, 115/230 V ac (U.S.A. and Canada)	1	each	45600-00
COD Reactor, 115/230 V ac (Europe)	1	each	45600-02
Cylinder, graduated, 10-mL	1	each	508-38
Flask, Erlenmeyer, 50-mL	1	each	505-41
Magnetic Stirrer	1	each	23436-00
Safety Shield, laboratory bench	1	each	50030-00

* These items are not sold separately.

** This item must be purchased separately.

ORGANIC CARBON, Total, continued

Test Tube Rack	1-3	each.....	18641-00
Pipet, TenSette®, 0.1 to 1.0 mL.....	1	each.....	19700-01
Pipet Tips, for 19700-01 TenSette® Pipet	2	50/pkg.....	21856-96
Stir Bar, Magnetic	1	each.....	45315-00
Wipes, Disposable, Kimwipes.....	1 pkg	280/pkg.....	20970-00

OPTIONAL REAGENTS

TOC Standard Solution (KHP Standard, 1000 mg/L C)	5/pkg.....	27915-05
Potassium Acid Phthalate.....	500 g.....	315-34
Sulfuric Acid Reagent Solution, 5.25 N.....	100 mL MDB.....	2449-32

OPTIONAL APPARATUS

Analytical Balance	each.....	26103-00
Flask, volumetric, 1000-mL	each.....	14574-53
Flask, volumetric, 100-mL	each.....	14574-42
Pipet, Class A, 10.00-mL	each.....	14515-38
Pipet, Class A, 15.00-mL	each.....	14515-39



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Method 10128

Direct Method*

HR (100 to 700 mg/L C)

Scope and Application: For wastewater and industrial waters

* Patent pending



1. Turn on the COD reactor. Heat to 103-105 °C. Place the plastic shield in front of the reactor.

Note: Ensure safety devices are in place to protect the analyst should leakage occur.

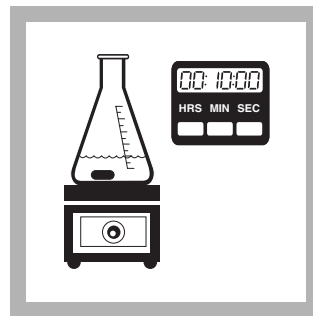


2. Use a graduated cylinder to add 10 mL of sample to a 50-mL erlenmeyer flask containing a stir bar.



3. Add 0.4 mL of Buffer Solution, pH 2.0.

Note: Use pH paper to make sure the sample pH is 2.

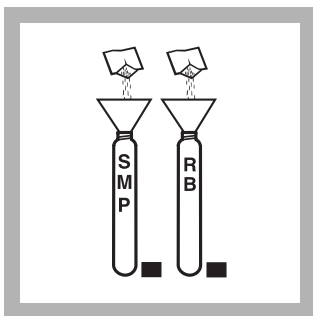


4. Place the flask on a stir plate and stir at a moderate speed for 10 minutes.

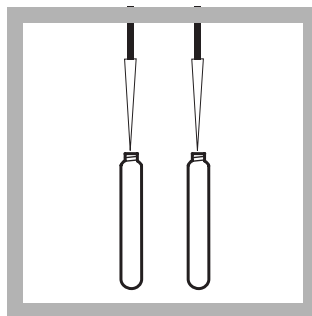


5. Label two High Range Acid Digestion vials: **sample** and **reagent blank**.

Note: A reagent blank is required for each series of samples.



6. Using a funnel, add the contents of one TOC Persulfate Powder Pillow to each Acid Digestion vial (colorless liquid).

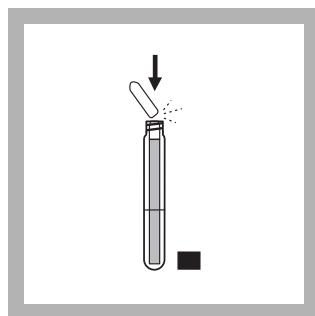


7. Use a TenSette Pipet to add 0.3 mL of **organic-free water** to the **reagent blank** vial and 0.3 mL of **prepared sample** to the **sample** vial. Do not cap; swirl gently to mix.



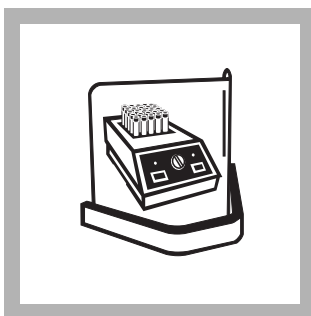
8. Rinse two blue Indicator Ampules with deionized water and wipe them with a soft, lint-free wipe.

Note: Do not touch the ampules on the sides after wiping. Pick them up by the top.

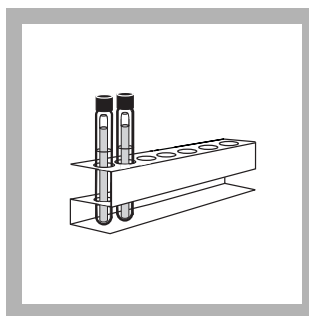


9. Lower one unopened ampule into each Acid Digestion vial. When the score mark on the ampule is level with the top of the Acid Digestion vial, snap the top off the ampule and allow it to drop into the Acid Digestion vial.

Note: Do not invert or tilt the vial after inserting the ampule to prevent the Indicator Reagent from mixing with the contents of the acid digestion vial.



10. Cap the vial assemblies tightly and place them in the COD reactor for 2 hours at 103-105 °C.

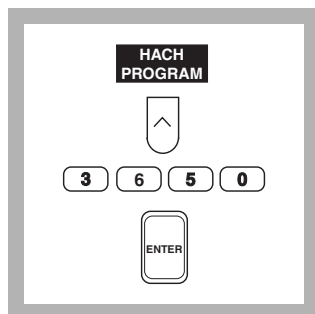


11. Carefully remove the vial assemblies from the reactor. Place them in a test tube rack.

Allow the vials to cool for **one hour** for accurate results.



12. Insert the Test 'N Tube™ adapter into the sample cell module by sliding it under the thumb screw and into the alignment grooves. Fasten with the thumb screw.



13. Press the soft key under **HACH PROGRAM**.

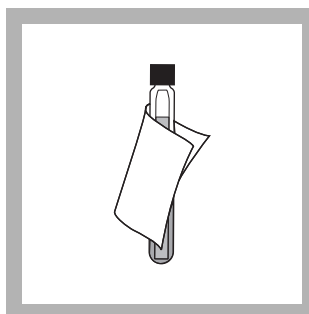
Select the stored program number for the High Range TOC method by pressing **3650** with the numeric keys.

Press: **ENTER**



14. The display will show:
HACH PROGRAM: 3650 TOC, HR

The instrument automatically selects multi-wavelength settings at 598 and 430 nm.

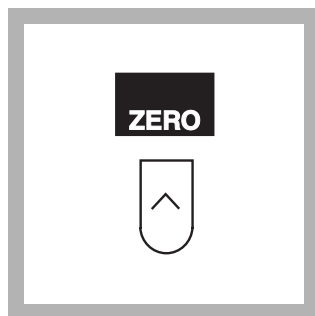


15. Wipe the reagent blank with a damp towel, followed by a dry one, to remove fingerprints or other marks.

Note: The liquid in the reagent blank vial should be dark blue.



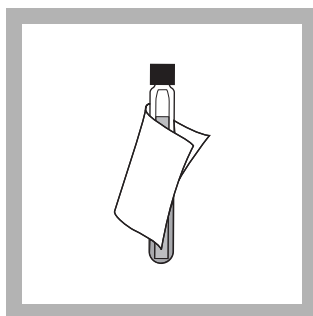
16. Place the **reagent blank** vial assembly in the adapter. Close the light shield.



17. Press the soft key under **ZERO**.

The display will show:

0 mg/L C



18. Wipe the sample vial assembly with a damp towel, followed by a dry one, to remove fingerprints or other marks.



19. Place the sample vial assembly in the adapter. Close the light shield. The result in mg/L C will be displayed.

Sampling and Storage

Collect samples in clean glass bottles. Rinse the sample bottle several times with the sample to be collected. Fill the bottle with minimum headspace before capping. Test samples as soon as possible. Acid preservation is not recommended. Homogenize samples containing solids to assure representative samples.

Accuracy Check

Standard Solutions Method

- a. Prepare a 1000 mg/L organic carbon stock standard by dissolving 2.1254 g dry primary standard Potassium Acid Phthalate in Organic-Free Reagent Water and dilute to 1000 mL. This stock standard is stable for about 1 month at room temperature.

Alternatively, open one ampule of TOC Standard Solution (Cat. No. 27915-05).

- b. Prepare a 300 mg/L C standard by transferring 15.00 mL of the stock standard to a 50-mL Class A volumetric flask. Dilute to volume using Organic-Free Reagent Water. Stopper and mix thoroughly. Prepare this standard fresh daily.

Standard Additions Method

- a. Prepare a 300 mg/L C standard by transferring 15.00 mL of 1000 mg/L C stock solution to a 50-mL Class A volumetric flask. Dilute to volume with organic-free water. Mix.
- b. Use the TenSette Pipet to add 0.1, 0.2, and 0.3 mL of the 300 mg/L C standard to each of three Acid Digestion vials.
- c. Add the contents of one TOC Persulfate powder pillow to each vial.
- d. Add 0.3 mL of sample to each vial. Swirl to mix.
- e. Proceed with the procedure starting at *step 8*.
- f. The mg/L C concentration should increase by 100 mg/L for each 0.1 mL increment.

Method Performance

Precision

at mg/L C	95% Confidence Limits
100	±14 mg/L
250	±14 mg/L
400	±13 mg/L
550	±11 mg/L
700	±6 mg/L

For more information on determining precision data and method detection limits, refer to *Method Performance*.

Estimated Detection Limit

To test TOC levels below 100 mg/L C, use Method Number 10173.

Sensitivity

Program Number: 3650

Portion of Curve	Δ Abs	Δ Concentration
At 100 mg/L C	0.010	4.9 mg/L
At 350 mg/L C	0.010	4.1 mg/L
At 650 mg/L C	0.010	5.6 mg/L

See *Sensitivity Explained* for more information.

Interferences

The following have been tested for interference and found not to interfere up to the indicated levels:

Table 1 Non-interfering Substances (Maximum Level Tested)

Substance	Maximum Level Tested
Aluminum	10 mg/L
Ammonia Nitrogen	1000 mg/L as N
ASTM Wastewater	No effect
Bromide	500 mg/L Br
Bromine	25 mg/L Br ₂
Calcium	2000 mg/L as CaCO ₃
Chloride	5000 mg/L
Chlorine	10 mg/L Cl ₂
Chlorine Dioxide	6 mg/L ClO ₂
Copper	10 mg/L
Cyanide	10 mg/L CN
Iodide	50 mg/L
Iron (II)	10 mg/L
Iron (III)	10 mg/L
Magnesium	2000 mg/L as CaCO ₃
Manganese (VII)	1 mg/L
Monochloramine	14 mg/L NH ₂ Cl as Cl ₂
Nitrite	500 mg/L NO ₂ ⁻
Ozone	2 mg/L O ₃
Phosphate	3390 mg/L PO ₄ ³⁻
Silica	100 mg/L SiO ₂
Sulfate	5000 mg/L SO ₄ ²⁻
Sulfide	20 mg/L S ²⁻
Sulfite	50 mg/L SO ₃ ²⁻
Zinc	5 mg/L

If the sample contains greater than 1000 mg/L CaCO₃ alkalinity, lower the sample pH to less than 7 before testing by adding sulfuric acid solution.

Most sample turbidity is either dissolved during the digestion stage or settled during the cooling period. Sample turbidities up to 900 NTU have been tested without interference.

Summary of Method

The total organic carbon (TOC) is determined by first sparging the sample under slightly acidic conditions to remove the inorganic carbon. In the outside vial, organic carbon in the sample is digested by persulfate and acid to form carbon dioxide. During digestion, the carbon dioxide diffuses into a pH indicator reagent in the inner ampule. The adsorption of carbon dioxide into the indicator forms carbonic acid. Carbonic acid changes the pH of the indicator solution which, in turn, changes the color. The amount of color change is related to the original amount of carbon present in the sample.

ORGANIC CARBON, Total, continued

REQUIRED REAGENTS

Total Organic Carbon Direct Method High Range

Test 'N Tube Reagent Set.....50 vials.....27604-45

Includes:

Description	Quantity Required		Cat. No.
	Per Test	Unit	
Acid Digestion Solution Vials, High Range TOC.....	1	50/pkg	*
Buffer Solution, Sulfate.....	0.4 mL	25 mL	452-33
Funnel, micro	1	each	25843-35
Indicator Ampules, Mid/High Range TOC	1	10/pkg	*
TOC Persulfate Powder Pillows	1	50/pkg	*
Water, Organic-free**	3.0 mL	500 mL	26415-49

REQUIRED APPARATUS

COD Reactor, 115/230 V ac (U.S.A. and Canada)	1	each	45600-00
COD Reactor, 115/230 V ac (Europe)	1	each	45600-02
Cylinder, graduated, 10-mL	1	each	508-38
Flask, Erlenmeyer, 50-mL.....	1	each	505-41
Magnetic Stirrer.....	1	each	23436-00
Safety Shield, laboratory bench	1	each	50030-00
Test Tube Rack	1-3	each	18641-00
Pipet, TenSette®, 0.1 to 1.0 mL.....	1	each	19700-01
Pipet Tips, for 19700-01 TenSette® Pipet	2	50/pkg	21856-96
Stir Bar, Magnetic	1	each	45315-00
Wipes, Disposable, Kimwipes.....	1 pkg	280/pkg	20970-00

OPTIONAL REAGENTS

TOC Standard Solution (KHP Standard, 1000 mg/L C)	5/pkg	27915-05
Potassium Acid Phthalate.....	500 g	315-34
Sulfuric Acid Reagent Solution, 5.25 N.....	100 mL MDB	2449-32

OPTIONAL APPARATUS

Analytical Balance	each	26103-00
Flask, volumetric, 1000-mL	each	14574-53
Flask, volumetric, 100-mL	each	14574-42
Pipet, Class A, 10.00-mL	each	14515-38
Pipet, Class A, 15.00-mL	each	14515-39

* These items are not sold separately.

** This item must be purchased separately.



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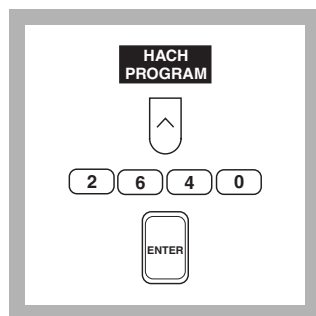
Telephone: (970) 669-3050

FAX: (970) 669-2932



Scope and Application: To indicate the total concentration of UV-absorbing organic compounds in drinking water. No estimated detection limit exists because it is a non-specific measurement. A DR4000U model is required.

* Adapted from *Standard Methods*, 19th. Ed., Method 5910



1. Press the soft key under **HACH PROGRAM**.

Select the stored program number for the Organics, UV-254 method, by pressing **2640** with the numeric keys.

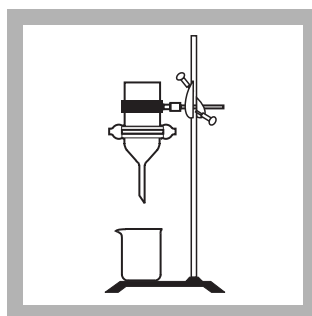
Press: **ENTER**



2. The display will show:
HACH PROGRAM: 2640 Organics, UV-254

The wavelength (λ), **253.7 nm**, is automatically selected.

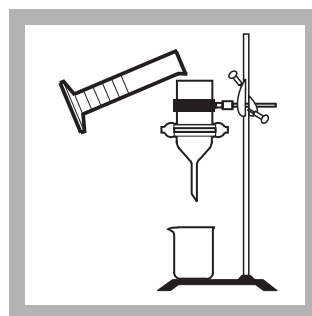
Note: If the UV lamp has been "OFF," allow a few minutes for full warm-up and stabilization.



3. Assemble the filter apparatus which includes the glass filter funnel, PTFE support plate, and install one 70-mm glass fiber filter. Be sure to use the white PTFE support plate. Place the filter with the wrinkled surface upward. Mount the apparatus into a support stand and place a clean glass beaker underneath it.

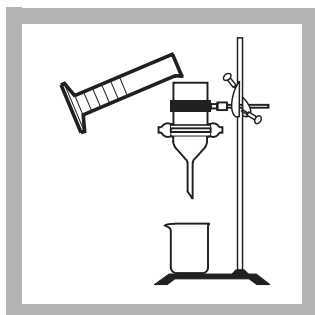
Note: Any non-plastic filter assembly can be used. Use a 0.45- μm or glass fiber filter of nominal pore size (1–1.5 μm) without organic binder.

Note: A 0.45- μm filter must be utilized if the results are to be used for SUVA calculations.



4. Prewash the filter assembly by pouring at least 50 mL of Organic-Free Reagent Water through the filter. Discard the filtered water.

Note: Pre-rinsing removes any soluble impurities from the filter.

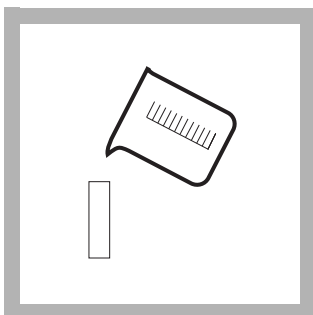


5. Pour 50 mL of sample through the filter and collect the filtered sample.

Note: The sample pH should be between 4 and 10. If not, see the Interferences section.

Note: Samples used for SUVA calculations must **not** be pH adjusted

Note: See Sample Collection, Storage and Preservation following these steps.

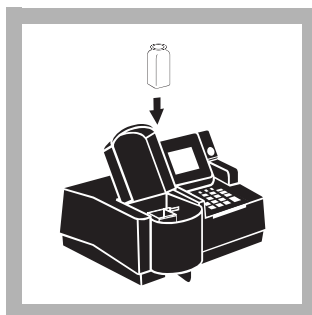


6. Rinse a clean 1-cm quartz cell several times with Organic-Free Reagent Water. Fill the cell with Organic-Free Reagent Water (the blank). Wipe the cell walls thoroughly.

Note: Use only Organic-Free Reagent Water to zero the instrument.

Note: A 1-inch or 1-cm Flow Cell Module or Sipper Module is highly recommended for best results. Be sure to satisfy the minimum volume requirements for each module type.

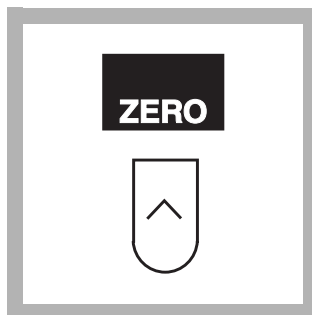
Note: Organic-Free Reagent Water, filtered at 0.45 μm , must be used as the blank for SUVA calculations.



7. Insert a 1-cm cell adapter into the cell compartment. Place the blank into the 1-cm adapter with the clear windows aligned with the light beam. Close the light shield.

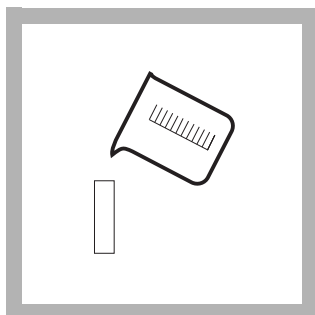
Note: Handle the cell only on the frosted sides.

Note: Occasionally clean cells using chromic acid to remove trace organic contamination. See Cell Cleaning following these steps.

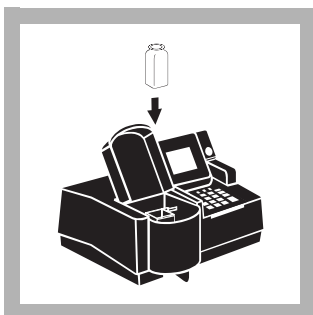


8. Press the soft key under **ZERO**.

The display will show
0.000 cm^{-1}



9. Discard the blank water from the cell and rinse the cell several times with filtered sample. After rinsing, fill the cell with filtered sample. Wipe the cell walls to remove fingerprints.



10. Place the cell containing the sample into the cell holder. Close the light shield. Results in absorbance per centimeter (cm^{-1}) will be displayed.

Note: For optimum results, the cm^{-1} value should fall between 0.005 and 0.900. If less than 0.005 absorbance using a 1-cm cell, use either a 1" Flow-Thru cell, 5-cm or 10-cm quartz cells. Press the soft keys under **OPTIONS** and then **FORM**:. Press the soft key under **FORM**: to scroll to the cell pathlength you wish to use. The display will indicate which cell pathlength you have selected. The displayed results (in absorbance per centimeter) will be corrected for the cell pathlength selected. If cm^{-1} results are greater than 0.900, accurately dilute the sample with Organic-Free Reagent Water. Correct the test result by the appropriate dilution factor.

Interferences

Table 1 Interfering Substances and Suggested Treatments

Interfering Substance	Interference Levels
Sample pH outside 4–10	Add either 1 N Sodium Hydroxide or 1 N Sulfuric Acid to the sample to adjust sample pH 4-10.
UV-absorbing inorganics (bromide, ferrous iron, nitrate, nitrites)	Follow the UV scanning procedure below.
UV-absorbing Oxidants and reductants (chloramines, chlorates, chlorites, ozone, thiosulfates)	Follow the UV scanning procedure below.

To determine the presence of interferences, a scan of the filtered sample versus Organic-Free Reagent Water is recommended on a regular basis:

1. From the main menu, press the soft key under **SCAN** λ .
2. Press the soft key under **OPTIONS**.
3. Press the soft key under λ **MIN:** and enter **2 0 0**. Press **ENTER**.
4. Press the soft key under λ **MAX:** and enter **4 0 0**. Press **ENTER**.
5. Press the soft key under λ **STEP;** then press the soft key under **1.0 NM**. Press **ENTER**.
6. Press **EXIT**.
7. Place the cell containing the Organic-Free Reagent Water (the blank) into the cell compartment and close the light shield.
8. Press the soft key under **BASELINE**. The baseline scan from 400 to 200 nm will begin.
9. After the baseline scan is recorded, place the cell containing the filtered sample into the adapter and close the light shield.
10. Press the soft key under **START SCAN**.

If the sample scan shows relatively sharp peaks, interferences may be present. Generally, natural organic matter will show a relatively featureless curve in the UV region with increasing absorption as the wavelength decreases. If the sharp peaks are indicated, an alternate wavelength should be selected and reported.

Sample Collection, Storage and Preservation

Collect samples in cleaned glass containers. Do not use plastic containers. Analyze samples as soon as possible after collection.

Cell Cleaning

New or dirty cells should be soaked with Chromic Acid Cleaning Solution (Cat. No. 1233-49) to remove trace organic contamination. Allow to soak overnight or up to 12 hours. After soaking, rinse with at least 10 volumes of Organic-Free Reagent Water. Treatment of cells with chromic acid is required only occasionally if cells are rinsed with Organic-Free Reagent Water after use.

Method Performance

Precision

Standard: There is no primary standard or calibration for the UV-254 method. Using a Potassium Acid Phthalate solution equivalent to 30-mg/L as carbon, the following reproducibility data was obtained using one instrument:

Program	95% Confidence Limits
2640	0.431–0.433 cm ⁻¹

Estimated Detection Limit

Because it is a non-specific measurement for organic constituents, there is no estimated detection limit for Program 2640.

Sensitivity

There is no calibration for Program 2640, so sensitivity data is not available.

Summary of Method

Filtered sample is measured at 253.7 nm against organic-free water as a indicator of organic constituents in the sample water. Results are automatically reported in absorbance per centimeter (cm⁻¹). The results can be used in calculating Specific Ultraviolet Absorbance (SUVA).

Safety

The Chromic Acid Cleaning Solution (Cat. No. 1233-49) is very toxic. Avoid inhalation and wear protective equipment as prescribed in the *Material Safety Data Sheet*. Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the standards and reagents used. For additional information, refer to Section I.

Pollution Prevention and Waste Management

The Chromic Acid Cleaning Solution (Cat. No. 1233-49) is regulated as a hazardous waste for chromium (D007) and corrosivity (D002) when disposed per Federal RCRA. The 1.0 M Hydrochloric Acid Solution (Cat. No. 23213-53) and the 1.00 N Sodium Hydroxide Standard Solution (Cat. No. 1045-32) are regulated as hazardous waste for corrosivity when disposed per Federal RCRA. Elementary neutralization of these two solutions will be an option for most users. For further information on pollution prevention and waste management, refer to Section I.

ORGANIC CONSTITUENTS, UV Absorbing (UV-254), continued

REQUIRED REAGENTS AND STANDARDS

Description	Quantity Required Per Test	Unit	Cat. No.
Organic-Free Reagent Water	varies	1 L	26415-53

REQUIRED EQUIPMENT AND SUPPLIES

DR/4000 1-cm Quartz Cells	1	each	26244-10
DR/4000 1-cm Cell Adapter	1	each	48584-00
Filter Funnel Assembly, 7-cm	1	each	21641-00
Filter Plate, PTFE, for 21641-00	1	each	21642-00
Filter, glass fiber, 70-mm	1	100/pkg	2530-53
Beaker, 100-mL	1	each	500-42
Clamp Holder	1	each	326-00
Clamp, 3-Prong	1	each	422-00
Buret Stand	1	each	329-00

OPTIONAL REAGENTS AND STANDARDS

Hydrochloric Acid Solution, 1.0 M	1 L	23213-53
Sodium Hydroxide Standard Solution, 1.00 N	100 mL * MDB	1045-32
Chromic Acid Cleaning Solution	500mL	1233-49

OPTIONAL EQUIPMENT AND SUPPLIES

Cylinder, graduated, 50-mL	each	508-41
DR/4000 Carousel Module	each	48070-02
DR/4000 1-inch Flow Cell Module	each	48070-04
DR/4000 1-inch Sipper Module	each	48090-03
DR/4000 1-cm Flow Cell Module	each	48070-05
DR/4000 1-cm Sipper Module	each	48090-06
DR/4000 5-cm Cell Adapter	each	48186-00
DR/4000 10-cm Cell Adapter	each	48118-00
Filter, membrane, 47-mm; 0.45-microns (SUVA)	each	13530-00
Filter Holder, glass for vacuum filtration (SUVA)	each	2340-00
Flask, filtering, glass, 1000-mL (SUVA)	each	546-53
pH Paper, pH 1.0 to 11.0	5 rolls/pkg	391-33
pH Meter, <i>sensio</i> TM 1, portable	each	51700-02
Quartz Sample Cell, 5-cm	each	26244-50
Quartz Sample Cell, 10-cm	each	26244-01
Tubing, rubber	12ft	560-19

* Contact Hach for larger sizes.



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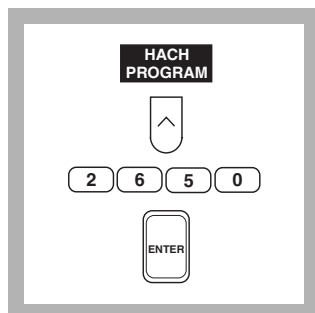
Method 8316

Indigo Carmine Method

AccuVac® Ampuls

LR (0 to 1000 µg/L O₂)

Scope and Application: For boiler feedwater. The estimated detection limit for program number 2650 is 10 µg/L.



1. Press the soft key under **HACH PROGRAM**.

Select the stored program number for low range dissolved oxygen by pressing **2650** with the numeric keys.

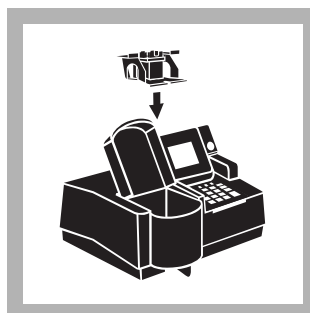
Press: **ENTER**

Note: Samples must be analyzed on site and cannot be stored; see Sample Collection, Preservation and Storage following this procedure.

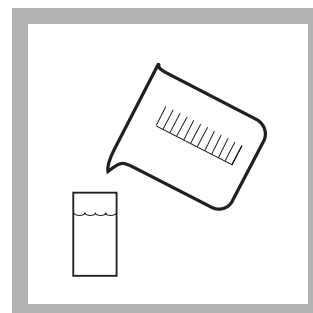


2. The display will show:
**HACH PROGRAM: 2650
O, Dissol. LR AV**

The wavelength (λ), **610 nm**, is automatically selected.



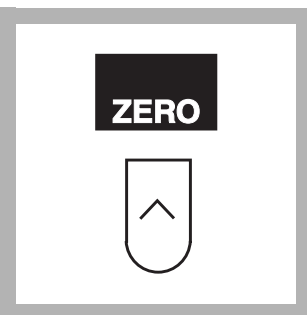
3. Insert the AccuVac Ampul Adapter into the sample cell module by sliding it under the thumb screw and into the alignment grooves. Fasten with the thumb screw.



4. Fill a zeroing vial (the blank) with at least 10 mL of sample.



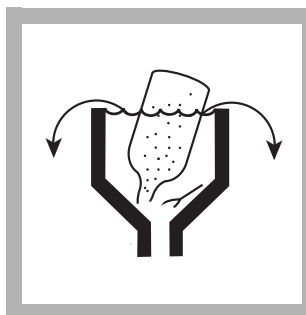
5. Place the blank into the cell holder. Close the light shield.



6. Press the soft key under **ZERO**.
The display will show:

0 µg/L O₂

Note: For alternate concentration units press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.

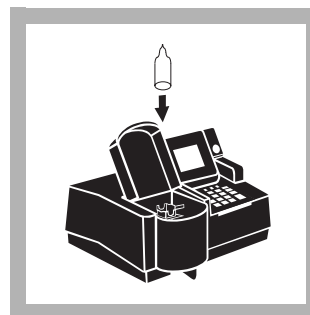


7. Fill a Low Range Dissolved Oxygen AccuVac Ampul with sample.

Note: Keep the tip immersed while the ampul fills completely.

Note: One measure of accuracy is to verify the zero concentration of the blank. Follow the steps given in the Accuracy Check.

Note: The ampuls will contain a small piece of wire to maintain reagent quality. The solution color will be yellow.



8. Immediately place the AccuVac Ampul into the vial adapter. Close the light shield. Result in µg/L dissolved oxygen (or chosen units) will be displayed.

Note: Use the initial reading. The reading is stable for 30 seconds. After 30 seconds, the ampul solution will absorb oxygen from the air.

Interferences

Interfering Substance	Interference Level and Treatment
Hydrazine	100,000 fold excess will begin to reduce the oxidized form of the indicator solution.
Sodium hydrosulfite	Reduces the oxidized form of the indicator solution and will cause a significant interference

Excess amounts of thioglycolate, ascorbate, ascorbate + sulfite, ascorbate + cupric sulfate, nitrite, sulfite, thiosulfate, and hydroquinone will not reduce the oxidized form of the indicator and do not cause significant interference.

Sample Collection, Preservation and Storage

The main consideration in this procedure is to prevent contaminating the sample with atmospheric oxygen. Sampling from a stream of water that is hard plumbed to the sample source is ideal. Use a funnel to maintain a continual flow of sample and yet collect enough sample to immerse the ampul. It is important not to introduce air in place of the sample. Rubber tubing, if used, will introduce unacceptable amounts of oxygen into the sample unless the length of tubing is minimized and the flow rate is maximized. Flush the sampling system with sample for at least 5 minutes.

Accuracy Check

The reagent blank for this test can be checked by following these steps:

- a. Fill a 50-mL beaker with sample and add approximately 50 mg Sodium Hydrosulfite.
- b. Immerse the tip of a Low Range Dissolved Oxygen AccuVac Ampul in the sample into the tip. Aspirate the sample into the ampul.
- c. Determine the dissolved oxygen concentration according to the preceding procedure. The result should be $0 \pm 1 \mu\text{g/L}$.

Method Performance

Precision

Standard: 500 $\mu\text{g/L O}_2$

Program	95% Confidence Limits
2650	499–501 $\mu\text{g/L O}_2$

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
2650	10 $\mu\text{g/L O}_2$

For more information on derivation and use of Hach's estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, appendix B, see Section 1.5.1.

Sensitivity

Program Number: 2650

Portion of Curve	ΔAbs	$\Delta\text{Concentration}$
Entire Range	0.010	7.0 $\mu\text{g/L}$

See Section 1.5.3 *Sensitivity Explained* for more information.

Summary of Method

The Low Range Dissolved Oxygen AccuVac Ampul contains reagent vacuum sealed in a 12-mL ampul. When the AccuVac Ampul is broken open in a sample containing dissolved oxygen, the yellow solution will turn blue. The blue color development is proportional to the concentration of dissolved oxygen.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to Section 1.

Pollution Prevention and Waste Management

For information on pollution prevention and waste management, refer to Section 1.

OXYGEN, Dissolved, continued

REQUIRED REAGENTS AND STANDARDS

Description	Quantity Required per test	Unit	Cat. No.
Low Range Dissolved Oxygen AccuVac Ampuls	1	25/pkg	25010-25

REQUIRED EQUIPMENT AND SUPPLIES

DR/4000 AccuVac Ampul Adapter.....	1	each.....	48187-00
Sample Cell, 10-mL with cap (zeroing vial).....	1	each.....	21228-00

OPTIONAL REAGENTS AND EQUIPMENT

Beaker, 50-mL.....		each.....	500-41
Sodium Hydrosulfite, technical-grade.....		500 g.....	294-34



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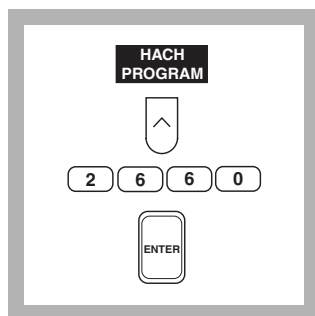
Method 8166

HRDO Method

HR (0 to 15.0 mg/L O₂)

Scope and Application: For water and wastewater.

The estimated detection limit for program number 2660 is 0.1 mg/L O₂.



1. Press the soft key under **HACH PROGRAM**.

Select the stored program number for high range dissolved oxygen by pressing **2660** with the numeric keys.

Press: **ENTER**

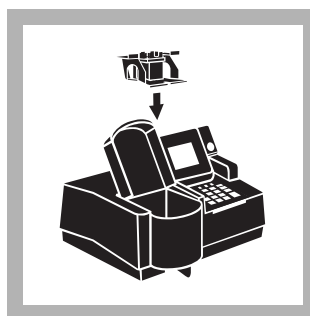
Note: Samples must be analyzed on site and cannot be stored; see Sample Collection, Preservation and Storage following these steps.



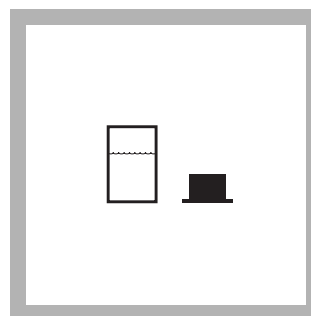
2. The display will show:

**HACH PROGRAM: 2660
O, Dissol. HR AV**

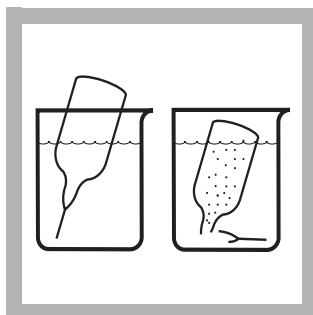
The wavelength (λ), **535 nm**, is automatically selected.



3. Insert the AccuVac Ampul Adapter into the sample cell module by sliding it under the thumb screw and into the alignment grooves. Fasten with the thumb screw.

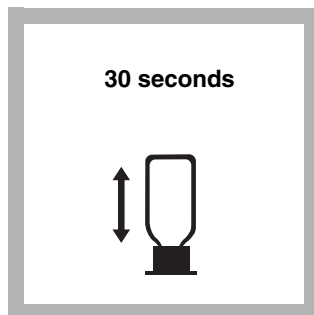


4. Fill a zeroing vial (the blank) with at least 10 mL of sample. Fill a blue ampul cap with sample.



5. Fill a High Range Dissolved Oxygen AccuVac Ampul with sample.

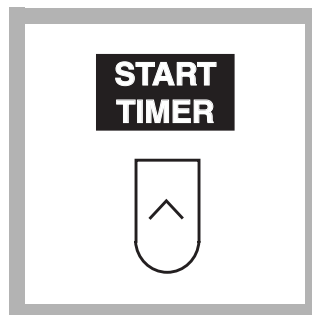
Note: Keep the tip immersed while the ampul fills completely.



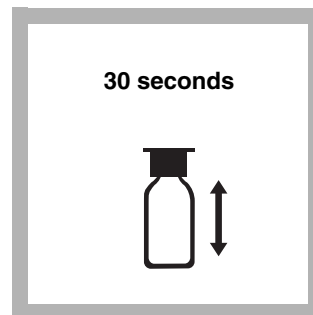
6. Without inverting the ampul, immediately place the ampul cap that has been filled with sample securely over the tip of the ampul. Shake the ampul for approximately 30 seconds.

Note: A small amount of the undissolved HRDO Reagent does not affect results.

Note: The cap prevents contamination with atmospheric oxygen.



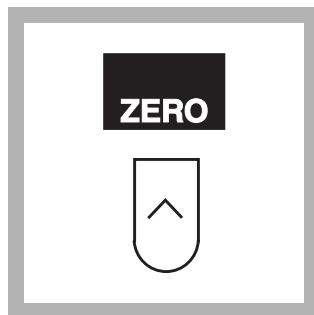
7. Press the soft key under **START TIMER**. This 2-minute reaction period enables oxygen, which was degassed during aspiration, to redissolve and react.



8. When the timer beeps, shake the ampul for 30 seconds.



9. Place the blank into the cell holder. Close the light shield.



10. Press the soft key under **ZERO**.

The display will show:

0.0 mg/L O₂

Note: For alternate concentration units press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



11. Place the AccuVac Ampul into the cell holder. Close the light shield. Wait approximately 30 seconds for the air bubbles to disperse from the light path. Results in mg/L (or chosen units) dissolved oxygen will be displayed.

Interferences

Interfering Substance	Interference Level and Treatment
Cr ³⁺	Greater than 10 mg/L
Cu ²⁺	Greater than 10 mg/L
Fe ²⁺	Greater than 10 mg/L
Mg ²⁺	Magnesium is commonly present in seawater and causes a negative interference. If the sample contains more than 50% seawater, the oxygen concentration obtained by this method will be 25% less than the true oxygen concentration. If the sample contains less than 50% seawater, the interference will be less than 5%.
Mn ²⁺	Greater than 10 mg/L
Ni ²⁺	Greater than 10 mg/L
NO ₂ ⁻	Greater than 10 mg/L

Sample Collection, Preservation and Storage

The main consideration in sampling with the High Range Dissolved Oxygen Ampul is to prevent the sample from becoming contaminated with atmospheric oxygen between breaking open the ampul and reading the absorbance. This is accomplished by capping the ampul with an ampul cap. If the ampul is securely capped, the ampul should be safe from contamination for several hours. The absorbance will decrease by approximately 3% during the first hour and will not change significantly afterwards.

Sampling and sample handling are important considerations in obtaining meaningful results. The dissolved oxygen content of the water being tested may change with depth, turbulence, temperature, sludge deposits, light, microbial action, mixing, travel time and other factors. A single dissolved oxygen test rarely reflects the accurate over-all condition of a body of water. Several samples taken at different times, locations and depths are recommended for most reliable results. Samples must be tested immediately upon collection, although only a small error results if the absorbance reading is taken several hours later.

Accuracy Check

The results of this procedure may be compared with the results of a titrimetric procedure (request Lit. Code 8042) or Portable Dissolved Oxygen Meter (Cat. No. 50175-00).

Method Performance

Precision

Standard: 8.0 mg/L O₂

Program	95% Confidence Limits
2660	7.9–8.1 mg/L O ₂

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
2660	0.1 mg/L O ₂

OXYGEN, Dissolved, continued

For more information on derivation and use of Hach's estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, Appendix B, see Section 1.5.1.

Sensitivity

Program Number: 2660

Portion of Curve	Δ Abs	Δ Concentration
0.010 Abs	0.010	0.12 mg/L
7.5 mg/L	0.010	0.08 mg/L
13.5 mg/L	0.010	0.08 mg/L

See Section 1.5.3 *Sensitivity Explained* for more information.

Summary of Method

The High Range Dissolved Oxygen AccuVac Ampul contains reagent vacuum sealed in a 12-mL ampul. When the AccuVac Ampul is broken open in a sample containing dissolved oxygen, it forms a yellow color which turns purple. The purple color development is proportional to the concentration of dissolved oxygen.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to Section 1.

Pollution Prevention and Waste Management

For information on pollution prevention and waste management, refer to Section 1.

REQUIRED REAGENTS AND STANDARDS

Description	Quantity Required per test	Unit	Cat. No.
High Range Dissolved Oxygen AccuVac Ampuls, 0–10 mg/L with 2 reusable ampul caps	1 ampul	25/pkg	25150-25

REQUIRED EQUIPMENT AND SUPPLIES

Beaker, 50-mL	1	each	500-41
Caps, ampul, blue	1	25/pkg	1731-25
DR/4000 AccuVac Ampul Adapter	1	each	48187-00
Sample Cell, 10-mL with cap (zeroing vial)	1	each	21228-00

OPTIONAL REAGENTS AND EQUIPMENT

AccuVac Ampul Dissolved Oxygen Sampler	each	24051-00
AccuVac Snapper	each	24052-00
BOD bottle and stopper, 300-mL	each	621-00
sens ^{ion} ™6 Dissolved Oxygen Meter, with probe	each	51850-10

Note: Dissolved oxygen may also be determined by titrimetric methods.
Request Publication 8042 for additional information.



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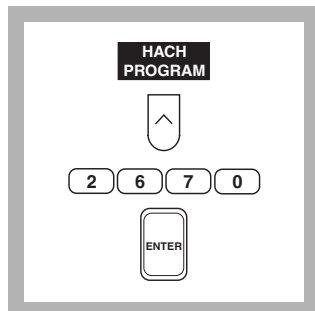


Method 8333

Ultra High Range Method

UHR (0 to 40.0 mg/L O₂)

Scope and Application: For aquaculture. The estimated detection limit for program number 2670 is 0.2 mg/L O₂.



- 1.** Press the soft key under **HACH PROGRAM**.

Select the stored program number for ultra high range dissolved oxygen by pressing **2670** with the numeric keys.

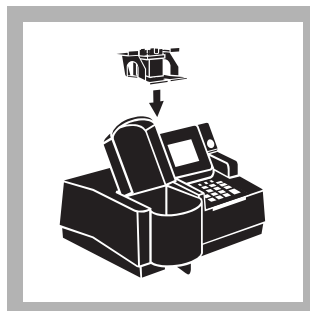
Press: **ENTER**

Note: Samples must be analyzed on site and cannot be stored; see Sample Collection, Preservation and Storage following these steps.

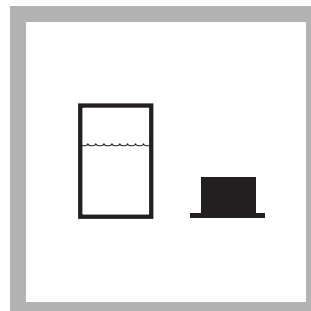


- 2.** The display will show:
**HACH PROGRAM: 2670
O, Dissol. UHR AV**

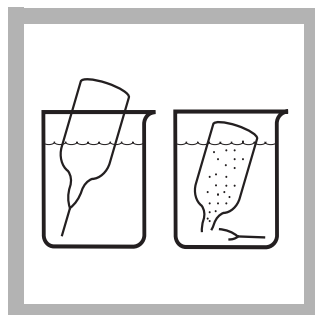
The wavelength (λ), **680 nm**, is automatically selected.



- 3.** Insert the AccuVac Ampul Adapter into the sample cell module by sliding it under the thumb screw and into the alignment grooves. Fasten with the thumb screw.

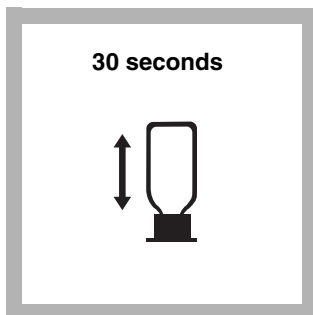


- 4.** Fill a zeroing vial (the blank) with at least 10 mL of sample. Fill a blue ampul cap with sample.



5. Fill a High Range Dissolved Oxygen AccuVac Ampul with sample.

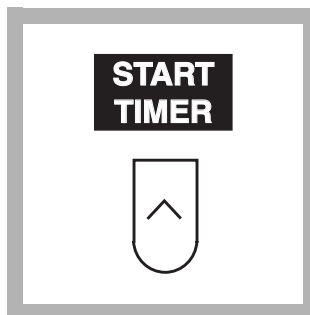
Note: Keep the tip immersed while the ampul fills completely.



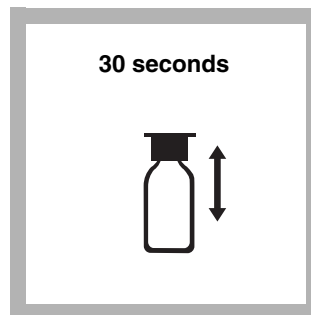
6. Without inverting the ampul, immediately place the ampul cap that has been filled with sample securely over the tip of the ampul. Shake the ampul for approximately 30 seconds.

Note: A small amount of undissolved reagent does not affect results.

Note: The cap prevents contamination with atmospheric oxygen.



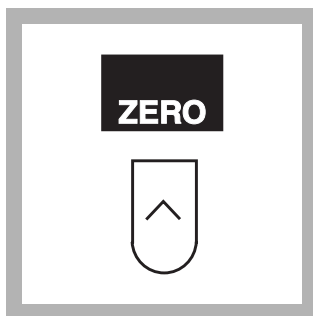
7. Press the soft key under **START TIMER**. This 2-minute reaction period enables oxygen, which was degassed during aspiration, to redissolve and react.



8. When the timer beeps, shake the ampul for 30 seconds.



9. Place the blank into the cell holder. Close the light shield.



10. Press the soft key under **ZERO**.

The display will show:

0.0 mg/L O₂

Note: For alternate concentration units press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



11. Place the AccuVac Ampul into the cell holder. Close the light shield. Results in mg/L dissolved oxygen (or chosen units) will be displayed.

Interferences

Interfering Substance	Interference Level and Treatment
Cr ³⁺	Greater than 10 mg/L
Cu ²⁺	Greater than 10 mg/L
Fe ²⁺	Greater than 10 mg/L
Mg ²⁺	Magnesium is commonly present in seawater and interferes. If the sample contains more than 50% seawater, the oxygen concentration observed will be 25% lower than the true oxygen concentration. If the sample contains less than 50% seawater, the interference will be less than 5%.
Mn ²⁺	Greater than 10 mg/L
Ni ²⁺	Greater than 10 mg/L
NO ₂ ⁻	Greater than 10 mg/L

Sample Collection, Preservation and Storage

The main consideration in sampling with the High Range Dissolved Oxygen AccuVac Ampul is to prevent the sample from becoming contaminated with atmospheric oxygen between breaking open the ampul and reading the absorbance. This is accomplished by capping the ampul with an ampul cap. If the ampul is securely capped, the ampul should be safe from contamination for several hours. The absorbance will decrease by approximately 3% during the first hour and will not change significantly afterwards.

Sampling and sample handling are important considerations in obtaining meaningful results. The dissolved oxygen content of the water being tested may change with depth, turbulence, temperature, sludge deposits, light, microbial action, mixing, travel time and other factors. A single dissolved oxygen test rarely reflects the accurate over-all condition of a body of water. Several samples taken at different times, locations and depths are recommended for most reliable results. Samples must be tested immediately upon collection although only a small error results if the absorbance reading is taken several hours later.

Accuracy Check

The results of this procedure may be compared with the results of a titrimetric procedure (request Lit. Code 8042) or Dissolved Oxygen Meter (Cat. No. 50175-00).

Method Performance

Precision

Standard: 25.0 mg/L O₂

Program	95% Confidence Limits
2670	24.9–25.1 mg/L O ₂

For more information on determining precision data and method detection limits, refer to Section 1.5.

OXYGEN, Dissolved, continued

Estimated Detection Limit

Program	EDL
2670	0.2 mg/L O ₂

For more information on derivation and use of Hach's estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, appendix B, see Section 1.5.1.

Sensitivity

Program Number: 2670

Portion of Curve	Δ Abs	Δ Concentration
Entire Range	0.010	0.35 mg/L

See Section 1.5.3 *Sensitivity Explained* for more information.

Summary of Method

The High Range Dissolved Oxygen AccuVac Ampul contains reagent vacuum sealed in a 12-mL ampul. When the AccuVac Ampul is broken open in a sample containing dissolved oxygen, it forms a yellow color which turns purple. The purple color development is proportional to the concentration of dissolved oxygen.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to Section 1.

Pollution Prevention and Waste Management

For information on pollution prevention and waste management, refer to Section 1.

REQUIRED REAGENTS AND STANDARDS

Description	Quantity Required per test	Unit	Cat. No.
High Range Dissolved Oxygen AccuVac Ampuls, 0–10 mg/L with 2 reusable ampul caps	1 ampul	25/pkg	25150-25

REQUIRED EQUIPMENT AND SUPPLIES

DR/4000 AccuVac Ampul Adapter	1	each	48187-00
Beaker, 50-mL	1	each	500-41
Sample Cell, 10-mL with cap (zeroing vial)	1	each	21228-00

OPTIONAL EQUIPMENT AND SUPPLIES

AccuVac Ampul Dissolved Oxygen Sampler	each	24051-00
AccuVac Snapper	each	24052-00
BOD bottle and stopper, 300-mL	each	621-00
Caps, ampul, blue	25/pkg	1731-25
sens ^{ion} ™6 Dissolved Oxygen Meter, with probe	each	51850-10



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WORLD HEADQUARTERS
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FAX: (970) 669-2932



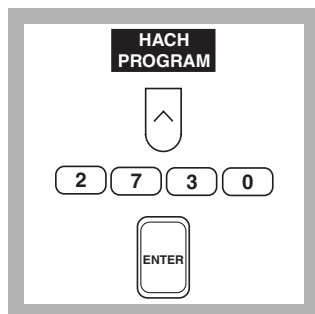
Method 10067

Manganese III Reactor Digestion Method (without chloride removal)*

(30-1000 mg/L)

Scope and Application: For water and wastewater.

* U.S. Patent 5,556,787 on method



1. Press the soft key under **HACH PROGRAM**.

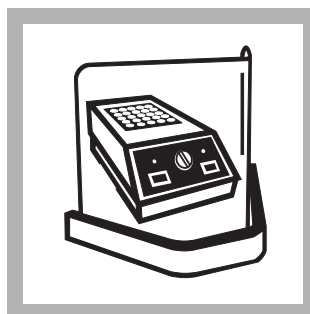
Select the stored program number for Manganese III COD by pressing **2730** with the numeric keys.

Press: **ENTER**



2. The display will show:
**HACH PROGRAM: 2730
COD, Mn III**

The wavelength (λ), **510 nm**, is automatically selected.



3. Turn on the COD Reactor and heat to 150 °C. Place the shield in front of the reactor.

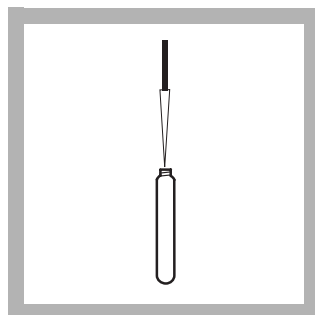
Note: To determine if the sample contains chloride, use Quantab Titrator Strips for Low range Chloride. If the sample contains chloride, use the chloride removal method (follows this method).



4. Homogenize 100 mL of sample for 30 seconds in a blender.

Note: Continue mixing the sample while pipetting if suspended solids are present.

Note: To store samples, see Sample Collection, Preservation and Storage in the Manganese III Digestion Method (with optional chloride removal) following this procedure.



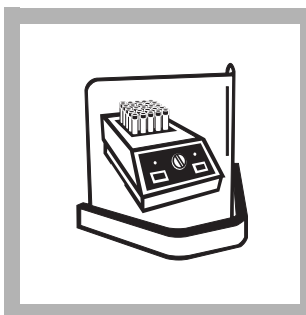
5. Pipet 0.50 ml of homogenized sample into a Mn III COD vial. Cap and invert several times to mix.

Note: If the sample COD value is not between 30-1000 mg/L, dilute the sample with deionized water to obtain this range. Multiply the final result by the dilution factor.

PREPARE BLANK

6. Prepare a blank by substituting 0.50 mL of deionized water for the sample.

Note: The reagent blank is stable and can be reused. Verify reagent blank quality by measuring the absorbance of the blank vs. a clean COD vial filled with deionized water. The absorbance range should be about 1.4–1.5.

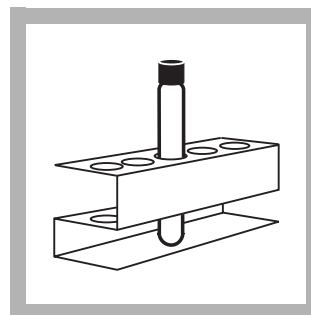


7. Place the vials in the COD Reactor preheated to 150 °C. Digest for 1 hour.

Note: Boiling sample in the vials during digestion indicates the vial is not properly sealed; test results will be invalid.

Note: To oxidize more resistant organics, digest samples up to 4 hours. Treat the blank in the same manner.

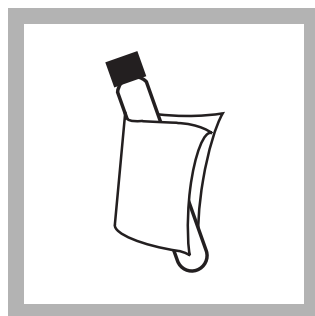
Note: Ensure safety devices are in place to protect the analyst from splattering if leaks occur. Spilled reagent will affect test accuracy and is hazardous. Do not run tests with vials which have been spilled.



8. Remove the vials and place them in a cooling rack for two minutes to air cool. Then cool the vials to room temperature in a cool water bath or running tap water. This usually takes about 3 minutes.

Note: Occasionally a vial will develop a colorless upper layer and a purple lower layer. Invert the vial several times to mix and proceed.

Note: The Hach COD Vial Lifter allows the transfer of several vials at once.



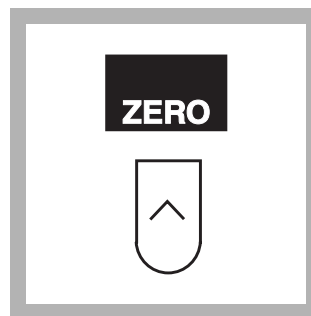
9. Remove the vials from the water and wipe with a clean, dry paper towel. Invert the vials several times to mix.



10. Insert the COD Vial Adapter into the sample cell module by sliding it under the thumb screw and into the alignment grooves. Fasten with the thumb screw.



11. Place the blank into the sample cell. Close the light shield.

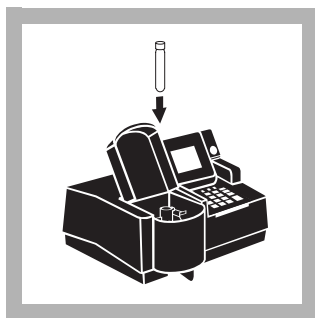


12. Press the soft key under **ZERO**.

The display will show:

0 mg/L COD

Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



13. Place the sample vial in the adapter. Close the light shield. Results in mg/L COD (or chosen units) will be displayed.

Note: Results may be expressed as mg/L COD or mg/L O₂. Press the soft keys under **OPTIONS**, then under **FORM**: to scroll through the available choices.

Additional Information

For information about sampling, storage, accuracy checks, interferences, method summary, reagents and apparatus, see the *Manganese III Digestion Method (with optional chloride removal)* following this procedure.



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FAX: (970) 669-2932



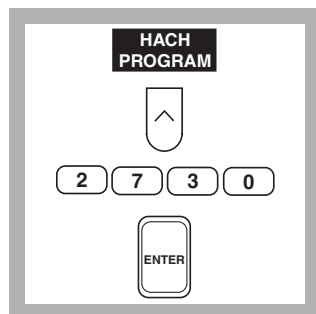
Method 10067

Manganese III Digestion Method* (with optional chloride removal)

(20 to 1000 mg/L COD)

Scope and Application: For water and wastewater.

* U.S. Patent 5,556,787



1. Press the soft key under **HACH PROGRAM**.

Select the stored program number for Manganese III COD by pressing **2730** with the numeric keys.

Press: **ENTER**

Note: If samples cannot be analyzed immediately, see *Sample Collection, Preservation and Storage* following these steps.



2. The display will show:
**HACH PROGRAM: 2730
COD, Mn III**

The wavelength (λ), **510 nm**, is automatically selected.



3. Turn on the COD Reactor and heat to 150 °C while preparing the sample and blank.

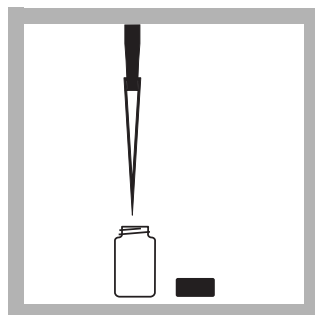


4. Homogenize 100 mL of sample for 30 seconds in a blender.

Note: Blending promotes even distribution of solids and improves accuracy and reproducibility.

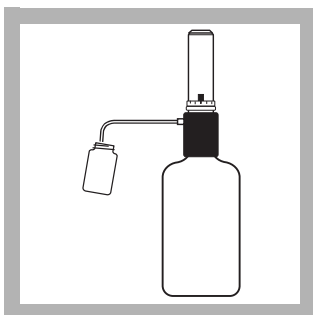
Note: Continue mixing the sample while pipetting if suspended solids are present.

Caution: Some of the chemicals and apparatus used in this procedure may be hazardous to the health and safety of the user if inappropriately handled or accidentally misused. Please read all warnings and the safety section of this manual. Wear appropriate eye protection and appropriate clothing. If contact occurs, flush the affected area with running water. Follow all instructions carefully.



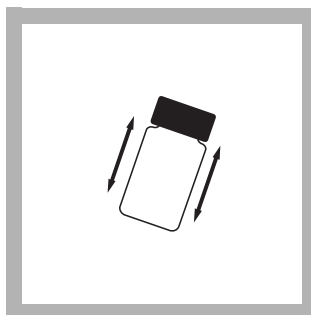
5. Using a TenSette Pipet or a pipet and safety bulb, pipet 9.0 mL of homogenized sample into an empty glass mixing cell. If the sample COD exceeds 1000 mg/L, dilute the sample as described in Table 1.

Note: Continue mixing the sample while pipetting samples with suspended solids.



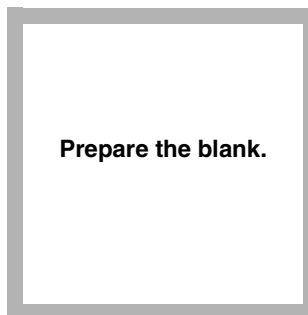
6. Using an automatic dispenser or TenSette Pipet, add 1.0 mL of concentrated sulfuric acid to the mixing cell.

Note: Mixing concentrated sulfuric acid and water is not additive. Adding 1.0 mL of concentrated sulfuric acid to 9.0 mL of sample does not result in a final volume of 10.0 mL. This factor is built into the calibration curve.



7. Cap the cell tightly and invert it several times. The solution will become hot. Cool to room temperature before proceeding.

Note: Acidified samples are stable for several months when refrigerated at 4 °C.



8. Prepare a blank (see note) by repeating steps 6-8, using 9.0 mL of deionized water for the sample.

Note: Use a clean pipet or rinse it thoroughly.

Note: One blank must be run with each lot of reagents. Run all samples and blanks with the same lot of vials (lot number is on the container label).

Note: The reagent blank is stable and can be re-used. Verify reagent blank quality by measuring the absorbance of the reagent blank versus a clean COD vial filled with deionized water. The absorbance for the blank should be about 1.41-1.47.

Table 1 Dilution Table

Sample (mL)	Deionized Water (mL)	Range (mg/L COD)	Multiplication Factor
6.0	3.0	30-1500	1.5
3.0	6.0	60-3000	3
1.0	8.0	180-9000	9
0.5	8.5	360-18000	18

All dilutions require that the ratio of sample to sulfuric acid remain at 9:1. For other dilutions that are not listed in Table 1, simply add the sample volume + deionized water and divide by the sample volume to obtain the multiplication factor.

Example:

Dilute the sample to a range of 90 to 4500 mg/L COD

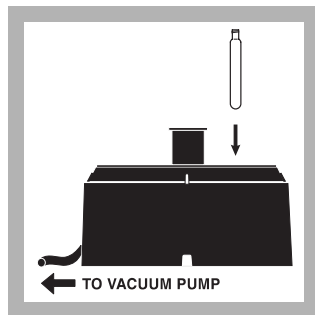
$$\text{Sample Volume (2.0 mL)} + \text{Deionized water (7.0 mL)} = \text{Total Volume (9.0 mL)}$$

$$\text{Multiplication Factor} = \frac{\text{Total Volume}}{\text{Sample Volume}} = \frac{9.0 \text{ mL}}{2.0 \text{ mL}} = 4.5$$

Standard test range is 50 to 1000 mg/L COD.

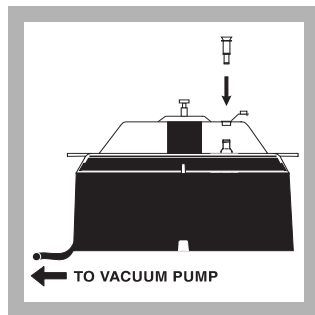
$$\text{Example Test Range} = 4.5 (50) \text{ to } 4.5 (1000) = 225 \text{ to } 4500 \text{ mg/L COD}$$

It is best to use 0.5 mL or more of sample for diluting. If sample values exceed 18,000 mg/L COD, use a separate sample dilution before performing the sample chloride removal procedure.

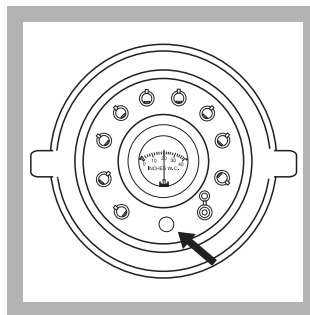


9. Label each Mn III COD vial and remove the cap. Place the vials in one of the numbered holes in the Vacuum Pretreatment Device (VPD)* base.

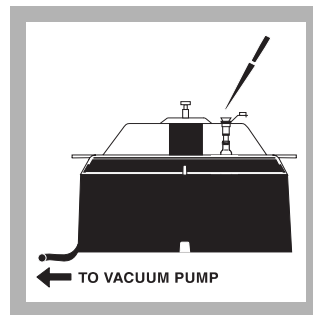
Note: The VPD must be attached to a vacuum pump (not an aspirator-type vacuum) that can create a vacuum of 20–25 inches of mercury.



10. Place the VPD top on the base. Insert a fresh Chloride Removal Cartridge (CRC)** directly above each Mn III COD Reagent Vial. Plug any open holes in the VPD top using the stoppers provided.



11. Turn the vacuum pump on and adjust the vacuum regulator valve on top of the VPD until the internal gauge reads 20 inches of water.

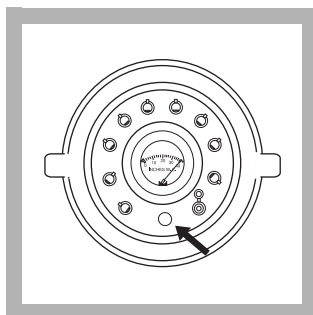


12. Pipet 0.60 mL of acidified sample (prepared in steps 6–8) into the CRC. Pipet 0.60 mL of acidified blank into another CRC. It should take 30–45 seconds to draw the liquid through the CRC into each vial.

Note: If the sample does not flow through the CRC, increase the vacuum until flow starts, then reduce the vacuum down to 20 inches of water. Proceed as usual.

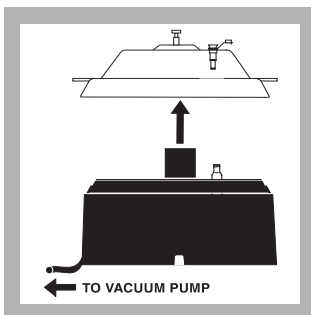
*Patent Pending

**U.S. Patents 5,667,754; 5,683,914

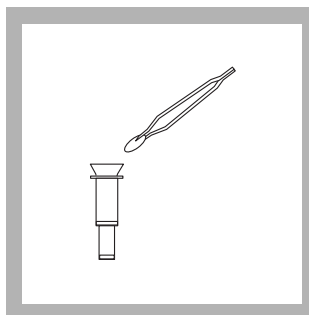


13. Close the vacuum regulator valve completely to achieve full vacuum. After 1 minute of full vacuum, slide the VRD back and forth several times to dislodge any drops clinging to the cartridge.

Note: The maximum range of the VRD vacuum gauge is 40 inches of water; it will not indicate the full vacuum level obtained. Full vacuum is 20-25 inches of mercury; this can be measured at the vacuum pump with a gauge calibrated for inches of mercury.



14. Open the VRD regulator valve to release the vacuum. Turn the pump off. Remove the VRD top and set it beside the base.

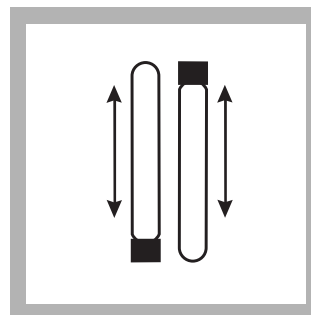


15. Use forceps to remove the filter from the top of each CRC. Place each filter in the corresponding Mn III COD Vial (use the numbers on the VRD as a guide).

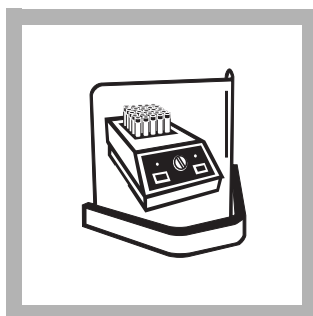
Note: To avoid cross contamination, clean forcep tips between samples by wiping with a clean towel or rinsing with deionized water.

Note: If the sample does not contain suspended solids, it is not necessary to transfer the filter to the digestion vial.

Note: Dispose of the used Chloride Removal Cartridge. Do not reuse it.



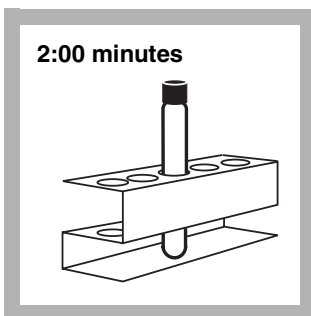
16. Remove the Mn III COD vial from the vacuum chamber and replace the original cap. Screw the cap on tightly. Invert several times to mix.



17. Place the vials in the COD Reactor that is preheated to 150 °C. Digest for 1 hour.

Note: Boiling sample in the vials during digestion indicates the vial is not properly sealed; test results will be invalid.

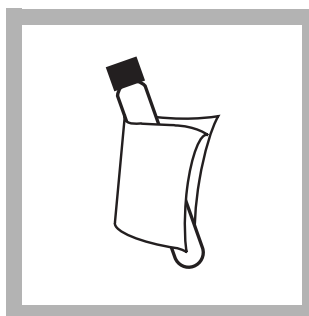
Note: Samples can be digested up to 4 hours to oxidize more resistant organics. The prepared blank must be treated in the same manner.



18. Place the vials in a cooling rack for two minutes. Then cool the vials to room temperature in a cool water bath or tap water (takes about three minutes).

Note: If the solution develops a colorless upper layer and a purple lower layer, invert the vial several times and proceed. This will not affect test results.

Note: Use the Hach COD Lifter to transfer several vials at once.

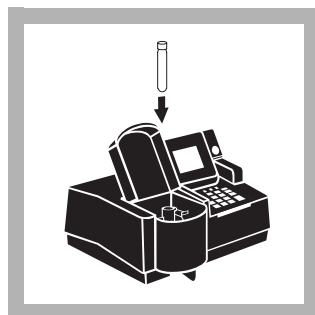


19. Remove the vials from the water and wipe with a clean, dry paper towel.

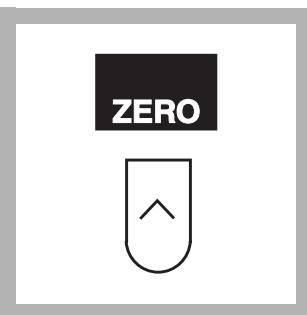
Invert the vials several times to mix.



20. Insert the COD Vial Adapter into the sample cell module by sliding it under the thumb screw and into the alignment grooves. Fasten with the thumb screw.



21. Place the blank into the sample cell compartment. Close the light shield.

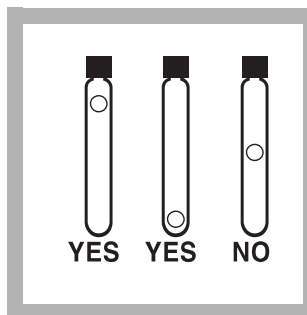


22. Press the soft key under **ZERO**.

The display will show:

0 mg/L COD

Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



23. If the chloride removal was done, make sure the filter disc is not suspended in the middle of the vial; it can interfere with the instrument reading. Move it with gentle swirling or by lightly tapping the vial on the table top.



24. Place the sample vial in the adapter. Close the light shield. Results in mg/L COD (or chosen units) will be displayed.

Note: Adjust the result for any sample dilution in steps 4 or 6.

Note: Results may be expressed as mg/L COD or mg/L O₂. Press the soft keys under **OPTIONS**, then under **FORM**: to scroll through the available choices.

Interferences

Inorganic materials may also be oxidized by trivalent manganese and constitute a positive interference when present in significant amounts. Chloride is the most common interference and is removed by sample pretreatment with the Chloride Removal Cartridge. If chloride is known to be absent or present in insignificant levels, the pretreatment can be omitted. A simple way to determine if chloride will affect test results is to run routine samples with and without the chloride removal, then compare results. Other inorganic interferences (i.e., nitrite, ferrous iron, sulfide) are not usually present in significant amounts. If necessary, these interferences can be corrected for after determining their concentrations with separate methods and adjusting the final COD test results accordingly.

Ammonia nitrogen is known to interfere in the presence of chloride; it does not interfere if chloride is absent.

Sample Collection, Preservation and Storage

Collect samples in clean glass bottles. Use plastic bottles only if they are known to be free of organic contamination. Test biologically active samples as soon as possible. Homogenize samples containing solids to assure representative samples. Samples treated with concentrated sulfuric acid to a pH of less than 2 (about 2 mL per liter) and refrigerated at 4 °C may be stored up to 28 days. Correct results for volume additions; see Section 1.2.2 *Correcting for Volume Additions*.

Accuracy Check

Standard Solution Method

Prepare an 800-mg/L COD standard solution by adding 0.6808 g of dried (103 °C, overnight) potassium acid phthalate (KHP) to 1 liter of deionized water. Use 0.50 mL of this solution (0.60 mL for the chloride removal procedure) as the sample volume. The result should be 800 ±24 mg/L COD. An 800-mg/L COD Standard Solution can also be purchased directly from Hach (see *OPTIONAL APPARATUS*).

To adjust the calibration curve using the reading obtained with the 800-mg/L COD standard solution, press the soft keys under **METHOD OPTIONS, (MORE)** then **STD:OFF**. Press **ENTER** to accept the value and return to the read screen. The instrument will only allow adjustment if the entered concentration is within 10% of the measured concentration. See Section 1.5.5 *Adjusting the Standard Curve*.

Method Performance

(Data is for Manganese III COD without the chloride removal procedure)

Precision

Standard: 500 mg/L COD

Program	95% Confidence Limits
2730	497–503 mg/L COD

For more information on determining precision data and method detection limits, see Section 1.5.

Estimated Detection Limit (EDL)

Program	EDL
2730	4 mg/L COD

For more information on derivation and use of Hach's estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, appendix B, see Section 1.5.1.

Sensitivity

Program Number: 2730

Portion Of Curve	ΔAbs	ΔConcentration
Entire range	0.010	8 mg/L

See Section 1.5.3 *Sensitivity Explained* for more information.

Calibration Standard Preparation

To perform a calibration using the manganese III method, prepare a 10,000-mg/L COD stock solution by diluting 0.8510 grams of dried (120 °C, overnight) KHP to 100 mL with deionized water using Class A glassware. Mix thoroughly.

Prepare calibration standards containing 100, 300, 500, 800 and 1000 mg/L COD as follows:

- a. Into five different 100-mL volumetric flasks, pipet 1.00, 3.00, 5.00, 8.00 and 10.00 mL of the 10,000-mg/L COD stock solution using Class A glassware.
- b. Dilute to the mark with deionized water. Stopper the flasks and invert each of them 10 times to mix.
- c. Using the Manganese III COD method and the calibration procedure described in the *User-Entered Programs* section of the *DR/4000 Spectrophotometer Instrument Manual*, generate a calibration curve from the standards prepared above.

Summary Of Method

Chemical oxygen demand (COD) is defined as "... a measure of the oxygen equivalent of the organic matter content of a sample that is susceptible to oxidation by a strong chemical oxidant" (APHA Standard Methods, 19th ed., 1995). Trivalent manganese is a strong, non-carcinogenic chemical oxidant that changes quantitatively from purple to colorless when it reacts with organic matter. It typically oxidizes about 80% of the organic compounds. Studies have shown that the reactions are highly reproducible and test results correlate closely to Biochemical Oxygen Demand (BOD) values and hexavalent chromium COD tests. None of the oxygen demand tests provide 100% oxidation of all organic compounds.

A calibration is provided which is based on the oxidation of Potassium Acid Phthalate (KHP). A different response may be seen in analyzing various wastewaters. The KHP calibration is adequate for most applications. The highest degree of accuracy is obtained when test results are correlated to a standard reference method such as BOD or one of the chromium COD methods. Special waste streams or classes will require a separate calibration to obtain a direct mg/L COD reading or to generate a correction factor for the precalibrated KHP response. The sample digestion time can be extended up to four hours for samples that are difficult to oxidize.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheets* for information specific to the reagents used. For additional information, refer to Section 1.

OXYGEN DEMAND, Chemical, continued

REQUIRED REAGENTS

Description	Quantity Required		Cat. No.
	Per Test	Unit	
Chloride Removal Cartridges (CRC)	1	25/pkg	26618-25
Manganese III COD Reagent Vials, 20-1000 mg/L COD	1	25/pkg	26234-25
Sulfuric Acid, concentrated, ACS	1 mL	2.5 L	979-09
Water, deionized	varies	4 L	272-56

REQUIRED APPARATUS

Blender, 120 VAC	1	each	26747-00
Blender Container, 50–250 mL	1	2/pkg	26748-00
Cap, with inert Teflon liner, for mixing bottle	varies	12/pkg	24018-12
COD Reactor, 115-230 VAC, 50-60 Hz	1	each	45600-00
<i>or</i>			
COD Reactor, 230 VAC, 50 Hz	1	each	45600-02
DR/4000 Test Tube Adapter	1	each	48189-00
Forceps, extra fine point	1	each	26696-00
Pipet, TenSette, 1.0 to 10.0 mL	1	each	19700-10
Pipet Tips, for 19700-10 TenSette Pipet	2	50/pkg	21997-96
Pipet, TenSette, 0.1 to 1.0 mL	1	each	19700-01
Pipet Tips, for 19700-01 TenSette	2	50/pkg	21856-96
Safety Shield	1	each	23810-00
Test Tube Rack, COD	1	each	18641-00
Vacuum Pretreatment Device (VPD)	1	each	49000-00
Vacuum Pump	1	each	14697-00
Vial, glass, for sample + acid	2	each	24277-00

OPTIONAL REAGENTS

COD Standard Solution, 800-mg/L COD	200 mL	26726-29
Potassium Acid Phthalate, ACS	500 g	315-34

OPTIONAL APPARATUS

Dispenser for sulfuric acid, 0.5-5.9 mL, digital	each	25631-37
Pipet Tips, for 19700-01 TenSette	1000/pkg	21856-28



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Method 8000

Reactor Digestion Method*

(0 to 40.0** mg/L COD)

Scope and Application: For water, wastewater and seawater; digestion is required.
The estimated detection limit for program number 2700 is 0.2 mg/L COD.

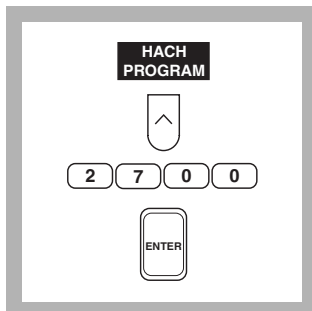
* Jirka, A.M.; Carter, M.J., *Analytical Chemistry*, 1975, 47(8), 1397

** Ultra low range vials are not USEPA approved and may be used only with spectrophotometers with 350 nm capability.

Colorimetric Measurement, 0 to 40 mg/L COD



1. Perform the digestion for this method as described in “Oxygen Demand, Chemical, Digestion Procedure” which precedes the COD colorimetric procedures.



2. Press the soft key under **HACH PROGRAM**.
Select the stored program number for ultra low range COD by pressing **2700** with the numeric keys.
Press: **ENTER**

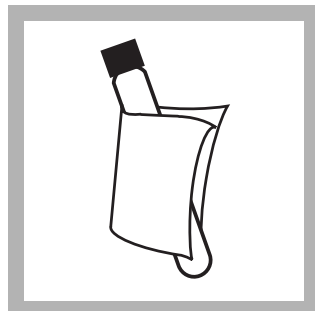


3. The display will show:
HACH PROGRAM: 2700 COD, ULR
The wavelength (λ), **350 nm**, is automatically selected.



4. Insert the Test ‘N Tube Adapter into the sample cell module by sliding it under the thumb screw and into the alignment grooves. Fasten with the thumb screw.

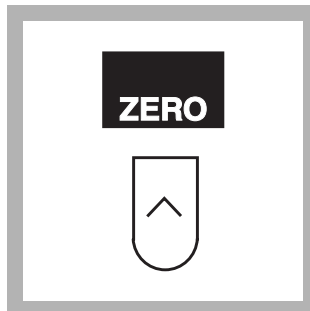
Note: The Test Tube Adapter is NOT designed to allow readings on hot vials (150 °C).



5. Clean the outside of the blank with a towel.
Note: Wiping with a damp towel, followed by a dry one, will remove fingerprints or other marks.



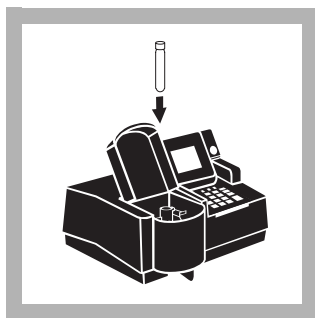
6. Place the blank into the adapter with the Hach logo facing the front of the instrument. Close the light shield.
Note: Preparation of the blank is described in the digestion procedure.
Note: The blank is stable when stored in the dark; see Blanks for Colorimetric Measurement following these procedures.



7. Press the soft key under **ZERO**.
The display will show:
0.0 mg/L COD
Note: For alternate concentration units press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



8. Clean the outside of the sample vial with a towel.



9. Place the sample vial into the adapter with the Hach logo facing the front of the instrument. Close the light shield. Results in mg/L COD (or chosen units) will be displayed.

Note: Results may be expressed as mg/L COD or mg/L O_2 . Press the soft keys under **OPTIONS** and then press **FORM** to scroll through the available choices.

Note: If the display shows **45 mg/L COD** and/or **OVER!**, the upper limit of the range has been exceeded. Repeat the test with a dilute sample or use a Low Range or High Range COD Reagent Vial.

Interferences

Chloride

Chloride is the primary interference when determining COD concentration. Each COD vial contains mercuric sulfate that will eliminate chloride interference up to the level specified in column 2. Samples with higher chloride concentrations should be diluted. Dilute the sample enough to reduce the chloride concentration to the level given in column 3.

Table 1 Interfering Substances and Suggested Treatments

Vial Type Used	Maximum Cl ⁻ concentration in sample (mg/L)	Suggested Cl ⁻ concentration of diluted sample (mg/L)	Maximum Cl ⁻ concentration in sample with 0.5 g HgSO ₄ Added (mg/L)
Ultra Low Range	2000	1000	NA
Low Range	2000	1000	8000
High Range	2000	1000	4000
Ultra High Range	20,000	10,000	40,000

If sample dilution will cause the COD concentration to be too low for accurate measurement, add 0.50 g of mercuric sulfate (HgSO₄) to each COD vial before the sample is added. The additional mercuric sulfate will raise the maximum chloride concentration allowable to the level given in column 4.

Bromide

Bromide interference will not be controlled by mercuric sulfate.

Sample Collection, Preservation and Storage

Collect samples in glass bottles. Use plastic bottles only if they are known to be free of organic contamination. Test biologically active samples as soon as possible. Homogenize samples containing solids to assure representative samples. Samples treated with sulfuric acid to a pH of less than 2 (about 2 mL per liter) and refrigerated at 4 °C can be stored up to 28 days. Correct results for volume additions; see Section 1.2.2 *Correcting for Volume Additions*.

Accuracy Check

Standard Solution Method

Check the accuracy of the 0 to 40 mg/L range with a 30 mg/L standard. Using Class A glassware, prepare a 1000-mg/L solution by diluting 850 mg of dried (120 °C, overnight) potassium acid phthalate (KHP) in 1000 mL of organic-free deionized water. Prepare a 30 mg/L dilution by diluting 3.00 mL of this solution into a 100.0 mL volumetric flask. Dilute to volume with deionized water, stopper, and invert 10 times to mix.

Method Performance

Precision

Standard: 30.0 mg/L COD

Program	95% Confidence Limits
2700	29.9–30.1 mg/L COD

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
2700	0.2 mg/L COD

For more information on derivation and use of Hach's estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, Appendix B, see Section 1.5.1.

Sensitivity

Program Number: 2700

Portion of Curve	Δ Abs	Δ Concentration
Entire Range	0.010	-0.52 mg/L

See Section 1.5.3 *Sensitivity Explained* for more information.

Preparing Organic-Free Water

Preparing organic-free water with no measurable COD:

1. Pour 1.0 liter of deionized water with low COD in a 2-liter erlenmeyer flask.
2. Add the contents of one Potassium Persulfate Powder Pillow to the flask. Swirl to dissolve.

3. Suspend a UV lamp in the flask so the glass portion of the bulb is immersed and the black bakelite portion is above the solution. Follow the safety and operation instructions recommended in the UV lamp kit. Safety UV goggles should be worn for eye protection.
4. Irradiate the solution with UV light for at least two hours (overnight is fine).
5. Remove the lamp from the solution. Add one level 0.05-gram scoop of Nickel Sulfate to the solution.
6. Heat the water to a boil. Remove the flask from the hot plate and cover it with a watch glass.
7. Let the flask cool to room temperature. The water will have zero oxygen demand. Seal the flask top with aluminum foil to prevent organic contamination. The water should stay free of oxygen demand for one week if properly sealed.

Blanks for Colorimetric Measurement

The blank may be used repeatedly for measurements using the same lot of vials. Store it in the dark. Monitor decomposition by measuring the absorbance at 350 nm. Zero the instrument in the absorbance mode, using a culture tube (see *OPTIONAL EQUIPMENT AND SUPPLIES*) containing 5 mL of deionized water. Measure the absorbance of the blank and record the value. Prepare a blank when the absorbance has changed by about 0.010 absorbance units.

Calibration Standard Preparation

To perform an ultra low range calibration using the reactor digestion method, prepare a 500-mg/L COD stock solution by pipetting 50.00 mL of a 1000-mg/L COD Standard Solution (Cat. No. 22539-29) into a 100-mL volumetric flask using Class A glassware. Dilute to the mark with organic-free deionized water and mix thoroughly.

Prepare calibration standards containing 5, 15, 25, 35 and 40 mg/L COD as follows:

- a. Into five different Class A 100-mL volumetric flasks, pipet 1.0, 3.0, 5.0, 7.0 and 8.0 mL of the 500-mg/L COD stock solution using Class A glassware.
- b. Dilute to the mark with deionized water. Stopper and invert 10 times to mix.
- c. Using the ultra low range reactor digestion method and the calibration procedure described in the *User-Entered Programs* section of the *DR/4000 Spectrophotometer Instrument Manual*, generate a calibration curve from the standards prepared above.

Summary of Method

The mg/L COD results are defined as the mg of O₂ consumed per liter of sample under conditions of this procedure. In this procedure, the sample is heated for two hours with a strong oxidizing agent, potassium dichromate. Oxidizable organic compounds react, reducing the dichromate ion (Cr₂O₇²⁻) to green chromic ion (Cr³⁺). When the 0–40 or 0–150 mg/L colorimetric or titrimetric method is used, the amount of Cr⁶⁺ remaining is determined. When the 0–1,500 mg/L or 0–15,000 mg/L

colorimetric method is used, the amount of Cr^{3+} produced is determined. The COD reagent also contains silver and mercury ions. Silver is a catalyst, and mercury is used to complex chloride interferences.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to Section 3.

Pollution Prevention and Waste Management

Final samples will contain mercury (D009), silver (D011), and chromium (D007) at concentration levels regulated as hazardous waste by the Federal RCRA. Please see Section 1 for further information on proper disposal of these materials.

REQUIRED REAGENTS AND STANDARDS

Description	Quantity Required per test	Unit	Cat. No.
Ultra Low Range COD Digestion Vials, 0 to 40 mg/L COD	1 to 2 vials.....	25/pkg	24158-25
Water, deionized	varies	4 liters.....	272-56

REQUIRED EQUIPMENT AND SUPPLIES

COD Reactor, 115/230 VAC, North American plug	1	each.....	45600-00
COD Reactor, 230 VAC, 50 Hz, European plug	1	each.....	45600-02
Description	per test	Unit	Cat. No.
DR/4000 Test Tube Adapter.....	1	each.....	48189-00
Pipet, volumetric, Class A, 2.00-mL	1	each.....	14515-36
Pipet Filler, safety bulb.....	1	each.....	14651-00
Test Tube Rack	1-2	each.....	18641-00

OPTIONAL REAGENTS AND STANDARDS

COD Digestion Reagent Vials, 0 to 40 mg/L COD	150/pkg	24158-15
COD Digestion Reagent Vials, 0 to 150 mg/L COD	150/pkg	21258-15
COD Digestion Reagent Vials, 0 to 1,500 mg/L COD	150/pkg	21259-15
COD Standard Solution, 300-mg/L.....	200 mL.....	12186-29
COD Standard Solution, 1000-mg/L.....	200 mL.....	22539-29
Mercuric Sulfate, ACS	28 g*	1915-20
Nickel Sulfate, ACS	25 g	11264-24
Potassium Acid Phthalate, ACS	500 g	315-34
Potassium Persulfate Powder Pillows	100/pkg	20847-69
Sulfuric Acid, ACS, concentrated	500 mL *	979-49

* Contact Hach for larger sizes.

OXYGEN DEMAND, Chemical, continued

OPTIONAL EQUIPMENT AND SUPPLIES

Description	Unit	Cat. No.
Beaker, 250 mL	each.....	500-46
Culture Tube, 16 x 100 mm, borosilicate glass	each.....	23800-00
Culture Tube Cap (for 23800-00).....	each.....	22411-00
Cylinder, graduated, 5-mL	each.....	508-37
Flask, Erlenmeyer, 2000-mL	each.....	505-54
Flask, volumetric, Class A, 100-mL	each.....	14574-42
Flask, volumetric, Class A, 1000-mL	each.....	14574-53
Goggle, safety.....	each.....	21134-00
Hot Plate, 120 VAC	each.....	23441-00
Hot Plate, 240 VAC	each.....	23441-02
pH Paper, pH 1.0 to 11.0	5 rolls/pkg.....	391-33
Pipet, serological, 5-mL	each.....	532-37
Pipet, volumetric, Class A, 1.00-mL	each.....	14515-35
Pipet, volumetric, Class A, 3.00-mL	each.....	14515-03
Pipet, volumetric, Class A, 5.00-mL	each.....	14515-37
Pipet, volumetric, Class A, 7.00-mL	each.....	14515-07
Pipet, volumetric, Class A, 8.00-mL	each.....	14515-08
Pipet, volumetric, Class A, 10.00-mL	each.....	14515-38
Pipet, volumetric, Class A, 50.00-mL	each.....	14515-41
Safety shield, for COD reactor	each.....	23810-00
Spoon, measuring, 0.5-g.....	each.....	907-00
UV Lamp Kit, 115 VAC	each.....	20828-00
UV Lamp Kit, 230 VAC	each.....	20828-02

RELATED LITERATURE—Ask for your copy by literature code number.

Title	Literature Code No.
COD Disposal Information Brochure	4144



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✓ Method 8000

Reactor Digestion Method*

(0 to 150.0 mg/L COD)

Scope and Application: For water, wastewater and seawater; digestion is required;

USEPA Approved** for wastewater analyses. The estimated detection limit for program number 2710 is 1.1 mg/L COD.

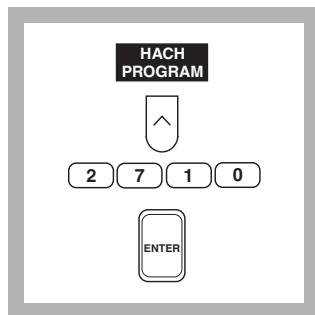
* Jirka, A.M.; Carter, M.J., *Analytical Chemistry*, 1975, 47(8), 1397

** *Federal Register*, April 21, 1980, 45(78), 26811–26812

Colorimetric Measurement



1. Perform the digestion for this method as described in “Oxygen Demand, Chemical, Digestion Procedure” which precedes the COD colorimetric procedures.



2. Press the soft key under **HACH PROGRAM**.

Select the stored program number for low range COD by pressing **2710** with the numeric keys.

Press: **ENTER**

Note: If samples cannot be digested immediately, see *Sample Collection, Preservation and Storage* following these steps.



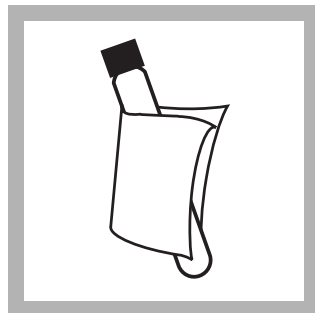
3. The display will show: **HACH PROGRAM: 2710 COD, LR**

The wavelength (λ), **420 nm**, is automatically selected.



4. Insert the Test ‘N’ Tube Adapter into the sample cell module by sliding it under the thumb screw and into the alignment grooves. Fasten with the thumb screw.

Note: The Test Tube Adapter is **NOT** designed to allow readings on hot vials (150 °C).



5. Clean the outside of the blank with a towel.

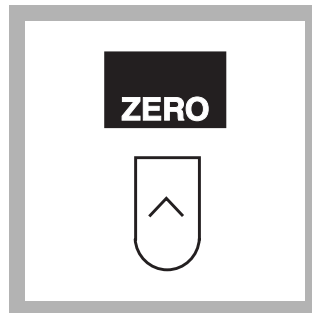
Note: Wiping with a damp towel, followed by a dry one, will remove fingerprints or other marks.



6. Place the blank into the adapter with the Hach logo facing the front of the instrument. Close the light shield.

Note: Preparation of the blank is described in the digestion procedure.

Note: The blank is stable when stored in the dark; see *Blanks For Colorimetric Measurement* following these procedures.



7. Press the soft key under **ZERO**.

The display will show:

0.0 mg/L COD

Note: For alternate concentration units press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



8. Clean the outside of the sample vial with a towel.



9. Place the sample vial into the adapter with the Hach logo facing the front of the instrument. Close the light shield. Results in mg/L COD (or chosen units) will be displayed.

Note: Results may be expressed as mg/L COD or mg/L O₂. Press the soft keys under **OPTIONS** and then **FORM**: to scroll through the available choices.

Note: For most accurate results with samples near 150 mg/L COD, repeat the analysis with a diluted sample. If the display shows **165.0 mg/L COD** and an **OVER!** warning, the working range has been exceeded. Repeat analysis with a diluted sample or the High Range reagent vials.

Interferences

Chloride

Chloride is the primary interference when determining COD concentration. Each COD vial contains mercuric sulfate that will eliminate chloride interference up to the level specified in column 2. Samples with higher chloride concentrations should be diluted. Dilute the sample enough to reduce the chloride concentration to the level given in column 3.

Table 1 Interfering Substances and Suggested Treatments

Vial Type Used	Maximum Cl ⁻ concentration in sample (mg/L)	Suggested Cl ⁻ concentration of diluted sample (mg/L)	Maximum Cl ⁻ concentration in sample with 0.5 g HgSO ₄ Added (mg/L)
Ultra Low Range	2000	1000	NA
Low Range	2000	1000	8000
High Range	2000	1000	4000
Ultra High Range	20,000	10,000	40,000

If sample dilution will cause the COD concentration to be too low for accurate measurement, add 0.50 g of Mercuric Sulfate (HgSO₄) to each COD vial before the sample is added. The additional mercuric sulfate will raise the maximum chloride concentration allowable to the level given in column 4.

Bromide

Bromide interference will not be controlled by Mercuric Sulfate.

Sample Collection, Preservation and Storage

Collect samples in glass bottles. Use plastic bottles only if they are known to be free of organic contamination. Test biologically active samples as soon as possible. Homogenize samples containing solids to assure representative samples. Samples treated with sulfuric acid to a pH of less than 2 (about 2 mL per liter) and refrigerated at 4 °C can be stored up to 28 days. Correct results for volume additions; see Section 1.2.2 *Correcting for Volume Additions*.

Accuracy Check

Standard Solution Method

Check the accuracy of the 0 to 150 mg/L range with a 100-mg/L KHP standard. Prepare by dissolving 85 mg of dried (120 °C, overnight) Potassium Acid Phthalate (KHP) in 1 liter of deionized water. Use 2 mL as the sample volume or, alternatively, dilute 10 mL of 1000-mg/L COD Standard Solution to 100 mL to produce a 100-mg/L standard.

To adjust the calibration curve using the reading obtained with the 100-mg/L standard solution, press the soft keys under **OPTIONS, MORE** then **STD: OFF**. Press **ENTER** to accept the displayed concentration, the value of which depends on the selected units. If an alternate concentration is used, enter the actual concentration and press **ENTER** to return to the read screen. See Section 1.5.5 *Adjusting the Standard Curve* for more information.

Method Performance

Precision

Standard: 100.0 mg/L COD

Program	95% Confidence Limits
2710	99.4–100.6 mg/L O ₂

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
2710	1.1 mg/L COD

For more information on derivation and use of Hach's estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, appendix B, see Section 1.5.1.

Sensitivity

Program Number: 2710

Portion of Curve	ΔAbs	ΔConcentration
Entire Range	0.010	-3.45 mg/L

See Section 1.5.3 *Sensitivity Explained* for more information.

Blanks For Colorimetric Measurement

The blank may be used repeatedly for measurements using the same lot of vials. Store it in the dark. Monitor decomposition by measuring the absorbance at 420 nm. Zero the instrument in the absorbance mode, using a culture tube (see *OPTIONAL EQUIPMENT AND SUPPLIES*) containing 5 mL of deionized water. Measure the absorbance of the blank and record the value. Prepare a blank when the absorbance has changed by about 0.010 absorbance units.

Calibration Standard Preparation

To perform a low range (0–150 mg/L) calibration using the reactor digestion method, use a 1000-mg/L COD Standard Solution (Cat. No. 22539-29).

Prepare calibration standards containing 20, 60, 100, 140 and 160 mg/L COD as follows:

- a. Into five different 100-mL volumetric flasks, pipet 2, 6, 10, 14 and 16 mL of the 1000-mg/L COD Standard Solution using Class A glassware.
- b. Dilute to the mark with deionized water. Stopper and invert 10 times to mix.
- c. Using the low range reactor digestion method and the calibration procedure described in the *User-Entered Programs* section of the *DR/4000 Spectrophotometer Instrument Manual*, generate a calibration curve from the standards prepared above.

Alternate Reagents—COD2 Reagent Vials

For non-reporting purposes, COD2 Reagent can provide a mercury-free testing option, eliminating mercury waste and saving on disposal costs.

COD2 Reagent Vials use the same COD procedures and the same COD calibration curves programmed into the DR/4000.

COD2 Reagent is not acceptable for USEPA reporting purposes. Request Literature Code 1356 for applications where COD 2 Reagent Vials may be suitable.

Summary of Method

The mg/L COD results are defined as the mg of O₂ consumed per liter of sample under conditions of this procedure. In this procedure, the sample is heated for two hours with a strong oxidizing agent, potassium dichromate. Oxidizable organic compounds react, reducing the dichromate ion (Cr₂O₇²⁻) to green chromic ion (Cr³⁺). When the 0–150 mg/L colorimetric is used, the amount of Cr⁶⁺ remaining is determined. When the 0–1,500 mg/L or 0–15,000 mg/L colorimetric method is used, the amount of Cr³⁺ produced is determined. The COD reagent also contains silver and mercury ions. Silver is a catalyst, and mercury is used to control chloride interferences.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to Section 1.

Pollution Prevention and Waste Management

Final samples will contain mercury (D009), silver (D011), and chromium (D007) at concentration levels regulated as hazardous waste by the Federal RCRA. Please see Section 1 for further information on proper disposal of these materials.

REQUIRED REAGENTS AND STANDARDS

Description	Quantity Required per test	Unit	Cat. No.
Low Range COD Digestion Vials, 0 to 150 mg/L COD	1 to 2 vials.....	25/pkg.....	21258-25
Water, deionized	varies.....	4 liters.....	272-56

REQUIRED EQUIPMENT AND SUPPLIES

COD Reactor, 115/230 VAC, North American plug	1	each.....	45600-00
COD Reactor, 230 VAC, 50 Hz, European plug	1	each.....	45600-02
Pipet, volumetric, Class A, 2.00-mL	1	each.....	14515-36
Pipet Filler, safety bulb.....	1	each.....	14651-00
Test Tube Rack	1 to 2 racks	each.....	18641-00
DR/4000 Test Tube Adapter.....	1	each.....	48189-00

OPTIONAL REAGENTS AND STANDARDS

Description	Unit	Cat. No.
COD Digestion Reagent Vials, 0 to 150 mg/L COD	150/pkg.....	21258-15
COD Digestion Reagent Vials, 0 to 1,500 mg/L COD	150/pkg.....	21259-15
COD Standard Solution, 300-mg/L.....	236 mL.....	12186-31
COD Standard Solution, 1000-mg/L.....	236 mL.....	22539-31
COD2 Reagent Vials, Low Range, 0–150 mg/L	25/pkg.....	25650-25
Mercuric Sulfate, ACS	28 g*.....	1915-20
Potassium Acid Phthalate, ACS	500 g.....	315-34
Sulfuric Acid, ACS, concentrated	500 mL*.....	979-49

OPTIONAL EQUIPMENT AND SUPPLIES

Beaker, 250-mL	each.....	500-46H
Cylinder, graduated, 5-mL	each.....	508-37
Culture Tube, 16 x 100 mm, borosilicate glass	6/pkg.....	22758-06
Culture Tube Cap (for 22758-06).....	6/pkg.....	22411-06
Flask, volumetric, Class A, 100-mL	each.....	14574-42
Flask, volumetric, Class A, 1000-mL	each.....	14574-53
pH Paper, pH 1.0 to 11.0	5 rolls/pkg.....	391-33
Pipet, serological, 5-mL	each.....	532-37
Pipet, volumetric, Class A, 2.00-mL	each.....	14515-36
Pipet, volumetric, Class A, 6.00-mL	each.....	14515-06
Pipet, volumetric, Class A, 7.00-mL	each.....	14515-07
Pipet, volumetric, Class A, 8.00-mL	each.....	14515-08
Safety shield, for COD reactor	each.....	50030-00
Spoon, measuring, 0.5-g.....	each.....	907-00

RELATED LITERATURE—Ask for your copy by literature code number.

Title	Literature Code No.
COD Disposal Information Brochure	4144
COD2 Reagent Vials Information Brochure	1356

* Contact Hach for larger sizes.



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HACH COMPANY
WORLD HEADQUARTERS
Telephone: (970) 669-3050
FAX: (970) 669-2932



✓ Method 8000

Reactor Digestion Method*

(0 to 1500 and 0 to 15,000 mg/L COD)

Scope and Application: For water, wastewater and seawater; digestion required; 0–1500 mg/L range is USEPA Approved** for wastewater analyses. The detection limit is 3 mg/L or 30 mg/L COD for the 0–1500 mg/L and the 0–15,000 mg/L range, respectively.

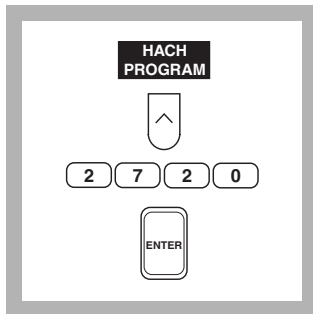
* Jirka, A.M.; Carter, M.J., *Analytical Chemistry*, 1975, 47(8), 1397

** *Federal Register*, April 21, 1980, 45(78), 26811-26812. The 0–15,000 mg/L range is NOT USEPA approved.

Colorimetric Measurement, 0 to 1,500 and 0 to 15,000 mg/L COD



1. Perform the digestion for this method as described in “Oxygen Demand, Chemical, Digestion Procedure” which precedes the COD colorimetric procedures.



2. Press the soft key under **HACH PROGRAM**.
Select the stored program number for high range and high range plus COD by pressing **2720** with the numeric keys.

Press: **ENTER**

Note: If samples cannot be analyzed immediately, see *Sample Collection, Preservation and Storage* following these steps.



3. The display will show:
**HACH PROGRAM:
2720
COD, HR, HR PLUS**
The wavelength (λ), **620 nm**, is automatically selected.



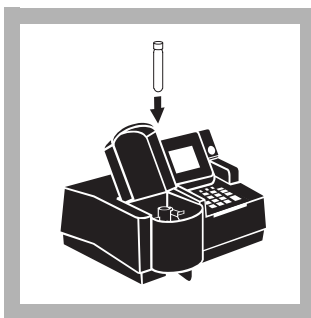
4. Insert the Test ‘N Tube Adapter into the sample cell module by sliding it under the thumb screw and into the alignment grooves. Fasten with the thumb screw.

Note: The Test Tube Adapter is NOT designed to allow readings on hot vials (150 °C).



5. Clean the outside of the blank with a towel.

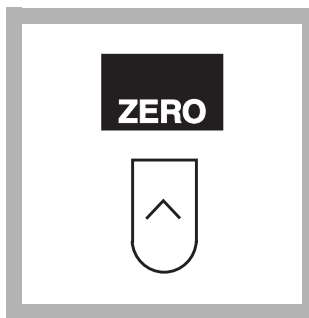
Note: Wiping with a damp towel, followed by a dry one, will remove fingerprints or other marks.



6. Place the blank into the adapter with the Hach logo facing the front of the instrument. Close the light shield.

Note: Preparation of the blank is described in the digestion procedure.

Note: The blank is stable when stored in the dark; see Blanks for Colorimetric Measurement following these procedures.



7. Press the soft key under **ZERO**.

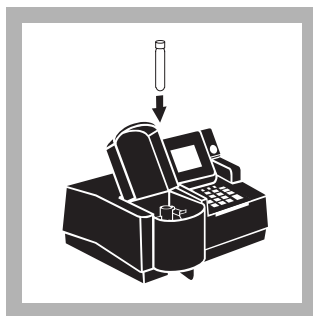
The display will show:

0 mg/L COD

Note: For alternate concentration units press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



8. Clean the outside of the sample vial with a towel.



9. Place the sample vial into the adapter with the Hach logo facing the front of the instrument. Close the light shield. Results in mg/L COD (or chosen units) will be displayed.

Note: When High Range Plus COD Digestion Reagent Vials are used, multiply the displayed value by ten.

Note: For most accurate results with samples near 1,500 or 15,000 mg/L COD, repeat the analysis with a diluted sample.

Note: Results may be expressed as mg/L COD or mg/L O₂. Press the soft keys under **OPTIONS** and then press **FORM**: to scroll through the available choices.

Interferences

Chloride

Chloride is the primary interference when determining COD concentration. Each COD vial contains mercuric sulfate that will eliminate chloride interference up to the level specified in column 2. Samples with higher chloride concentrations should be diluted. Dilute the sample enough to reduce the chloride concentration to the level given in column 3.

Table 1 Interfering Substances and Suggested Treatments

Vial Type Used	Maximum Cl ⁻ concentration in sample (mg/L)	Suggested Cl ⁻ concentration of diluted sample (mg/L)	Maximum Cl ⁻ concentration in sample with 0.5 g HgSO ₄ Added (mg/L)
Ultra Low Range	2000	1000	NA
Low Range	2000	1000	8000
High Range	2000	1000	4000
Ultra High Range	20,000	10,000	40,000

If sample dilution will cause the COD concentration to be too low for accurate measurement, add 0.50 g of mercuric sulfate (HgSO₄) to each COD vial before the sample is added. The additional mercuric sulfate will raise the maximum chloride concentration allowable to the level given in column 4.

Bromide

Bromide interference will not be controlled by mercuric sulfate.

Sample Collection, Preservation and Storage

Collect samples in glass bottles. Use plastic bottles only if they are known to be free of organic contamination. Test biologically active samples as soon as possible. Homogenize samples containing solids to assure representative samples. Samples treated with sulfuric acid to a pH of less than 2 (about 2 mL per liter) and refrigerated at 4 °C can be stored up to 28 days. Correct results for volume additions; see Section 1.2.2 *Correcting for Volume Additions*.

Accuracy Check

Standard Solution Method

0–1500 mg/L range: Check the accuracy of the 0 to 1,500 mg/L range by using either a 300-mg/L or 1000-mg/L COD Standard Solution. Use 2 mL of one of these solutions as the sample volume; the expected result will be 300 or 1000 mg/L COD respectively.

Or, prepare a 500-mg/L standard by dissolving 425 mg of dried (120 °C, overnight) KHP in 1000 mL of deionized water.

0–15,000 mg/L range: Check the accuracy of the 0 to 15,000 mg/L range by using a 10,000-mg/L COD standard solution. Prepare the 10,000-mg/L solution by dissolving 8.500 g of dried (120 °C, overnight) KHP in 1 liter of deionized water. Use 0.2 mL of this solution as the sample volume; the expected result will be 10,000 mg/L COD (display x 10).

To adjust the calibration curve using the reading obtained with 1000-mg/L COD Standard Solution, press the soft keys under **METHOD OPTIONS, (MORE)** then **STD:OFF**. Press **ENTER** to accept the value and return to the read screen. The instrument will only allow adjustment if the entered concentration is within 10% of the measured concentration. See Section 1.5.5 *Adjusting the Standard Curve* for more information.

Method Performance

Precision

0–1500 mg/L range

Standard: 1000 mg/L COD

Program	95% Confidence Limits
2720	998-1002 mg/L COD

0–15,000 mg/L range

Standard: 10,000 mg/L COD

Program	95% Confidence Limits
2720	9980-10,020 mg/L COD

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
2720 (0-1500 mg/L)	3 mg/L COD
2720 (0-15,000 mg/L)	30 mg/L COD

For more information on derivation and use of Hach's estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, appendix B, see Section 1.5.1.

Sensitivity

Program Number: 2720

Portion of Curve	Δ Abs	Δ Concentration
Entire Range	0.010	23.5 mg/L

See Section 1.5.3 *Sensitivity Explained* for more information.

Blanks for Colorimetric Measurement

The blank may be used repeatedly for measurements using the same lot of vials. Store it in the dark. Monitor decomposition by measuring the absorbance at 620 nm. Zero the instrument in the absorbance mode using a culture tube (see *OPTIONAL EQUIPMENT AND SUPPLIES*) containing 5 mL of deionized water. Measure the absorbance of the blank and record the value. Prepare a blank when the absorbance has changed by about 0.01 absorbance units.

Calibration Standard Preparation

High Range:

To perform a high range (0–1500 mg/L) calibration using the reactor digestion method, prepare a 10,000-mg/L COD stock solution by diluting 0.85 g of dried (120 °C, overnight) KHP to 100-mL with deionized water using Class A glassware. Mix thoroughly.

Prepare calibration standards containing 200, 600, 1000, 1400 and 1600 mg/L COD as follows:

- a. Into five different 100-mL volumetric flasks, pipet 2.0, 6.0, 10.0, 14.0, and 16.0 mL of the 10,000-mg/L COD stock solution using Class A glassware.
- b. Dilute to the mark with deionized water. Stopper and invert 10 times to mix.
- c. Using the COD Reactor Digestion Method and the calibration procedure described in the *User-Entered Programs* section of the *DR/4000 Spectrophotometer Instrument Manual*, generate a calibration curve from the standards prepared above.

High Range Plus:

To perform a super high range (0–15,000 mg/L) calibration using the reactor digestion method, prepare a 50,000-mg/L COD stock solution by diluting 8.5 g of dried (120 °C, overnight) KHP to 200-mL with deionized water using class A glassware. Mix thoroughly.

Prepare calibration standards containing 2000, 6000, 10000, and 14000 mg/L COD as follows:

- a. Into four different 100-mL volumetric flasks, pipet 4.0, 12.0, 20.0 and 28.0 mL of the 50,000-mg/L COD stock solution using Class A glassware.
- b. Dilute to the mark with deionized water. Mix thoroughly.
- c. Using the COD Reactor Digestion Method (with High Range Plus COD vials) and the calibration procedure described in the *User-Entered Programs* section of the *DR/4000 Spectrophotometer Instrument Manual*, generate a calibration curve from the standards prepared above.

Alternatively, use the High Range procedure and multiply the results by 10.

Alternate Reagents—COD2 Reagent Vials

For non-reporting purposes, COD2 Reagent can provide a mercury-free testing option, eliminating mercury waste and saving on disposal costs.

COD2 Reagent Vials use the same COD procedures and the same COD calibration curves programmed into the DR/4000.

COD2 Reagent is not acceptable for USEPA reporting purposes. Request Literature Code 1356 for applications where COD 2 Reagent Vials may be suitable.

Summary of Method

The mg/L COD results are defined as the mg of O₂ consumed per liter of sample under the conditions of this procedure. In this procedure, the sample is heated for two hours with a strong oxidizing agent, potassium dichromate. Oxidizable organic compounds react, reducing the dichromate ion (Cr₂O₇²⁻) to green chromic ion (Cr³⁺). When the 0-150 mg/L colorimetric or titrimetric method is used, the amount of Cr⁶⁺ remaining is determined. When the 0–1,500 mg/L or 0–15,000 mg/L colorimetric method is used, the amount of Cr³⁺ produced is determined. The COD reagent also contains silver and mercury ions. Silver is a catalyst, and mercury is used to complex chloride interferences.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to *SECTION 1 WASTE MANAGEMENT AND SAFETY*.

Pollution Prevention and Waste Management

Final samples will contain mercury (D009), silver (D011), and chromium (D007) at concentration levels regulated as hazardous waste by the Federal RCRA. Please see *SECTION 1 WASTE MANAGEMENT AND SAFETY* for further information on proper disposal of these materials.

REQUIRED REAGENTS AND STANDARDS

Description	Quantity Required per test	Unit	Cat. No.
Select the appropriate COD Digestion Reagent Vial:			
High Range, 0 to 1,500 mg/L COD	1 to 2 vials.....	25/pkg.....	21259-25
High Range Plus, 0 to 15,000 mg/L COD.....	1 to 2 vials.....	25/pkg.....	24159-25
Water, deionized.....	varies.....	4 liters.....	272-56

REQUIRED EQUIPMENT AND SUPPLIES

COD Reactor, 115/230 VAC, North American plug	1	each.....	45600-00
COD Reactor, 230 VAC, 50 Hz, European plug	1	each.....	45600-02
DR/4000 Test Tube Adapter.....	1	each.....	48189-00
Pipet, TenSette, 0.1 to 1.0 mL	1	each.....	19700-01
Pipet Tips, for 19700-01 TenSette Pipet	1	50/pkg*.....	21856-96
Pipet, volumetric, Class A, 2 mL	1	each.....	14515-36
Pipet Filler, safety bulb.....	1	each.....	14651-00
Test Tube Rack.....	1 to 2 racks	each.....	18641-00

OPTIONAL REAGENTS AND STANDARDS

COD Digestion Reagent Vials, 0 to 150 mg/L COD	150/pkg.....	21258-15
COD Digestion Reagent Vials, 0 to 1,500 mg/L COD	150/pkg.....	21259-15
COD Standard Solution, 300-mg/L.....	200 mL.....	12186-29
COD Standard Solution, 1000-mg/L.....	200 mL.....	22539-29
COD2 Reagent Vials, High Range, 0–1500 mg/L	25/pkg.....	25651-25
COD2 Reagent Vials, High Range, 0–1500 mg/L	150/pkg.....	25651-15
COD2 Reagent Vials, Ultra High Range, 0–15,000 mg/L	25/pkg.....	28343-25
Mercuric Sulfate, ACS	28 g*.....	1915-20
Potassium Acid Phthalate, ACS	500 g.....	315-34
Sulfuric Acid, ACS, concentrated	500 mL *.....	979-49

OPTIONAL EQUIPMENT AND SUPPLIES

Description	Unit	Cat. No.
Beaker, 250-mL.....	each.....	500-46H
Culture Tube, 16 x 100 mm.....	6/pkg.....	22758-06
Culture Tube Cap (for 22758-06).....	6/pkg.....	22411-06
Cylinder, graduated, 5-mL	each.....	508-37
Flask, volumetric, Class A, 100-mL	each.....	14574-42
Flask, volumetric, Class A, 250-mL	each.....	14574-46
Flask, volumetric, Class A, 1000-mL	each.....	14574-53
pH -Paper, pH 1.0 to 11.0.....	5 rolls/pkg.....	391-33
Pipet, serological, 5-mL	each.....	532-37
Pipet, volumetric, Class A, 2.00-mL	each.....	14515-04
Pipet, volumetric, Class A, 6.00-mL	each.....	14515-06
Pipet, volumetric, Class A, 8.00-mL	each.....	14515-08
Pipet, volumetric, Class A, 10.00-mL	each.....	14515-38
Pipet, volumetric, Class A, 20.00-mL	each.....	14515-20
Safety shield, for COD reactor	each.....	50030-00
Spoon, measuring, 0.5-g.....	each.....	907-00

RELATED LITERATURE—Ask for your copy by literature code number.

Title	Literature Code No.
COD Disposal Information Brochure	4144
COD2 Reagent Vials Information Brochure	1356

* Contact Hach for larger sizes.



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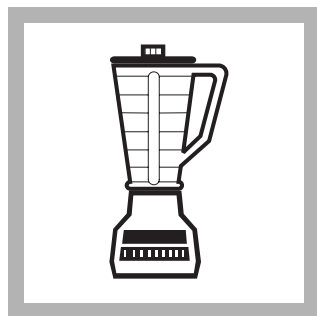


Scope and Application: For water, wastewater and seawater. USEPA Approved** for wastewater analyses

* Jirka, A.M.; Carter, M.J., *Analytical Chemistry*, 1975, 47(8), 1397

** *Federal Register*, April 21, 1980, 45(78), 26811–26812

Digestion



1. Homogenize 100 mL of sample for 30 seconds in a blender.

Note: 0 to 15,000 mg/L: Homogenize 100 mL of sample. Pour the homogenized sample into a 250-mL beaker and stir with a magnetic stirrer.

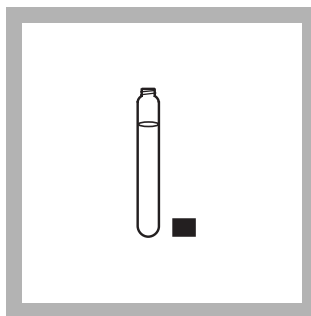
Note: Mix the sample prior to homogenization. To improve accuracy and reproducibility, pour the homogenized sample into a 250-mL beaker and gently stir with a magnetic stir plate. For samples containing large amounts of solids, increase the homogenization time.

Note: If samples cannot be analyzed immediately, see Sample Collection, Preservation and Storage following these procedures.



2. Turn on the COD Reactor. Preheat to 150 °C. Place the plastic shield in front of the reactor.

Note: Ensure safety devices are in place to protect analyst from splattering should reagent leaking occur.

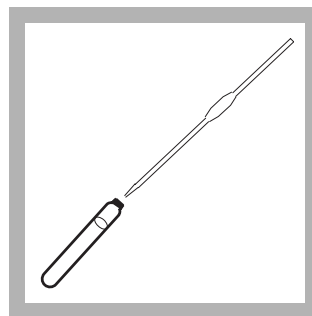


3. Remove the cap of a COD Digestion Reagent Vial for the appropriate range.

Sample Concentration Range (mg/L)	COD Digestion Reagent Vial Type
-----------------------------------	---------------------------------

0 to 40	Ultra Low Range
0 to 150	Low Range
0 to 1,500	High Range
0 to 15,000	Ultra High Range

Note: The reagent mixture is light-sensitive. Keep unused vials in the opaque shipping container, in a refrigerator if possible. The amount of light striking the vials during the test will not affect results.



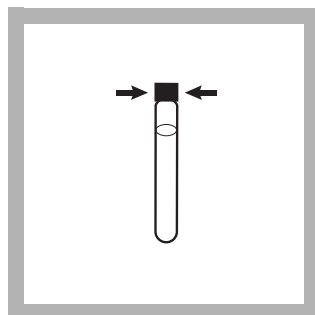
4. Hold the vial at a 45-degree angle. Pipet 2.00 mL (0.2 mL for the 0 to 15,000 mg/L range) of sample into the vial.

Note: Pipet only 0.20 mL of sample, not 2.00 mL, using a TenSette Pipet. For greater accuracy a minimum of three replicates should be analyzed and the results averaged.

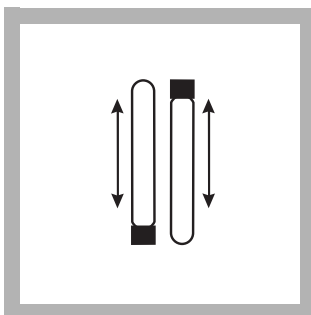
Note: Spilled reagent will affect test accuracy and is hazardous to skin and other materials. Do not run tests with vials which have been spilled. If some spills, wash with running water.

Note: For proof of accuracy, use COD standard solutions (preparation given in the Accuracy Check section of the individual colorimetric procedure) in place of the sample.

Caution: Some of the chemicals and apparatus used in this procedure may be hazardous to the health and safety of the user if inappropriately handled or accidentally misused. Please read all warnings and the safety section of this manual. Appropriate eye protection and clothing should be used for adequate user protection. If contact occurs, flush the affected area with running water. Follow instructions carefully.

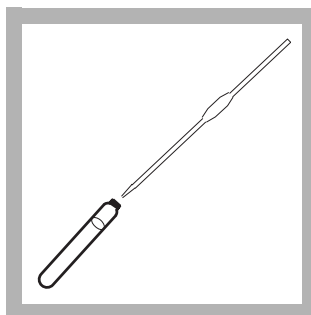


5. Replace the vial cap tightly. Rinse the COD vial with deionized water and wipe the vial clean with a paper towel.



6. Hold the vial by the cap and over a sink. Invert gently several times to mix the contents. Place the vial in the preheated COD Reactor.

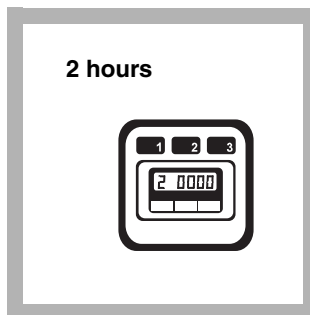
Note: The vial will become very hot during mixing.



7. Prepare a blank by repeating steps 3 to 6, substituting 2.00 mL (0.2 mL for the 0 to 15,000 mg/L range) deionized water for the sample. Place the blank in the COD Reactor.

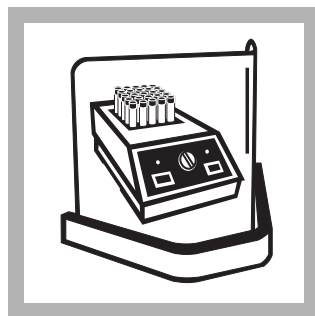
Note: Use a clean or well-rinsed pipet.

Note: One blank must be run with each set of samples. All tests (samples and blank) should be run with the same lot of vials. The lot number appears on the container label.

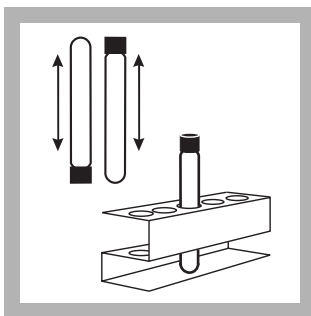


8. Heat the vials for 2 hours.

Note: Although many wastewater samples are digested completely in less than two hours, the DR/4000 Test Tube Adapter is NOT designed to measure hot vials (150 °C). If preliminary readings are desired, cool vial before reading.

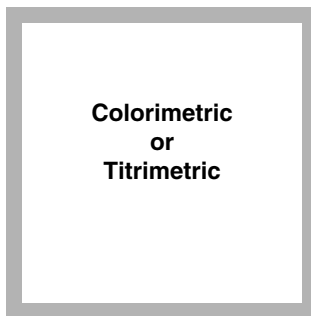


9. Turn the reactor off. Wait about 20 minutes for the vials to cool to 120 °C or less.



10. Invert each vial several times while still warm. Place the vials into a rack. Wait until the vials have cooled to room temperature.

Note: If a pure green color appears in the reacted sample, the reagent capacity may have been exceeded. Measure the COD and, if necessary, repeat the test with a diluted sample or alternate reagent vial.



11. Use one of the analytical techniques in *Method 8140, Iron Reduction Method for Oxygen Scavengers* to determine the sample concentration:

Colorimetric measurement,
0 to 40 mg/L COD

Colorimetric measurement,
0 to 150 mg/L COD

Colorimetric measurement,
0 to 1,500 mg/L COD and
0 to 15,000 mg/L COD

Note: A titrimetric procedure is also available. Contact Hach Customer Service for details.

Sample Collection, Preservation and Storage

Refer to the appropriate COD colorimetric measurement procedure.

Summary of Method

Refer to the appropriate COD colorimetric measurement procedure.

REQUIRED REAGENTS AND STANDARDS

Description	Quantity Required per test	Unit	Cat. No.
Select the appropriate COD Digestion Reagent Vial:			
Ultra Low Range, 0 to 40 mg/L	1 to 2 vials.....	25/pkg.....	24158-25
Low Range, 0 to 150 mg/L COD	1 to 2 vials.....	25/pkg.....	21258-25
High Range, 0 to 1,500 mg/L COD	1 to 2 vials.....	25/pkg.....	21259-25
High Range Plus, 0 to 15,000 mg/L COD.....	1 to 2 vials.....	25/pkg.....	24159-25
Water, deionized	varies.....	4 liters.....	272-56

REQUIRED EQUIPMENT AND SUPPLIES

Description	Quantity Required per test	Unit	Cat. No.
Blender, 2 speed, 120 VAC	1	each.....	26161-00
Blender, 2 speed, 240 VAC	1	each.....	26161-02
Cap Tool, COD.....	1	each.....	45587-00
COD Reactor, 115/230 VAC, North American Plug.....	1	each.....	45600-00
COD Reactor, 230 VAC, 50 Hz, European plug	1	each.....	45600-02
Pipet, TenSette, 0.1 to 1.0 mL	1	each.....	19700-01
Pipet, volumetric, Class A, 2-mL	1	each.....	14515-36
Pipet Filler, safety bulb.....	1	each.....	14651-00
Test Tube Rack	1 to 2 racks	each.....	18641-00

OPTIONAL REAGENTS AND STANDARDS

COD Digestion Reagent Vials, 0 to 40 mg/L COD	150/pkg.....	24158-15
COD Digestion Reagent Vials, 0 to 150 mg/L COD	150/pkg.....	21258-15
COD Digestion Reagent Vials, 0 to 1,500 mg/L COD	150/pkg.....	21259-15
COD Digestion Reagent Vials, 0 to 15,000 mg/L COD	150/pkg.....	24159-15
COD Standard Solution, 300-mg/L.....	200 mL.....	12186-29
COD Standard Solution, 1000-mg/L.....	200 mL.....	22539-29
Mercuric Sulfate, ACS	28 g*.....	1915-20
Potassium Acid Phthalate, ACS	500 g.....	315-34
Potassium Persulfate Powder Pillows	100/pkg.....	20847-69
Sulfuric Acid, ACS, concentrated	500 mL*.....	979-49

OPTIONAL EQUIPMENT AND SUPPLIES

Beaker, 250-mL	each.....	500-46
Cylinder, graduated, 5-mL	each.....	508-37
pH Indicator Paper, 1 to 11 pH.....	5 rolls/pkg.....	391-33
Pipet, serological, 5-mL	each.....	532-37
Pipet Tips, for 19700-01 TenSette Pipet	50/pkg*.....	21856-96
Pipet, volumetric, Class A, 10-mL	each.....	14515-38
Safety shield, for COD reactor	each.....	23810-00
Spoon, measuring, 0.5-g.....	each.....	907-00

* Contact Hach for larger sizes.



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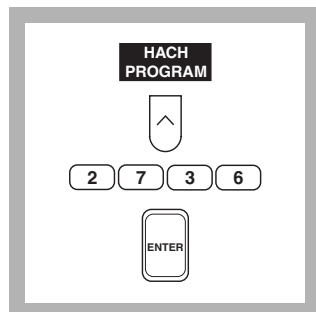
Method 8140

Iron Reduction Method for Oxygen Scavengers*

0-600 µg/L carbohydrazide;
0-500 µg/L DEHA; 0-1000 µg/L hydroquinone;
0-1500 µg/L iso-ascorbic acid;
0-1000 µg/L methylethyl ketoxime (MEKO)

Scope and Application: For testing residual corrosion inhibitors (oxygen scavengers) in boiler feed water or condensate.

* Adapted from Ishii and Koh, *Bunseki Kagaku*, 28 473 (1979)



1. Press the soft key under **HACH PROGRAM**.

Select the stored program number for the desired oxygen scavenger using the numeric keys.

Oxygen Scavenger	Program Number
Carbohydrazide	2736
DEHA	2738
Hydroquinone	2740
Iso-Ascorbic Acid	2742

Press: **ENTER**

Note: Samples must be analyzed immediately and cannot be stored for later analysis.

Note: The Flow Cell and Sipper Modules can be used for this procedure.



2. The display will show:
**HACH PROGRAM: 2736
O Scav-Carbohy.**

**HACH PROGRAM: 2736
O Scav-Carbohy.**

OR
**HACH PROGRAM: 2738
O Scav-DEHA**

**HACH PROGRAM: 2738
O Scav-DEHA**

OR
**HACH PROGRAM: 2740
O Scav-Hydroquin**

**HACH PROGRAM: 2740
O Scav-Hydroquin.**

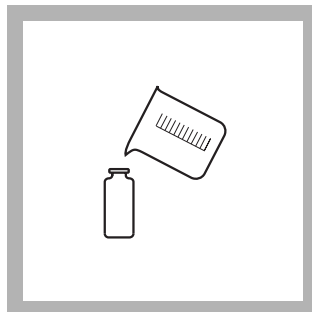
OR
**HACH PROGRAM: 2742
O Scav-Iso-As.**

**HACH PROGRAM: 2742
O Scav-Iso-As.**

OR
**HACH PROGRAM: 2744
O Scav-MEKO**

**HACH PROGRAM: 2744
O Scav-MEKO**

The wavelength (λ),
562 nm, is automatically
selected.

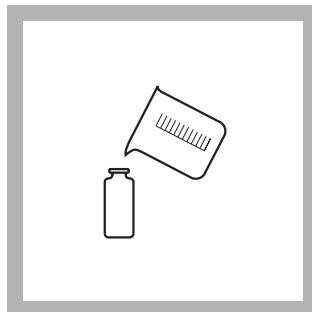


3. Fill a sample cell (the prepared sample) with 25 mL of sample.

Note: Soak glassware with 1:1 Hydrochloric Acid Solution. Rinse several times with deionized water. These two steps will remove iron deposits which can cause slightly high results.

Note: The sample temperature should be 25 ± 3 °C (77 ± 5 °F).

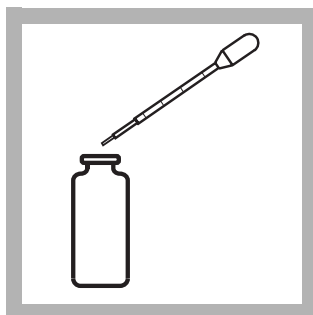
Note: When determining those oxygen scavengers which react quickly with oxygen at room temperature, it may be necessary to stopper the cell containing the prepared sample in steps 3-8 to exclude air.



4. Fill a second sample cell (the blank) with 25 mL of deionized water.

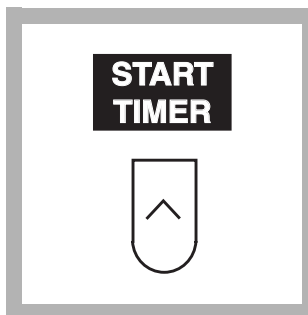


5. Add the contents of one DEHA Reagent 1 Powder Pillow to each sample cell. Swirl to mix.



6. Add exactly 0.5 mL of DEHA Reagent 2 Solution to each sample cell. Mix. Place both sample cells in the dark.

Note: A purple color will develop if an oxygen scavenger is present.

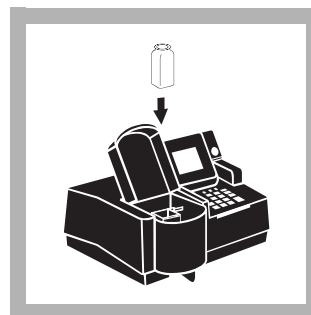


7. Immediately press the soft key under **START TIMER**. A 10-minute reaction period (or a 2-minute period for hydroquinone) will begin.

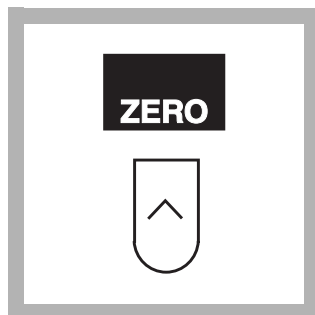
Note: Both sample cells must remain in the dark during the color reaction period.

Note: Temperature and reaction time affect the results. Be sure these factors are controlled as described.

Note: Read the MEKO result at exactly 10 minutes for most accurate results.



8. When the timer beeps, place the blank into the cell holder. Close the light shield.



9. Press the soft key under **ZERO**.

The display will show:

0 µg/L Carbo.

or

0 µg/L DEHA

or

0 µg/L Hydro.

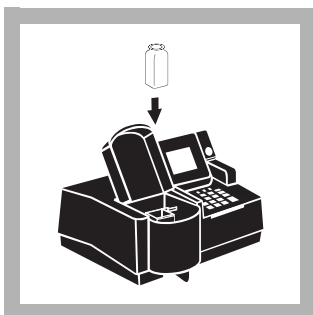
or

0 µg/L ISA

or

0 µg/L MEKO

Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



10. Immediately place the prepared sample into the cell holder. Close the light shield. Results in µg/L (or chosen units) will be displayed.

Note: Repeat the above procedure, omitting the addition of DEHA Reagent 2 (Step 6), to determine the ferrous iron concentration in the sample. Correct for the ferrous iron concentration by pressing the soft keys under **OPTION, (MORE)** and then **BLANK:OFF**. Enter the reading attributed to the ferrous iron concentration and press **ENTER**. The net result is the actual oxygen scavenger concentration.

Interferences

Interfering Substance	Interference Level and Treatment
Borate (as Na ₂ B ₄ O ₇)	Greater than 500 mg/L
Cobalt	Greater than 0.025 mg/L
Copper	Greater than 8.0 mg/L
Ferrous Iron	All levels
Hardness (as CaCO ₃)	Greater than 1000 mg/L
Light	Light may interfere. Keep sample cells in the dark during color development.
Lignosulfonates	Greater than 0.05 mg/L
Manganese	Greater than 0.8 mg/L
Molybdenum	Greater than 80 mg/L
Nickel	Greater than 0.8 mg/L
Phosphate	Greater than 10 mg/L
Phosphonates	Greater than 10 mg/L
Sulfate	Greater than 1000 mg/L
Temperature	Sample temperatures below 22 °C or above 28 °C (72 °F or 82 °F) may affect test accuracy.
Zinc	Greater than 50 mg/L

Substances which reduce ferric iron will interfere. Substances which complex iron strongly may also interfere.

Sample Collection, Preservation and Storage

Collect samples in clean, dry plastic or glass containers. Avoid excessive agitation or exposure to sunlight when sampling. Rinse the container several times with the sample. Allow the container to overflow and cap the container so there is no headspace above the sample. Rinse the sample cell several times with sample, then carefully fill to the 25-mL mark. Perform the analysis immediately.

Method Performance

Precision

Program	Standard Level	Confidence Limits
2736	300 µg/L	298–302 µg/L
2738	250 µg/L	248–252 µg/L
2740	500 µg/L	498–502 µg/L
2742	750 µg/L	743–757 µg/L
2744	500 µg/L	488–511 µg/L

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
2736	7 µg/L
2738	5 µg/L
2740	4 µg/L
2742	18 µg/L
2744	27 µg/L

For more information on derivation and use of Hach's estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, appendix B, see Section 1.5.1.

Sensitivity

Program Number: 2736

Portion of Curve	ΔAbs	ΔConcentration
Entire Range	0.010	4.2 µg/L

Program Number: 2738

Portion of Curve	ΔAbs	ΔConcentration
Entire Range	0.010	3.2 µg/L

Program Number: 2740

Portion of Curve	ΔAbs	ΔConcentration
Entire Range	0.010	8.4 µg/L

Program Number: 2742

Portion of Curve	Δ Abs	Δ Concentration
Entire Range	0.010	12.5 μ g/L

Program Number: 2744

Portion of Curve	Δ Abs	Δ Concentration
0.010 Abs	0.010	14.4 μ g/L
500 μ g/L	0.010	13.7 μ g/L
900 μ g/L	0.010	13.2 μ g/L

See Section 1.5.3 *Sensitivity Explained* for more information.

Summary of Method

Diethylhydroxylamine (DEHA) or other oxygen scavengers present in the sample react with ferric iron in DEHA Reagent 2 Solution to produce ferrous ion in an amount equivalent to the DEHA concentration. This solution then reacts with DEHA 1 Reagent, which forms a purple color with ferrous iron.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to Section 1.

Pollution Prevention and Waste Management

For information on pollution prevention and waste management, refer to Section 1.

OXYGEN SCAVENGERS, continued

REQUIRED REAGENTS AND STANDARDS

Description	Quantity Required per test	Unit	Cat. No.
Oxygen Scavenger Reagent Set (50 tests).....			24466-00
Includes: (2) 21679-69, (1) 21860-42			
DEHA Reagent 1 Powder Pillows.....	2 pillows	100/pkg	21679-69
DEHA Reagent 2 Solution	1 mL	500 mL	21680-49
Water, deionized	25 mL	4 liters	272-56

REQUIRED EQUIPMENT AND SUPPLIES

Dropper, 0.5 and 1.0-mL marks	1	20/pkg	21247-20
DR/4000 1-Inch Cell Adapter	1	each	48190-00

OPTIONAL REAGENTS AND STANDARDS

Hydrochloric Acid, 1:1 (6.0 N)	500 mL		884-49
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OPTIONAL EQUIPMENT AND SUPPLIES

Cylinder, graduated, polypropylene, 25-mL	each		1081-40
DR/4000 Carousel Module Kit	each		48070-02
DR/4000 Flow Cell Module Kit, 1-inch.....	each		48070-04
DR/4000 Flow Cell Module Kit, 1-cm.....	each		48070-05
DR/4000 Sipper Module Kit, 1-inch	each		48090-03
Stopper, hollow, poly, No. 1	6/pkg		14480-00
Thermometer, -10 to 110 °C.....	each		1877-01



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Method 8311

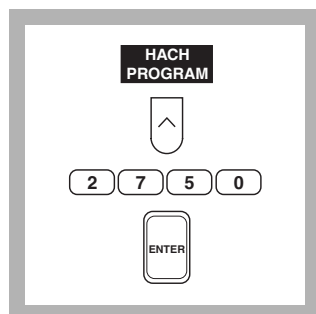
Indigo Method

AccuVac® Ampuls

(0 to 0.25 mg/L, 0 to 0.75 mg/L or 0 to 1.50 mg/L O₃)

Scope and Application: For water.

The estimated detection limit for program numbers 2750, 2760 and 2770 is 0.01 mg/L O₃.



- 1.** Press the soft key under **HACH PROGRAM**.

Select the stored program number of the correct range of ozone (O₃) by pressing the numeric keys:

Range Number	Program
Low range	2750
Mid range	2760
High range	2770

Press: **ENTER**

Note: Samples must be analyzed immediately and cannot be preserved for later analysis.



- 2.** The display will show:
**HACH PROGRAM: 2750
Ozone, LR AV**

or

**HACH PROGRAM: 2760
Ozone, MR AV**

or

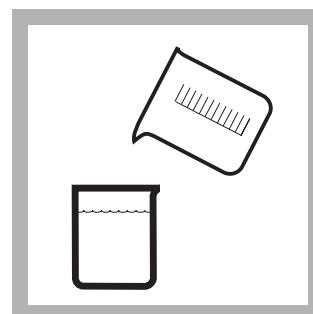
**HACH PROGRAM: 2770
Ozone, HR AV**

**HACH PROGRAM: 2770
Ozone, HR AV**

The wavelength (λ),
600 nm, is automatically selected.

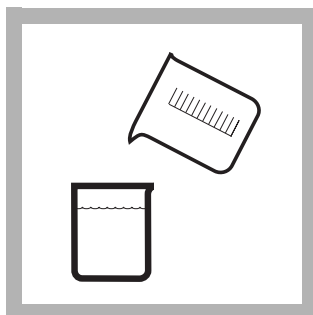


- 3.** Insert the AccuVac Ampul Adapter into the sample cell module by sliding it under the thumb screw and into the alignment grooves. Fasten with the thumb screw.



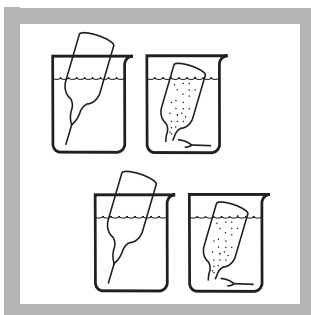
- 4.** Gently collect at least 40 mL of sample in a 50-mL beaker.

Note: See *Sample Collection, Storage and Preservation for proper collection*.



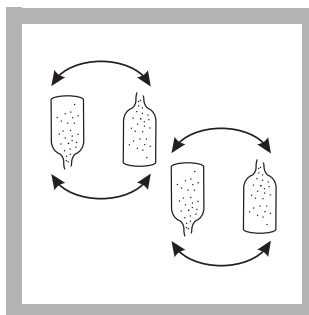
5. Collect at least 40 mL of ozone-free water (blank) in another 50-mL beaker.

Note: Ozone-free water used for the blank may be deionized water or tap water.



6. Fill one Indigo Ozone Reagent AccuVac Ampul with the sample and one Ampul with the blank.

Note: Keep the tip immersed while the ampul fills.

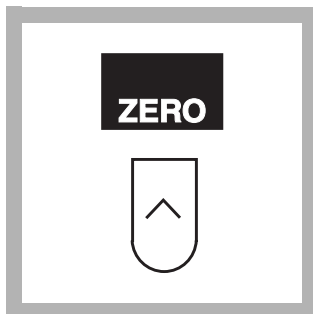


7. Quickly invert both ampuls several times to mix. Wipe off any liquid or fingerprints with a Kimwipe.

Note: Part of the blue color will be bleached if ozone is present.



8. Place the sample AccuVac Ampul into the cell holder. Close the light shield.



9. Press the soft key under **ZERO**.

The display will show:

0.00 mg/L O₃

Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



10. Place the AccuVac Ampul containing the blank into the cell holder. Close the light shield. Results in mg/L ozone (O₃) (or chosen units) will be displayed.

Note: The sequence of measuring blank and sample is reversed in this procedure.

Sample Collection, Storage and Preservation

The chief consideration when collecting a sample is to prevent the escape of ozone from the sample. The sample should be collected gently and analyzed immediately. Warming the sample, or disturbing the sample by stirring or shaking, will result in ozone loss. After collecting the sample, do not transfer it from one container to another unless absolutely necessary.

Stability of Indigo Reagent

Because indigo is light-sensitive, the AccuVac Ampuls should be kept in the dark at all times. The indigo solution, however, decomposes slowly under room light after filling with sample. The blank ampul can be used for multiple measurements during the same day.

Method Performance

Precision

Standard: 0.15 mg/L for program number 2750
 0.45 mg/L for program number 2760
 1.00 mg/L for program number 2770

Program	95% Confidence Limits
2750	0.14–0.16 mg/L O ₃
2760	0.44–0.46 mg/L O ₃
2770	0.99–1.01 mg/L O ₃

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
2750	0.01 mg/L O ₃
2760	0.01 mg/L O ₃
2770	0.01 mg/L O ₃

For more information on derivation and use of Hach's estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, appendix B, see Section 1.5.1.

Sensitivity

Program Number: 2750

Portion of Curve	Δ Abs	Δ Concentration
Entire Range	0.010	0.011 mg/L

Program Number: 2760

Portion of Curve	Δ Abs	Δ Concentration
Entire Range	0.010	0.011 mg/L

Program Number: 2770

Portion of Curve	Δ Abs	Δ Concentration
Entire Range	0.010	0.011 mg/L

See Section 1.5.3 *Sensitivity Explained* for more information.

Summary of Method

The reagent formulation adjusts the sample pH to 2.5 after the ampul has filled. The indigo reagent reacts immediately and quantitatively with ozone. The blue color of indigo is bleached in proportion to the amount of ozone present in the sample. Other reagents in the formulation prevent chlorine interference. No transfer of sample is needed in the procedure. Therefore, ozone loss due to sampling is eliminated.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to Section 1.

Pollution Prevention and Waste Management

For information on pollution prevention and waste management, refer to Section 1.

REQUIRED REAGENTS AND STANDARDS

Description	Quantity Required		Unit	Cat. No.
	Per Test			
Select one or more Ozone AccuVac Ampuls based on range:				
0–0.25 mg/L.....	2 ampuls	25/pkg	25160-25	
0–0.75 mg/L.....	2 ampuls	25/pkg	25170-25	
0–1.50 mg/L.....	2 ampuls	25/pkg	25180-25	

REQUIRED EQUIPMENT AND SUPPLIES

DR/4000 AccuVac Ampul Adapter.....	1	each.....	48187-00
Beaker, 50-mL.....	2	each.....	500-41

OPTIONAL EQUIPMENT AND SUPPLIES

AccuVac Snapper	each.....	24052-00
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Scope and Application: For soil and water

* Test is semi-quantitative. Results are expressed as greater or less than the threshold value used.

This method analyzes for PCB that has been extracted from soil samples. Sample extracts, calibrators, and reagents are added to cuvettes coated with PCB-specific antibodies. The color that develops is then measured and compared with the color measurements of the calibrators. The test requires about 20 minutes for complete analysis. As many as 10 cuvettes can be run simultaneously.

Tips and Techniques

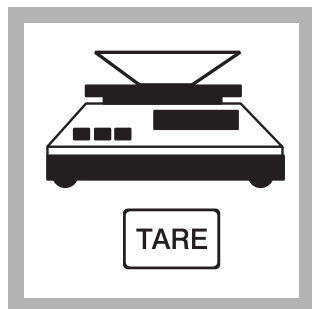
- **Read the entire procedure before starting.** Identify and have ready all the necessary reagents, cuvettes, and other apparatus before beginning the analysis.
- **Timing is critical;** follow instructions carefully.
- **A consistent technique when mixing the cuvettes is critical to this test.** The best results come from using the cuvette rack and mixing as described in *Using the 1-cm MicroCuvette Rack*. Cuvettes can be mixed individually, but test results may not be as consistent.
- Handle the cuvettes carefully. Scratches on the inside or outside may cause erroneous results. Carefully clean the outside of the cuvettes with a clean absorbent cloth or tissue before placing them into the instrument. (Kimwipe® tissues are provided with the kit.)
- Antibody cuvettes and enzyme conjugate are made in matched lots. Do not mix reagent lots.
- Twenty Antibody Cuvettes are provided with each reagent set. One Antibody Cuvette will be used for each calibrator or sample. Antibody Cuvettes are not reusable.
- To avoid damaging the Color Developing Solution, do not expose it to direct sunlight.
- There are two protocols in this procedure, one for levels of 1 ppm and 5 ppm, and another for 10 ppm and 50 ppm. Each uses a different quantity of calibrator and sample extract as follows:

Range (as Arochlor 1248)	Volume of calibrator and sample extract used
1 ppm and 5 ppm	50 µL
10 ppm and 50 ppm	10 µL

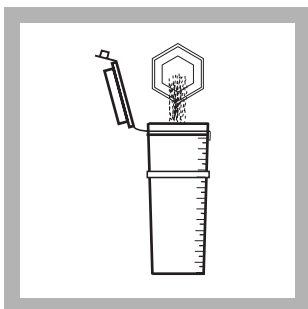
- To test across ranges, such as 1 and 50 ppm, test the lower concentration first. If the result is positive then test at the higher level. If the result of the test at the lower concentration is negative, the higher range test will be negative also, and need not be performed.
- The same filtered extract can be used for both protocols if it is tightly capped between assays. The maximum time between assays cannot exceed one-half hour.
- Store the reagents at 4 °C when they are not in use. Allow the reagents to reach room temperature before using them in an analysis. Actual testing may be done at temperatures ranging from 1 °C to 38 °C.
- The Soil Extractant contains methyl alcohol which is poisonous and flammable. Before using this and other reagents, read the Material Safety Data Sheet (MSDS) for proper use of protective equipment and other safety information.

Note: Hach Company recommends wearing protective nitrile gloves for this procedure.

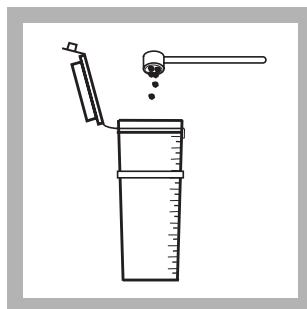
Soil Extraction Procedure



1. Weigh out 5 g of soil in the plastic weighing boat.



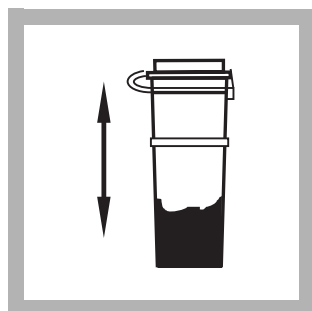
2. Carefully pour the soil into an extraction vial.



3. Use the 5-gram scoop to add one scoop of sodium sulfate to the extraction vial.



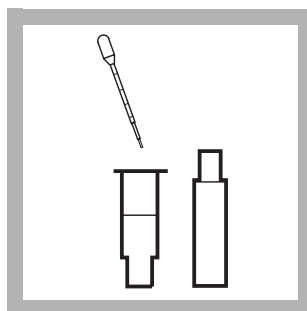
4. Use the graduated cylinder to transfer 10 mL of Soil Extractant into the extraction vial.



5. Cap the extraction vial tightly and shake vigorously for one minute.



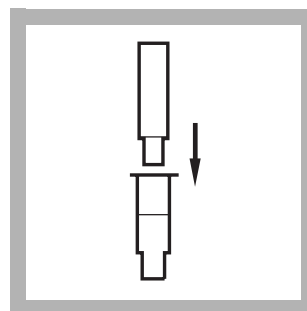
6. Allow to settle for at least one minute. Carefully open the extraction vial.



7. Using the disposable bulb pipet, withdraw 1.0–1.5 mL from the liquid layer at the top of the extraction vial.

Transfer it into the filtration barrel (the bottom part of the filtering assembly into which the plunger inserts).

Note: Do not use more than 1.5 mL. The bulb is marked in 0.25-mL increments

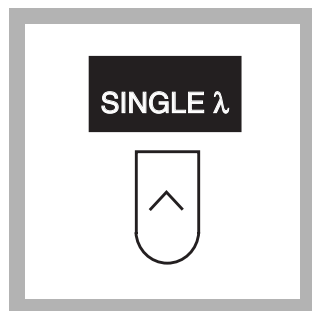


8. Insert the filtration plunger into the filtration barrel. Press firmly on the plunger until the sample extract is forced upward into the center of the plunger.

Use the resultant filtrate for the immunoassay in the *Immunoassay Procedure for Soil Extracts*

Note: It may be necessary to place the filtration assembly on a table and press down on the plunger.

Immunoassay Procedure for Soil Extracts



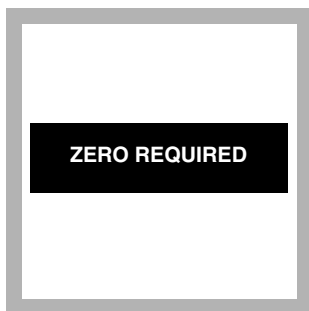
1. Press the soft key under **SINGLE λ**.

Press the soft key under **GO TO λ**.

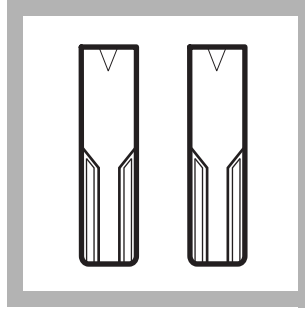
Select **450 nm** by pressing the numeric keys **4 5 0**.

Press: **ENTER**

Note: The Flow Cell and Sipper Modules cannot be used with this procedure.

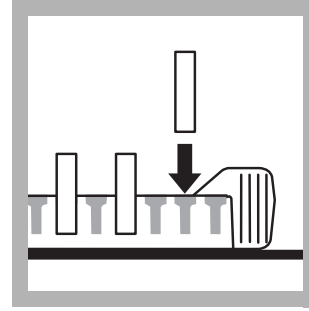


2. The display will show:
ZERO REQUIRED

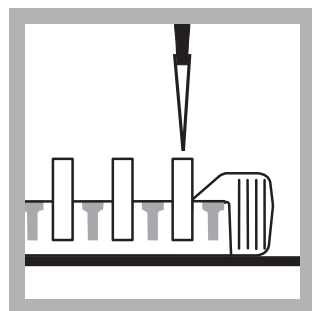


3. Label an Antibody Cuvette for each calibrator and each sample to be tested.

Note: As many as 10 cuvettes may be tested at one time and may comprise any combination of samples and calibrators.



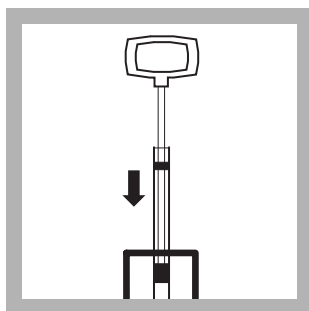
4. Place the cuvettes into the rack snugly.



5. Pipet 0.5 mL of Diluent Solution into each cuvette.

Note: The same pipette tip can be used repeatedly for this step.

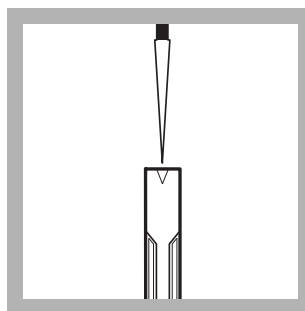
Note: Have the necessary apparatus at hand for the next four steps as they must be done without delay.



6. Use a Wiretrol® pipet to transfer the appropriate volume of calibrator or sample extract into each cuvette.

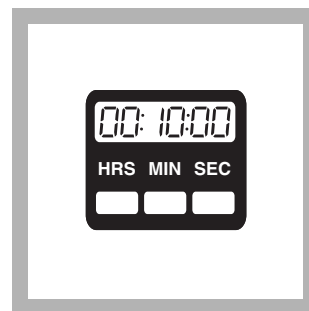
Note: When testing at the 1 ppm and/or 5 ppm levels, use 50 µL of calibrator and sample extract. When testing at the 10 ppm and/or 50 ppm levels, use 10 µL of calibrator and sample extract.

Note: Use a separate capillary tube for each solution.



7. Immediately pipet 0.5 mL of PCB Enzyme Conjugate into each calibrator and sample cuvette.

Note: The same pipette tip can be used repeatedly for this step.

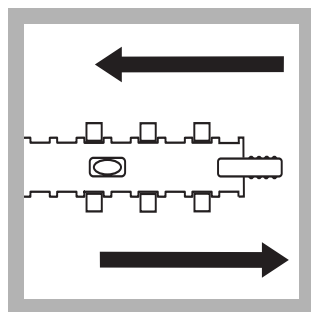


8. Key **1000** to bring up a 10-minute timer.

Press **START TIMER**.

A 10-minute reaction time will begin. Proceed immediately to the next step.

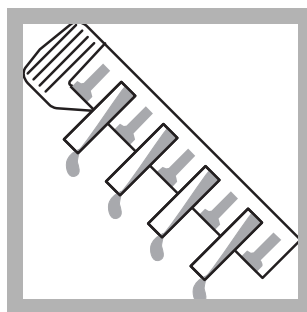
PCB (Polychlorinated Biphenyls), continued



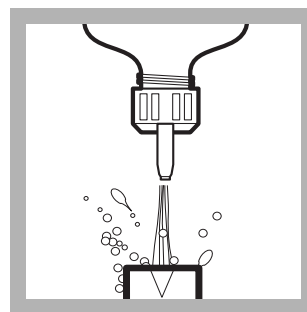
9. Mix the contents of the cuvettes for 30 seconds using the technique described in *Using the 1-cm MicroCuvette Rack*.



10. After 5 minutes, mix the contents of the rack a second time for a period of 30 seconds using the same technique.



11. At the end of the 10-minute period, discard the contents of all the cuvettes into an appropriate waste container.

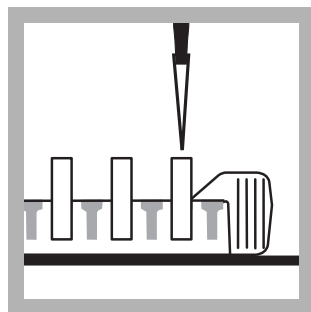


12. Wash each cuvette forcefully and thoroughly four times with deionized water. Empty the rinse water into the waste container.

Note: Ensure most of the water is drained from the cuvettes by turning the cuvettes upside down and tapping them lightly on a paper towel

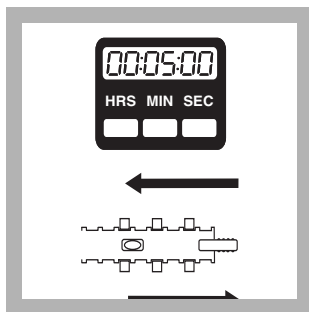
Color Development

Note: Timing is critical; follow instructions carefully



13. With the cuvettes still held snugly in the rack, pipet 0.5 mL of Color Developing Solution into each Antibody Cuvette.

Note: Use a new pipette tip for each cuvette.

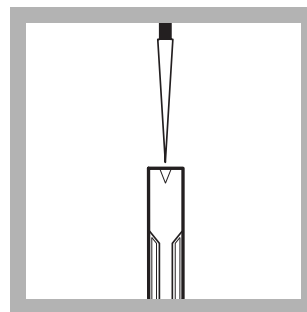


14. Key 500. Press the soft key under **START TIMER**.

A 5-minute reaction period will begin. Mix following the instructions in *Using the 1-cm MicroCuvette Rack*.



15. After 2.5 minutes, mix the contents of the rack a second time for a period of 30 seconds using the same technique.



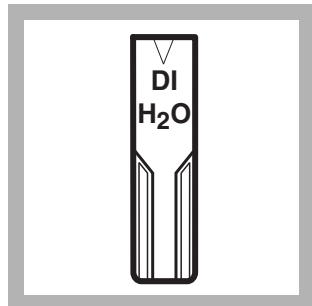
16. At the end of the 5-minute reaction period, pipette 0.5 mL of Stop Solution into each cuvette in the same order as the Color Developing Solution was added in step 13.

Slide the rack for 20 seconds using the technique described in *Using the 1-cm MicroCuvette Rack*.

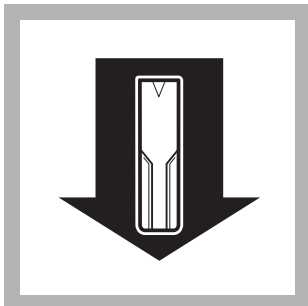
Note: Blue solutions will turn yellow with the addition of the Stop Solution.

Note: The same pipette tip can be used repeatedly for this step.

Measuring the Color

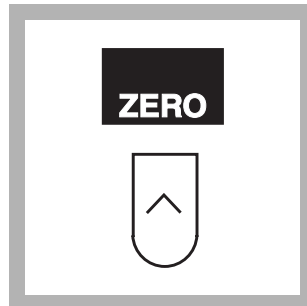


17. Label and fill a Zeroing Cuvette with deionized water. Wipe the outside of all the cuvettes with a tissue to remove water, smudges, and fingerprints.



18. Place the filled zeroing cuvette into the cell holder with the arrow pointing left.

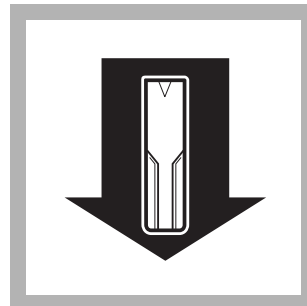
Orient the arrow in the same direction for all cuvettes.



19. Press the soft key under **ZERO**.

The display will show:

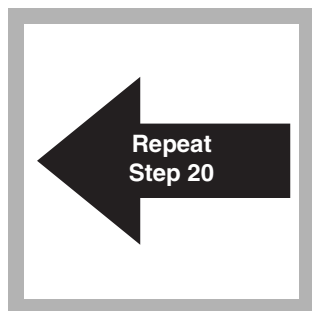
0.000 ABS



20. Place the prepared sample into the cell holder. Read the results.

The display will give an absorbance reading. Record the results for each calibrator and sample.

Note: See the *Instrument Manual* for more information on taking a reading.



21. Repeat *step 20* for all remaining calibrators and samples.

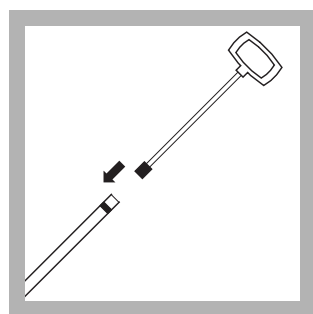
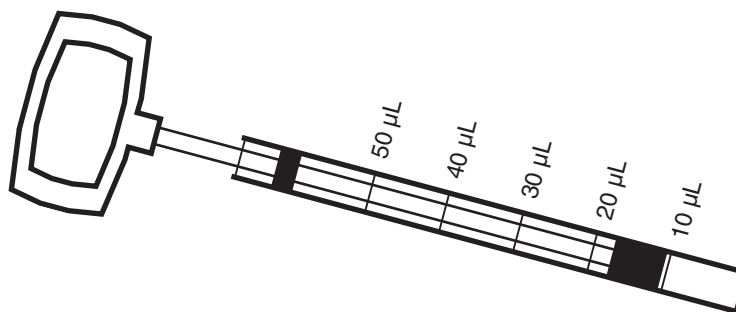
See *Interpreting and Reporting Results* for help with interpretation of results.

Using the Wiretrol® Pipet

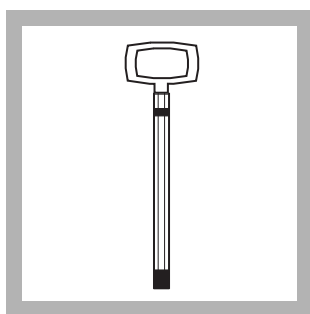
The Wiretrol Pipet can accurately measure small quantities of liquids. It consists of two parts: a Teflon®-tipped plunger and a calibrated capillary tube. Use *Figure 1* to determine the quantity measured at each line on the capillary tube.

The plunger can be re-used; the capillary tubes must be discarded after one use.

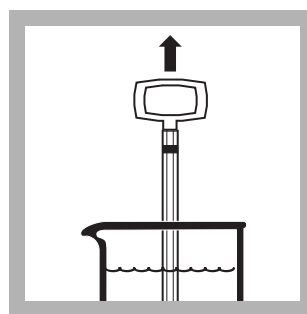
Figure 1 Wiretrol Pipet



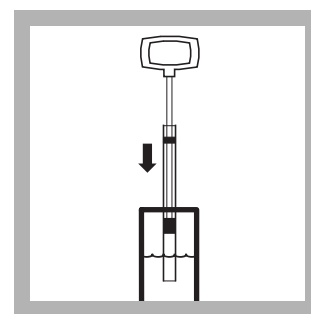
1. Wet the orange Teflon® tip of the Wiretrol plunger in the sample and carefully insert it into the end of the capillary tube with the colored band on it.



2. Push the tip to the other end of the capillary tube until it barely extends beyond the end of the capillary tube.



3. Submerge the capillary tube below the surface of the liquid to be pipetted. Slowly and smoothly draw the Wiretrol plunger up until the bottom of the plunger tips reaches the appropriate volume line.



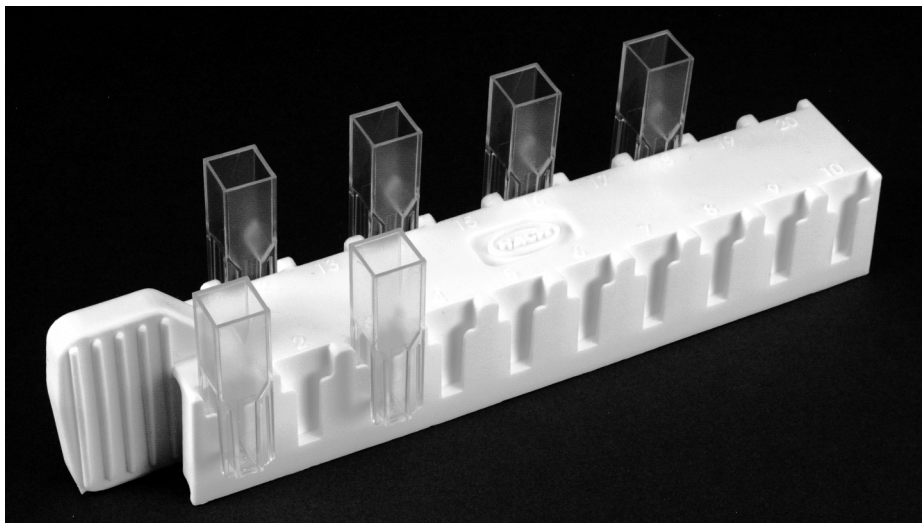
4. To discharge the pipet, place the tip of the capillary tube below the surface of the solution and push the Wiretrol plunger down in one smooth motion. Change capillary tubes for each calibrator and sample.

Note: Touch the end of the tube to the side of the vessel to release drops on the capillary tube tip.

Using the 1-cm MicroCuvette Rack

This rack (see *Figure 2*) has been designed specifically to aid in achieving precise and accurate results when using the immunoassay technique to analyze several samples at the same time.

Figure 2 **The 1-cm MicroCuvette Rack**



Loading the Rack — The cuvette rack is designed so that it may be inverted with the cuvettes in place. Identify each cuvette with a sample or calibrator number and place all the cuvettes in the rack before beginning the procedure. Fit the cuvettes snugly into the rack, but do not force them or they may be difficult to remove and their contents may spill. The cuvettes should remain in place when the rack is inverted and tapped lightly.

Mixing — Set the rack on a hard, flat surface that is at least twice the length of the rack. Hold the rack by one end and vigorously slide it back and forth along its long axis for 30 seconds. The rack should move through a distance equal to its own length in each direction.

Interpreting and Reporting Results

There is an inverse relationship between the concentration of PCB and the reading. In other words, the higher the reading, the lower the concentration of PCB.

If the sample reading is...	the sample TPH Concentration is...
...less than calibrator reading	...greater than the calibrator concentration
...greater than calibrator reading	...less than the calibrator concentration

Example

Readings:

1 ppm PCB Calibrator: **0.775 Abs**

5 ppm PCB Calibrator: **0.430 Abs**

Sample #1: **0.200 Abs**

Sample #2: **0.600 Abs**

Sample #3: **0.900 Abs**

Interpretation

Interpretation for a soil sample:

Sample #1 — Sample reading is less than the readings for both calibrators. Therefore the sample concentration of PCB is greater than both 1 ppm and 5 ppm as Aroclor 1248.

Sample #2 — Sample reading is between the readings for the 1 ppm and 5 ppm PCB calibrators. Therefore the sample concentration of PCB is between 1 ppm and 5 ppm as Aroclor 1248.

Sample #3 — Sample reading is greater than the readings for both calibrators. Therefore the sample concentration of PCB is less than both 5 ppm and 1 ppm as Aroclor 1248.

Storing and Handling Reagents

- Wear protective gloves and eyewear.
- When storing reagent sets for extended periods of time, keep them out of direct sunlight. Store reagents at a temperature of 4 °C when not in use.
- Keep the foil pouch containing the PCB Antibody Cuvettes sealed when not in use.
- If Stop Solution comes in contact with eyes, wash thoroughly for 15 minutes with cold water and seek immediate medical help.

Sensitivity

The PCB immunoassay cannot differentiate between the various Aroclors, but it detects their presence in differing degrees.

Table 1 Various PCBs in Soil

Compound	Concentration (ppm) to give a positive result at			
	1 ppm	5 ppm	10 ppm	50 ppm
1248	1	5	10	50
1016	2	9	20	67
1242	1.2	6	14	50
1254	1.4	4.6	11	28
1260	1.1	4.9	11	38

The following compounds are not detectable at 1000 ppm.

Biphenyl	2,4,6-trichlorophenyl	1,3-dichlorobenzene
2,4-dichlorophenyl	pentachlorophenol	1,4-dichlorobenzene
2,4,5-trichlorophenyl	1,2-dichlorobenzene	1,2,4-trichlorobenzene

Sample Collection and Storage

Analyze the samples as soon as possible after collection. If the samples must be stored, collect them in glass or Teflon® containers that have been washed with soap and water and rinsed with methanol. The container should be capped with a Teflon-lined cap. If a Teflon cap is not available, aluminum foil rinsed in methanol may be used as a substitute cap liner.

Summary of Method

Hach immunoassay tests use antigen/antibody reactions to test for specific organic compounds in water and soil. Antibodies specific for PCB are attached to the walls of plastic cuvettes. They selectively bind and remove PCB from complex sample matrices. A prepared sample and a reagent containing enzyme-conjugate molecules (analyte molecules attached to molecules of an enzyme) are added to the Antibody Cuvettes. During incubation, enzyme-conjugate molecules and PCB compete for binding sites on the antibodies. Samples with higher levels of analyte will have more antibody sites occupied by PCB and fewer antibody sites occupied by the enzyme-conjugate molecules.

After incubation, the sample and unbound enzyme conjugate are washed from the cuvette and a color-development reagent is added. The enzyme in the conjugate catalyzes the development of color. Therefore, there is an inverse relationship between color intensity and the amount of PCB in the sample. The resulting color is then compared with a calibrator to determine whether the PCB concentration in the sample is greater or less than the threshold levels. The PCB concentration is inversely proportional to the color development: the lighter the color, the higher the PCB concentration.

Required Reagents

Description	Unit	Cat. No.
Reagent Set, PCB *	20 cuvettes	27735-00
Deionized water	500 mL	272-48

Required Apparatus

Adapter, 1-cm MicroCell	each	48588-00
Caps, flip spout	2/pkg	25818-02
Marker, laboratory	each	20920-00
TenSette®, Pipet, 0.1–1.0 mL	each	19000-01
Tips, for TenSette®, Pipet	1000/pkg	21856-28
Rack, for 1-cm Micro Cuvettes	each	48799-00
Wipes, disposable	box	20970-00

For Soil Extraction only:

Soil Scoop, 5-g, 4.25-cc	each	26572-05
Soil Extraction Refill Kit	each	27752-00
Includes:		
Dropper, LDPE, 0.5 and 1.0-mL	20/pkg	21247-20
Filter and Barrel Assembly	20/pkg	25676-20
Sodium Sulfate, anhydrous	250 g	7099-29
Soil Extractant Solution	200 mL	25677-29
Soil Sample Container	20/pkg	25929-20
Weighing Boat, 8.9-cm, square	20/pkg	21790-20

* Immunoassay components are manufactured for Hach Company by Beacon Analytical Systems, Inc.



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HACH COMPANY
WORLD HEADQUARTERS
Telephone: (970) 669-3050
FAX: (970) 669-2932



✓ Method 8047

4-Aminoantipyrine Method*

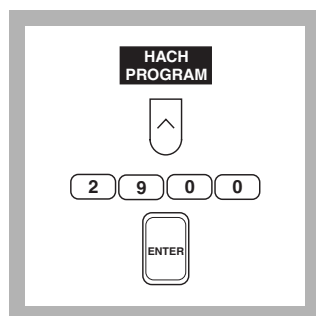
(0 to 0.200 mg/L)

Scope and Application: For water, wastewater and seawater;

USEPA Accepted (distillation required)**. The estimated detection limit for program number 2900 is 0.001 mg/L phenol.

* Adapted from *Standard Methods for the Examination of Water and Wastewater*

** Procedure is equivalent to USEPA method 420.1 for wastewater.



1. Press the soft key under **HACH PROGRAM**.

Select the stored program number for phenols by pressing **2900** with the numeric keys.

Press: **ENTER**

Note: Samples should be analyzed within four hours to avoid oxidation.

Note: The Flow Cell and Sipper Modules cannot be used with this procedure.

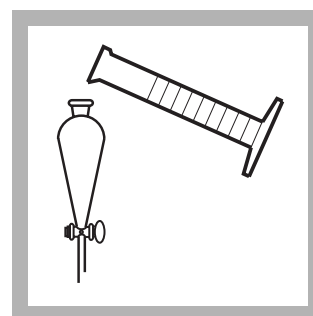


2. The display will show: **HACH PROGRAM: 2900 Phenols**

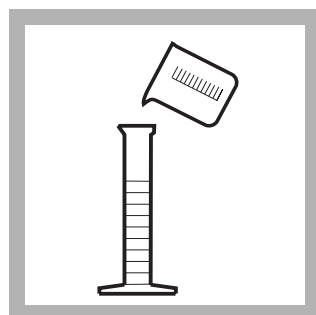
The wavelength (λ), **460 nm**, is automatically selected.



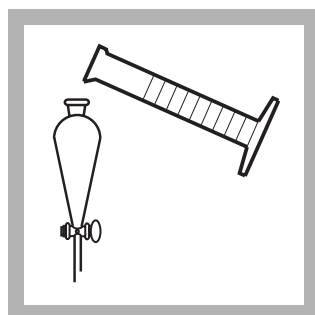
3. Measure 300 mL of deionized water in a 500-mL graduated cylinder.



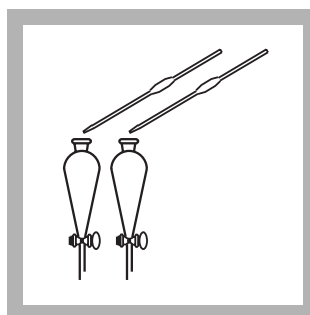
4. Pour the measured deionized water into a 500-mL separatory funnel (the blank).



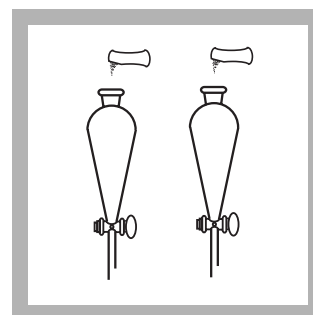
5. Measure 300 mL of sample in a 500-mL graduated cylinder.



6. Pour the sample into another 500-mL separatory funnel (the prepared sample).

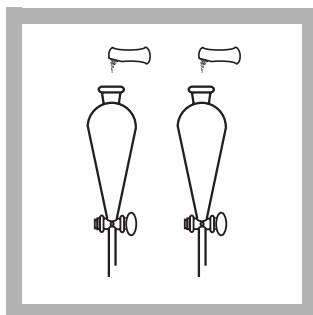


7. Add 5 mL of Hardness 1 Buffer to each separatory funnel. Stopper. Shake to mix.

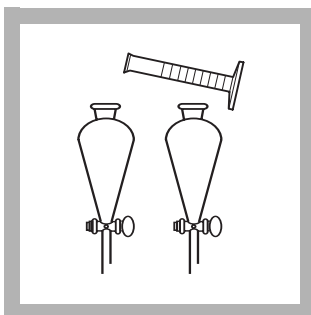


8. Add the contents of one Phenol Reagent Powder Pillow to each separatory funnel. Stopper. Shake to dissolve.

Note: Spilled reagent affects test accuracy and is hazardous to skin and other materials.

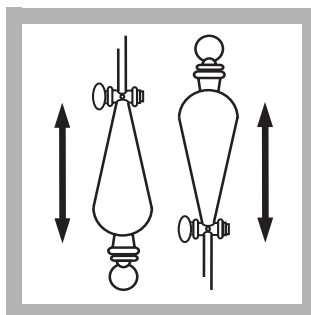


9. Add the contents of one Phenol 2 Reagent Powder Pillow to each separatory funnel. Stopper. Shake to dissolve.

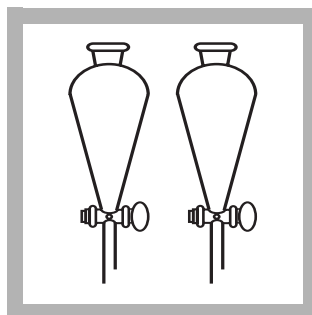


10. Add 30 mL of chloroform to each separatory funnel. Stopper each funnel.

Note: Use chloroform only with proper ventilation.

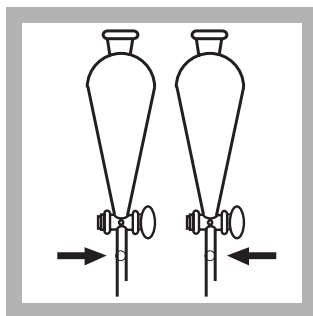


11. Invert each funnel and temporarily vent. Shake each funnel briefly and vent. Then vigorously shake each funnel for a total of 30 seconds (venting if necessary).

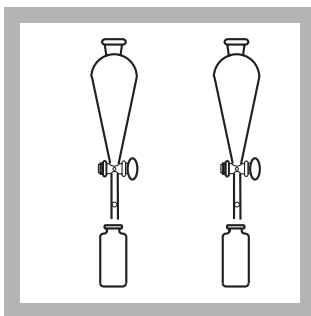


12. Remove the stoppers. Allow both funnels to stand until the chloroform settles to the bottom of the funnel.

Note: The chloroform layer will be yellow to amber if phenol is present.



13. Insert a large pea-sized cotton plug into the delivery tube of each funnel.

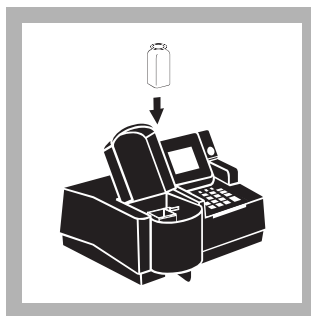


14. Drain the chloroform layers into separate sample cells (one for the blank, one for each sample).

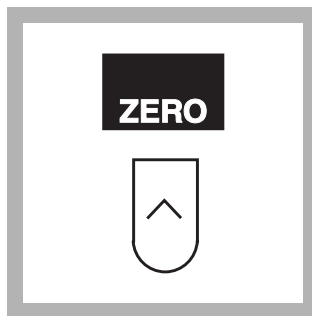
Note: Filtering the chloroform layer through the cotton removes suspended water or particles. The volume of chloroform extract will be about 25 mL.

Note: Proceed promptly through the rest of the procedure since the chloroform will evaporate, causing high readings. Glass-stoppered sample cells are recommended.

Note: The water phase contains chloroform, which is a hazardous waste. See Pollution Prevention and Waste Management following these steps.



15. Place the blank into the cell holder. Close the light shield.

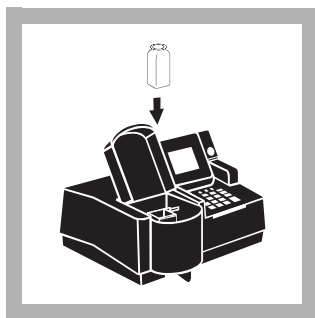


16. Press the soft key under **ZERO**.

The display will show:

0.000 mg/L Phenol

Note: For alternate concentration units press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



17. Place the prepared sample into the cell holder. Close the light shield. Result in mg/L phenols (or chosen units) will be displayed.

Interferences

Table 1 Interfering Substances and Suggested Treatments

Interfering Substance	Interference Levels and Treatments
pH	The sample pH must be between 3 and 11.5 for best results.
Oxidizing or reducing agents	May interfere. Distill samples (see procedure below).
Sulfides or suspended matter	Distillation or the following pretreatment is necessary: <ol style="list-style-type: none">1. Fill a clean 500-mL graduated cylinder with 350 mL of sample. Pour the sample into a clean 500-mL erlenmeyer flask.2. Add the contents of one Sulfide Inhibitor Reagent Powder Pillow. Swirl to mix.3. Filter 300 mL of the sample through a folded filter paper. Use this solution in Step 6.

Sample Collection, Storage and Preservation

Most reliable results are obtained when samples are analyzed within four hours after collection. Use the following storage instructions only if prompt analysis is not possible. Collect 500 mL of sample in clean glass containers and add the contents of two Copper Sulfate Powder Pillows. Adjust the pH to 4 or less with 10% Phosphoric Acid Solution. Store at 4 °C (39 °F) or lower and analyze within 24 hours.

Accuracy Check

Standard Solution Method

For greater accuracy, analyze standard solutions when new lots of reagent are first used.

1. Weigh out 1.00 g of Phenol, ACS. Transfer to a 1000-mL volumetric flask. Dilute to the mark with freshly boiled and cooled deionized water. This is a 1000-mg/L stock solution.
2. Pipet 10.0 mL of the 1000-mg/L stock solution to a 1000-mL volumetric flask. Dilute to the mark with deionized water. This is a 10-mg/L working solution.
3. Prepare a 0.200-mg/L standard solution by pipeting 10.0 mL of the working solution into a 500-mL volumetric flask. Dilute to the mark with deionized water.
4. Perform the phenol procedure described above using the prepared standard.
5. To adjust the calibration curve using the reading obtained with the standard solution, press the soft keys under **OPTIONS, (MORE)** then **STD:OFF**. Press **ENTER** to accept the default concentration, the value of which will depend on the selected units. If an alternate concentration is used, enter the actual concentration and press **ENTER** to return to the read screen. See Section 1.5.5 *Adjusting the Standard Curve* for more information.

Method Performance

Precision

Standard: 0.200 mg/L phenol

Program	95% Confidence Limits
2900	0.199-0.201 mg/L phenol

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
2900	0.001 mg/L phenol

For more information on derivation and use of Hach's estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, Appendix B, see Section 1.5.1.

Sensitivity

Program Number: 2900

Portion of Curve	Δ Abs	Δ Concentration
Entire Range	0.010	0.0020 mg/L

See Section 1.5.3 *Sensitivity Explained* for more information.

Distillation

This procedure is in the *Hach Distillation Apparatus Manuals* in step-by-step illustrated format.

1. Set up the Hach Distillation Apparatus by assembling the general purpose apparatus as shown in the Distillation Apparatus Manual. Use the 500-mL erlenmeyer flask to collect the distillate. It may be necessary to use a laboratory jack to elevate the flask.
2. Place a stirring bar into the flask.
3. Measure 300 mL of water sample in a clean 500-mL graduated cylinder. Pour it into the distillation flask.
4. For proof of accuracy, use a 0.200-mg/L phenol standard (see *Accuracy Check*) in addition to the sample.
5. Using a serological pipet, add 1 mL of Methyl Orange Indicator to the distillation flask.
6. Turn on the stirrer power switch. Set the stir control to 5.
7. Add 10% Phosphoric Acid Solution drop-wise until the indicator changes from yellow to orange.
8. Add the contents of one Copper Sulfate Powder Pillow and allow to dissolve (omit this step if copper sulfate was used to preserve the sample). Cap the distillation flask.
9. Turn the water on and adjust it so a constant flow is maintained through the condenser. Set the heat control to 10.
10. Collect 275 mL of distillate in the Erlenmeyer flask, then turn the heat off.
11. Fill a 25-mL graduated cylinder to the 25-mL mark with deionized water. Add the water to the distillation flask.
12. Turn the still back on. Heat until another 25 mL of distillate is collected.
13. Using a clean graduated cylinder, re-measure the distillate to verify that 300 mL has been collected. The distillate is ready for analysis.

Calibration Standard Preparation

To perform a phenol calibration using the 4-aminoantipyrine method, prepare a 1000-mg/L phenol stock solution by adding 1.00 g Phenol, ACS to a 1000-mL volumetric flask. Dilute to the mark with freshly boiled and cooled deionized water. Prepare a 10.0-mg/L working solution by pipetting 10.0 mL of the 1000-mg/L stock solution into a 1000-mL volumetric flask using Class A glassware. Dilute to the mark with deionized water and mix thoroughly. Prepare standards containing 0.02, 0.08, 0.16, and 0.20 mg/L phenol as follows:

- a. Into four different 500-mL volumetric flasks, pipet 1.0, 4.0, 8.0, and 10.0 mL of the 10 mg/L working solution using Class A glassware.
- b. Dilute to the mark with deionized water. Mix thoroughly.

- c. Using the 4-aminoantipyrine method and the calibration procedure described in the *User-Entered Programs* section of the *DR/4000 Spectrophotometer Instrument Manual*, generate a calibration curve from the standards prepared above.

Summary of Method

The 4-aminoantipyrine method measures all ortho- and meta- substituted phenols. These phenols react with 4-aminoantipyrine in the presence of potassium ferricyanide to form a colored antipyrine dye. The dye is then extracted from the aqueous phase with chloroform and the color is measured at 460 nm. The sensitivity of the method varies with the type of phenolic compound. Because water samples may contain various types of phenolic compounds, the test results are expressed as the equivalent concentration of phenol.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to Section 1.

Pollution Prevention and Waste Management

Phenol 2 Reagent Powder Pillows contain potassium ferricyanide. Both chloroform (D022) and cyanide (D001) solutions are regulated as hazardous waste by the Federal RCRA. Do not pour these materials down the drain. Chloroform solutions and the cotton plug used in the delivery tube of the separatory funnel should be collected for disposal as a reactive waste. Be sure that cyanide solutions are stored in a caustic solution with a pH >11 to prevent release of hydrogen cyanide gas. See Section 1 for further information on proper disposal of these materials.

REQUIRED REAGENTS AND STANDARDS

	Cat. No
Phenols Reagent Set (100 tests)	22439-00
Includes: (3) 424-49, (2) 14458-17, (2) 1836-99, (2) 872-99	

Description	Quantity Required		Cat. No.
	Per Test	Unit	
Chloroform, ACS	60 mL	4 L	14458-17
Hardness 1 Buffer Solution, pH 10.1	10 mL	500 mL	424-49
Phenol 2 Reagent Powder Pillows.....	2	100/pkg	1836-99
Phenol Reagent Powder Pillows.....	2	100/pkg	872-99
Water, deionized	300 mL	4 liters	272-56

REQUIRED EQUIPMENT AND SUPPLIES

Clippers, for opening powder pillows	1	each	968-00
Cotton Balls.....	1	100/pkg	2572-01
Cylinder, graduated, 50-mL	1	each	508-41
Cylinder, graduated, 500-mL	1	each	508-49
Funnel, separatory, 500-mL	2	each	520-49
Pipet, volumetric, Class A, 5.00-mL	1	each	14515-37
Ring, support, 4-inch.....	2	each	580-01
Sample Cells, glass-stoppered, 1-inch, matched pair.....	1	2/pkg	26126-02
Support, ring stand, 5 x 8 inch base	1	each	563-00

OPTIONAL REAGENTS AND STANDARDS

Copper Sulfate Powder Pillows.....	50/pkg	14818-66
Methyl Orange Indicator Solution, 0.5-g/L.....	100 mL MDB	148-32
Phenol, ACS	113 g	758-14
Phosphoric Acid Solution, 10%	100 mL MDB	14769-32
Sulfide Inhibitor Reagent Powder Pillows	100/pkg	2418-99

OPTIONAL EQUIPMENT AND SUPPLIES

Cylinder, graduated, 25-mL	each	508-40
Distillation Heater and Support Apparatus, 115 VAC.....	each	22744-00
Distillation Heater and Support Apparatus, 230 VAC.....	each	22744-02
Distillation Apparatus Set, general purpose	each	22653-00
DR/4000 Carousel Module Kit	each	48070-02
Filter Paper, folded, 12.5-cm.....	100/pkg	1894-57
Flask, Erlenmeyer, 500-mL.....	each	505-49
Flask, volumetric, Class A, 500-mL	each	14574-49
Flask, volumetric, Class A, 1000-mL	each	14574-53
Funnel, poly, 65-mm	each	1083-67
Jack, laboratory, 15 X 15 cm.....	each	22743-00
pH Paper, pH 1.0 to 11.0	5 rolls/pkg	391-33
Pipet Filler, safety bulb.....	each	14651-00
Pipet, serological, 1.0-mL	each	532-35
Pipet, volumetric, Class A, 1.00-mL	each	14515-35
Pipet, volumetric, Class A, 2.00-mL	each	14515-36
Pipet, volumetric, Class A, 3.00-mL	each	14515-03
Pipet, volumetric, Class A, 4.00-mL	each	14515-04
Pipet, volumetric, Class A, 10.00-mL	each	14515-38



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WORLD HEADQUARTERS
Telephone: (970) 669-3050
FAX: (970) 669-2932



Method 8007

Persulfate UV Oxidation Method*

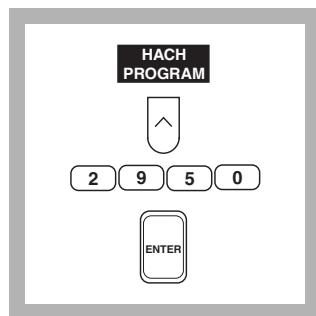
Powder Pillows

(0-2.50 to 0-125 mg/L)

Scope and Application: For boiler and cooling water, water, wastewater and seawater.

The estimated detection limit for program number 2950 depends on the sample volume. See Method Performance.

* Adapted from Blystone, P., Larson, P., *A Rapid Method for Analysis of Phosphonate Compounds*, International Water Conference, Pittsburgh, PA. (Oct 26–28, 1981)



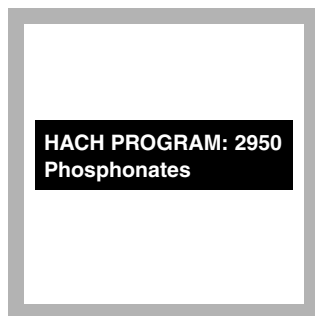
1. Press the soft key under **HACH PROGRAM**.

Select the stored program number for phosphonates by pressing **2950** with the numeric keys.

Press: **ENTER**

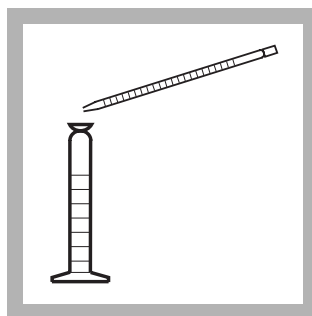
Note: If samples cannot be analyzed immediately, see *Sample Collection, Storage and Preservation* following these steps.

Note: The Flow Cell and Sipper Modules can be used with this procedure. Use a 25-mL sample and reagents with the Flow Cell Module.



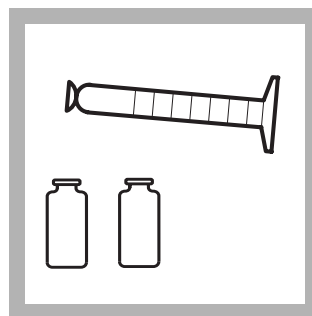
2. The display will show: **HACH PROGRAM: 2950 Phosphonates**

The wavelength (λ), **890 nm**, is automatically selected.



3. Choose the appropriate sample size from *Table 1* below. Pipet the chosen volume into a 50-mL mixing graduated cylinder. If necessary, dilute the sample to 50 mL with deionized water and mix well.

Note: Clean glassware with 1:1 hydrochloric acid, followed by a distilled water rinse. Do not use a commercial detergent.



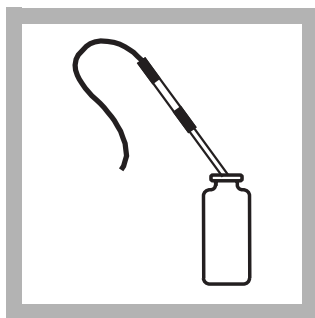
4. Fill a 1-inch sample cell to the 10-mL mark with diluted sample from Step 3 (this is the blank). Fill a second, 1-inch sample cell to the 25-mL mark with diluted sample from Step 3.

Table 1

Expected range (mg/L phosphonate)	Sample Volume (mL)
0–2.5	50
0–5	25
0–12.5	10
0–25	5
0–125	1



5. Add the contents of one Potassium Persulfate for Phosphonate Powder Pillow to the cell containing 25 mL of sample. Swirl to mix.

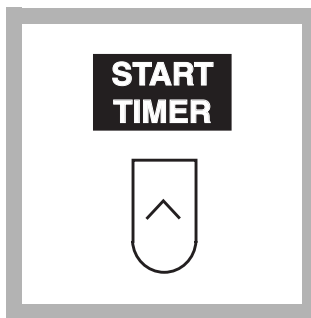


6. Insert the ultraviolet (UV) lamp into the sample cell.

Note: Wear UV safety goggles while the lamp is on.

Note: Do not handle the lamp surface. Fingerprints will etch the glass. Wipe lamp with a soft, clean tissue between samples. Do not use phosphate detergents to wash glassware.

Note: A specially designed cord adapter (Cat. No. 19485-00) is available so two digestions can be performed at once using one power supply. A second UV lamp is required.



7. Turn the UV lamp on. Press the soft key under **START TIMER**.

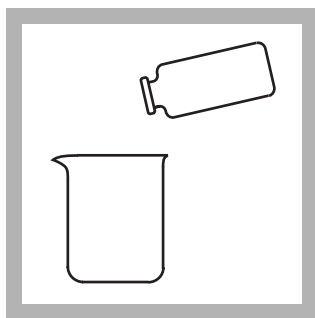
A 10-minute reaction period will begin.

Note: Phosphonates are converted to orthophosphate in this step.

Note: The digestion step is normally completed in less than 10 minutes. Contaminated samples or a weak lamp, however, can cause incomplete conversion to phosphate. Check conversion efficiency by running a longer digestion and seeing if readings increase.

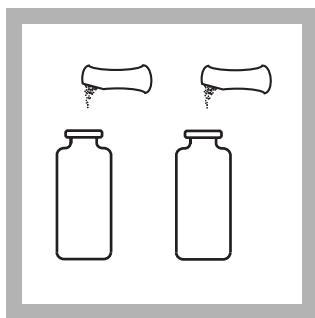


8. When the timer beeps, turn the UV lamp off and remove it from the sample.



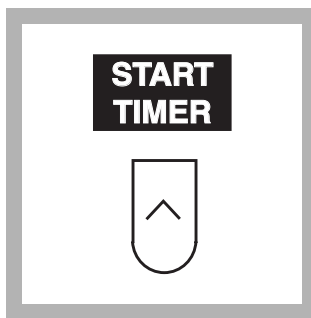
9. Pour off about 15 mL of sample into a 50-mL beaker so that 10 mL remains in the 1-inch sample cell. This is the prepared sample.

Note: Use a beaker, rather than a sink or waste container, in case too much sample is poured out initially.



10. Add the contents of one PhosVer 3 Phosphate Reagent Powder Pillow to the blank and prepared sample. Swirl immediately to mix.

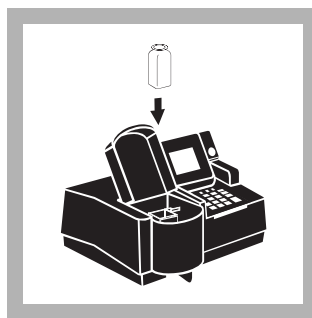
Note: A blue color will develop if phosphate is present. Both sample and blank cells may develop color; the increase in sample color is proportional to the phosphonate concentration.



11. Press the soft key under **START TIMER**.

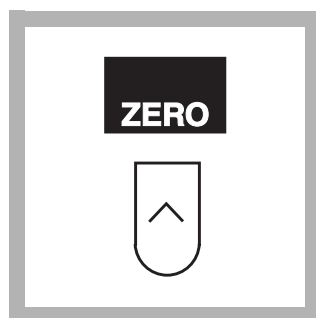
A 2-minute reaction period will begin.

Note: If sample is colder than 15 °C, four minutes are required for color development.



12. When the timer beeps, place the blank (undigested sample) into the cell holder. Close the light shield.

Note: Do steps 13 and 14 within three minutes after the timer beeps.



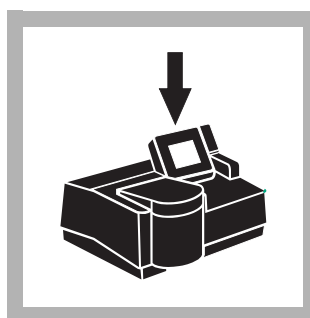
13. Press the soft key under **ZERO**.

The display will show:

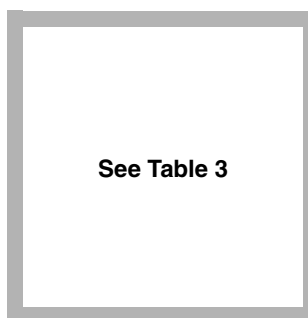
0.00 mg/L PO₄³⁻



14. Place the prepared sample in the cell holder. Close the light shield.



15. Read the mg/L phosphate from the display. Multiply this value by the appropriate multiplier in *Table 2* to obtain the actual concentration of phosphates in the sample.



See *Table 3*

16. Results can be expressed as active phosphonate by using the appropriate conversion factor from *Table 3*.

Active phosphonate (mg/L)
= Phosphate concentration
(Step 15) x Conversion
Factor

Table 2

Sample volume	Multiplier
50	0.1
25	0.2
10	0.5
5	1.0
1	5.0
phosphonate concentration = instrument reading x multiplier	

Table 3

Phosphonate type	Conversion factor
PBTC	2.84
NTP	1.050
HEDPA	1.085
EDTMPA	1.148
HMDTMPA	1.295
DETPMPA	1.207
HPA	1.49
The result from Step 16 is the active phosphonate. To determine the concentration of the product being used (e.g., NTP), divide the active phosphonate value by the percent active phosphonate value on the product label.	

Interferences

When testing a 5-mL sample volume, the following may interfere if present in concentrations exceeding those listed below:

Table 4 Interfering Substances and Suggested Treatments

Interfering Substance	Interference Levels and Treatments
Aluminum	100 mg/L
Arsenate	Interferes at all levels
Benzotriazole	10 mg/L
Bicarbonate	1000 mg/L
Bromide	100 mg/L
Calcium	5000 mg/L
CDTA	100 mg/L
Chloride	5000 mg/L

Table 4 Interfering Substances and Suggested Treatments (Continued)

Interfering Substance	Interference Levels and Treatments
Chromate	100 mg/L
Copper	100 mg/L
Cyanide	100 mg/L. Increase the UV digestion to 30 minutes.
Diethanoldithiocarbamate	50 mg/L
EDTA	100 mg/L
Iron	200 mg/L
Nitrate	200 mg/L
NTA	250 mg/L
Orthophosphate	15 mg/L
Phosphites and organophosphorus compounds	React quantitatively. Meta- and polyphosphates do not interfere.
Silica	500 mg/L
Silicate	100 mg/L
Sulfate	2000 mg/L
Sulfide	Interferes at all levels
Sulfite	100 mg/L
Thiourea	10 mg/L
Highly buffered samples or extreme sample pH	May exceed the buffering capacity of the reagents and require sample pretreatment.

The interference levels will decrease as the sample size increases. For example, copper does not interfere at or below 100 mg/L for a 5.00 mL sample. If the sample volume is increased to 10 mL, copper will begin to interfere above 50 mg/L.

Sample Collection, Storage and Preservation

Collect samples in acid-cleaned (1:1 HCl) plastic or glass bottles that have been rinsed with distilled water. Do not use a commercial detergent. If prompt analysis is impossible, preserve the sample by adjusting to pH 2 or less with sulfuric acid (about 2 mL per liter). Store at 4 °C (39 °F). Preserved samples may be stored at least 24 hours. Correct the test result for volume additions; see Section 1.2.2 *Correcting for Volume Additions*.

Accuracy Check

Ideally, a solution containing the phosphonate product being used should be prepared. This will check the UV conversion of phosphonate to orthophosphate. Or, a phosphate standard can be used to check the accuracy or the colorimetric part of the method.

Standard Solution

A 1-mg/L Phosphate Standard Solution (available from Hach) can be used to check accuracy. Use 10 mL of this standard in place of the prepared sample in Step 9. Use deionized water for the blank. A multiplier value from *Table 2* is not needed. The result should be 10.0 mg/L phosphate, due to a factor of 10 in calibration.

Method Performance

Precision

Standard: 1.00 mg/L PO_4^{3-}

Program	95% Confidence Limits
2950	0.98–1.02 mg/L PO_4^{3-}

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

The EDL depends on the sample volume.

Range (mg/L)	Volume (mL)	EDL (mg/L)
0–2.5	50	0.045
0–5	25	0.09
0–12.5	10	0.23
0–25	5	0.45
0–125	1	2.25

EDL is expressed as PO_4^{3-} in this table. Use *Table 3* to express as a specific phosphonate.

For more information on derivation and use of Hach's estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, appendix B, see Section 1.5.1.

Sensitivity

Program Number: 2950

Portion of Curve	ΔAbs	$\Delta\text{Concentration}$
0.010 Abs	0.010	0.16813 mg/L
12.5 mg/L	0.010	0.177409 mg/L
22.5 mg/L	0.010	0.184591 mg/L

Sensitivity is expressed as PO_4^{3-} in this table. Use *Table 3* to convert to a specific phosphonate.

See Section 1.5.3 *Sensitivity Explained* for more information.

Calibration Standard Preparation

To perform a phosphonate calibration using the Persulfate UV Oxidation method, phosphate standard solutions are substituted for digested phosphonate samples in Step 9 of the procedure. Use a 10-mg/L Phosphate Standard Solution (Cat. No 14204-16). Prepare calibration standards containing 0.300, 1.500 and 2.400 mg/L phosphate as follows:

- a. Into three different 100-mL Class A volumetric flasks, pipet 3.00, 15.00 and 24.00 mL of the 10-mg/L Phosphate Standard Solution using Class A glassware.
- b. Dilute to the mark with deionized water. Mix thoroughly.

Because of the factor of 10 built into the procedure (*i.e.* the multiplier for an undiluted sample in Step 3 is 0.1), the 0.3, 1.5 and 2.4 mg/L standards should be entered as 3.0, 15.0, and 24.0 mg/L PO_4^{3-} .

Using the above standards in place of the sample in Step 9 and deionized water as the blank, generate a calibration curve following the calibration procedure described in the *User-Entered Programs* section of the *DR/4000 Spectrophotometer Instrument Manual*.

Summary of Method

This method is directly applicable to boiler and cooling tower samples. The procedure is based on a UV catalyzed oxidation of phosphonate to orthophosphate. The orthophosphate reacts with the molybdate in the PhosVer 3 reagent to form a phosphomolybdate complex. This complex is reduced by the ascorbic acid in the PhosVer 3, yielding a blue color which is proportional to the phosphonate present in the original sample.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to Section 3.

Pollution Prevention and Waste Management

For information on pollution prevention and waste management, refer to Section 3.

REQUIRED REAGENTS AND STANDARDS

	Cat. No
Phosphonate Reagent Set for 10 mL sample (100 tests).....	24297-00
Includes: (1) 21060-69, (1) 20847-69, (1) 171-02	

Description	Quantity Required		Unit	Cat. No.
	Per Test			
PhosVer 3 Phosphate Reagent Powder Pillows, 10-mL	2 pillows	100/pkg		21060-69
Potassium Persulfate Powder Pillow for Phosphonate.....	1 pillow	100/pkg		20847-69
Water, deionized	varies	4 liters		272-56

REQUIRED EQUIPMENT AND SUPPLIES

Beaker, 50-mL	1	each	500-41
Cylinder, mixing, graduated, 50-mL	1	each	1896-41
DR/4000 1-Inch Cell Adapter	1	each	48190-00
Goggles, UV safety	1	each	21134-00
Pipet, serological, 10-mL	1	each	532-38
Safety bulb.....	1	each	14651-00
UV Lamp with power supply, 115 VAC.....	1	each	20828-00
<i>or</i>			
UV Lamp with power supply, 230 VAC.....	1	each	20828-02

OPTIONAL REAGENTS AND STANDARDS

Hydrochloric Acid Standard Solution, 6.0 N (1:1)	500 mL.....	884-49
Sulfuric Acid, ACS, concentrated	500 mL.....	979-49
Phosphate Standard Solution, 10-mg/L.....	946 mL.....	14204-16

OPTIONAL EQUIPMENT AND SUPPLIES

Cord Adapter, single to dual UV lamp.....	each.....	19485-00
DR/4000 Carousel Module Kit	each.....	48070-02
DR/4000 Flow Cell Module Kit, 1-inch.....	each.....	48070-04
DR/4000 Flow Cell Module Kit, 1-cm.....	each.....	48070-05
DR/4000 Sipper Module Kit, 1-inch	each.....	48090-03
Flask, volumetric, Class A, 100-mL	each.....	14574-42
pH Paper, pH 1.0 to 11.0	5 rolls/pkg	391-33
Pipet, serological, 2-mL	each.....	532-36
Pipet, volumetric, Class A, 3.00-mL	each.....	14515-03
Pipet, volumetric, Class A, 9.00-mL	each.....	14515-09
Pipet, volumetric, Class A, 15.00-mL	each.....	14515-39
Pipet Filler, safety bulb.....	each.....	14651-00
UV Lamp without power supply	each.....	20823-00



FOR TECHNICAL ASSISTANCE, PRICE INFORMATION AND ORDERING:

In the U.S.A. – Call toll-free 800-227-4224

Outside the U.S.A. – Contact the HACH office or distributor serving you.

On the Worldwide Web – www.hach.com; E-mail – techhelp@hach.com

HACH COMPANY
WORLD HEADQUARTERS
Telephone: (970) 669-3050
FAX: (970) 669-2932



Method 8180

PhosVer 3 with Acid Hydrolysis

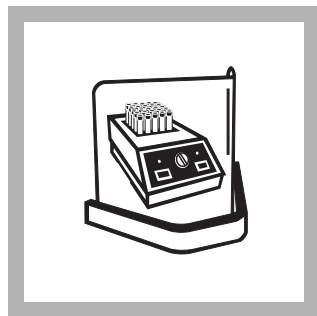
(0.00 to 5.00 mg/L PO_4^{3-})

Test 'N Tube™ Vials

(0.00 to 1.60 mg/L P)

Scope and Application: For water, wastewater and seawater.

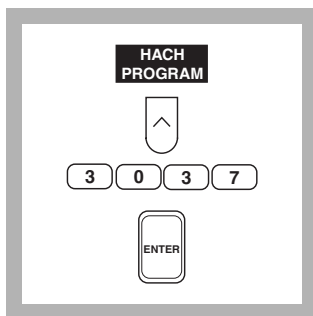
The estimated detection limit for program number 3037 is 0.17 mg/L PO_4^{3-} .



1. Turn on the COD Reactor. Heat to 150 °C. Place the plastic shield in front of the reactor.

Note: Ensure safety devices are in place to protect the analyst from splattering should leakage occur.

Note: See COD Reactor Manual for temperature adjustment instructions.



2. Press the soft key under **HACH PROGRAM**. Select the stored program for acid hydrolyzable phosphorus by pressing **3037** with the numeric keys.

Press: **ENTER**

Note: If samples cannot be analyzed immediately, see Sample Collection, Storage and Preservation following these steps.

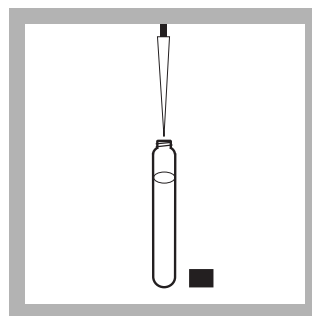


3. The display will show:
HACH PROGRAM: 3037
P A-H As. TNT

The wavelength (λ), **890 nm**, is automatically selected.

Note: Clean glassware with 1:1 Hydrochloric Acid Standard Solution. Rinse with deionized water. Do not use phosphate detergents to clean glassware.

Note: For best results, determine a reagent blank for each new lot of reagent as follows. Prepare a reagent blank by repeating steps 4 through 21, using deionized water as the sample. Zero the instrument on deionized water by pressing the soft key under **ZERO**. Insert the reagent blank and the blank value will be displayed. Correct for the reagent blank by pressing the soft keys under **OPTIONS**, **(MORE)**, and then **BLANK: OFF**. Enter the reagent blank value and press **ENTER**. Repeat for each new lot of reagent.

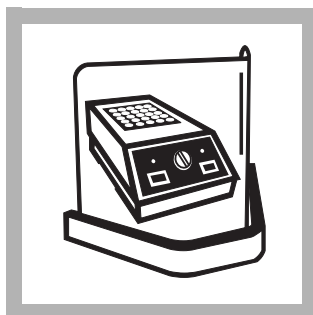


4. Use a TenSette Pipet to add 5 mL of sample to a Total and Acid Hydrolyzable Test Vial. Cap and mix.

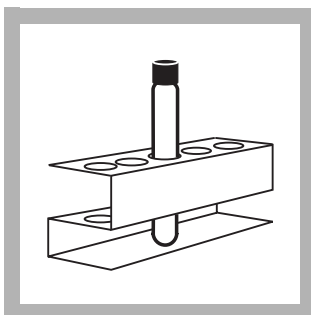
Note: For proof of accuracy, use a 1.0-mg/L Phosphate (0.33-mg/L P) Standard Solution in place of the sample (see **OPTIONAL REAGENTS AND STANDARDS**).

Note: For non-preserved samples with extreme pH.

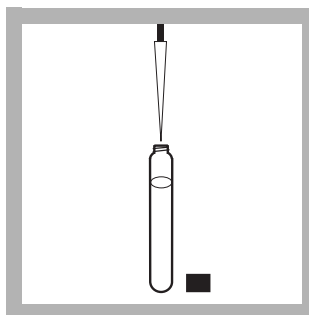
PHOSPHORUS, Acid Hydrolyzable, continued



5. Place the vial in the COD Reactor, and start a 30-minute heating period by pressing the soft key under **START TIMER**.



6. After the timer beeps, carefully remove the vial from the reactor. Place it in a test tube rack and allow to cool to room temperature.



7. Using a TenSette Pipet, add 2 mL of 1.00 N Sodium Hydroxide to the vial. Cap tightly and shake to mix.

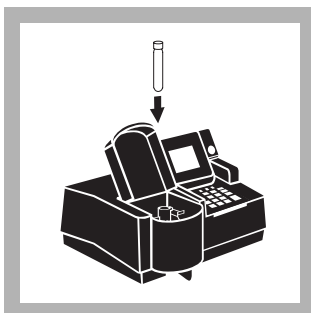


8. Insert the Test Tube Adapter into the sample cell module by sliding it under the thumb screw and into the alignment grooves. Fasten with the thumb screw.

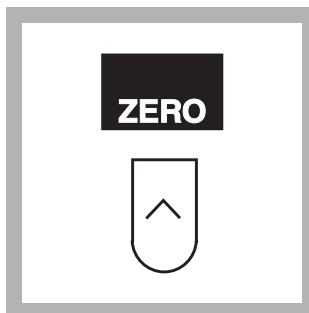


9. Clean the outside of the vial with a towel.

Note: Wiping with a damp towel, followed by a dry one, will remove fingerprints or other marks.



10. Place the sample vial in the cell holder and close the light shield.



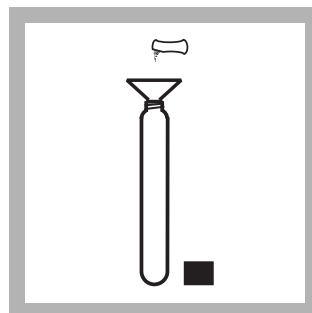
11. Press the soft key under **ZERO**.

The display will show:

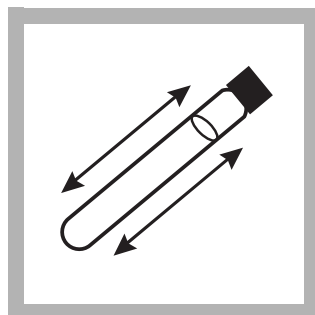
0.00 mg/L PO₄³⁻

Note: If you are using a reagent blank correction, the display will show the correction.

Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.

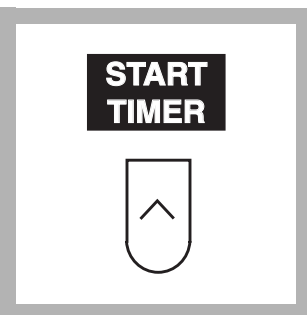


12. Using a funnel, add the contents of one PhosVer 3 Powder Pillow to the vial.



13. Cap tightly and shake to mix for 10-15 seconds.

Note: The powder will not completely dissolve.



14. Press the soft key under **START TIMER**.

A 2-minute reaction period will begin.



15. Clean the outside of the sample vial with a towel.

Note: Wiping with a damp towel, followed by a dry one, will remove fingerprints or other marks.

Note: Read the samples 2-8 minutes after the addition of the PhosVer 3 Reagent.



16. Place the prepared sample vial into the cell holder and close the light shield. Results in mg/L PO_4^{3-} (or chosen units) will be displayed.

Note: Results may be expressed as phosphorus (P) or as phosphorus pentoxide (P_2O_5). Press the soft keys under **OPTIONS** and then **FORM** to scroll through the available options.

Interferences

Interfering Substance	Interference Levels and Treatments
Aluminum	Greater than 200 mg/L
Arsenate	All levels
Chromium	Greater than 100 mg/L
Copper	Greater than 10 mg/L
Iron	Greater than 100 mg/L
Nickel	Greater than 300 mg/L
Silica	Greater than 50 mg/L
Silicate	Greater than 10 mg/L
Sulfide	Greater than 9 mg/L. Remove sulfide interference as follows: <ol style="list-style-type: none"> 1. Measure 25 mL of sample into a 50-mL beaker. 2. Swirling constantly, add Bromine Water drop-wise until a permanent yellow color appears. 3. Swirling constantly, add Phenol Solution drop-wise just until the yellow color disappears. Proceed with step 1.
Turbidity	Large amounts may cause inconsistent results in the test because the acid present in the powder pillows may dissolve some of the suspended particles and because of variable desorption of orthophosphate from the particles.
Zinc	Greater than 80 mg/L
Highly buffered samples or extreme sample pH	May exceed the buffering capacity of the reagents and require sample pretreatment

Store the PhosVer 3 Phosphate Reagent Powder Pillows in a cool, dry environment.

Sample Collection, Storage and Preservation

Collect samples in plastic or glass bottles that have been acid washed with 1:1 Hydrochloric Acid Solution and rinsed with deionized water. Do not use commercial detergents containing phosphate for cleaning glassware used in this test.

Analyze samples immediately after collection for best results. If prompt analysis is impossible, preserve samples up to 24 hours by storing at 4 °C. Warm samples to room temperature before analysis.

Accuracy Check

Standard Additions Method

- a. Leave the unspiked sample in the sample compartment. Verify that the units displayed are in mg/L. Select standard additions mode by pressing the soft keys under **OPTIONS, (MORE)** and then **STD ADD**.
- b. Press **ENTER** to accept the default sample volume (mL), 25.
- c. Press **50.0** to change the standard concentration to (mg/L), 50.0. Then press **ENTER**.
- d. Press the soft key under **ENTRY DONE**.
- e. Snap the neck off a Phosphate 2-mL Ampule Standard, 50-mg/L as PO_4^{3-} .
- f. Use the TenSette Pipet to add 0.1 mL, 0.2 mL and 0.3 mL of standard, respectively to three 25-mL samples and mix each thoroughly.
- g. Analyze each standard addition sample as described above (use a 5-mL aliquot of the spiked sample as the sample). Accept the standard additions reading by pressing the soft key under **READ** each time. Each addition should reflect approximately 100% recovery.
- h. After completing the sequence, the display will show the extrapolated concentration value and the “best-fit” line through the standard additions data points, accounting for matrix interferences.
- i. See Section 1.4.1 *Standard Additions* for more information.

Method Performance

Precision

Standard: 3.00 mg/L PO_4^{3-}

Program	95% Confidence Limits
3037	2.96–3.04 mg/L PO_4^{3-}

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
3037	0.07 mg/L PO ₄ ³⁻

For more information on derivation and use of Hach's estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, appendix B, see Section 1.5.1.

Sensitivity

Program Number: 3037

Portion of Curve	Δ Abs	Δ Concentration
Entire Range	0.010	0.061 mg/L

See Section 1.5.3 *Sensitivity Explained* for more information.

Calibration Standard Preparation

To perform a phosphate calibration using the Test 'N Tube method, prepare calibration standards containing 1.00, 2.00, 3.00, 4.00, and 5.00 mg/L phosphate as follows:

- Into five different 100-mL Class A volumetric flasks, pipet 2.00, 4.00, 6.00, 8.00 and 10.00 mL of a 50-mg/L Phosphate Standard Solution (Cat. No. 171-49) using Class A glassware.
- Dilute to the mark with deionized water. Mix thoroughly.
- Using the Test 'N Tube method and the calibration procedure described in the *User-Entered Programs* section of the *DR/4000 Spectrophotometer Instrument Manual*, generate a calibration curve from the standards prepared above.

Summary of Method

Phosphates present in condensed inorganic forms (meta-, pyro-, or other polyphosphates) must be converted to reactive orthophosphate before analysis. Pretreating the sample with acid and heat hydrolyzes the condensed inorganic forms to orthophosphate.

Orthophosphate reacts with molybdate in an acid medium to produce a Phosphomolybdate complex. Ascorbic acid then reduces the complex, giving an intense molybdenum blue color.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to *Section 1*.

Pollution Prevention and Waste Management

Final samples will contain molybdenum. In addition, final samples will have a pH less than 2 and are considered corrosive (D002) by the Federal RCRA. Please see *Section 1* for more information on proper disposal of these materials.

PHOSPHORUS, Acid Hydrolyzable, continued

REQUIRED REAGENTS AND STANDARDS

Description	Quantity Required Per Test	Unit	Cat. No
Total and Acid Hydrolyzable Phosphorus Reagent Set.....		50 tests.....	27427-45
Includes:			
PhosVer 3 Phosphate Reagent Powder Pillows	1 pillow	50/pkg	21060-46
Potassium Persulfate Powder Pillows	1	50/pkg	20847-66
Sodium Hydroxide Standard Solution, 1.00 N.....	2 mL	100 mL	1045-42
Total and Acid Hydrolyzable Test Vials.....	1 vial	50/pkg	*

REQUIRED EQUIPMENT AND SUPPLIES

COD Reactor, 115/230 VAC, North American plug	1	each.....	45600-00
COD Reactor, 115/230 VAC, European plug	1	each.....	45600-02
DR/4000 Test Tube Adapter.....	1	each.....	48189-00
Funnel, micro	1	each.....	25843-35
Pipet, volumetric, Class A, 2.00-mL	1	each.....	14515-36
Pipet, volumetric, Class A, 5.00-mL	1	each.....	14515-37
Pipet Filler, safety bulb.....	1	each.....	14651-00
Pipet, TenSette, 1 to 10 mL	1	each.....	19700-10
Pipet Tips, for 19700-10 TenSette Pipet	1	1000/pkg.....	21997-28
Safety Shield, laboratory bench	1	each.....	23810-00
Test Tube Rack	1-3	each.....	18641-00

OPTIONAL REAGENTS AND STANDARDS

Bromine Water, 30-g/L.....	29 mL.....	2211-20
Hydrochloric Acid Standard Solution, 6.0 N (1:1)	500 mL.....	884-49
Phosphate Standard Solution, 1-mg/L as PO_4^{3-}	500 mL.....	2569-49
Phosphate Standard Solution, 50-mg/L.....	500 mL.....	171-49
Phosphate Standard Solution, 2-mL PourRite Ampule, 50-mg/L as PO_4	20/pkg.....	171-20H
Sodium Hydroxide Standard Solution, 5.0 N.....	1 L.....	2450-53
Water, deionized	4 liters.....	272-56

OPTIONAL EQUIPMENT AND SUPPLIES

pH Paper, pH 1.0 to 11.0	5 rolls/pkg	391-33
pH Meter, <i>sension™</i> I, portable	each.....	51700-00
Pipet, volumetric, Class A, 2.00-mL	each.....	14515-36
Pipet, volumetric, Class A, 4.00-mL	each.....	14515-04
Pipet, volumetric, Class A, 6.00-mL	each.....	14515-06
Pipet, volumetric, Class A, 8.00-mL	each.....	14515-08
Pipet, volumetric, Class A, 10.00-mL	each.....	14515-38
PourRite™ Ampule Breaker	each.....	24846-00
Pipet, TenSette, 0.1 to 1 mL	each.....	19700-01
Pipet Tips, for 19700-01 TenSette Pipet	50/pkg.....	21856-96

* Not sold separately.



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Telephone: (970) 669-3050
FAX: (970) 669-2932



Method 8178

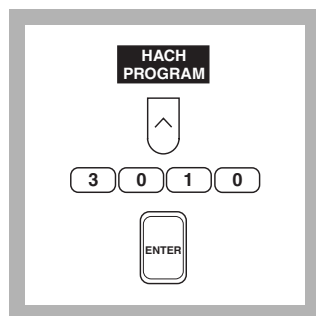
Amino Acid Method*

(0 to 30.00 mg/L PO_4^{3-})

Scope and Application: For water, wastewater, seawater.

The estimated detection limit for program number 3010 is 0.04 mg/L PO_4^{3-} .

* Adapted from *Standard Methods for the examination of Water and Wastewater*



1. Press the soft key under **HACH PROGRAM**.

Select the stored program number for reactive phosphorus, amino acid method by pressing **3010** with the numeric keys.

Press: **ENTER**

Note: If samples cannot be analyzed immediately, see *Sample Collection, Storage and Preservation* following these steps. Adjust the pH of preserved samples before analysis.

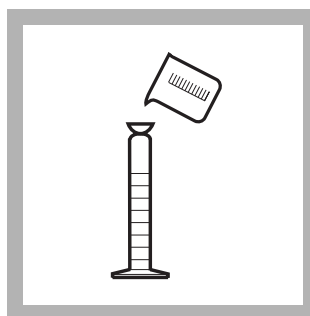
Note: The Flow Cell and Sipper Modules can be used with this procedure.



2. The display will show:
HACH PROGRAM: 3010
P. React. Amino. HR

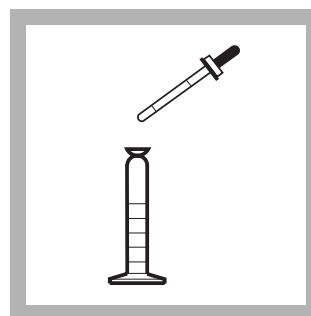
The wavelength (λ), **530 nm**, is automatically selected.

Note: For best results, determine a reagent blank for each new lot of reagent as follows. Prepare a reagent blank by repeating steps 3 through 10, using phosphate-free deionized water as the sample. Zero the instrument on deionized water by pressing the soft key under **ZERO**. Insert the reagent blank and the blank value will be displayed. Correct for the reagent blank by pressing the soft keys under **OPTIONS, (MORE)**, and then **BLANK:OFF**. Enter the reagent blank value and press **ENTER**. Repeat for each new lot of reagent.



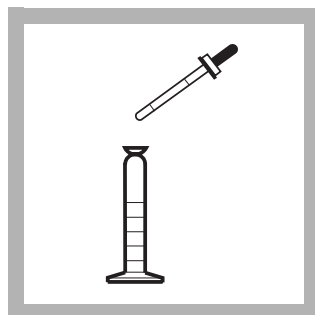
3. Fill a 25-mL mixing graduated cylinder with 25 mL of sample.

Note: For proof of accuracy, use a 10.0-mg/L as PO_4^{3-} (3.3-mg/L as P) phosphorus standard solution (see *Accuracy Check*) in place of the sample.



4. Add 1 mL of Molybdate Reagent using a 1-mL calibrated dropper.

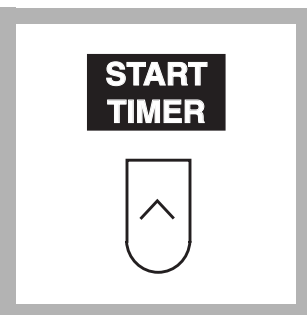
PHOSPHORUS, Reactive (Orthophosphate), continued



5. Add 1 mL of Amino Acid Reagent Solution. Stopper and invert several times to mix (the prepared sample).

Note: A blue color will form if phosphate is present.

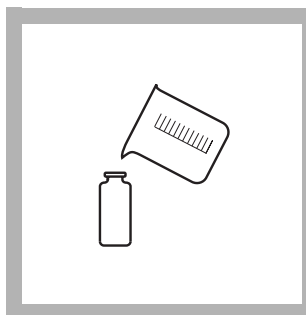
Note: Substitute the contents of one Amino Acid Reagent Powder Pillow for 1 mL of amino acid reagent solution if desired.



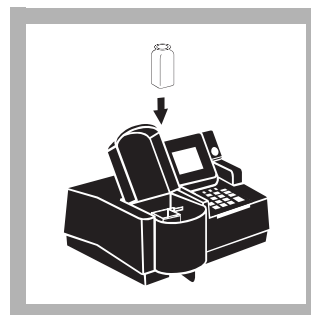
6. Press the soft key under **START TIMER**.

A 10-minute reaction period will begin.

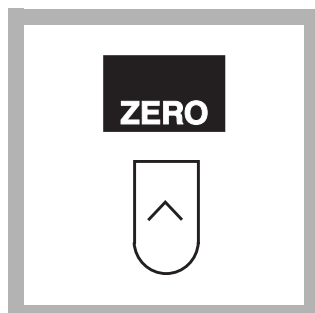
Note: Do Step 7 while the timer is running.



7. Pour 25 mL of sample (the blank) into a sample cell.



8. When the timer beeps, place the blank into the cell holder. Close the light shield.



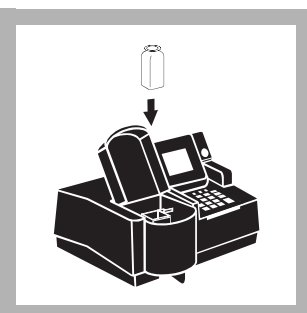
9. Press the soft key under **ZERO**.

The display will show:

0.00 mg/L PO₄³⁻

Note: If you are using a reagent blank correction, the display will show the correction.

Note: For alternate concentration units press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



10. Place the prepared sample into the cell holder. Close the light shield. Results in mg/L PO₄³⁻ (or chosen units) will be displayed.

Note: Results may be expressed as phosphorus (P), or as phosphorus pentoxide (P₂O₅). Press the soft keys under **OPTIONS** and then **FORM** to scroll through the available options.

Interferences

Interfering Substance	Interference Levels and Treatments
Calcium (Ca^{2+})	Greater than 10,000 mg/L as CaCO_3
Chloride	Greater than 150,000 mg/L Cl^-
Colored samples	Add 1 mL of 10 N Sulfuric Acid Standard Solution to another 25-mL sample. Use this instead of untreated sample as the blank to zero the instrument. Use a pipet and pipet filler to measure the sulfuric acid standard.
High salt levels (Na^+)	May cause low results. To eliminate this interference, dilute the sample until two successive dilutions yield about the same result.
Magnesium	Greater than 40,000 mg/L as CaCO_3
Nitrites (NO_2^-)	Bleach the blue color. Remove nitrite interference by adding 0.50 g of sulfamic acid to the sample. Swirl to mix. Continue with Step 4.
Phosphates, high levels (PO_4^{3-})	As the concentration of phosphate increases, the color changes from blue to green, then to yellow and finally to brown. The brown color may suggest a concentration as high as 100,000 mg/L PO_4^{3-} . If a color other than blue is formed, dilute the sample and retest.
Sulfide (S^{2-})	Sulfide interferes. For samples with sulfide concentration less than 5 mg/L sulfide interference may be removed by oxidation with Bromine Water as follows: <ol style="list-style-type: none">1. Measure 25 mL of sample into a sample cell.2. Add Bromine Water drop-wise with constant swirling until permanent yellow color develops.3. Add Phenol Solution drop-wise until the yellow color just disappears. Proceed with Step 4.
Temperature	For best results, sample temperature should be $21 \pm 3^\circ\text{C}$ ($70 \pm 5^\circ\text{F}$).
Turbidity	May give inconsistent results for two reasons. Some suspended particles may dissolve because of the acid used in the test. Also, desorption of orthophosphate from particles may occur. For highly turbid samples, add 1 mL of 10 N Sulfuric Acid Standard Solution to another 25-mL sample. Use this instead of untreated sample as the blank to zero the instrument. Use a pipet and pipet filler to measure the sulfuric acid standard.
Highly buffered samples or extreme sample pH	May exceed the buffering capacity of the reagents and require sample pretreatment

Sample Collection, Storage and Preservation

Collect samples in clean plastic or glass bottles that have been cleaned with 1:1 Hydrochloric Acid Solution and rinsed with deionized water. Do not use a commercial phosphate-based detergent for cleaning glassware because the phosphate content will contaminate the sample.

Analyze samples immediately after collection for best results. If samples cannot be analyzed the same day, adjust the pH to 2 or less by adding about 2 mL of sulfuric acid per liter of sample. Store the sample at 4°C (39°F) or below. Samples can be stored up to 24 hours.

Adjust the acidified sample to about pH 7 just before running the test by adding 5 N Sodium Hydroxide Standard Solution. Mix thoroughly. Warm to room temperature before analyzing.

PHOSPHORUS, Reactive (Orthophosphate), continued

Accuracy Check

Standard Additions Method

- Leave the unspiked sample in the sample compartment. Verify that the units displayed are in mg/L. Select standard additions mode by pressing the soft keys under **OPTIONS, (MORE)** and then **STD ADD**.
- Press **ENTER** to accept the default sample volume (mL), 25.0.
- Press **ENTER** to accept the default standard concentration (mg/L), 500.0.
- Press the soft key under **ENTRY DONE**.
- Snap the neck off a Phosphate 2-mL Ampule Standard, 500-mg/L PO_4^{3-} .
- Use the TenSette Pipet to add 0.1 mL, 0.2 mL and 0.3 mL of standard, respectively to three 25-mL samples and mix each thoroughly.
- Analyze each standard addition sample as described above. Accept the standard additions reading by pressing the soft key under **READ** each time. Each addition should reflect approximately 100% recovery.
- After completing the sequence, the display will show the extrapolated concentration value and the “best-fit” line through the standard additions data points, accounting for matrix interferences.
- See Section 1.4.1 *Standard Additions* for more information.

Standard Solution Method

Use a 10-mg/L Phosphate Standard Solution (Cat. No. 14204-16). Perform the amino acid procedure as described above.

Precision

Standard: 10.00 mg/L PO_4^{3-}

Program	95% Confidence Limits
3010	9.98–10.02 mg/L PO_4^3

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
3010	0.04 mg/L PO_4^3

For more information on derivation and use of Hach’s estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, appendix B, see Section 1.5.1.

Sensitivity

Program Number: 3010

Portion of Curve	ΔAbs	$\Delta\text{Concentration}$
0.010 Abs	0.010	0.201 mg/L
15 mg/L	0.010	0.207 mg/L
27 mg/L	0.010	0.212 mg/L

See Section 1.5.3 *Sensitivity Explained* for more information.

Calibration Standard Preparation

To perform a phosphate calibration using the amino acid method, use a 100-mg/L Phosphate Standard Solution (Cat. No. 14368-16).

Prepare calibration standards containing 5.0, 10.0, 15.0, 20.0, 25.0, and 30.0 mg/L phosphate as follows:

- a. Into six different 100-mL Class A volumetric flasks, pipet 5, 10, 15, 20, 25, and 30 mL of the 100-mg/L Phosphate Standard Solution using Class A glassware.
- b. Dilute to the mark with deionized water. Mix thoroughly.
- c. Using the amino acid method and the calibration procedure described in the *User-Entered Programs* section of the *DR/4000 Spectrophotometer Instrument Manual*, generate a calibration curve from the standards prepared above.

Summary of Method

In a highly acidic solution, ammonium molybdate reacts with orthophosphate to form molybdophosphoric acid. This complex is then reduced by the amino acid reagent to yield an intensely colored molybdenum blue compound.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to *Section 1*.

Pollution Prevention and Waste Management

Please see *Section 1* for more information on proper disposal of these materials.

PHOSPHORUS, Reactive (Orthophosphate), continued

REQUIRED REAGENTS AND STANDARDS

	Cat. No.
High Range Reactive Phosphorus Reagent Set (100 tests)	22441-00
Includes: (1) 1934-32, (1) 2236-32	

Description	Quantity Required		Cat. No.
	Per Test	Unit	
Amino Acid Reagent	1 mL ... 100 mL	MDB*	1934-32
Molybdate Reagent	1 mL ... 100 mL	MDB*	2236-32

REQUIRED EQUIPMENT AND SUPPLIES

Cylinder, 25-mL, graduated, mixing	1	each	1896-40
DR/4000 1-Inch Cell Adapter	1	each	48190-00

OPTIONAL REAGENTS AND STANDARDS

Amino Acid Reagent Powder Pillows	100/pkg	804-99
Bromine Water, 30-g/L	29 mL *	2211-20
Hydrochloric Acid Solution, 1:1 (6.0 N)	500 mL	884-49
Phenol Solution, 30-g/L	29 mL	2112-20
Phosphate Standard Solution, 10-mg/L	946 mL	14204-16
Phosphate Standard Solution, 100-mg/L PO ₄ ³⁻	946 mL	14368-16
Phosphate Standard Solution, 2-mL PourRite Ampule, 500-mg/L PO ₄ ³⁻	20/pkg	14242-20
Sodium Chloride Standard Solution, 246-mg/L NaCl	100 mL	23074-42
Sodium Hydroxide Standard Solution, 5.0 N	100 mL MDB*	2450-32
Sulfamic Acid, ACS	113 g	2344-14
Sulfuric Acid Standard Solution, 10 N	1000 mL	931-53
Water, deionized	4 liters	272-56

OPTIONAL EQUIPMENT AND SUPPLIES

DR/4000 Carousel Module Kit	each	48070-02
DR/4000 Flow Cell Module Kit, 1-inch	each	48070-04
DR/4000 Flow Cell Module Kit, 1-cm	each	48070-05
DR/4000 Sipper Module Kit, 1-inch	each	48090-03
Flask, volumetric, Class A, 100-mL	each	14574-42
Pipet, volumetric, Class A, 5.00-mL	each	14515-37
Pipet, volumetric, Class A, 10.00-mL	each	14515-38
Pipet, volumetric, Class A, 15.00-mL	each	14515-39
Pipet, volumetric, Class A, 20.00-mL	each	14515-20
Pipet, volumetric, Class A, 25.00-mL	each	14515-40
PourRite Ampule Breaker	each	24846-00

* Larger sizes available from Hach.



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Method 8114

Molybdovanadate Method*

Reagent Solution or AccuVac® Ampuls

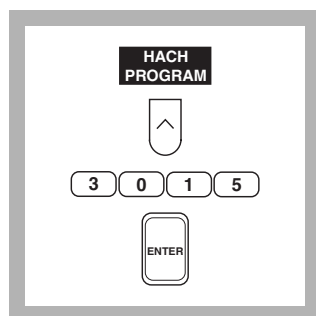
(0 to 45.00 mg/L PO₄³⁻)

Scope and Application: For water and wastewater.

The estimated detection limits for program numbers 3015 and 3020 are 0.09 and 0.24 mg/L PO₄³⁻, respectively.

* Adapted from *Standard Methods for the Examination of Water and Wastewater*.

Using Reagent Solution



- 1.** Press the soft key under **HACH PROGRAM**.

Select the stored program number for reactive phosphorus, molybdovan-adate method by pressing **3015** with the numeric keys.

Press: **ENTER**

Note: If samples cannot be analyzed immediately, see Sample Collection, Storage and Preservation following these steps. Adjust the pH of preserved samples before analysis.

Note: The Flow Cell and Sipper Modules can be used with this procedure.



- 2.** The display will show:
HACH PROGRAM: 3015
P React. Mo. HR

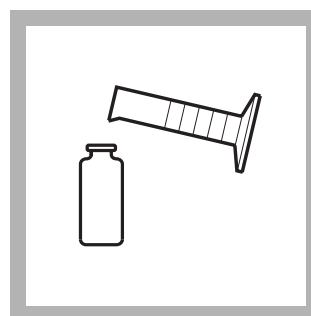
The wavelength (λ), **430 nm**, is automatically selected.



- 3.** Fill a sample cell (the blank) with 25 mL of deionized water with a 25-mL graduated cylinder.

Note: For non-preserved samples with extreme pH, see the Interferences section.

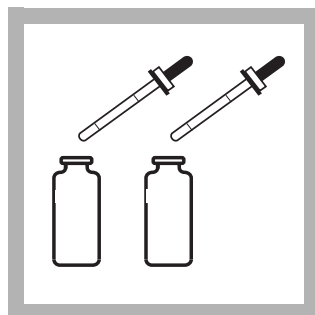
Note: For best results, sample temperature should be 20–25 °C.



- 4.** Fill a sample cell (the prepared sample) with 25 mL of sample with a 25-mL graduated cylinder.

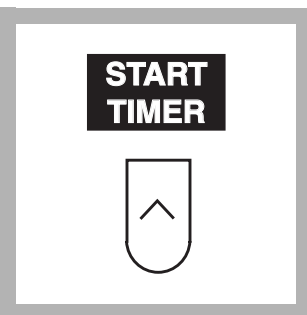
Note: For proof of accuracy, use a 10.0-mg/L phosphate (3.3-mg/L phosphorus) standard solution (see the Accuracy Check section) in place of the sample.

PHOSPHORUS, Reactive (Orthophosphate), continued



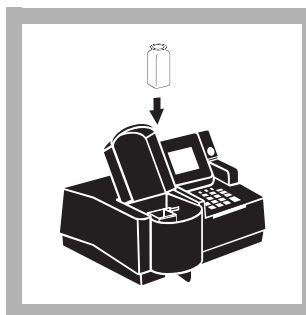
5. Add 1.0 mL of Molybdovanadate Reagent to each sample cell. Swirl to mix.

Note: A yellow color will form if phosphate is present. A small amount of yellow will be present in the blank because of the reagent.

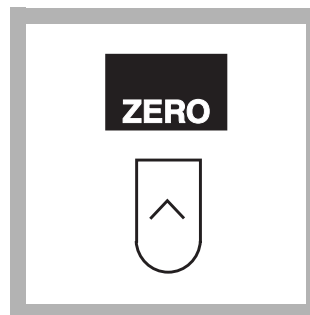


6. Press the soft key under **START TIMER**.

A 5-minute reaction period will begin.



7. When the timer beeps, place the blank into the cell holder. Close the light shield.

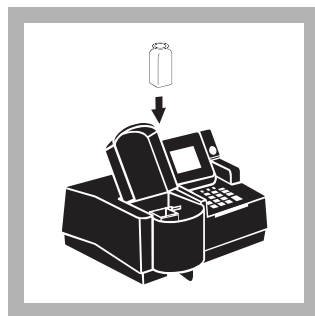


8. Press the soft key under **ZERO**.

The display will show:

0.00 mg/L PO₄³⁻

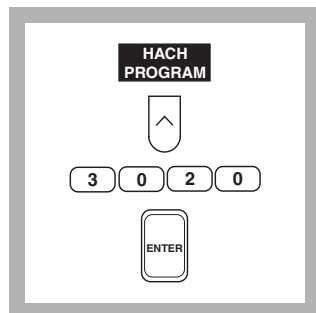
Note: For alternate concentration units press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen



9. Place the prepared sample into the cell holder. Close the light shield. Result in mg/L PO₄³⁻ (or chosen units) will be displayed.

Note: The results can be expressed as PO₄³⁻, P, or P₂O₅. Press the soft keys under **OPTIONS**, then **FORM**: to scroll through the available options.

Using AccuVac Ampuls



1. Press the soft key under **HACH PROGRAM**.

Select the stored program number for reactive phosphorus, molybdovan-adate method by pressing **3020** with the numeric keys.

Press: **ENTER**

Note: If samples cannot be analyzed immediately, see Sample Collection, Storage and Preservation following these steps. Adjust the pH of preserved samples before analysis.



2. The display will show:
HACH PROGRAM:
3020

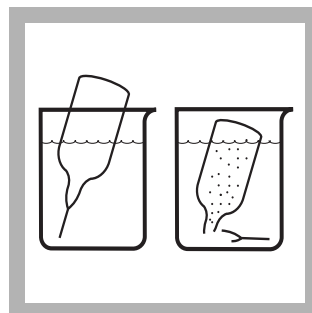
P React. Mo. HR AV

The wavelength (λ), **430 nm**, is automatically selected.



3. Collect at least 40 mL of sample in a 50-mL beaker. Pour at least 40 mL of deionized water into a second beaker.

Note: For best results, sample temperature should be 20-25 °C.

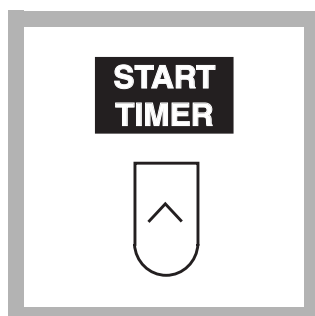


4. Fill a Molyb-dovanadate Reagent AccuVac Ampul with sample by breaking the tip on the bottom of the beaker. Fill a second AccuVac Ampul with deionized water (the blank) in the same manner.

Note: Keep the tip immersed while the ampul fills completely.

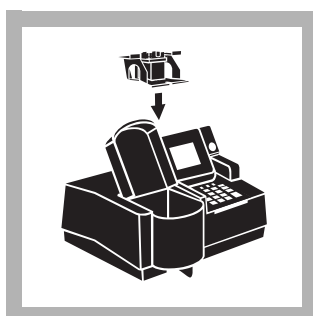
Note: For proof of accuracy, use a 10.0-mg/L phosphate (3.3-mg/L phosphorus) standard solution (see the Accuracy Check section) in place of the sample.

Note: A yellow color will form if phosphate is present. A small amount of yellow will be present in the blank because of the reagent.

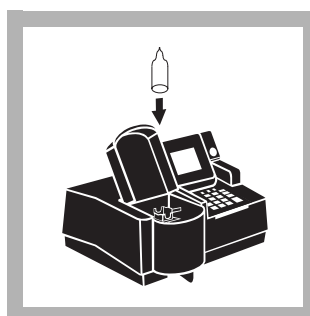


5. Press the soft key under **START TIMER**.

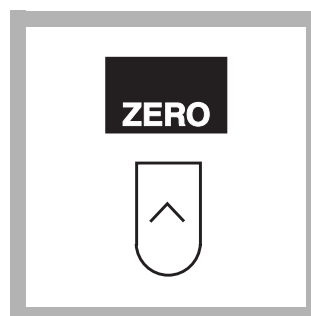
A 5-minute reaction period will begin.



6. During the reaction period, insert the 1-Inch Cell Adapter into the sample cell module by sliding it under the thumb screw and into the alignment grooves. Fasten with the thumb screw.



7. When the timer beeps, place the blank into the cell holder. Close the light shield.

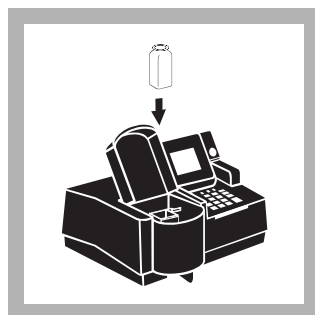


8. Press the soft key under **ZERO**.

The display will show:

0.00 mg/L PO₄³⁻

Note: For alternate concentration units press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



9. Place the prepared sample into the cell holder. Close the light shield. Result in mg/L PO₄³⁻ (or chosen units) will be displayed.

Note: The results can be expressed as PO₄³⁻, P, or P₂O₅. Press the soft keys under **OPTIONS**, then **FORM**: to scroll through the available options.

Interferences

Interfering Substance	Interference Levels and Treatments
Arsenate	Only interferes if sample is heated
Iron, ferrous	Blue color caused by ferrous iron does not interfere if concentration is less than 100 mg/L.
Molybdate	Causes negative interference above 1000 mg/L
Silica	Only interferes if sample is heated
Sulfide	Causes a negative interference. <ol style="list-style-type: none"> 1. Measure 50 mL of sample into an Erlenmeyer flask. 2. Add Bromine Water drop-wise with constant swirling until a permanent yellow color develops. 3. Add Phenol Solution drop-wise until the yellow color just disappears. Proceed with Step 4 (Step 3 if using AccuVac procedure).
pH, extreme or highly buffered samples	May exceed buffering capacity of reagents. See Section 1.3.1 <i>pH Interference</i> . May require pretreatment. pH should be about 7.
Fluoride, thorium, bismuth, thiosulfate or thiocyanate	Cause negative interference
Pyrophosphate, tetraborate selenate, benzoate, citrate, oxalate, lactate, tartrate, formate, salicylate, Al ³⁺ , Fe ³⁺ , Mg ²⁺ , Ca ²⁺ , Ba ²⁺ , Sr ²⁺ , Li ⁺ , Na ⁺ , K ⁺ , NH ₄ ⁺ , Cd ²⁺ , Mn ²⁺ , NO ₃ ⁻ , NO ₂ ⁻ , SO ₄ ²⁻ , SO ₃ ²⁻ , Pb ²⁺ , Hg ⁺ , Hg ²⁺ , Sn ²⁺ , Cu ²⁺ , Ni ²⁺ , Ag ⁺ , U ⁴⁺ , Zr ⁴⁺ , AsO ₃ ⁻ , Br ⁻ , CO ₃ ²⁻ , ClO ₄ ⁻ , CN ⁻ , IO ₃ ⁻ , SiO ₄ ⁴⁻ .	Do not interfere in concentrations up to 1000 mg/L

Sample Collection, Storage and Preservation

Collect samples in clean plastic or glass bottles that have been cleaned with 1:1 Hydrochloric Acid Solution and rinsed with deionized water. Do not use a commercial detergent because the phosphate content will contaminate the sample.

If samples cannot be analyzed the same day, adjust the pH to 2 or less by adding about 2 mL of concentrated sulfuric acid per liter of sample. Store the sample at 4 °C (39 °F) or below. Samples can be stored up to 24 hours.

Just before analysis, warm to room temperature and adjust the acidified sample to about pH 7 by adding 5 N Sodium Hydroxide Standard Solution. Mix thoroughly.

Accuracy Check

Standard Additions Method

- a. Leave the unspiked sample in the sample compartment. Verify that the units displayed are in mg/L. Select standard additions mode by pressing the soft keys under **OPTIONS, (MORE)** and then **STD ADD**.
- b. Press **ENTER** to accept the default sample volume (mL), 25.0.
- c. Press **ENTER** to accept the default standard concentration (mg/L) 500.00.
- d. Press the soft key under **ENTRY DONE**.
- e. Snap the neck off a Phosphate 2-mL Ampule Standard, 500-mg/L PO_4^{3-} .
- f. Use the TenSette Pipet to add 0.1 mL, 0.2 mL and 0.3 mL of standard, respectively to three 25-mL samples and mix each thoroughly.
- g. Analyze each standard addition sample as described above. Accept the standard additions reading by pressing the soft key under **READ** each time. Each addition should reflect approximately 100% recovery.
- h. After completing the sequence, the display will show the extrapolated concentration value and the “best-fit” line through the standard additions data points, accounting for matrix interferences.
- i. See Section 1.4.1 *Standard Additions* for more information.

Standard Solution Method

Prepare a 10-mg/L phosphate standard solution by pipetting 5.0 mL of Phosphate Standard Solution, 100-mg/L, into a 50-mL volumetric flask. Dilute to the mark with deionized water. Prepare this solution daily. Perform the molybdovanadate procedure as described above.

Method Performance

Precision

Standard: 10.0 mg/L PO_4^{3-}

Program	95% Confidence Limits
3015	9.80–10.20 mg/L PO_4^{3-}
3020	9.50–10.50 mg/L PO_4^{3-}

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
3015	0.09 mg/L PO_4^{3-}
3020	0.24 mg/L PO_4^{3-}

For more information on derivation and use of Hach’s estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, appendix B, see Section 1.5.1.

Sensitivity

Program Number: 3015

Portion of Curve	Δ Abs	Δ Concentration
0.010 Abs	0.010	0.318 mg/L
22.5 mg/L	0.010	0.305 mg/L
40.5 mg/L	0.010	0.349 mg/L

Program Number: 3020

Portion of Curve	Δ Abs	Δ Concentration
0.010 Abs	0.010	0.313 mg/L
22.5 mg/L	0.010	0.350 mg/L
40.5 mg/L	0.010	0.378 mg/L

See Section 1.5.3 *Sensitivity Explained* for more information.

Calibration Standard Preparation

To perform a phosphate calibration using the molybdovanadate method, prepare calibration standards containing 5.0, 10.0, 15.0, 20.0, 25.0, and 50.0 mg/L phosphorus as follows:

- Into six different 100-mL Class A volumetric flasks, pipet 5, 10, 15, 20, 25, and 50 mL of the 100-mg/L Phosphate Standard Solution (Cat. No. 14368-16) using Class A glassware.
- Dilute to the mark with deionized water. Mix thoroughly.
- Using the molybdovanadate method and the calibration procedure described in the *User-Entered Programs* section of the *DR/4000 Spectrophotometer Instrument Manual*, generate a calibration curve from the standards prepared above.

Summary of Method

In the molybdovanadate method, orthophosphate reacts with molybdate in an acid medium to produce a phosphomolybdate complex. In the presence of vanadium, yellow vanadomolybdophosphoric acid is formed. The intensity of the yellow color is proportional to the phosphate concentration.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to *Section 1*.

Pollution Prevention and Waste Management

Please see *Section 1* for more information on proper disposal of these materials.

PHOSPHORUS, Reactive (Orthophosphate), continued

REQUIRED REAGENTS AND STANDARDS (Using Solution)

Description	Quantity Required		Unit	Cat. No.
	Per Test			
Molybdovanadate Reagent	2.0 mL.....	100 mL*	MDB	20760-32
Water, deionized	25 mL	4 liters.....		272-56

REQUIRED EQUIPMENT AND SUPPLIES (Using Solution)

Cylinder, graduated, 25-mL	1	each.....	508-40
DR/4000 1-Inch Cell Adapter	1	each.....	48190-00

REQUIRED REAGENTS AND STANDARDS (Using AccuVac Ampuls)

Molybdovanadate Reagent AccuVac Ampuls.....	2	25/pkg.....	25250-25
---	---------	-------------	----------

REQUIRED EQUIPMENT AND SUPPLIES (Using AccuVac Ampuls)

Beaker, 50-mL.....	2	each.....	500-41
DR/4000 AccuVac Ampul Adapter.....	1	each.....	48187-00

OPTIONAL REAGENTS AND STANDARDS

Bromine Water	29 mL*	2211-20
Hydrochloric Acid Solution, 1:1 (6.0 N).....	500 mL.....	884-49
Phenol Solution, 30-g/L	29 mL.....	2112-20
Phosphate Standard Solution, 10-mg/L as PO_4^{3-}	946 mL.....	14204-16
Phosphate Standard Solution, 100-mg/L as PO_4^{3-}	946 mL.....	14368-16
Phosphate Standard Solution, 2-mL PourRite Ampule, 500-mg/L as PO_4^{3-}	20/pkg.....	14242-20
Sodium Chloride, ACS.....	454 g.....	182-01
Sodium Hydroxide Standard Solution, 5.0 N.....	100 mL MDB.....	2450-32
Sulfuric Acid, ACS, concentrated	500 mL*	979-49

OPTIONAL EQUIPMENT AND SUPPLIES

AccuVac Snapper Kit	each.....	24052-00
DR/4000 Carousel Module Kit	each.....	48070-02
DR/4000 Flow Cell Module Kit, 1-inch.....	each.....	48070-04
DR/4000 Flow Cell Module Kit, 1-cm.....	each.....	48070-05
DR/4000 Sipper Module Kit, 1-inch.....	each.....	48090-03
Dispenser, fixed-volume, 1.0-mL Repipet Jr.....	each.....	21113-02
Flask, Erlenmeyer, 125-mL.....	each.....	505-43
Flask, volumetric, Class A, 100-mL	each.....	14574-42
pH Paper, pH 1.0 to 11.0	5 rolls/pkg.....	391-33
pH Meter, EC10, portable	each.....	50050-00
Pipet, serological, 2.0-mL	each.....	532-36
Pipet, TenSette, 0.1 to 1.0 mL	each.....	19700-01
Pipet Tips, for 19700-01 TenSette Pipet	50/pkg.....	21856-96
Pipet, volumetric, Class A, 5.00-mL	each.....	14515-37
Pipet, volumetric, Class A, 10.00-mL	each.....	14515-38
Pipet, volumetric, Class A, 15.00-mL	each.....	14515-39
Pipet, volumetric, Class A, 20.00-mL	each.....	14515-20
Pipet, volumetric, Class A, 25.00-mL	each.....	14515-40
Pipet, volumetric, Class A, 50.00-mL	each.....	14515-41
Pipet Filler.....	each.....	12189-00
PourRite Ampule Breaker	each.....	24846-00
Spoon, measuring, 0.1-g.....	each.....	511-00

* Contact Hach for larger sizes.



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Telephone: (970) 669-3050
FAX: (970) 669-2932



Method 8114

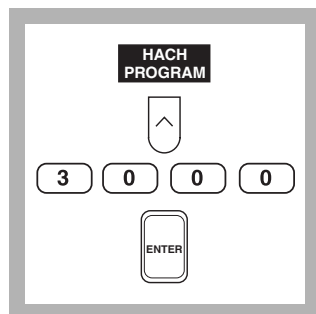
Molybdovanadate Method*

Test 'N Tube™ Vials

HR (0.0 to 100.0 mg/L PO₄³⁻)

Scope and Application: For water and wastewater

* Adapted from *Standard Methods for the Examination of Water and Wastewater*.



1. Press the soft key under **HACH PROGRAM**.

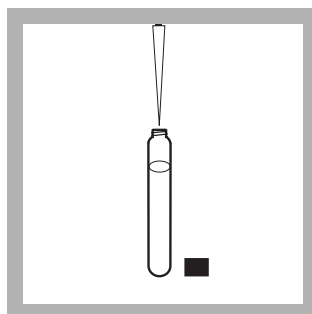
Select the stored program number for phosphorus, reactive high range, Test 'N Tube.

Press: **3000 ENTER**



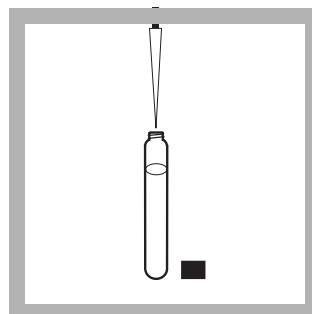
2. The display will show:
**HACH PROGRAM: 3000
P React. HR TNT**

The wavelength (λ), **420 nm**, is automatically selected.



3. Use a TenSette Pipet to add 5.0 mL of deionized water to a Reactive High Range Phosphorus Test 'N Tube Vial (the blank).

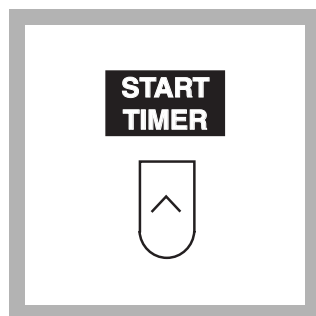
Cap and invert to mix.



4. Use a TenSette Pipet to add 5.0 mL of sample to a Reactive High Range Phosphorus Test 'N Tube Vial (the sample).

Cap and invert to mix.

Note: For non-preserved samples with extreme pH, see Interferences following these steps.



5. Press the soft key under **START TIMER**.

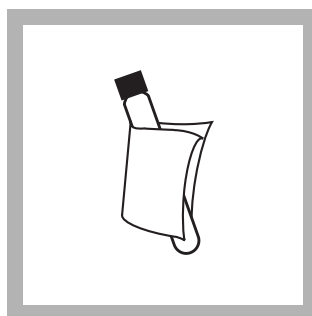
A 7-minute reaction time will begin.

Note: Read samples between 7 and 9 minutes.

Note: This reaction time is for samples at 23 °C (73 °F). If the sample temperature is 13 °C (55 °F), wait 15 minutes. If the sample temperature is 33 °C (91 °F), wait two minutes.

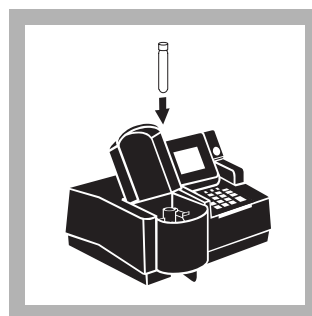


6. Insert the Test Tube Adapter into the sample cell module by sliding it under the thumb screw and into the alignment grooves. Fasten with the thumb screw.

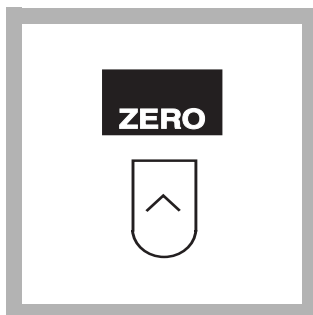


7. Clean the outside of the vials with a towel.

Note: Wipe with a damp towel, followed by a dry one, to remove fingerprints or other marks.



8. When the timer sounds, place the blank vial into the cell holder and close the light shield.



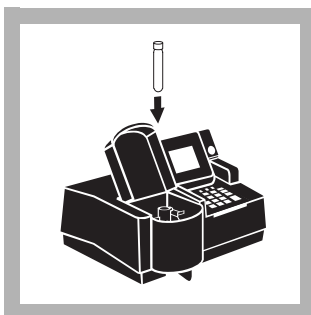
9. Press the soft key under **ZERO**.

The display will show:

0.0 mg/L PO₄³⁻

Note: For alternate concentration units press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.

Note: Reagent blanks for each lot of reagents may be used more than once. At room temperature, the reagent blank is stable for as long as three weeks; then prepare a new one.



10. Place the sample vial into the cell holder and close the light shield. The results in mg/L PO₄³⁻ (or chosen units) will be displayed.

Note: Results may be expressed as phosphorus (P) or as phosphorus pentoxide (P₂O₅). Press the soft keys under **OPTIONS** and then **FORM**: to scroll through the available options.

Interferences

Large amounts of sample turbidity may cause inconsistent results in the test because the acid present in the reagents may dissolve some of the suspended particles and because of variable desorption of orthophosphate from the particles. The following may interfere when present in concentrations exceeding the values listed in the following table:

Table 1 Interfering Substances and Suggested Treatments

Interfering Substance	Interference Level and Treatment
Arsenate	Only interferes if the sample is heated.*
Iron, ferrous	Blue color caused by ferrous iron does not interfere if iron concentration is less than 100 mg/L.
Molybdate	Causes negative interference above 1000 mg/L.
Silica	Only interferes if the sample is heated.*
Sulfide	Causes a negative interference. Remove interference as follows: <ol style="list-style-type: none">1. Measure 25 mL of sample into a 50-mL beaker.2. Add Bromine Water drop-wise with constant swirling until a permanent yellow color develops.3. Add Phenol Solution drop-wise until the yellow color just disappears. Proceed with step 1.

Table 1 Interfering Substances and Suggested Treatments (Continued)

Interfering Substance	Interference Level and Treatment
Extreme pH or highly buffered samples	May exceed buffering capacity of the reagents. See <i>pH Interferences</i> . Samples may require pretreatment. Sample pH should be about 7.
Fluoride, thorium, bismuth, thiosulfate or thiocyanate	Cause a negative interference.
Temperature, Cold (less than 20 °C)	Causes a negative interference.
Temperature, Hot (greater than 25 °C)	Causes a positive interference.
The following do not interfere in concentrations up to 1000 mg/L: Pyrophosphate, tetraborate, selenate, benzoate, citrate, oxalate, lactate, tartrate, formate, salicylate, Al^{3+} , Fe^{3+} , Mg^{2+} , Ca^{2+} , Ba^{2+} , Sr^{2+} , Li^{+} , Na^{+} , K^{+} , NH_4^{+} , Cd^{2+} , Mn^{2+} , NO_3^{-} , NO_2^{-} , SO_4^{2-} , SO_3^{2-} , Pb^{2+} , Hg^{+} , Hg^{2+} , Sn^{2+} , Cu^{2+} , Ni^{2+} , Ag^{+} , U^{4+} , Zr^{4+} , AsO_3^{-} , Br^{-} , CO_3^{2-} , ClO_4^{-} , CN^{-} , IO_3^{-} , SiO_4^{4-} .	

* Gentle warming of the sample to reach room temperature will not cause this substance to interfere.

Sampling and Storage

Collect sample in plastic or glass bottles that have been cleaned with 1:1 Hydrochloric Acid Solution and rinsed with deionized water. Do not use commercial detergents containing phosphate for cleaning glassware used in this test.

For best results, analyze the samples immediately after collection. If prompt analysis is impossible, preserve the samples for up to 48 hours by filtering immediately and storing at 4 °C. The sample should have a neutral (6–8) pH and be at room temperature before analysis.

Accuracy Check

Standard Additions Method

Note: Clean glassware with 1:1 Hydrochloric Acid Solution. Rinse again with deionized water. Do not use detergents containing phosphate to clean glassware.

- Leave the unspiked sample (from *step 10* in the procedure) in the sample compartment. Verify that the units displayed are in mg/L. Select standard additions mode by pressing the soft keys under **OPTIONS, (MORE)** and then **STD ADD**.
- Press **ENTER** to accept the default sample volume (mL), 10.
- Press **ENTER** to accept the default standard concentration, 500-mg/L PO_4^{3-} .
- Press the soft key under **ENTRY DONE**.
- Fill each of three 10-mL graduated mixing cylinders with 10 mL of sample.
- Snap the neck off a 10-mL Voluette Ampule of Phosphate Standard Solution, 500-mg/L PO_4^{3-} (Cat. No. 14242-10).
- Use the TenSette Pipet to add 0.1, 0.2 mL and 0.3 mL of standard, respectively to three 10-mL samples prepared in *step e*. Mix well.

- h. Analyze each standard addition sample from *step g* as described in the procedure. Accept the standard additions reading by pressing the soft key under **READ** each time. Each addition should reflect approximately 100% recovery, or an increase of 5 mg/L PO_4^{3-} for each 0.1 mL of standard added.
- i. After completing the sequence, the display will show the extrapolated concentration value and the “best-fit” line through the standard additions data points, accounting for matrix interferences.
- j. See *Section 1.4.1, Standard Additions*, for more information.

Standard Solution Method

To check accuracy, prepare a 80-mg/L PO_4^{3-} standard by pipetting 8.0 mL of a 500-mg/L as PO_4^{3-} Voluette Ampule Phosphate Standard Solution into an acid-cleaned 50-mL Class A volumetric flask. Fill to the line with deionized water. Substitute this standard for the sample and perform the procedure as described.

Standard Adjust

To adjust the calibration curve using the reading obtained with the 80-mg/L PO_4^{3-} Phosphate Standard Solution, press the soft keys under **OPTIONS, (MORE)** then **STD:OFF**. Press **ENTER** to accept the default concentration, 80.0 mg/L PO_4^{3-} . If an alternate concentration is used, enter the actual concentration and press **ENTER** to return to the read screen. (For more information, see *Section 1.5.5, Adjusting the Standard Curve*.)

Method Performance

Precision

Standard: 80.0 mg/L PO_4^{3-}

Program	95% Confidence Limits
3000	78.0–82.0 mg/L PO_4^{3-}

For more information on determining precision data and method detection limits, refer to *Section 1.5*.

Estimated Detection Limit

Program	EDL
3000	5.0 mg/L PO_4^{3-}

For more information on derivation and use of Hach’s estimated detection limit, see *Section 1.5.2*. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, appendix B, see *Section 1.5.1*.

Sensitivity

Program Number: 3000

Portion of Curve	Δ Abs	Δ Concentration
Entire Range	0.010	0.7 mg/L

See *Section 1.5.3, Sensitivity Explained*, for more information.

Calibration Standard Preparation

To perform a phosphate calibration using the Reactive High Range Phosphorus Test 'N Tube method, prepare calibration standards containing 10, 25, 50, 75, and 100 mg/L phosphate as follows:

- a. Into a 500-mL Class A volumetric flask, pipet 10.0 mL of 500-mg/L Phosphate Standard Solution using a Class A pipet.
- b. Into four different 100-mL Class A volumetric flasks, pipet 5.0, 10.0, 15.0, and 20.0 mL of a 500 mg/L Phosphate Standard Solution (Cat. No. 14242-32) using Class A glassware.
- c. Dilute to the mark with deionized water. Mix thoroughly.
- d. Using the Reactive High Range Phosphorus Test 'N Tube method and the calibration procedure described in the *User-Entered Programs Section* of the *DR/4000 Spectrophotometer Instrument Manual*, generate a calibration curve from the standards prepared above.

Sample Disposal Information

Final samples will contain molybdenum. In addition, final samples will have a pH less than 2 and are considered corrosive (D002) by the Federal RCRA. Consult the Material Data Safety Data Sheet for information specific to the reagents used.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the Material Safety Data Sheet for information specific to the reagents used.

Summary of Method

Orthophosphate reacts with molybdate in an acid medium to produce a phosphomolybdate complex. In the presence of vanadium, yellow vanadomolybdophosphoric acid forms. The intensity of the yellow color is proportional to the phosphate concentration.

PHOSPHORUS, Reactive, continued

REQUIRED REAGENTS

High Range Reactive Phosphorus Test 'N Tube™ Reagent Set 50 vials 27673-45
Includes: (50) Reactive High Range Phosphorus Test 'N Tube Vials*, (2) 272-42

Description	Quantity Required		Cat. No.
	Per Test	Unit	
Reactive High Range Phosphorus Test 'N Tube™ Vials	1	50/pkg	*
Water, deionized		100 mL	272-42

REQUIRED APPARATUS

Dropper, LDPE, 0.5–1.0 mL	1	20/pkg	21247-20
DR/4000 Test Tube Adapter	1	each	48189-00
Pipet, TenSette®, 1 to 10 mL	1	each	19700-10
Pipet Tips, for 19700-10 TenSette Pipet	1	50/pkg	21997-96
Test Tube Rack	1–3	each	18641-00

OPTIONAL REAGENTS

Bromine Water, 30-g/L	29 mL**	2211-20
Hydrochloric Acid Standard Solution, 6.0 N (1:1)	500 mL	884-49
Phenol Solution, 30-g/L	29 mL	2112-20
Phosphate Standard Solution, 500-mg/L as PO ₄ ³⁻ ,	100 mL	14242-32
Phosphate Standard Solution, PourRite™ ampule, 500-mg/L as PO ₄ ³⁻ , 2-mL	20/pkg	14242-20
Phosphate Standard Solution, Voluette™ ampule, 500-mg/L as PO ₄ ³⁻ , 10-mL	16/pkg	14242-10

OPTIONAL APPARATUS

Ampule Breaker Kit	each	21968-00
Aspirator, vacuum	each	2131-00
Cylinder, graduated, mixing, 10-mL, 3 required	each	20886-38
Filter Holder, 47-mm, 300-mL, graduated	each	13529-00
Filter, membrane, 47-mm, 0.45-microns	100/pkg	13530-00
Flask, filtering, 500-mL	each	546-49
Flask, volumetric, Class A, 50-mL	each	14574-41
Flask, volumetric, Class A, 100-mL	each	14574-42
Flask, volumetric, Class A, 500-mL	each	14574-49
pH Indicator Paper, 1 to 11 pH units	5 rolls/pkg	391-33
pH Meter, <i>sensio</i> ™1, portable	each	51700-10
Pipet, TenSette®, 0.1 to 1.0 mL	each	19700-01
Pipet Tips, for 19700-01 TenSette® Pipet	50/pkg	21856-96
Pipet Tips, for 19700-10 TenSette® Pipet	250/pkg	21997-25
Pipet, volumetric, Class A, 5.00-mL	each	14515-37
Pipet, volumetric, Class A, 8.00-mL	each	14515-08
Pipet, volumetric, Class A, 10.0-mL	each	14515-38
Pipet, volumetric, Class A, 15.0-mL	each	14515-39
Pipet, volumetric, Class A, 20.0-mL	each	14515-20
PourRite™ Ampule Breaker	each	24846-00

* These items are not sold separately.

** Larger sizes available.



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✓ Method 8048

PhosVer 3 (Ascorbic Acid) Method*

Powder Pillows or AccuVac® Ampuls

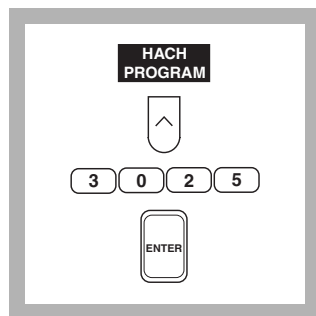
(0 to 2,500 mg/L PO_4^{3-})

Scope and Application: For water, wastewater, seawater; USEPA Accepted for reporting for wastewater analyses**. The estimated detection limits for program numbers 3025 and numbered 3030 are 0.045 and 0.031 mg/L PO_4^{3-} respectively.

* Adapted from *Standard Methods for the Examination of Water and Wastewater*

** Procedure is equivalent to USEPA method 365.2 and Standard Method 4500-P-E for wastewater.

Using Powder Pillows



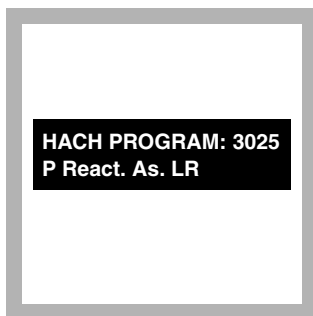
- 1.** Press the soft key under **HACH PROGRAM**.

Select the stored program number for phosphorus, ascorbic acid method by pressing **3025** with the numeric keys.

Press: **ENTER**

Note: If samples cannot be analyzed immediately, see *Sample Collection, Storage and Preservation* following these steps.

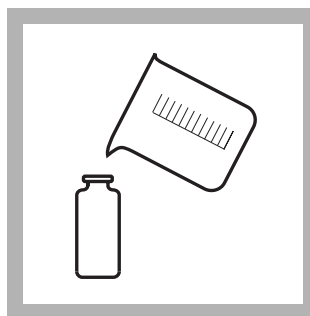
Note: The Flow Cell and Sipper Modules can be used with this procedure. Use a 25-mL sample and reagents with the Flow Cell Module.



- 2.** The display will show:
**HACH PROGRAM: 3025
P React. As. LR**

The wavelength (λ), **890 nm**, is automatically selected.

Note: For best results, determine a reagent blank for each new lot of reagent as follows. Prepare a reagent blank by repeating steps 3 through 8, using deionized water as the sample. Zero the instrument on deionized water by pressing the soft key under **ZERO**. Insert the reagent blank and the blank value will be displayed. Correct for the reagent blank by pressing the soft keys under **OPTIONS, (MORE)**, and then **BLANK:OFF**. Enter the reagent blank value and press **ENTER**. Repeat for each new lot of reagent.



- 3.** Fill a sample cell with 10-mL of sample.

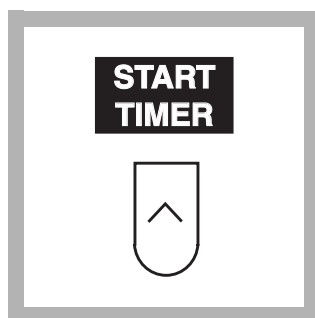
Note: For proof of accuracy, use a 1.0 mg/L Phosphate (0.33 mg/L P) Standard Solution listed under **OPTIONAL REAGENTS AND STANDARDS** in place of the sample.



- 4.** Add the contents of one PhosVer 3 phosphate Powder Pillow to the cell (the prepared sample). Swirl immediately to mix.

Note: A blue color will form if phosphate is present.

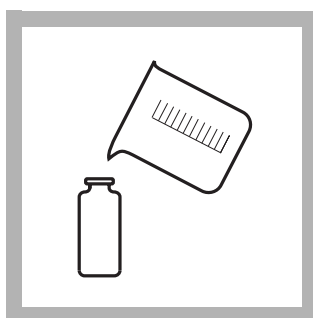
PHOSPHORUS, Reactive (Orthophosphate), continued



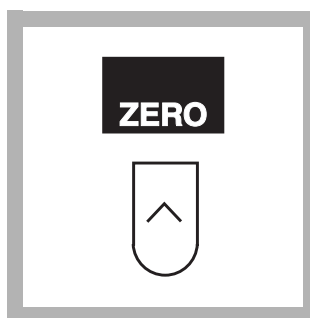
5. Press the soft key under **START TIMER**.

A 2-minute reaction period will begin.

Note: If the sample has been digested using the Acid Persulfate digestion in this manual, this step requires 10 minutes.



6. Fill another sample cell (the blank) with 10 mL of sample. Place it into the cell holder.



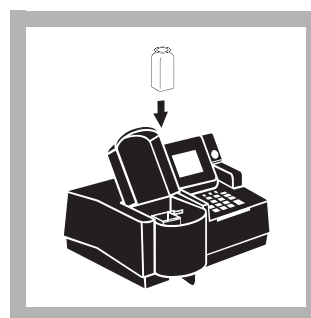
7. When the timer beeps press the soft key under **ZERO**.

The display will show:

0.000 mg/L PO₄³⁻

Note: If you are using a reagent blank correction, the display will show the correction.

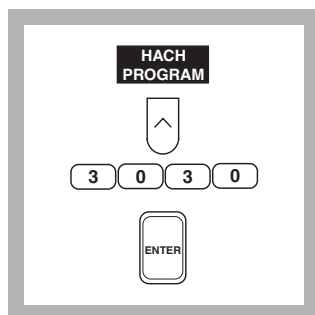
Note: For alternate concentration units press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



8. Place the prepared sample into the cell holder. Close the light shield. Results in mg/L PO₄³⁻ (or chosen units) will be displayed.

Note: The results can be expressed as PO₄³⁻, P or P₂O₅. Press the soft keys under **OPTIONS**, and then **FORM** to scroll through the available options.

Using AccuVac Ampuls



1. Press the soft key under **HACH PROGRAM**.

Select the stored program number for phosphorus, ascorbic acid method by pressing **3030** with the numeric keys.

Press: **ENTER**

Note: If samples cannot be analyzed immediately, see *Sample Collection, Storage and Preservation* following these steps.

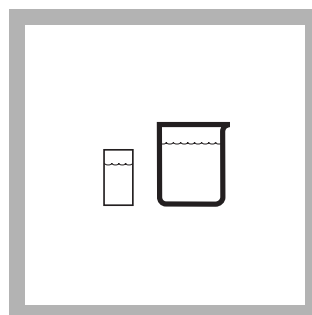
Note: The Flow Cell and Sipper Modules can be used with this procedure.



2. The display will show:
HACH PROGRAM: 3030
P React. As. LR AV

The wavelength (λ), **890 nm**, is automatically selected.

Note: For best results, determine a reagent blank for each new lot of reagent as follows. Prepare a reagent blank by repeating steps 3 through 10, using deionized water as the sample. Zero the instrument on deionized water by pressing the soft key under **ZERO**. Insert the reagent blank and the blank value will be displayed. Correct for the reagent blank by pressing the soft keys under **OPTIONS, (MORE)**, and then **BLANK:OFF**. Enter the reagent blank value and press **ENTER**. Repeat for each new lot of reagent.

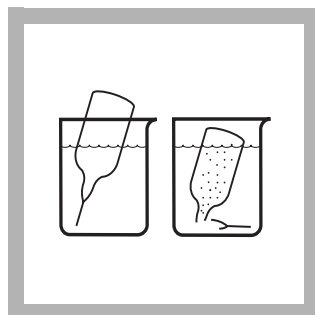


3. Fill a zeroing vial (the blank) with at least 10 mL of sample. Collect at least 40 mL of sample in a 50-mL beaker.



4. Insert the AccuVac Ampul Adapter into the sample cell module by sliding it under the thumb screw and into the alignment grooves. Fasten with the thumb screw.

PHOSPHORUS, Reactive (Orthophosphate), continued



5. Fill a PhosVer 3 Phosphate AccuVac Ampul with sample.

Note: Keep the tip immersed while the ampul fills completely.

30 seconds



6. Place an ampul cap securely over the tip of the ampul. Shake the ampul for approximately 30 seconds. Wipe off any liquid and fingerprints.

Note: A blue color will form if phosphate is present.

Note: Accuracy is unaffected by undissolved powder.

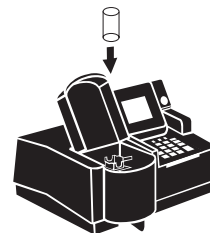
START
TIMER



7. Press the soft key under **START TIMER**.

A 2-minute reaction period will begin.

Note: If the sample has been digested using the Acid Persulfate digestion in this manual, this step requires 10 minutes.



8. When the timer beeps, place the zeroing vial into the cell holder. Close the light shield.

ZERO



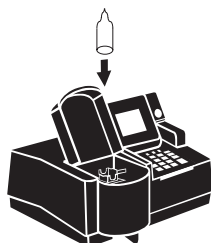
9. Press the soft key under **ZERO**.

The display will show:

0.000 mg/L PO₄³⁻

Note: If you are using a reagent blank correction, the display will show the correction.

Note: For alternate concentration units press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



10. Place the AccuVac Ampul into the cell holder. Close the light shield. Results in mg/L PO₄³⁻ (or chosen units) will be displayed.

Note: The results can be expressed as PO₄³⁻, P or P₂O₅. Press the soft keys under **OPTIONS**, and then **FORM**: to scroll through the available options.

Interferences

Interfering Substance	Interference Levels and Treatments
Aluminum	Greater than 200 mg/L
Arsenate	Interferes at any level
Chromium	Greater than 100 mg/L
Copper	Greater than 10 mg/L
Hydrogen Sulfide	Interferes at any level
Iron	Greater than 100 mg/L
Nickel	Greater than 300 mg/L
pH, excess buffering	Highly buffered samples or extreme sample pH may exceed the buffering capacity of the reagents and require sample pretreatment. pH 2–10 is recommended.
Silica	Greater than 50 mg/L
Silicate	Greater than 10 mg/L
Turbidity (large amounts) or color	May cause inconsistent results because the acid in the powder pillow may dissolve some of the suspended particles and because of variable desorption of orthophosphate from the particles. For highly turbid or colored samples, add the contents of one Phosphate Pretreatment Powder Pillow to 25 mL of sample. Mix well. Use this solution to zero the instrument.
Zinc	Greater than 80 mg/L

Store the PhosVer 3 Phosphate Reagent Powder Pillows and AccuVac Ampuls in a cool, dry environment.

Sample Collection, Storage and Preservation

Collect sample in plastic or glass bottles that have been cleaned with 1:1 Hydrochloric Acid Solution and rinsed with deionized water. Do not use commercial detergents containing phosphate for cleaning glassware used in phosphate analysis.

Most reliable results are obtained when samples are analyzed as soon as possible after collection. If prompt analysis is impossible, preserve samples up to 24 hours by storing at or below 4 °C.

Accuracy Check

Standard Additions Method

- Leave the unspiked sample in the sample compartment. Verify that the units displayed are in mg/L. Select standard additions mode by pressing the soft keys under **OPTIONS, (MORE)** and then **STD ADD**.
- Press **ENTER** to accept the default sample volume (mL), 25.
- Press **ENTER** to accept the default standard concentration (mg/L) 50.
- Press the soft key under **ENTRY DONE**.
- Snap the neck off a Phosphate 2-mL Ampule Standard, 50-mg/L PO_4^{3-} .
- Use the TenSette Pipet to add 0.1, 0.2 mL and 0.3 mL of standard, respectively to three 25-mL samples and mix each thoroughly (for AccuVac Ampuls, use 50-mL beakers).

PHOSPHORUS, Reactive (Orthophosphate), continued

- g. Analyze each standard addition sample as described above (use 10-mL aliquots of the standard addition samples for the powder pillow method). Accept the standard additions reading by pressing the soft key under **READ** each time. Each addition should reflect approximately 100% recovery.
- h. After completing the sequence, the display will show the extrapolated concentration value and the “best-fit” line through the standard additions data points, accounting for matrix interferences.
- i. See Section 1.4.1 *Standard Additions* for more information.

Method Performance

Precision

Standard: 1.000 mg/L PO₄³⁻

Program	95% Confidence Limits
3025	0.979–1.021 mg/L PO ₄ ³⁻
3030	0.985–1.014 mg/L PO ₄ ³⁻

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
3025	0.045 mg/L PO ₄ ³⁻
3030	0.031 mg/L PO ₄ ³⁻

For more information on derivation and use of Hach’s estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, appendix B, see Section 1.5.1.

Sensitivity

Program Number: 3025

Portion of Curve	ΔAbs	ΔConcentration
0.010 Abs	0.010	0.0168 mg/L
1.25 mg/L	0.010	0.0177 mg/L
2.25 mg/L	0.010	0.0185 mg/L

Program Number: 3030

Portion of Curve:	ΔAbs	ΔConcentration
0.010 Abs	0.010	0.0160 mg/L
1.1 mg/L	0.010	0.0193 mg/L
1.98 mg/L	0.010	0.0184 mg/L

See Section 1.5.3 *Sensitivity Explained* for more information.

Calibration Standard Preparation

To perform a phosphate calibration using the ascorbic acid method, use a 10-mg/L Phosphate Standard Solution (Cat. No. 14204-16).

Prepare calibration standards containing 0.300, 1.500, and 2.400 mg/L Phosphate as follows:

- a. Into three different 100-mL Class A volumetric flasks, pipet 3, 15, and 24 mL of the 10-mg/L Phosphate Standard Solution using Class A glassware.
- b. Dilute to the mark with deionized water. Mix thoroughly.
- c. Using the ascorbic acid method and the calibration procedure described in the *User-Entered Programs* section of the *DR/4000 Spectrophotometer Instrument Manual*, generate a calibration curve from the standards prepared above.

Summary of Method

Orthophosphate reacts with molybdate in an acid medium to produce a Phosphomolybdate complex. Ascorbic acid then reduces the complex, giving an intense molybdenum blue color.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to *Section 1*.

Pollution Prevention and Waste Management

Please see *Section 1* for more information on proper disposal of these materials.

PHOSPHORUS, Reactive (Orthophosphate), continued

REQUIRED REAGENTS AND STANDARDS (Using Powder Pillows)

Description	Quantity Required Per Test	Unit	Cat. No.
PhosVer 3 Phosphate Reagent Powder Pillows, 10-mL.....	1 pillow	100/pkg.....	21060-69

REQUIRED REAGENTS AND STANDARDS (Using AccuVac Ampuls)

PhosVer 3 Phosphate Reagent AccuVac Ampuls.....	1 ampul.....	25/pkg.....	25080-25
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REQUIRED EQUIPMENT AND SUPPLIES (Using Powder Pillows)

DR/4000 1-Inch Cell Adapter	1	each.....	48190-00
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REQUIRED EQUIPMENT AND SUPPLIES (Using AccuVac Ampuls)

DR/4000 AccuVac Ampul Adapter.....	1	each.....	48187-00
Beaker, 50-mL.....	1	each.....	500-41
Cap, ampul, blue.....	1	25/pkg.....	1731-25
Sample Cell, 10-mL with cap (zeroing vial).....	1	each.....	21228-00

OPTIONAL REAGENTS AND STANDARDS

Hydrochloric Acid Standard Solution, 6.0 N (1:1)	500 mL.....	884-49
Phosphate Pretreatment Powder Pillows	50/pkg.....	14501-66
Phosphate Standard Solution, 1-mg/L as PO ₄	500 mL.....	2569-49
Phosphate Standard Solution, 10-mg/L as PO ₄	946 mL.....	14204-16
Phosphate Standard Solution, 2-mL PourRite Ampul, 50-mg/L as PO ₄	20/pkg.....	171-20H
Phosphate Standard Solution, 10-mL Voluette Ampul, 50-mg/L as PO ₄	16/pkg.....	171-10
Sodium Chloride, ACS.....	454 g.....	182-01
Sodium Hydroxide Standard Solution, 5.0 N.....	100 mL MDB*.....	2450-32
Water, deionized	4 liters.....	272-56

OPTIONAL EQUIPMENT AND SUPPLIES

AccuVac Snapper Kit	each.....	24052-00
Ampule Breaker Kit	each.....	21968-00
DR/4000 Carousel Module Kit	each.....	48070-02
DR/4000 Flow Cell Module Kit, 1-inch.....	each.....	48070-04
DR/4000 Flow Cell Module Kit, 1-cm.....	each.....	48070-05
DR/4000 Sipper Module Kit, 1-inch	each.....	48090-03
Flask, volumetric, Class, 100-mL	each.....	14574-42
pH Paper, pH 1.0 to 11.0	5 rolls/pkg.....	391-33
pH Meter, <i>sensio</i> TM 1, portable	each.....	51700-00
Pipet, 2-mL, serological	each.....	532-36
Pipet, TenSette, 0.1 to 1.0 mL	each.....	19700-01
Pipet Tips, for 19700-01	50/pkg.....	21856-96
Pipet, volumetric, Class A, 3.00-mL	each.....	14515-03
Pipet, volumetric, Class A, 6.00-mL	each.....	14515-06
Pipet, volumetric, Class A, 9.00-mL	each.....	14515-09
Pipet Filler, safety bulb.....	each.....	14651-00
PourRite Ampule Breaker	each.....	24846-00
Spoon, measuring, 0.1-g.....	each.....	511-00

* Larger sizes available from Hach.



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✓ Method 8048

PhosVer 3 Method

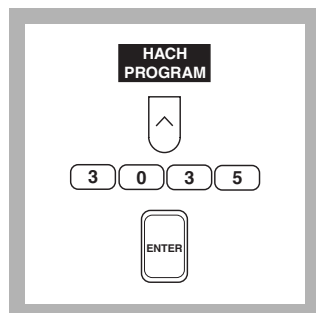
(0.00 to 5.00 mg/L PO_4^{3-})

Test 'N Tube™ Vials

(0.00 to 1.60 mg/L P)

Scope and Application: For water, wastewater and seawater. USEPA accepted for reporting wastewater analysis*. The estimated detection limit for program number 3035 is 0.02 mg/L PO_4^{3-} .

* Procedure is equivalent to USEPA Method 365.2 and Standard Method 4500-P E for wastewater.



1. Press the soft key under **HACH PROGRAM**.

Select the stored program number for Test 'N Tube reactive phosphorus by pressing **3035** with the numeric keys.

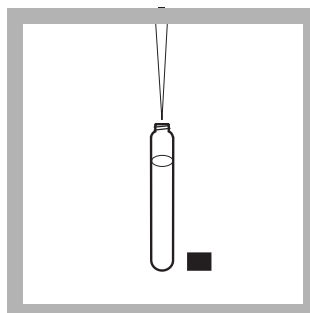
Press: **ENTER**



2. The display will show:
HACH PROGRAM: 3035
P React. As. TNT

The wavelength (λ), **890 nm**, is automatically selected.

Note: For best results, determine a reagent blank for each new lot of reagent as follows. Prepare a reagent blank by repeating steps 3 through 12, using deionized water as the sample. Zero the instrument on deionized water by pressing the soft key under **ZERO**. Insert the reagent blank and the blank value will be displayed. Correct for the reagent blank by pressing the soft keys under **OPTIONS**, (**MORE**), and then **BLANK:OFF**. Enter the reagent blank value and press **ENTER**. Repeat for each new lot of reagent.



3. Use a TenSette Pipet to add 5.0 mL of sample to a Reactive Phosphorus Test 'N Tube Dilution Vial. Cap and mix.

Note: For non-preserved samples with extreme pH, see Interferences.

Note: For proof of accuracy, use a 1.0-mg/L Phosphate (0.33-mg/L P) Standard Solution in place of the sample (see **OPTIONAL REAGENTS**).

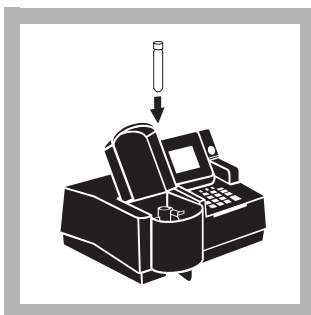


4. Insert the Test Tube Adapter into the sample cell module by sliding it under the thumb screw and into the alignment grooves. Fasten with the thumb screw.

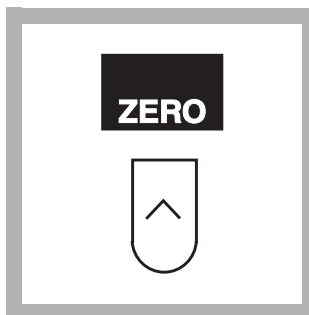


5. Clean the outside of the vial with a towel.

Note: Wiping with a damp towel, followed by a dry one, will remove fingerprints or other marks.



6. Place the vial into the cell holder and close the light shield.



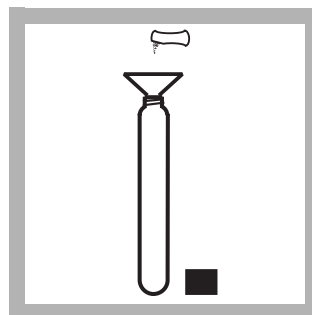
7. Press the soft key under **ZERO**.

The display will show:

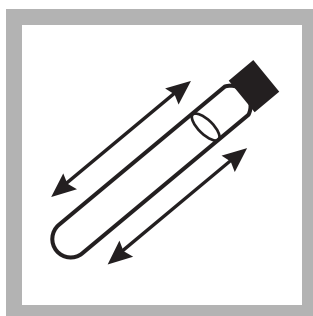
0.00 mg/L PO₄³⁻

Note: If you are using a reagent blank correction, the display will show the correction.

Note: For alternate concentration units press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.

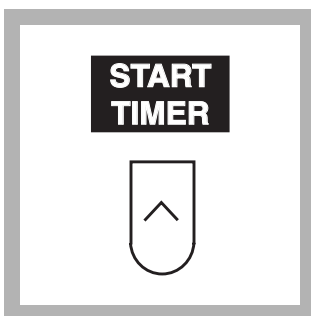


8. Using a funnel, add the contents of one PhosVer 3 Phosphate Powder Pillow to the vial.



9. Cap the vial tightly and shake for 10-15 seconds.

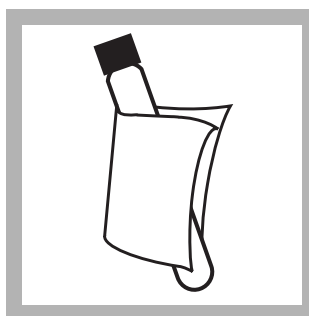
Note: The powder will not dissolve completely.



10. Press the soft key under **START TIMER**.

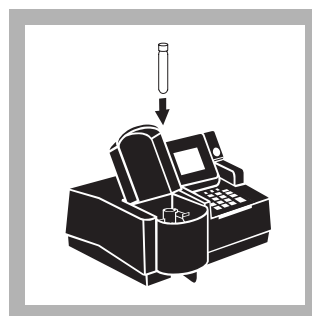
A 2-minute reaction time will begin.

Note: Read samples between 2 and 8 minutes after the addition of the PhosVer 3 reagent.



11. Clean the outside of the vial with a towel.

Note: Wiping with a damp towel, followed by a dry one, will remove fingerprints or other marks.



12. When the timer beeps, place the vial into the cell holder and close the light shield. The results in mg/L PO₄³⁻ (or chosen units) will be displayed.

Note: Results may be expressed as phosphorus (P) or as phosphorus pentoxide (P₂O₅). Press the soft keys under **OPTIONS** and then **FORM**: to scroll through the available options.

Interferences

Interfering Substance	Interference Levels and Treatments
Aluminum	Greater than 200 mg/L
Arsenate	All levels
Chromium	Greater than 100 mg/L
Copper	Greater than 10 mg/L
Iron	Greater than 100 mg/L
Nickel	Greater than 300 mg/L
Silica	Greater than 50 mg/L
Silicate	Greater than 10 mg/L
Sulfide	Greater than 6 mg/L. Remove sulfide interference as follows: <ol style="list-style-type: none"> 1. Measure 25 mL of sample into a 50-mL beaker. 2. Swirling constantly, add Bromine Water drop-wise until a permanent yellow color appears. 3. Swirling constantly, add Phenol Solution drop-wise just until the yellow color disappears. Proceed with <i>step 1</i> of the phosphorus procedure.
Turbidity	Large amounts may cause inconsistent results in the test because the acid present in the powder pillows may dissolve some of the suspended particles and because of variable desorption of orthophosphate from the particles.
Zinc	Greater than 80 mg/L
Highly buffered samples or extreme sample pH	May exceed the buffering capacity of the reagents and require sample pretreatment

The PhosVer 3 Phosphate Reagent Powder Pillows should be stored in a cool, dry environment.

Sample Collection, Storage and Preservation

Collect samples in plastic or glass bottles that have been acid cleaned with 1:1 Hydrochloric Acid Solution and rinsed with deionized water. Do not use commercial detergents containing phosphate for cleaning glassware used in this test.

Analyze samples immediately after collection for best results. If prompt analysis is impossible, preserve samples up to 48 hours by filtering immediately and storing at 4 °C. Warm samples to room temperature before analysis.

Accuracy Check

Standard Additions Method

Note: Clean glassware with 1:1 Hydrochloric Acid Solution. Rinse again with deionized water. Do not use phosphate detergents to clean glassware.

- a. Leave the unspiked sample in the sample compartment. Verify that the units displayed are in mg/L. Select standard additions mode by pressing the soft keys under **OPTIONS, (MORE)** and then **STD ADD**.
- b. Press **ENTER** to accept the default sample volume (mL), 25.0.
- c. Press **ENTER** to accept the default standard concentration (mg/L), 50.00.
- d. Press the soft key under **ENTRY DONE**.
- e. Snap the neck off a Phosphate 2-mL Ampule Standard, 50-mg/L as PO_4^{3-} .
- f. Use the TenSette Pipet to add 0.1 mL, 0.2 mL and 0.3 mL of standard, respectively to three 25-mL samples and mix each thoroughly.
- g. Analyze each standard addition sample as described above (use a 5-mL aliquot of the spiked sample as the sample). Accept the standard additions reading by pressing the soft key under **READ** each time. Each addition should reflect approximately 100% recovery.
- h. After completing the sequence, the display will show the extrapolated concentration value and the “best-fit” line through the standard additions data points, accounting for matrix interferences.
- i. See Section 1.4.1 *Standard Additions* for more information.

Method Performance

Precision

Standard: 5.00 mg/L PO_4^{3-}

Program	95% Confidence Limits
3035	4.20–5.80 mg/L PO_4^{3-}

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
3035	0.01 mg/L PO_4^{3-}

For more information on derivation and use of Hach’s estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, appendix B, see Section 1.5.1.

Sensitivity

Program Number: 3035

Portion of Curve	ΔAbs	$\Delta\text{Concentration}$
Entire Range	0.010	0.061 mg/L

See Section 1.5.3 *Sensitivity Explained* for more information.

Calibration Standard Preparation

To perform a phosphate calibration using the Test 'N Tube method, prepare calibration standards containing 1.0, 2.0, 4.0, and 5.0 mg/L phosphate as follows:

- a. Into four different 50-mL Class A volumetric flasks, pipet 1.0, 2.0, 4.0, and 5.0 mL of a 50-mg/L Phosphate Standard Solution (Cat. No. 171-49) using Class A glassware.
- b. Dilute to the mark with deionized water. Mix thoroughly.
- c. Using the Test 'N Tube method and the calibration procedure described in the *User-Entered Programs* section of the *DR/4000 Spectrophotometer Instrument Manual*, generate a calibration curve from the standards prepared above.

Summary Of Method

Orthophosphate reacts with molybdate in an acid medium to produce a Phosphomolybdate complex. Ascorbic acid then reduces the complex, giving an intense molybdenum blue color.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to *Section 1*.

Pollution Prevention and Waste Management

Final samples will contain molybdenum. In addition, final samples will have a pH less than 2 and are considered corrosive (D002) by the Federal RCRA.

PHOSPHORUS, Reactive, continued

REQUIRED REAGENTS

Reactive Phosphorus Test 'N Tube Reagent Set 50 tests 27425-45
Includes: (1) 21060-46, (50) Reactive Phosphorus Dilution Vials*

Description	Quantity Required		Unit	Cat. No.
	Per Test			
PhosVer 3 Phosphate Reagent Powder Pillows	1		50/pkg	21060-46
Reactive Phosphorus Test 'N Tube Dilution Vials	1		50/pkg	*

REQUIRED APPARATUS

DR/4000 Test Tube Adapter	1	each	48189-00
Funnel, micro	1	each	25843-35
Pipet, TenSette, 1 to 10 mL	1	each	19700-10
Pipet Tips, for 19700-10 TenSette Pipet	1	50/pkg	21997-96
Test Tube Rack	1-3	each	18641-00

OPTIONAL REAGENTS

Bromine Water, 30-g/L	29 mL**	2211-20
Hydrochloric Acid Standard Solution, 6.0 N (1:1)	500 mL	884-49
Phenol Solution, 30-g/L	29 mL	2112-20
Phosphate Standard Solution, 1-mg/L as PO_4^{3-}	500 mL	2569-49
Phosphate Standard Solution, PourRite™ Ampule, 50-mg/L as PO_4^{3-} , 2-mL	16/pkg	171-20H
Phosphate Standard Solution, PourRite™ Ampule, 50-mg/L as PO_4^{3-} , 10-mL	16/pkg	171-10
Phosphate Standard Solution, 50-mg/L	500 mL	171-49
Water, deionized	4 L	272-56

OPTIONAL APPARATUS

Flask, volumetric, Class A, 50-mL	each	14574-41
pH Paper, pH 1.0 to 11.0	5 rolls/pkg	391-33
pH Meter, EC10, portable	each	50050-00
Pipet, TenSette, 0.1 to 1.0 mL	each	19700-01
Pipet Tips, for 19700-01 TenSette® Pipet	50/pkg	21856-96
Pipet Tips, for 19700-10 TenSette® Pipet	1000/pkg	21997-28
Pipet, volumetric, Class A, 2.00-mL	each	14515-36
PourRite™ Ampule Breaker	each	24846-00

* These items are not sold separately.

** Larger sizes available.



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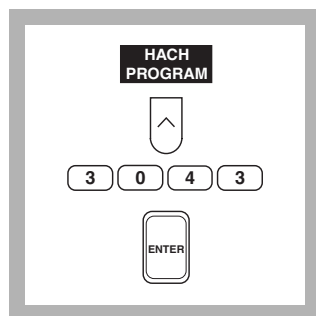


Ascorbic Acid Method

UniCell™ Vials

(0.0 to 15.0 mg/L PO₄³⁻)

Scope and Application: For water, wastewater, boiler water, surface water, and process control. The estimated detection limit for program number 3043 is 1.5 mg/L PO₄³⁻.



1. Press the soft key under **HACH PROGRAM**.

Select the stored program number for UniCell phosphorus by pressing **3043** with the numeric keys.

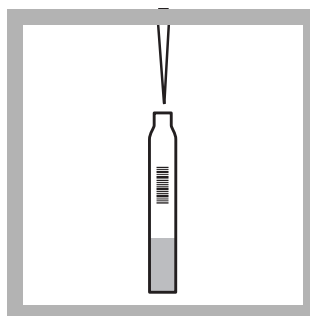
Press: **ENTER**



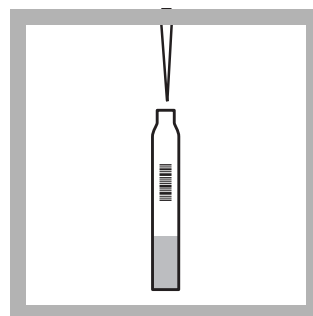
2. The display will show:
**HACH PROGRAM: 3043
Phosphate, HCT 121**

The wavelength (λ), **890 nm**, is automatically selected.

Note: Clean glassware with 1:1 Hydrochloric Acid Solution. Rinse again with deionized water. Do not use phosphate detergents to clean glassware.

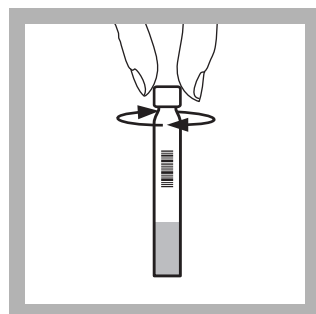


3. Pipet 1.0 mL of sample into a sample vial.

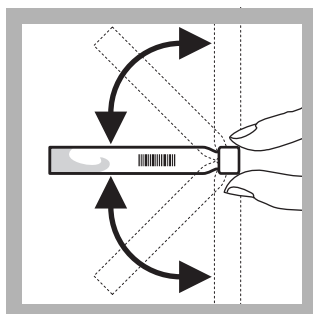


4. Pipet 0.4 mL of Reagent B (HCT 121/122 B) into the sample vial.

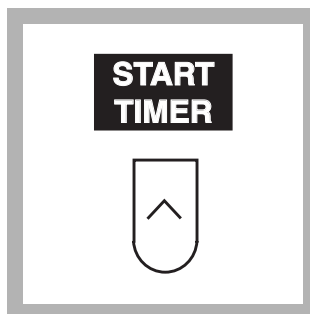
Close the Reagent B bottle **immediately** after use.



5. Screw a **light green** UniCap C (HCT 121C) onto the sample vial.



6. Cap tightly and invert the sample vial several times to mix.

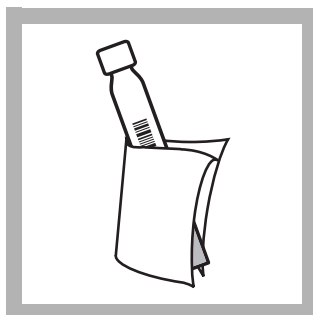


7. Press the soft key under **START TIMER**.
A 10-minute reaction time will begin.



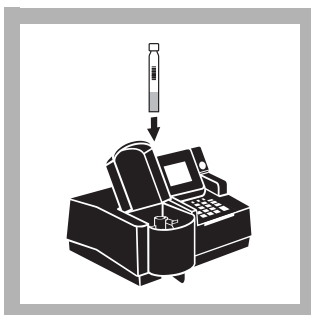
8. Insert the Test Tube Adapter into the sample cell module by sliding it under the thumb screw and into the alignment grooves. Fasten with the thumb screw.

PHOSPHORUS, Reactive, continued

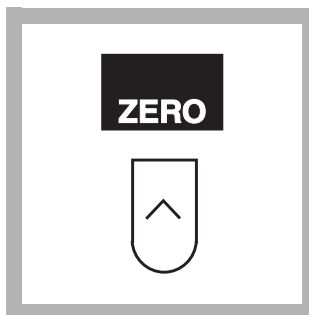


9. Clean the outside of the zero vial (white cap) with a towel.

Note: Wiping with a damp towel, followed by a dry one, will remove fingerprints or other marks.



10. Place the zero vial into the cell holder and close the light shield.

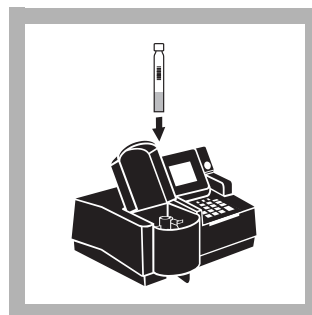


11. Press the soft key under **ZERO**.

The display will show:

0.0 mg/L PO₄³⁻

Note: For alternate concentration units press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



12. When the timer beeps, place the sample vial into the cell holder and close the light shield. The results in mg/L PO₄³⁻ (or chosen units) will be displayed.

Note: Results may also be expressed as phosphorus (P) or as phosphorus pentoxide (P₂O₅). Press the soft keys under **OPTIONS** and then **FORM**: to scroll through the available options.

Interferences

The ions listed in the following table have been individually tested up to the given concentrations. Cumulative effects and the influence of other ions have not been evaluated.

Ion	No interference up to:
SO ₄ ²⁻	20 g/L
Cl ⁻	10 g/L
K ⁺ , Na ⁺	4 g/L
Ca ²⁺	1 g/L
Mg ²⁺	400 mg/L
Co ²⁺ , Fe ²⁺ , Fe ³⁺ , Zn ²⁺ , Cu ²⁺ , Ni ²⁺ , NO ₂ ⁻ , Cd ²⁺ , NH ₄ ⁺ , Mn ²⁺ , Al ³⁺ , CO ₃ ²⁻	200 mg/L
I ⁻	100 mg/L
SiO ₂	50 mg/L
Hg ²⁺	40 mg/L
Sn ²⁺	25 mg/L
Pb ²⁺	20 mg/L
Ag ⁺ , Cr ³⁺	10 mg/L
Cr ⁶⁺	1 mg/L

Sample Collection, Storage, and Preservation

Analyze samples within 3 hours after collection for best results. Collect samples in plastic or glass bottles that have been acid cleaned with 1:1 Hydrochloric Acid

Solution and rinsed with deionized water. Store in a cool, dry place. Do not use commercial detergents containing phosphate to clean glassware used in this test.

If prompt analysis is not possible, preserve samples up to 48 hours by filtering immediately and storing at 4 °C. Do not use mercury compounds as preservatives. Warm samples to room temperature before analysis.

Accuracy Check

Standard Solutions Method

Use a 10 mg/L Phosphate Standard Solution listed under Optional Reagents. Perform the phosphate procedure as described.

Standard Adjust

To adjust the calibration curve using the reading obtained with the 10.0 mg/L PO_4^{3-} Phosphate Standard Solution, press the soft keys under **OPTIONS, (MORE)**, then **STD:OFF**. Press **ENTER** to accept the default concentration, 10.0 mg/L PO_4^{3-} . If an alternate concentration is used, enter the actual concentration and press **ENTER** to return to the read screen. For more information, see *Section 1.5.5, Adjusting the Calibration Curve*.

Standard Additions Method

Note: Clean glassware with 1:1 Hydrochloric Acid Solution. Rinse again with deionized water. Do not use phosphate detergents to clean glassware.

- a. Leave the unspiked sample in the sample compartment. Verify that the units displayed are in mg/L. Select standard additions mode by pressing the soft keys under **OPTIONS, (MORE)** and then **STD ADD**.
- b. Press **ENTER** to accept the default sample volume (mL), 100.0.
- c. Press **ENTER** to accept the default standard concentration (mg/L), 500.0.
- d. Press the soft key under **ENTRY DONE**.
- e. Use a pipet to add 0.2 mL, 0.4 mL, and 0.6 mL of standard (500.0-mg/L PO_4^{3-}), respectively, to three 100-mL samples in 100-mL mixing cylinders. Mix each sample thoroughly.
- f. Analyze each standard addition sample as described above (use a 1.0-mL aliquot of the spiked sample as the sample). Accept the standard additions reading by pressing the soft key under **READ** each time. Each addition should reflect approximately 100% recovery.
- g. After completing the sequence, the display will show the extrapolated concentration value and the “best-fit” line through the standard additions data points, accounting for matrix interferences.
- h. See *Section 1.4.1 Standard Additions* for more information.

Method Performance

Precision

Standard: 7.5 mg/L PO_4^{3-}

Program	95% Confidence Limits
3043	7.3–7.7 mg/L PO_4^{3-}

For more information on determining precision data and method detection limits, refer to *Section 1.5*.

PHOSPHORUS, Reactive, continued

Estimated Detection Limit

Program	EDL
3043	1.5 mg/L PO ₄ ³⁻

For more information on derivation and use of Hach's estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by 40 CFR part 136, appendix B, see Section 1.5.1.

Sensitivity

Program Number: 3043

Portion of Curve	Δ Abs	Δ Concentration
Entire Range	0.010	0.2 mg/L

See Section 1.5.3 *Sensitivity Explained* for more information.

Summary Of Method

Phosphate ions react with molybdate and antimony in an acidic solution to form an antimonyl phosphomolybdate complex. Ascorbic acid then reduces the complex to phosphomolybdenum blue.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to *Section 1*.

UniCap A— (HCT 121 A) contains: sodium peroxodisulfate.

Reagent B— (HCT 121/122 B) contains 16% sulfuric acid.

Pollution Prevention and Waste Management

Final samples will contain molybdenum. In addition, final samples will have a pH less than 2 and are considered corrosive (D002) by the Federal RCRA.

REQUIRED REAGENTS AND STANDARDS

Description	Quantity Required Per Test	Unit	Cat. No.
Phosphate PO ₄ -P, UniCell™ HCT 121		23/pkg	HCT 121

OPTIONAL REAGENTS

Hydrochloric Acid Standard Solution, 6.0 N (1:1)	500 mL	884-49
Phosphate Standard Solution, 500-mg/L as PO ₄	100 mL MDB	14242-32
Phosphate Standard Solution, 10-mg/L as PO ₄	946 mL	14204-16

OPTIONAL APPARATUS

Cylinder, mixing, 100-mL.....	each.....	20886-42
Digital Reactor Block 100.....	1 each.....	DRB 100
Pipettor, Jencons, 100–1000 μ L	1 each.....	27949-00
Replacement tips for 27949-00	400/pkg.....	27950-00



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Telephone: (970) 669-3050
FAX: (970) 669-2932

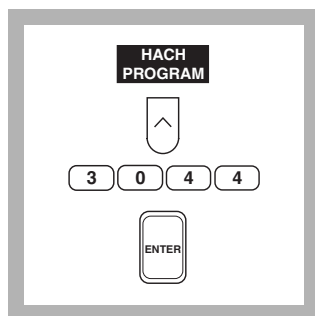


Ascorbic Acid Method

UniCell™ Vials

(0.0 to 60.0 mg/L PO₄³⁻)

Scope and Application: For water, wastewater, boiler water, surface water, and process control. The estimated detection limit for program number 3044 is 6.0 mg/L PO₄³⁻.



- 1.** Press the soft key under **HACH PROGRAM**.

Select the stored program number for UniCell phosphorus by pressing **3044** with the numeric keys.

Press: **ENTER**

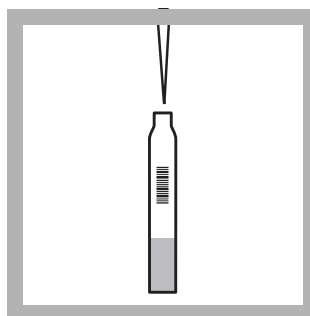
Note: If samples cannot be analyzed immediately, see Sample Collection, Storage, and Preservation following these steps. Adjust the pH of preserved samples before analysis.



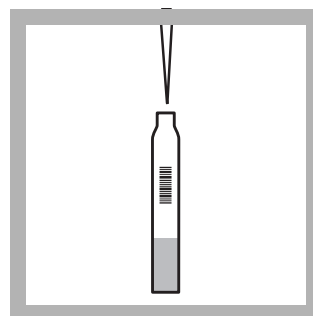
- 2.** The display will show:
**HACH PROGRAM: 3044
Phosphate, HCT 122**

The wavelength (λ), **890 nm**, is automatically selected.

Note: Clean glassware with 1:1 Hydrochloric Acid Solution. Rinse again with deionized water. Do not use phosphate detergents to clean glassware.

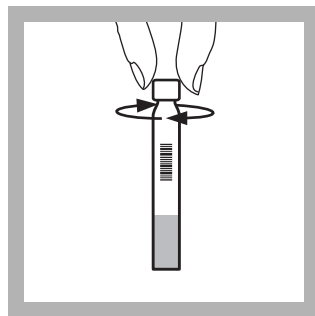


- 3.** Pipet 0.4 mL of sample into a sample vial.

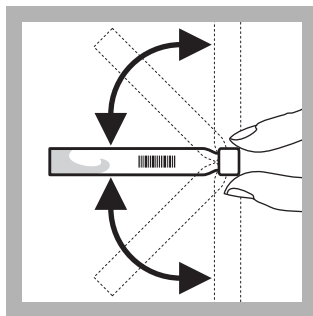


- 4.** Pipet 0.5 mL of Reagent B (HCT 121/122 B) into the sample vial.

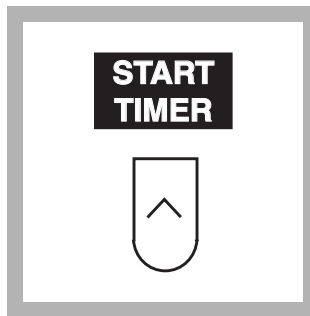
Close the Reagent B bottle **immediately** after use.



- 5.** Screw a **grey UniCap C (HCT 122C)** onto the sample vial.



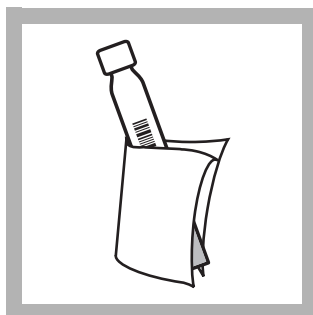
- 6.** Cap and invert the sample vial several times to mix.



- 7.** Press the soft key under **START TIMER**. A 10-minute reaction time will begin.



- 8.** Insert the Test Tube Adapter into the sample cell module by sliding it under the thumb screw and into the alignment grooves. Fasten with the thumb screw.

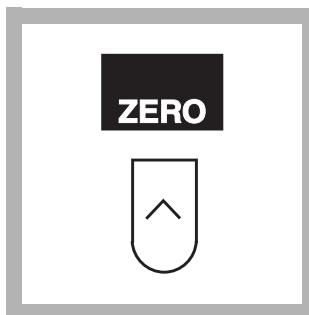


9. Clean the outside of the zero vial (white cap) with a towel.

Note: Wiping with a damp towel, followed by a dry one, will remove fingerprints or other marks.



10. Place the zero vial into the cell holder and close the light shield.



11. Press the soft key under **ZERO**.

The display will show:

0.0 mg/L PO₄³⁻

Note: For alternate concentration units press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



12. When the timer beeps, place the sample vial into the cell holder and close the light shield. The results in mg/L PO₄³⁻ (or chosen units) will be displayed.

Note: Results may also be expressed as phosphorus (P) or as phosphorus pentoxide (P₂O₅). Press the soft keys under **OPTIONS** and then **FORM**: to scroll through the available options.

Interferences

The ions listed in the following table have been individually tested up to the given concentrations. Cumulative effects and the influence of other ions have not been evaluated.

Ion	No interference up to:
SO ₄ ²⁻	5000 mg/L
Cl ⁻	2000 mg/L
K ⁺ , Na ⁺ , Ca ²⁺	1000 mg/L
Mg ²⁺ , NO ₃ ⁻	500 mg/L
Co ²⁺ , Fe ²⁺ , Fe ³⁺ , Zn ²⁺ , Cu ²⁺ , Ni ²⁺ , Cr ³⁺ , I ⁻ , NO ₂ ⁻ , Cd ²⁺ , Sn ²⁺ , NH ₄ ⁺ , Mn ²⁺ , Al ³⁺ , Hg ²⁺ , Pb ²⁺ , SiO ₂	50 mg/L
Ag ⁺	25 mg/L
Cr ⁶⁺	5 mg/L

Sample Collection, Storage, and Preservation

Analyze samples within 3 hours after collection for best results. Collect samples in plastic or glass bottles that have been acid cleaned with 1:1 Hydrochloric Acid Solution and rinsed with deionized water. Store in a cool, dry place. Do not use commercial detergents containing phosphate for cleaning glassware used in this test.

If prompt analysis is not possible, preserve samples up to 48 hours by filtering immediately and storing at 4 °C. Do not use mercury compounds as preservatives. Warm samples to room temperature before analysis.

Accuracy Check

Standard Solutions Method

Use a 30 mg/L Phosphate Standard Solution listed under Optional Reagents. Perform the phosphate procedure as described.

Standard Adjust

To adjust the calibration curve using the reading obtained with the 30.0 mg/L PO_4^{3-} Phosphate Standard Solution, press the soft keys under **OPTIONS, (MORE)** then **STD:OFF**. Press **ENTER** to accept the default concentration, 30.0 mg/L PO_4^{3-} . If an alternate concentration is used, enter the actual concentration and press **ENTER** to return to the read screen. For more information, see Section 1.5.5, *Adjusting the Calibration Curve*.

Standard Additions Method

Note: Clean glassware with 1:1 Hydrochloric Acid Solution. Rinse again with deionized water. Do not use phosphate detergents to clean glassware.

- Leave the unspiked sample in the sample compartment. Verify that the units displayed are in mg/L. Select standard additions mode by pressing the soft keys under **OPTIONS, (MORE)** and then **STD ADD**.
- Press **ENTER** to accept the default sample volume (mL), 25.0.
- Press **ENTER** to accept the default standard concentration (mg/L), 500.0.
- Press the soft key under **ENTRY DONE**.
- Use a pipet to add 0.1 mL, 0.2 mL and 0.3 mL of standard (500-mg/L PO_4^{3-}), respectively, to three 25-mL samples in 25-mL mixing cylinders. Mix each sample thoroughly.
- Analyze each standard addition sample as described above (use a 0.4-mL aliquot of the spiked sample as the sample). Accept the standard additions reading by pressing the soft key under **READ** each time. Each addition should reflect approximately 100% recovery.
- After completing the sequence, the display will show the extrapolated concentration value and the “best-fit” line through the standard additions data points, accounting for matrix interferences.
- See Section 1.4.1 *Standard Additions* for more information.

Method Performance

Precision

Standard: 30.0 mg/L PO_4^{3-}

Program	95% Confidence Limits
3044	29.5–30.5 mg/L PO_4^{3-}

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
3044	6.0 mg/L PO_4^{3-}

For more information on derivation and use of Hach’s estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by 40 CFR part 136, appendix B, see Section 1.5.1.

PHOSPHORUS, Reactive, continued

Sensitivity

Program Number: 3044

Portion of Curve	Δ Abs	Δ Concentration
Entire Range	0.010	0.5 mg/L

See Section 1.5.3 *Sensitivity Explained* for more information.

Summary Of Method

Phosphate ions react with molybdate and antimony in an acidic solution to form an antimonyl phosphomolybdate complex. Ascorbic acid then reduces the complex to phosphomolybdenum blue.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to *Section 1*.

UniCap A —(HCT 122 A) contains: sodium peroxodisulfate.

Reagent B —(HCT 121/122 B) contains 16% sulfuric acid.

Pollution Prevention and Waste Management

Final samples will contain molybdenum. In addition, final samples will have a pH less than 2 and are considered corrosive (D002) by the Federal RCRA.

REQUIRED REAGENTS AND STANDARDS

Phosphate PO₄-P, UniCell™ HCT 122 23/pkg..... HCT 122

OPTIONAL REAGENTS

Description	Quantity Required Per Test	Unit	Cat. No.
Hydrochloric Acid Standard Solution, 6.0 N (1:1)	500	mL.....	884-49
Phosphate Standard Solution, 500-mg/L as PO ₄	100	mL MDB.....	14242-32
Phosphate Standard Solution, 30-mg/L as PO ₄	946	mL.....	14367-16

OPTIONAL APPARATUS

Cylinder, graduated, 25-mL	3	each.....	20886-40
Digital Reactor Block 100.....	1	each.....	DRB 100
Pipettor, Jencons, 100–1000 μ L	1	each.....	27949-00
Replacement tips for 27949-00	400/pkg.....		27950-00



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✓ Method 8190

PhosVer 3 with Acid Persulfate Digestion

(0.00 to 3.50 mg/L PO_4^{3-})

Test 'N Tube™ Vials

(0.00 to 1.10 mg/L P)

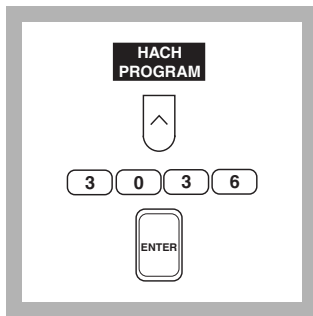
Scope and Application: For water, wastewater and seawater. USEPA accepted for reporting wastewater analyses. The estimated detection limit for program number 3036 is 0.06 mg/L PO_4^{3-} .



1. Turn on the COD Reactor. Heat to 150 °C. Place the plastic shield in front of the reactor.

Note: Ensure safety devices are in place to protect the analyst from splattering should leakage occur.

Note: See COD Reactor Manual for temperature adjustment instructions.



2. Press the soft key under **HACH PROGRAM**. Select the stored program for Test 'N Tube total phosphorus by pressing **3036** with the numeric keys.

Press: **ENTER**

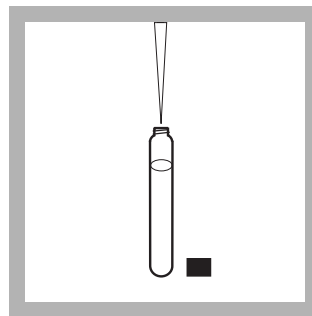
Note: If samples cannot be analyzed immediately, see Sample Collection, Storage and Preservation following these steps.



3. The display will show:
**HACH PROGRAM: 3036
P Total As. TNT**

The wavelength (λ), **890 nm**, is automatically selected.

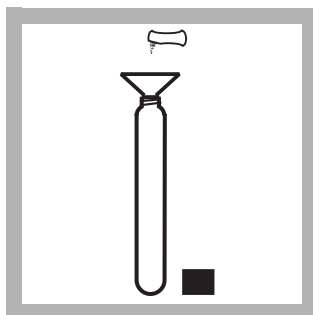
Note: Determine a reagent blank for each new lot of each reagent as follows: Prepare a reagent blank by repeating Steps 4 through 18, using deionized water as the sample. Insert the reagent blank and the blank value will be displayed. Correct for the reagent blank by pressing the soft keys under **OPTIONS, (MORE)**, and then **BLANK: OFF**. Enter the reagent blank value and press **ENTER**. Repeat for each new lot of reagent.



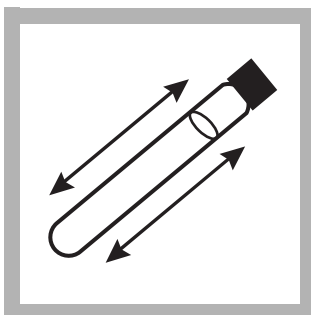
4. Use a TenSette Pipet to add 5.0 mL of sample to a Total and Acid Hydrolyzable Test Vial.

Note: For proof of accuracy, use a 1.0 mg/L Phosphate (0.33 mg/L P) Standard Solution in place of the sample (see **OPTIONAL REAGENTS AND STANDARDS**).

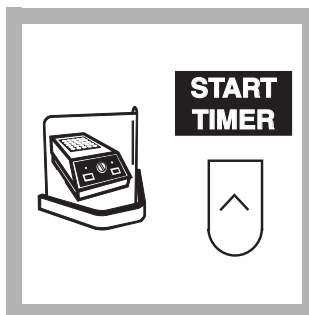
Note: For non-preserved samples with extreme pH, see Interferences.



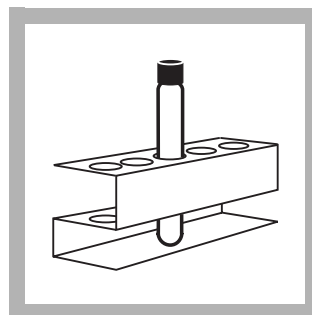
5. Using a funnel, add the contents of one Potassium Persulfate Powder Pillow for Phosphonate to the vial.



6. Cap tightly and shake to mix.

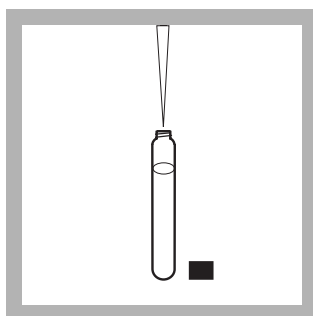


7. Place the vial in the COD Reactor, and start a 30-minute heating period by pressing the soft key under **START TIMER**.



8. Carefully remove the vial from the reactor. Place it in a test tube rack and allow to cool to room temperature.

Note: Tubes will be hot.



9. Using a TenSette Pipet, add 2 mL of 1.54 N Sodium Hydroxide Standard Solution to the vial. Cap and mix.

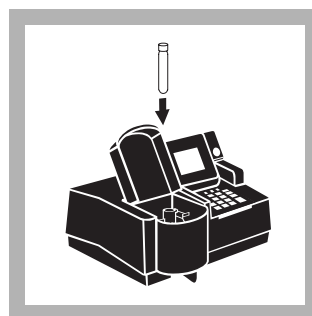


10. Insert the Test Tube Adapter into the sample cell module by sliding it under the thumb screw and into the alignment grooves. Fasten with the thumb screw.

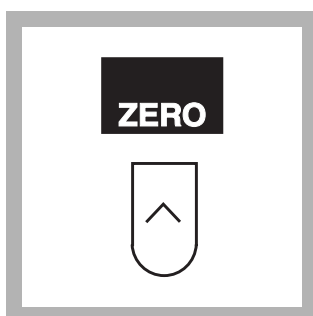


11. Clean the outside of the vial with a towel.

Note: Wiping with a damp towel, followed by a dry one, will remove fingerprints or other marks.



12. Place the sample vial in the cell holder and close the light shield.



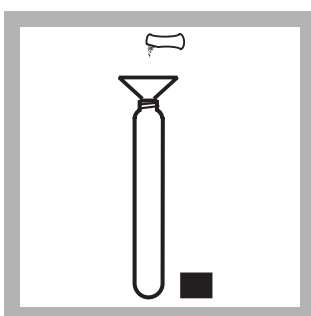
13. Press the soft key under **ZERO**.

The display will show:

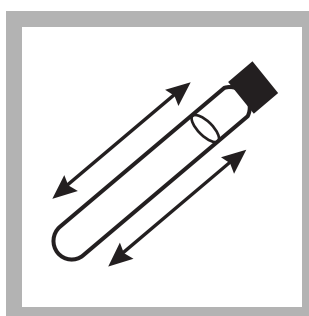
0.00 mg/L PO₄³⁻

Note: If you are using a reagent blank correction, the display will show the correction.

Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.

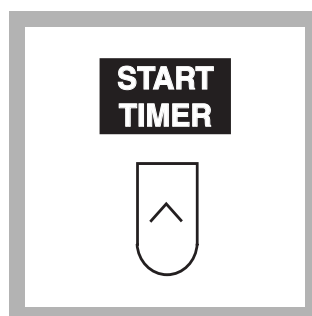


14. Using a funnel, add the contents of one PhosVer 3 Powder Pillow to the vial.



15. Cap tightly and shake to mix for 10-15 seconds.

Note: The powder will not dissolve completely.



16. Press the soft key under **START TIMER**.

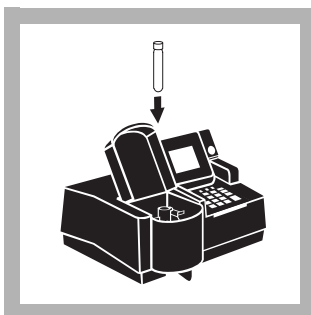
A 2-minute waiting period will begin.



17. After the timer beeps, clean the outside of the sample vial with a towel.

Note: Wiping with a damp towel, followed by a dry one, will remove fingerprints or other marks.

Note: Read the sample 2-8 minutes after the addition of the PhosVer 3 reagent.



18. Place the prepared sample vial into the cell holder and close the light shield. Results in mg/L PO_4^{3-} (or chosen units) will be displayed.

Note: Results may be expressed as phosphorus (P) or as phosphorus pentoxide (P_2O_5). Press the soft keys under **OPTIONS** and then **FORM**: to scroll through the available options.

Important Note: The test range for total phosphate is limited to 0 to 3.5 mg/L PO_4^{3-} . Values greater than 3.5 mg/L may be used to estimate dilution ratios, but should NOT be used for reporting purposes. If the value is greater than 3.5 mg/L, dilute the sample and repeat the digestion and colorimetric test.

Interferences

Interfering Substance	Interference Levels and Treatments
Aluminum	Greater than 200 mg/L
Arsenate	Interferes at any level
Chromium	Greater than 100 mg/L
Copper	Greater than 10 mg/L
Iron	Greater than 100 mg/L
Nickel	Greater than 300 mg/L
pH, excess buffering	Highly buffered samples or extreme sample pH may exceed the buffering capacity of the reagents and require sample pretreatment.
Silica	Greater than 50 mg/L
Silicate	Greater than 10 mg/L
Sulfide	Greater than 90 mg/L
Turbidity (large amounts) or color	May cause inconsistent results because the acid in the powder pillow may dissolve some of the suspended particles and because of variable desorption of orthophosphate from the particles.
Zinc	Greater than 80 mg/L

Store the PhosVer 3 Phosphate Reagent Powder Pillows in a cool, dry environment.

Sample Collection, Storage and Preservation

Collect samples in plastic or glass bottles that have been acid washed with 1:1 Hydrochloric Acid Solution and rinsed with deionized water. Do not use commercial detergents containing phosphate for cleaning glassware used in this test.

PHOSPHORUS, Total, continued

Analyze samples immediately after collection for best results. If prompt analysis is impossible, preserve samples up to 28 days by adjusting the pH to 2 or less with H₂SO₄ (2 mL per L) and storing at 4 °C. Before analyzing samples, warm to room temperature and neutralize.

Accuracy Check

Note: Clean glassware with 1:1 Hydrochloric Acid Standard Solution. Rinse again with deionized water. Do not use phosphate detergents to clean glassware.

Standard Additions Method

- Leave the unspiked sample in the sample compartment. Verify that the units displayed are in mg/L. Select standard additions mode by pressing the soft keys under **OPTIONS, (MORE)** and then **STD ADD**.
- Press **ENTER** to accept the default sample volume (mL), 25.
- Press **ENTER** to accept the default standard concentration (mg/L), 50.0.
- Press the soft key under **ENTRY DONE**.
- Snap the neck off a Phosphate 2-mL Ampule Standard, 50-mg/L as PO₄³⁻.
- Use the TenSette Pipet to add 0.1 mL, 0.2 mL and 0.3 mL of standard, respectively to three 25-mL samples and mix each thoroughly.
- Analyze each standard addition sample as described above (use a 5-mL aliquot of the spiked sample as the sample). Accept the standard additions reading by pressing the soft key under **READ** each time. Each addition should reflect approximately 100% recovery.
- After completing the sequence, the display will show the extrapolated concentration value and the “best-fit” line through the standard additions data points, accounting for matrix interferences.
- See Section 1.4.1 *Standard Additions* for more information.

Method Performance

Precision

Standard: 3.00 mg/L PO₄³⁻

Program	95% Confidence Limits
3036	294–3.06 mg/L PO ₄ ³⁻

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
3036	0.06 mg/L PO ₄ ³⁻

For more information on derivation and use of Hach’s estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, appendix B, see Section 1.5.1.

Sensitivity

Program Number: 3036

Portion of Curve	Δ Abs	Δ Concentration
Entire Range	0.010	0.061 mg/L

See Section 1.5.3 *Sensitivity Explained* for more information.

Calibration Standard Preparation

To perform a phosphate calibration using the Test 'N Tube method, prepare calibration standards containing 0.5, 1.0, 1.5, 2.0, and 2.5 mg/L phosphate as follows:

- a. Into five different 100-mL Class A volumetric flasks, pipet 1, 2, 3, 4, and 5 mL of a 50-mg/L Phosphate Standard Solution (Cat. No. 171-49) using Class A glassware.
- b. Dilute to the mark with deionized water. Mix thoroughly.
- c. Using the Test 'N Tube method and the calibration procedure described in the *User-Entered Programs* section of the *DR/4000 Spectrophotometer Instrument Manual*, generate a calibration curve from the standards prepared above.

Summary of Method

Orthophosphate reacts with molybdate in an acid medium to produce a Phosphomolybdate complex. Ascorbic acid then reduces the complex, giving an intense molybdenum blue color.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to *Section 1*.

Pollution Prevention and Waste Management

Final samples will contain molybdenum. In addition, final samples will have a pH less than 2 and are considered corrosive (D002) by the Federal RCRA.

PHOSPHORUS, Total, continued

REQUIRED REAGENTS AND STANDARDS

Total Phosphorus Test 'N Tube Reagent Set 50 tests 27426-45
Includes: (1) 272-42, (1) 20847-66, (1) 21060-46, (1) 27430-42, (50) Total and Acid Hydrolyzable Test Vials*

Description	Quantity Required		Cat. No.
	Per Test	Unit	
PhosVer 3 Phosphate Reagent Powder Pillows	1	50/pkg	21060-46
Potassium Persulfate powder Pillows	1	50/pkg	20847-66
Sodium Hydroxide Solution, 1.54 N	2 mL	100 mL	27430-42
Total and Acid Hydrolyzable Test Vials	1	50/pkg	*
Water, deionized	4 liters		272-56

REQUIRED EQUIPMENT AND SUPPLIES

COD Reactor, 115/230 VAC (U.S.A. and Canada)	1	each	45600-00
COD Reactor, 115/230 VAC (Europe)	1	each	45600-02
DR/4000 Test Tube Adapter	1	each	48189-00
Funnel, micro	1	each	25843-35
Pipet, TenSette, 1 to 10 mL	1	each	19700-10
Pipet Tips, for 19700-10 TenSette Pipet	1	1000/pkg	21997-28
Safety Shield, laboratory bench	1	each	23810-00
Test Tube Rack	1-3	each	18641-00

OPTIONAL REAGENTS AND STANDARDS

Hydrochloric Acid Standard Solution, 6.0 N (1:1)	500 mL	884-49
Phosphate Standard Solution, 1-mg/L as PO_4^{3-}	500 mL	2569-49
Phosphate Standard Solution, 2-mL PourRite Ampule, 50-mg/L as PO_4^{3-}	20/pkg	171-20H
Sodium Hydroxide Standard Solution, 5.0 N	1 liter	2450-53
Sulfuric Acid, ACS	500 mL	979-49
Total and Acid Hydrolyzable Test 'N Tube Reagent Set	50/pkg	27427-45

OPTIONAL EQUIPMENT AND SUPPLIES

Flask, volumetric, Class A, 50-mL	each	14574-41
pH Paper, pH 1.0 to 11.0	5 rolls/pkg	391-33
pH Meter, <i>sension</i> TM I, portable	each	51700-00
Pipet, volumetric, Class A, 0.5-mL	each	14515-34
Pipet, volumetric, Class A, 1.00-mL	each	14515-35
Pipet, volumetric, Class A, 3.00-mL	each	14515-03
Pipet, volumetric, Class A, 4.00-mL	each	14515-04
Pipet, volumetric, Class A, 5.00-mL	each	14515-37
Pipet Filler, safety bulb	each	14651-00
Pipet, TenSette, 0.1 to 1.0 mL	each	19700-01
Pipet Tips, for 19700-01 TenSette Pipet	50/pkg	21856-96

* These items are not sold separately.



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Method 10127

Molybdovanadate Method

with Acid Persulfate Digestion*

HR (0.0 to 100.0 mg/L PO₄³⁻)

Test 'N Tube™ Vials

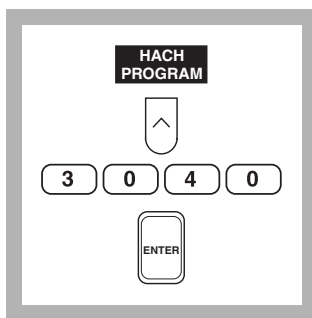
Scope and Application: For water and wastewater

* Adapted from *Standard Methods for the Examination of Water and Wastewater*.



1. Turn on the COD Reactor. Heat to 150 °C. Place the plastic shield in front of the reactor.

Note: Ensure safety devices are in place to protect the analyst if splattering or leakage occurs.



2. Press the soft key under **HACH PROGRAM**.

Select the stored program number for phosphorus, total, high range, Test 'N Tube.

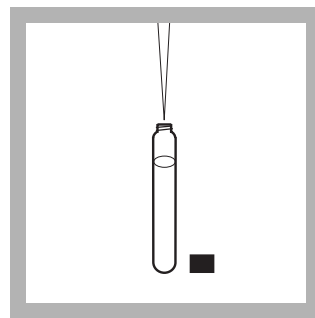
Press: **3040 ENTER**

Note: If samples cannot be analyzed immediately, see *Sampling and Storage* following these steps.

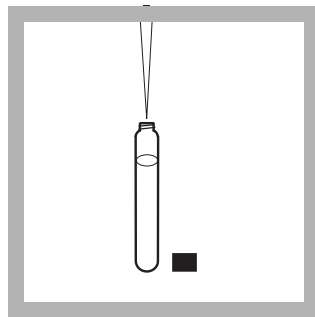


3. The display will show:
**HACH PROGRAM: 3040
P Total HR TNT**

The wavelength (λ), **420 nm**, is automatically selected.

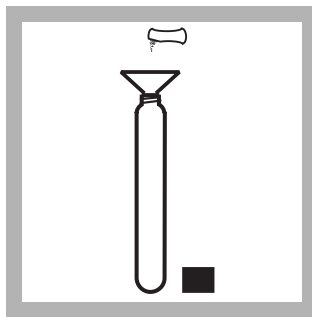


4. Use a TenSette® Pipet to add 5.0 mL of deionized water to a Total Phosphorus Test 'N Tube Vial (the blank).



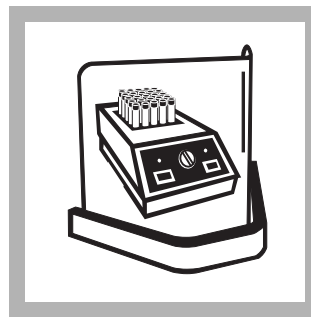
5. Use a TenSette Pipet to add 5.0 mL of sample to a Total Phosphorus Test 'N Tube Vial (the sample).

Note: For non-preserved samples with extreme pH, see *Interferences* following these steps.

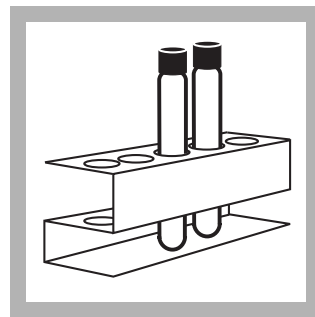


6. Use a funnel to add the contents of one Potassium Persulfate Powder Pillow for Phosphonate to each vial.

Cap tightly and shake to dissolve.



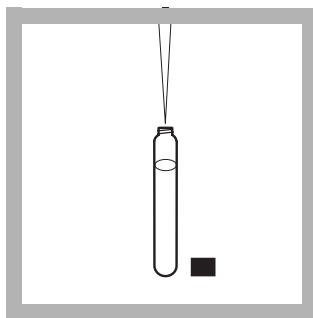
7. Place the vials in the COD Reactor, and start a 30-minute heating period by pressing the soft key under **START TIMER**.



8. Carefully remove the vials from the reactor. Place them in a test tube rack and allow to cool to room temperature (18-25 °C).

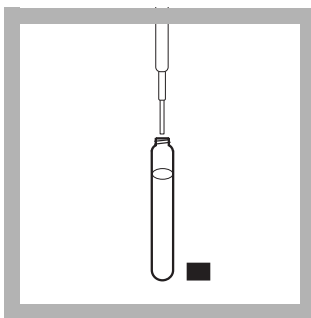
Note: Vials will be hot.

PHOSPHORUS, Total, continued



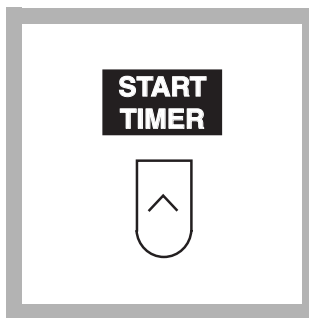
9. Use a TenSette Pipet to add 2.0 mL of 1.54 N sodium hydroxide to each vial.

Cap and invert to mix.



10. Use a polyethylene dropper to add 0.5 mL of Molybdovanadate Reagent to each vial.

Cap and invert to mix.



11. Press the soft key under **START TIMER**. A 7-minute reaction period will begin.

Note: Read the sample between 7 and 9 minutes.

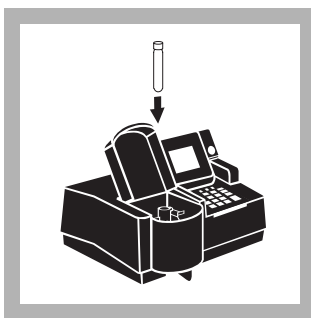


12. Insert the Test Tube Adapter into the sample cell module by sliding it under the thumb screw and into the alignment grooves. Fasten with the thumb screw.



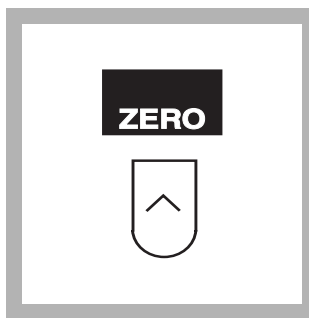
13. Clean the outside of the vials with a towel.

Note: Wipe with a damp towel, followed by a dry one, to remove fingerprints or other marks.



14. When the timer sounds, place the blank vial in the cell holder and close the light shield.

Note: Reagent blanks for each lot of reagents may be used more than once, but should not be used for longer than one day.

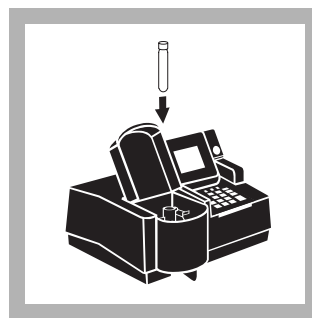


15. Press the soft key under **ZERO**.

The display will show:

0.0 mg/L PO₄³⁻

Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



16. Place the prepared sample vial into the cell holder and close the light shield. Results in mg/L PO₄³⁻ (or chosen units) will be displayed.

Note: Results may be expressed as phosphorus (P) or as phosphorus pentoxide (P₂O₅). Press the soft keys under **OPTIONS** and then **FORM** to scroll through the available options.

Interferences

Large amounts of sample turbidity may cause inconsistent results in the test because the acid present in the reagents may dissolve some of the suspended particles and because of variable desorption of orthophosphate from the particles.

The following may interfere when present in concentrations exceeding these listed below:

Interfering Substance	Interference Level and Treatment
Arsenate	Causes positive interference if the sample is heated.*
Iron, ferrous	Blue color caused by ferrous iron does not interfere if iron concentration is less than 100 mg/L.
Molybdate	Causes negative interference above 1000 mg/L.
Silica	Causes positive interference if the sample is heated.*
Extreme pH or highly buffered samples	May exceed buffering capacity of the reagents. See <i>pH Interference</i> in Section 1.3 of the <i>DR/4000 Procedures Manual</i> . Samples may require pretreatment. Sample pH should be about 7.
Fluoride, thorium, bismuth, thiosulfate or thiocyanate	Cause a negative interference.
Temperature, Cold (less than 18 °C)	Causes a negative interference.
Temperature, Hot (greater than 25 °C)	Causes a positive interference. Post-digestion samples should be brought to room temperature (18–25 °C) before the addition of the Molybdovanadate Reagent or sodium hydroxide.
The following do not interfere in concentrations up to 1000 mg/L: Pyrophosphate, tetraborate, selenate, benzoate, citrate, oxalate, lactate, tartrate, formate, salicylate, Al ³⁺ , Fe ³⁺ , Mg ²⁺ , Ca ²⁺ , Ba ²⁺ , Sr ²⁺ , Li ⁺ , Na ⁺ , K ⁺ , NH ₄ ⁺ , Cd ²⁺ , Mn ²⁺ , NO ₃ ⁻ , NO ₂ ⁻ , SO ₄ ²⁻ , SO ₃ ²⁻ , Pb ²⁺ , Hg ⁺ , Hg ²⁺ , Sn ²⁺ , Cu ²⁺ , Ni ²⁺ , Ag ⁺ , U ⁴⁺ , Zr ⁴⁺ , AsO ₃ ⁻ , Br ⁻ , CO ₃ ²⁻ , ClO ₄ ⁻ , CN ⁻ , IO ₃ ⁻ , SiO ₄ ⁴⁻ .	

* Gentle warming of the sample to reach room temperature will not cause this substance to interfere.

Sampling and Storage

Collect samples in plastic or glass bottles that have been acid washed with 1:1 Hydrochloric Acid Solution and rinsed with deionized water. Do not use commercial detergents containing phosphate for cleaning the glassware used in this test.

Analyze samples immediately after collection for best results. If prompt analysis is impossible, preserve samples up to 28 days by adjusting the pH to 2 or less with concentrated H₂SO₄ (about 2 mL per liter) and storing at 4 °C. Warm the sample to room temperature and neutralize with 5.0 N NaOH before analysis. Correct for volume additions; see *Section 1, Correcting for Volume Additions*.

Accuracy Check

Standard Additions Method

Note: Clean glassware with 1:1 Hydrochloric Acid Standard Solution. Rinse again with deionized water. Do not use detergents containing phosphate to clean glassware.

- a. Leave the unspiked sample (from *step 16* in the procedure) in the sample compartment. Verify that the units displayed are in mg/L. Select standard additions mode by pressing the soft keys under **OPTIONS, (MORE)** and then **STD ADD**.
- b. Press **ENTER** to accept the default sample volume (mL), 10.
- c. Press **ENTER** to accept the default standard concentration, 500 mg/L as PO₄³⁻.
- d. Press the soft key under **ENTRY DONE**.
- e. Fill each of three 10-mL graduated mixing cylinders with 10 mL of sample.
- f. Snap the neck off a 10-mL Voluette Ampule of Phosphate Standard Solution, 500 mg/L as PO₄³⁻ (Cat. No. 14242-10).

- g. Use the TenSette Pipet to add 0.1 mL, 0.2 mL and 0.3 mL of standard, respectively to three 10-mL samples from *step e*. Mix well.
- h. Analyze each standard addition sample from *step g* as described in the procedure. Accept the standard additions reading by pressing the soft key under **READ** each time. Each addition should reflect approximately 100% recovery, or an increase of 5 mg/L PO_4^{3-} for each 0.1 mL of standard added.
- i. After completing the sequence, the display will show the extrapolated concentration value and the “best-fit” line through the standard additions data points, accounting for matrix interferences.
- j. See *Section 1.4.1, Standard Additions*, for more information.

Standard Solution Method

To check accuracy, prepare a 80 mg/L standard by pipetting 8.0 mL of solution from a 10-mL Voluette Ampule of Phosphate Standard Solution, 500-mg/L as PO_4^{3-} , into an acid-cleaned, 50-mL Class A volumetric flask. Dilute to the mark with deionized water. Substitute this standard for the sample and perform the procedure as described.

Standard Adjust

To adjust the calibration curve using the reading obtained with the 80 mg/L PO_4^{3-} Phosphate Standard Solution, press the soft keys under **OPTIONS, (MORE)** then **STD:OFF**. Press **ENTER** to accept the default concentration, 80.0 mg/L PO_4^{3-} . If an alternate concentration is used, enter the actual concentration and press **ENTER** to return to the read screen. For more information, see *Section 1.5.5, Adjusting the Calibration Curve*.

Method Performance

Precision

Standard: 80.0 mg/L PO_4^{3-}

Program	95% Confidence Limits
3040	78.0–82.0 mg/L PO_4^{3-}

For more information on determining precision data and method detection limits, refer to *Section 1.5, Estimated Detection Limit*.

Estimated Detection Limit

Program	EDL
3040	5.0 mg/L PO_4^{3-}

For more information on derivation and use of Hach’s estimated detection limit, see *Section 1.5.2*. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, appendix B, see *Section 1.5.1*.

Sensitivity

Program Number: 3040

Portion of Curve	Δ Abs	Δ Concentration
Entire Range	0.010	0.9 mg/L

See Section 1.5.3, *Sensitivity Explained*, for more information.

Calibration Standard Preparation

To perform a phosphate calibration using the Total High Range Phosphorus Test 'N Tube method, prepare calibration standards containing 10, 25, 50, 75, and 100 mg/L phosphate as follows:

- a. Into a 500-mL Class A volumetric flask, pipet 10.0 mL of 500 mg/L Phosphate Standard Solution using a Class A pipet.
- b. Into four separate 100-mL Class A volumetric flasks, pipet 5.0, 10.0, 15.0 and 20.0 mL of a 500-mg/L Phosphate Standard Solution (Cat. No. 14242-32) using Class A glassware.
- c. Dilute to the mark with deionized water. Mix thoroughly.
- d. Using the Total High Range Phosphorus Test 'N Tube method and the calibration procedure described in the *User-Entered Programs Section* of the *DR/4000 Spectrophotometer Instrument Manual*, generate a calibration curve from the standards prepared above.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the Material Safety Data Sheet for information specific to the reagents used.

Sample Disposal Information

The final samples will contain molybdenum. In addition, the final samples will have a pH less than 2 and are considered corrosive (D002) by the Federal RCRA. Consult the Material Data Safety Data Sheet for information specific to the reagents used.

Summary of Method

Phosphates present in organic and condensed inorganic forms (meta-, pyro-, or other polyphosphates) must be converted to reactive orthophosphate before analysis. Pretreatment of the sample with acid and heat provides the conditions for hydrolysis of the condensed inorganic forms. Organic phosphates are converted to orthophosphates by heating with acid and persulfate.

Orthophosphate reacts with molybdate in an acid medium to produce a phosphomolybdate complex. In the presence of vanadium, yellow vanadomolybdophosphoric acid forms. The intensity of the yellow color is proportional to the phosphate concentration.

PHOSPHORUS, Total, continued

REQUIRED REAGENTS

Total High Range Phosphorus Test 'N Tube Reagent Set.....50 vials.....27672-45
Includes: (50) Total Phosphorus Test 'N Tube Vials*, (2) 272-42, (1) 20847-66, (1) 20760-26, (1) 27430-42

Description	Quantity Required		Cat. No.
	Per Test	Unit	
Molybdovanadate Reagent	0.5 mL.....	25 mL.....	20760-26
Potassium Persulfate powder Pillows.....	1	50/pkg.....	20847-66
Sodium Hydroxide Solution, 1.54 N.....	2 mL	100 mL.....	27430-42
Total Phosphorus Test Vials	1	50/pkg.....	*
Water, deionized		100 mL.....	272-42

REQUIRED APPARATUS

COD Reactor, 115/230 VAC (U.S.A. and Canada).....	1	each.....	45600-00
COD Reactor, 115/230 VAC (Europe)	1	each.....	45600-02
DR/4000 Test Tube Adapter.....	1	each.....	48189-00
Pipet, TenSette, 1 to 10 mL	1	each.....	19700-10
Pipet Tips, for 19700-10 TenSette Pipet	1	1000/pkg.....	21997-28
Safety Shield, laboratory bench	1	each.....	23810-00
Test Tube Rack	1-3	each.....	18641-00

OPTIONAL REAGENTS

Hydrochloric Acid Standard Solution, 6.0 N (1:1)	500 mL	884-49
Phosphate Standard Solution, PourRite Ampule, 500 mg/L as PO_4^{3-} , 2-mL	20/pkg.....	14242-20
Phosphate Standard Solution, Voluette ampule, 500 mg/L as PO_4^{3-} , 10 mL.....	16/pkg.....	14242-10
Phosphate Standard Solution, Voluette ampule, 500 mg/L as PO_4^{3-} ,	100 mL MDB.....	14242-32
Sodium Hydroxide Standard Solution, 5.0 N.....	1 liter.....	2450-53
Sulfuric Acid, ACS, concentrated	500 mL.....	979-49

OPTIONAL APPARATUS

Ampule Breaker Kit	each.....	21968-00
Aspirator, vacuum	each.....	2131-00
Cylinder, graduated, mixing, 10-mL, 3 required.....	each.....	20886-38
Filter Holder, 47-mm, 300-mL, graduated	each.....	13529-00
Filter Membrane, 47-mm, 0.45-microns	200/pkg.....	13530-01
Flask, filtering, 500-mL	each.....	546-49
Flask, volumetric, Class A, 50-mL	each.....	14574-41
Flask, volumetric, Class A, 100 mL.....	each.....	14574-42
Flask, volumetric, Class A, 500 mL.....	each.....	14574-49
pH Indicator Paper, 1 to 11 pH units	5 rolls/pkg.....	391-33
pH Meter, <i>sension</i> TM I, portable	each.....	51700-10
Pipet Filler, Safety Bulb	each.....	14651-00
Pipet, TenSette, 0 to 1.0 mL	each.....	19700-01
Pipet Tips, for 19700-01 TenSette Pipet	50/pkg.....	21856-96
Pipet, volumetric, Class A, 5.00 mL	each.....	14515-37
Pipet, volumetric, Class A, 8.00-mL	each.....	14515-08
Pipet, volumetric, Class A, 10.0-mL	each.....	14515-38
Pipet, volumetric, Class A, 15.0-mL	each.....	14515-39
Pipet, volumetric, Class A, 20.0-mL	each.....	14515-20

* These items are not sold separately.



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Scope and Application: For water, wastewater and seawater; USEPA accepted for wastewater analyses.

* Adapted from *Standard Methods for the Examination of Water and Wastewater* 4500-P B & E.



1. Measure 25 mL of sample into a 125-mL Erlenmeyer flask.

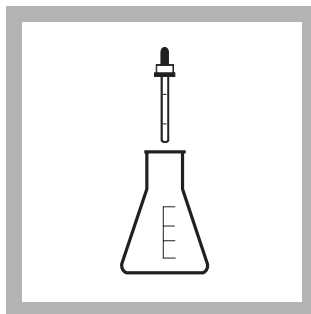
Note: Use a graduated cylinder to measure the sample.

Note: Rinse all glassware with 1:1 hydrochloric acid. Rinse again with deionized water.

Note: If prompt analysis is impossible, see Sample Collection, Storage and Preservation after these steps.

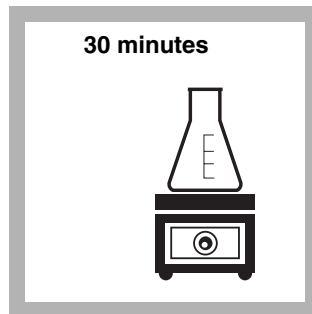


2. Add the contents of one Potassium Persulfate Powder Pillow. Swirl to mix.



3. Add 2.0 mL of 5.25 N Sulfuric Acid Solution.

Note: Use the 1-mL calibrated dropper provided.



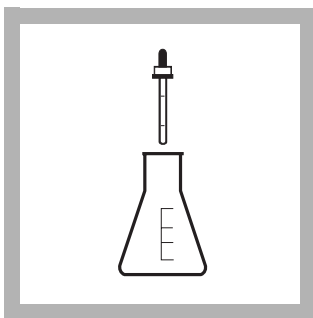
4. Place the flask on a hot plate. Boil gently for 30 minutes. Do not boil dry.

Note: Sample should be concentrated to less than 20 mL for best recovery. After concentration, maintain the volume near 20 mL by adding small amounts of deionized water. Do not exceed 20 mL.

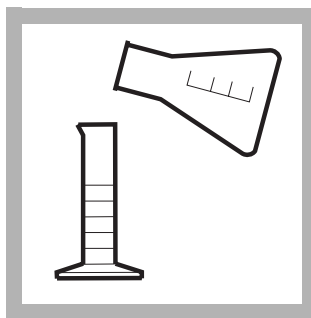
PHOSPHORUS, Total, Digestion, continued



5. Cool the sample to room temperature.



6. Add 2.0 mL of 5.0 N Sodium Hydroxide Solution. Swirl to mix.
Note: Use the 1-mL calibrated dropper provided.



7. Pour the sample into a 25-mL graduated cylinder. Adjust the volume to 25 mL. Proceed with a reactive phosphorus test of the expected total phosphorus concentration range. Extend the color development time to 10 minutes for the Ascorbic Acid method.
Note: Use deionized water rinsings from the flask to adjust the volume.

Note: Results of the reactive phosphorus test at this point will include the organic phosphate plus the orthophosphate and the acid-hydrolyzable (condensed) phosphate. The organic phosphate concentration is determined by subtracting results of an acid hydrolyzable phosphorus test from this result. Make sure that both results are in the same units, either mg/L PO_4 or mg/L P before subtracting.

Interferences

Interfering Substance	Interference Levels and Treatments
Alkaline or highly buffered samples	It may be necessary to add additional acid in Step 3 to drop the pH of the solution below 1.
Turbidity	Use 50 mL of sample and double the reagent quantities. Use 25 mL of the reacted sample to zero the instrument in the reactive phosphorus procedure. This compensates for any color or turbidity destroyed by this procedure.

Sample Collection, Storage and Preservation

Most reliable results are obtained when samples are analyzed immediately. If prompt analysis is not possible, samples may be preserved up to 28 days by adjusting the pH to 2 or less with H_2SO_4 (2 mL per L) and storing at 4 °C (39 °F). Warm to room temperature before testing.

Summary of Method

Phosphates present in organic and condensed inorganic forms (meta-, pyro- or other polyphosphates) must be converted to reactive orthophosphate before analysis. Pretreatment of the sample with acid and heat provides the conditions for hydrolysis of the condensed inorganic forms. Organic phosphates are converted to orthophosphate by heating with acid and persulfate. Organically bound phosphates are thus determined indirectly by subtracting the result of an acid hydrolyzable phosphorus test from the total phosphorus result.

This procedure must be followed by one of the reactive phosphorus (orthophosphate) analysis methods for determination of the phosphorus content

PHOSPHORUS, Total, Digestion, continued

of the sample. If the ascorbic acid (PhosVer 3) method is used to measure the reactive phosphorus, this method is USEPA accepted for NPDES reporting.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to *Section 1*.

Pollution Prevention and Waste Management

For information on pollution prevention and waste management, refer to *Section 1*.

REQUIRED REAGENTS AND STANDARDS

The following reagents and apparatus are required besides those required for the reactive phosphorus test.

Description	Quantity Required		Cat. No.
	Per Test	Unit	
Potassium Persulfate Powder Pillows	1 pillow	100/pkg	2451-99
Sodium Hydroxide Solution, 5.0 N	2 mL ... 100 mL	MDB*	2450-32
Sulfuric Acid Solution, 5.25 N	2 mL ... 100 mL	MDB*	2449-32

REQUIRED EQUIPMENT AND SUPPLIES

Cylinder, graduated, 25-mL	1	each	508-40
Flask, Erlenmeyer, 125-mL	1	each	505-43
Hot Plate, 4 inch diameter, 120 VAC	1	each	12067-01
Hot Plate, 4 inch diameter, 240 VAC	1	each	12067-02

OPTIONAL REAGENTS AND STANDARDS

Hydrochloric Acid, 6.0 N (1:1)	500 mL	884-49
Sodium Hydroxide Solution, 5.0 N	1 liter	2450-53
Water, deionized	4 liters	272-56

OPTIONAL EQUIPMENT AND SUPPLIES

Beads, glass	100/pkg	2596-00
Cylinder, graduated, 50-mL	each	508-41
Flask, Erlenmeyer, 125-mL	each	505-43
Pads, cooling, 4 x 4 inch	each	18376-00
pH Meter, <i>sension</i> TM 1, portable	each	51700-00
pH Paper, pH 1.0 to 11.0	5 rolls/pkg	391-33

* Contact Hach for larger sizes.



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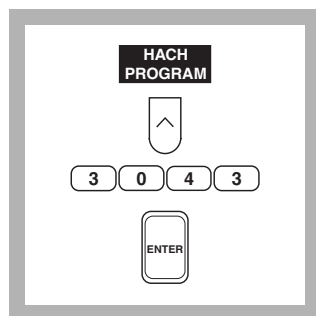


Ascorbic Acid with Acid Persulfate Digestion Method

UniCell™ Vials

(0.0 to 15.0 mg/L PO₄³⁻)

Scope and Application: For water, wastewater, boiler water, surface water, and process control. The estimated detection limit for program number 3043 is 1.5 mg/L PO₄³⁻.

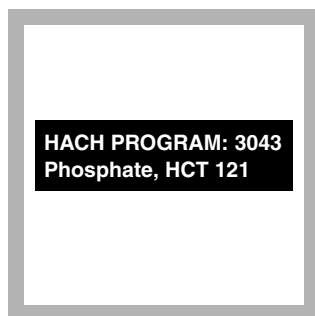


- 1.** Press the soft key under **HACH PROGRAM**.

Select the stored program number for UniCell phosphorus by pressing **3043** with the numeric keys.

Press: **ENTER**

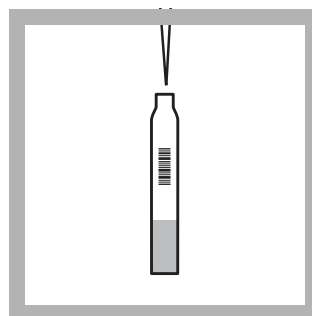
Note: If samples cannot be analyzed immediately, see Sample Collection, Storage, and Preservation following these steps.



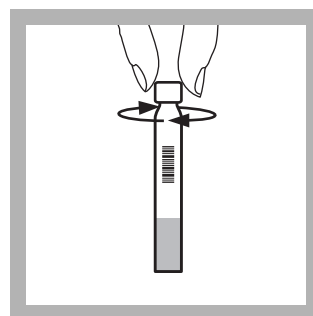
- 2.** The display will show:
HACH PROGRAM: 3043 Phosphate, HCT 121

The wavelength (λ), **890 nm**, is automatically selected.

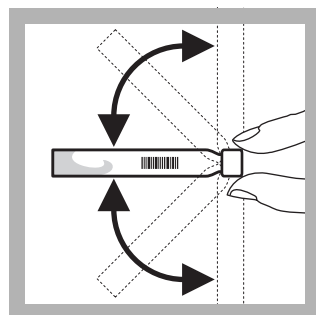
Note: Clean glassware with 1:1 Hydrochloric Acid Solution. Rinse again with deionized water. Do not use phosphate detergents to clean glassware.



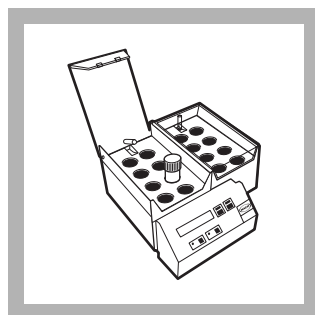
- 3.** Pipet 1.0 mL of sample into a sample vial.



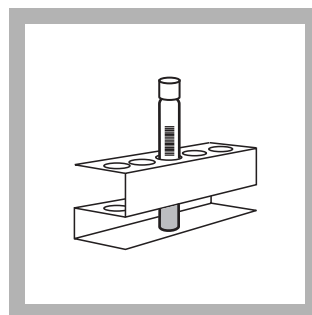
- 4.** Screw a **blue** UniCap A (HCT 121 A) onto a sample vial.



- 5.** Cap and invert the sample vial several times to mix.

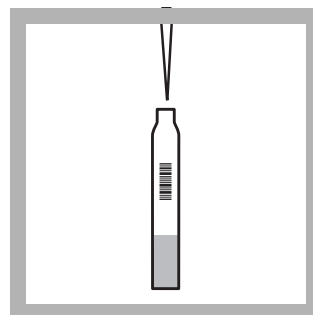


- 6.** Place the sample in the reactor block and heat for 60 minutes at 100 °C.



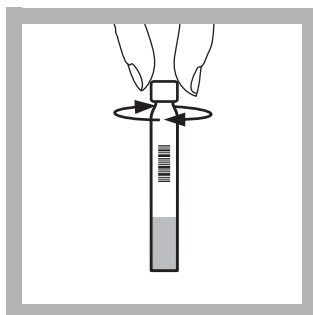
- 7.** After the 60-minute heating period, carefully remove the vial from the reactor. Place it in a test tube rack and allow to cool to room temperature.

Note: Tubes will be hot.

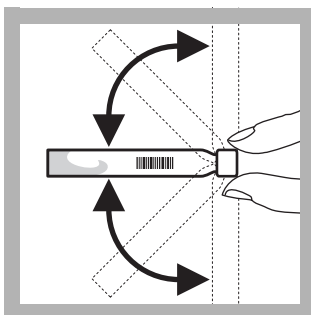


- 8.** After the sample cools, pipet 0.4 mL of Reagent B (HCT 121/122 B) into the sample vial.

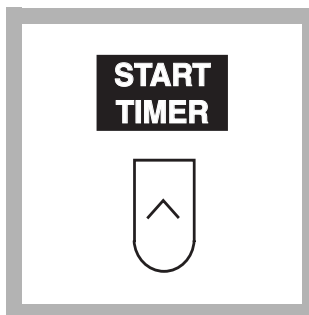
Close the Reagent B bottle **immediately** after use.



9. Screw a **light green** Unicap C (HCT 121 C) onto the sample vial.



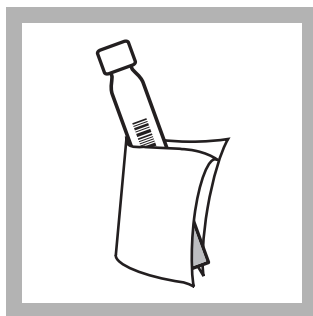
10. Cap tightly and invert the sample vial several times to mix.



11. Press the soft key under **START TIMER**. A 10-minute reaction time will begin.



12. Insert the Test Tube Adapter into the sample cell module by sliding it under the thumb screw and into the alignment grooves. Fasten with the thumb screw.

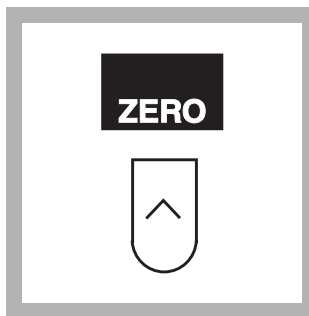


13. Clean the outside of the zero vial (white cap) with a towel.

Note: Wiping with a damp towel, followed by a dry one, will remove fingerprints or other marks.



14. Place the zero vial into the cell holder and close the light shield.

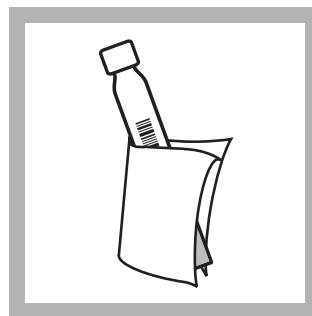


15. Press the soft key under **ZERO**.

The display will show:

0.0 mg/L PO₄³⁻

Note: For alternate concentration units press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



16. After the timer beeps, clean the outside of the sample vial with a towel.

Note: Wiping with a damp towel, followed by a dry one, will remove fingerprints or other marks.



17. Place the prepared sample vial into the cell holder and close the light shield. Results in mg/L PO_4^{3-} will be displayed.

Note: Results may also be expressed as phosphorus (P) or as phosphorus pentoxide (P_2O_5). Press the soft keys under **OPTIONS** and then **FORM**: to scroll through the available options.

Interferences

The ions listed in the following table have been individually tested up to the given concentrations. Cumulative effects and the influence of other ions have not been evaluated.

Ion	No interference up to:
SO_4^{2-}	20 g/L
Cl^-	10 g/L
K^+ , Na^+	4 g/L
Ca^{2+}	1 g/L
Mg^{2+}	400 mg/L
Co^{2+} , Fe^{2+} , Fe^{3+} , Zn^{2+} , Cu^{2+} , Ni^{2+} , NO_2^- , Cd^{2+} , NH_4^+ , Mn^{2+} , Al^{3+} , CO_3^{2-}	200 mg/L
I^-	100 mg/L
SiO_2	50 mg/L
Hg^{2+}	40 mg/L
Sn^{2+}	25 mg/L
Pb^{2+}	20 mg/L
Ag^+ , Cr^{3+}	10 mg/L
Cr^{6+}	1 mg/L

Sample Collection, Storage, and Preservation

Analyze samples within 3 hours after collection for best results. Collect samples in plastic or glass bottles that have been acid cleaned with 1:1 Hydrochloric Acid Solution and rinsed with deionized water. Store in a cool, dry place. Do not use commercial detergents containing phosphate for cleaning glassware used in this test.

If prompt analysis is impossible, preserve samples* up to 28 days by adjusting the pH to 2 or less with Sulfuric Acid, 5.25 N and storing the sample at 4 °C. The pH can be checked using pH paper. Warm the samples to room temperature and neutralize the pH before analysis.

Accuracy Check

Standard Solutions Method

Use a 10 mg/L Phosphate Standard Solution listed under Optional Reagents. Perform the phosphate procedure as described.

Standard Adjust

To adjust the calibration curve using the reading obtained with the 10 mg/L PO_4^{3-} Phosphate Standard Solution, press the soft keys under **OPTIONS, (MORE)** then **STD:OFF**. Press **ENTER** to accept the default concentration, 10.0 mg/L PO_4^{3-} . If an alternate concentration is used, enter the actual concentration and press **ENTER** to return to the read screen. For more information, see *Section 1.5.5, Adjusting the Calibration Curve*.

Standard Additions Method

Note: Clean glassware with 1:1 Hydrochloric Acid Solution. Rinse again with deionized water. Do not use phosphate detergents to clean glassware.

- a. Leave the unspiked sample in the sample compartment. Verify that the units displayed are in mg/L. Select standard additions mode by pressing the soft keys under **OPTIONS, (MORE)** and then **STD ADD**.
- b. Press **ENTER** to accept the default sample volume (mL), 100.0.
- c. Press **ENTER** to accept the default standard concentration (mg/L), 500.0.
- d. Press the soft key under **ENTRY DONE**.
- e. Use a pipet to add 0.2 mL, 0.4 mL and 0.6 mL of standard (500.0-mg/L PO_4^{3-}), respectively, to three 25-mL samples in 25-mL mixing cylinders. Mix each sample thoroughly.
- f. Analyze each standard addition sample as described above (use a 0.4-mL aliquot of the spiked sample as the sample). Accept the standard additions reading by pressing the soft key under **READ** each time. Each addition should reflect approximately 100% recovery.
- g. After completing the sequence, the display will show the extrapolated concentration value and the “best-fit” line through the standard additions data points, accounting for matrix interferences.
- h. See *Section 1.4.1 Standard Additions* for more information.

* See 40 CFR part 136.3.

Method Performance

Precision

Standard: 7.5 mg/L PO₄³⁻

Program	95% Confidence Limits
3043	7.3–7.7 mg/L PO ₄ ³⁻

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
3043	1.5 mg/L PO ₄ ³⁻

For more information on derivation and use of Hach's estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by 40 CFR part 136, appendix B, see Section 1.5.1.

Sensitivity

Program Number: 3043

Portion of Curve	ΔAbs	ΔConcentration
Entire Range	0.010	0.2 mg/L

See Section 1.5.3 *Sensitivity Explained* for more information.

Summary Of Method

Phosphate ions react with molybdate and antimony in an acidic solution to form an antimonyl phosphomolybdate complex. Ascorbic acid then reduces the complex to phosphomolybdenum blue. Total phosphate measurements include a digestion step.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to *Section 1*.

UniCap A — (HCT 121 A) contains: sodium peroxodisulfate.

Reagent B — (HCT 121/122 B) contains 16% sulfuric acid.

Pollution Prevention and Waste Management

Final samples will contain molybdenum. In addition, final samples will have a pH less than 2 and are considered corrosive (D002) by the Federal RCRA.

PHOSPHORUS, Total, continued

REQUIRED REAGENTS AND STANDARDS

Phosphate PO₄-P, UniCell™ HCT 121 23/pkg HCT 121

OPTIONAL REAGENTS

Description	Quantity Required Per Test	Unit	Cat. No.
Hydrochloric Acid Standard Solution, 6.0 N (1:1)	500	mL.....	884-49
Phosphate Standard Solution, 500-mg/L as PO ₄	100	mL MDB.....	14242-32
Phosphate Standard Solution, 30-mg/L as PO ₄	946	mL.....	14367-16

OPTIONAL APPARATUS

Cylinder, graduated, 25-mL	3	each.....	20886-40
Digital Reactor Block 100.....	1	each.....	DRB 100
Pipettor, Jencons, 100–1000 µL	1	each.....	27949-00
Replacement tips for 27949-00	400/pkg.....		27950-00



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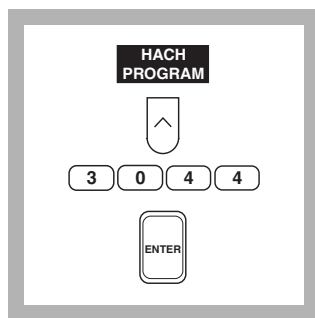


Ascorbic Acid Method

UniCell™ Vials

(0.0 to 60.0 mg/L PO_4^{3-})

Scope and Application: For water, wastewater, boiler water, surface water, and process control. The estimated detection limit for program number 3044 is 6.0 mg/L PO_4^{3-} .



- 1.** Press the soft key under **HACH PROGRAM**.

Select the stored program number for UniCell phosphorus by pressing **3044** with the numeric keys.

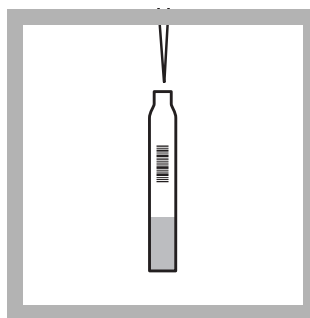
Press: **ENTER**



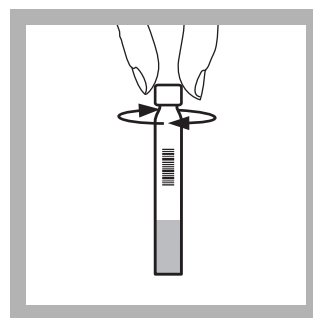
- 2.** The display will show:
**HACH PROGRAM: 3044
Phosphate, HCT 122**

The wavelength (λ), **890 nm**, is automatically selected.

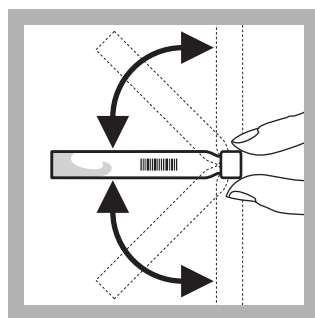
Note: Clean glassware with 1:1 Hydrochloric Acid Solution. Rinse again with deionized water. Do not use phosphate detergents to clean glassware.



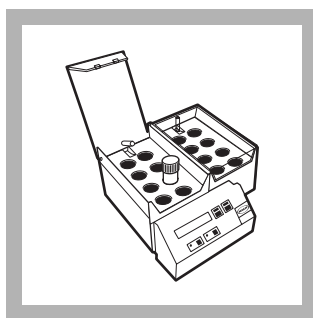
- 3.** Pipet 0.4 mL of sample into the sample vial.



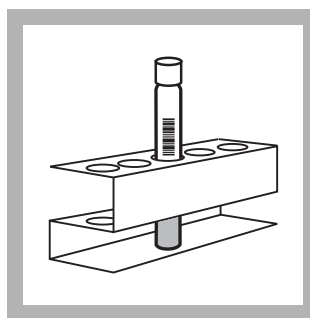
- 4.** Screw an **orange** UniCap A (HCT 122 A) onto the sample vial.



- 5.** Cap and invert the sample vial several times to mix.

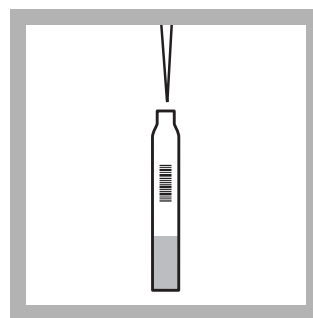


- 6.** Place the sample in the reactor block and heat for 60 minutes at 100 °C.



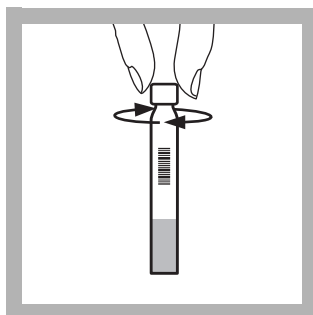
- 7.** After the 60-minute heating period, carefully remove the vial from the reactor. Place it in a test tube rack and allow to cool to room temperature.

Note: Tubes will be hot.

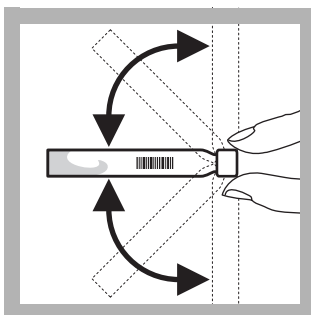


- 8.** After the sample cools, pipet 0.5 mL of Reagent B (HCT 121/122 B) into the sample vial.

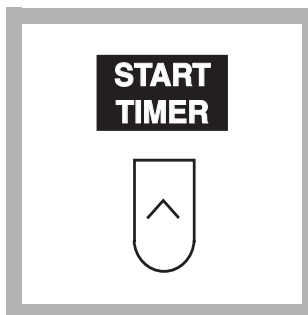
Close the Reagent B bottle **immediately** after use.



9. Screw a **grey** Unicap (HCT 122 C) onto the sample vial.



10. Cap tightly and invert several times to mix.

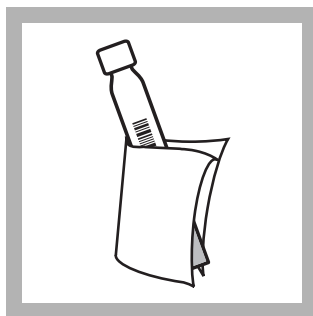


11. Press the soft key under **START TIMER**.

A 10-minute waiting period will begin.



12. Insert the Test Tube Adapter into the sample cell module by sliding it under the thumb screw and into the alignment grooves. Fasten with the thumb screw.

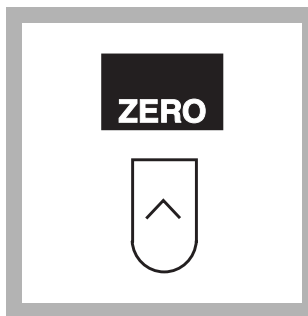


13. Clean the outside of the zero vial (white cap) with a towel.

Note: Wiping with a damp towel, followed by a dry one, will remove fingerprints or other marks.



14. Place the zero vial into the cell holder and close the light shield.



15. Press the soft key under **ZERO**.

The display will show:

0.0 mg/L PO₄³⁻

Note: For alternate concentration units press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



16. After the timer beeps, clean the outside of the sample vial with a towel.

Note: Wiping with a damp towel, followed by a dry one, will remove fingerprints or other marks.



17. Place the prepared sample vial into the cell holder and close the light shield. Results in mg/L PO_4^{3-} (or chosen units) will be displayed.

Note: Results may also be expressed as phosphorus (P) or as phosphorus pentoxide (P_2O_5). Press the soft keys under **OPTIONS** and then **FORM**: to scroll through the available options.

Interferences

The ions listed in the following table have been individually tested up to the given concentrations. Cumulative effects and the influence of other ions have not been evaluated.

Ion	No interference up to:
SO_4^{2-}	5000 mg/L
Cl^-	2000 mg/L
K^+ , Na^+ , Ca^{2+}	1000 mg/L
Mg^{2+} , NO_3^-	500 mg/L
Co^{2+} , Fe^{2+} , Fe^{3+} , Zn^{2+} , Cu^{2+} , Ni^{2+} , Cr^{3+} , I^- , NO_2^- , Cd^{2+} , Sn^{2+} , NH_4^+ , Mn^{2+} , Al^{3+} , Hg^{2+} , Pb^{2+} , SiO_2	50 mg/L
Ag^+	25 mg/L
Cr^{6+}	5 mg/L

Sample Collection, Storage and Preservation

Analyze samples within 3 hours after collection for best results. Collect samples in plastic or glass bottles that have been acid cleaned with 1:1 Hydrochloric Acid Solution and rinsed with deionized water. Store in a cool, dry place. Do not use commercial detergents containing phosphate for cleaning glassware used in this test.

If prompt analysis is impossible, preserve samples* up to 28 days by adjusting the pH to 2 or less with Sulfuric Acid, 5.25 N and storing the sample at 4 °C. The pH can be checked using pH paper. Samples can be stored up to 28 days. Warm the samples to room temperature and neutralize the pH before analysis.

Accuracy Check

Standard Solutions Method

Use a 30 mg/L Phosphate Standard Solution listed under Optional Reagents. Perform the phosphate procedure as described.

Standard Adjust

To adjust the calibration curve using the reading obtained with the 30 mg/L PO_4^{3-} Phosphate Standard Solution, press the soft keys under **OPTIONS, (MORE)** then **STD:OFF**. Press **ENTER** to accept the default concentration, 30.0-mg/L PO_4^{3-} . If an alternate concentration is used, enter the actual concentration and press **ENTER** to return to the read screen. For more information, see Section 1.5.5, *Adjusting the Calibration Curve*.

Standard Additions Method

Note: Clean glassware with 1:1 Hydrochloric Acid Solution. Rinse again with deionized water. Do not use phosphate detergents to clean glassware.

- a. Leave the unspiked sample in the sample compartment. Verify that the units displayed are in mg/L. Select standard additions mode by pressing the soft keys under **OPTIONS, (MORE)** and then **STD ADD**.
- b. Press **ENTER** to accept the default sample volume (mL), 25.0.
- c. Press **ENTER** to accept the default standard concentration (mg/L), 500.
- d. Press the soft key under **ENTRY DONE**.
- e. Use a pipet to add 0.1 mL, 0.2 mL and 0.3 mL of standard (500.0-mg/L PO_4^{3-}), respectively, to three 25-mL samples in 25-mL mixing cylinders. Mix each sample thoroughly.
- f. Analyze each standard addition sample as described above (use a 0.4-mL aliquot of the spiked sample as the sample). Accept the standard additions reading by pressing the soft key under **READ** each time. Each addition should reflect approximately 100% recovery.
- g. After completing the sequence, the display will show the extrapolated concentration value and the “best-fit” line through the standard additions data points, accounting for matrix interferences.
- h. See Section 1.4.1 *Standard Additions* for more information.

* See 40 CFR part 136.3

Method Performance

Precision

Standard: 30.0 mg/L PO₄³⁻

Program	95% Confidence Limits
3044	28.8–30.2 mg/L PO ₄ ³⁻

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
3044	6.0 mg/L PO ₄ ³⁻

For more information on derivation and use of Hach's estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by 40 CFR part 136, appendix B, see Section 1.5.1.

Sensitivity

Program Number: 3044

Portion of Curve	ΔAbs	ΔConcentration
Entire Range	0.010	0.5 mg/L

See Section 1.5.3 *Sensitivity Explained* for more information.

Summary Of Method

Phosphate ions react with molybdate and antimony in an acidic solution to form an antimonyl phosphomolybdate complex. Ascorbic acid then reduces the complex to phosphomolybdenum blue. Total phosphate measurements include a digestion step.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to *Section 1*.

UniCap A — (HCT 122 A) contains: sodium peroxodisulfate.

Reagent B — (HCT 121/122 B) contains 16% sulfuric acid.

Pollution Prevention and Waste Management

Final samples will contain molybdenum. In addition, final samples will have a pH less than 2 and are considered corrosive (D002) by the Federal RCRA.

PHOSPHORUS, Total, continued

REQUIRED REAGENTS AND STANDARDS

Phosphate PO₄-P, UniCell™ HCT 122 23/pkg HCT 122

OPTIONAL REAGENTS

Description	Quantity Required Per Test	Unit	Cat. No.
Hydrochloric Acid Standard Solution, 6.0 N (1:1)	500	mL.....	884-49
Phosphate Standard Solution, 500-mg/L as PO ₄	100	mL MDB.....	14242-32
Phosphate Standard Solution, 30-mg/L as PO ₄	946	mL.....	14367-16

OPTIONAL APPARATUS

Cylinder, graduated, 25-mL	3	each.....	20886-40
Digital Reactor Block 100.....	1	each.....	DRB 100
Pipettor, Jencons, 100–1000 µL	1	each.....	27949-00
Replacement tips for 27949-00	400/pkg.....		27950-00



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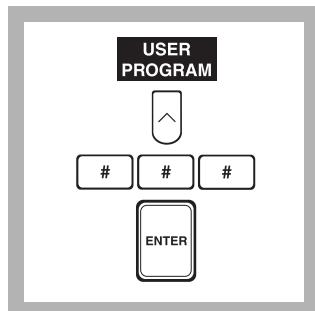


Method 8049

Tetraphenylborate Method

(0 to 7.0 mg/L)

Scope and Application: For water, wastewater and seawater.



1. If this test has not been run on this instrument, perform the *User Programming* procedure under *Calibration*.

Press the soft key under **USER PROGRAM**. Select the stored program number for potassium (K) using the numeric keys.

Press: **ENTER**

Note: If samples cannot be analyzed immediately, see *Sample Collection, Preservation and Storage* below.

Note: Because of potential variation between lots of Potassium 3 Reagent, perform a new calibration for each lot of reagent to obtain best accuracy. Prepare and store the calibration as directed under *Calibration* following these steps.

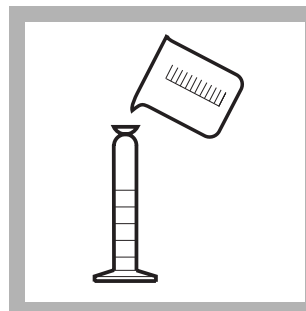
Note: The Flow Cell or Sipper Cell Modules may not be used for this procedure.



2. The display will show:
USER PROGRAM: ### Potassium

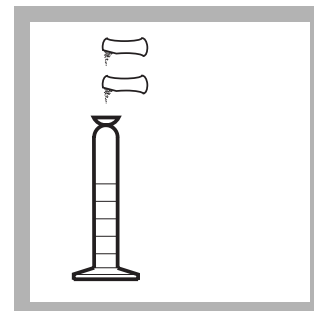
The wavelength (λ), **650 nm**, is automatically selected.

Note: ### refers to the number assigned during calibration.

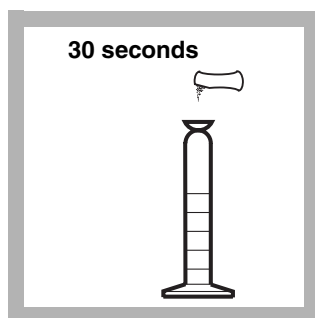


3. Fill a mixing graduated cylinder with 25 mL of sample.

Note: Filter highly colored or turbid samples. Use filtered sample here and in Step 8.

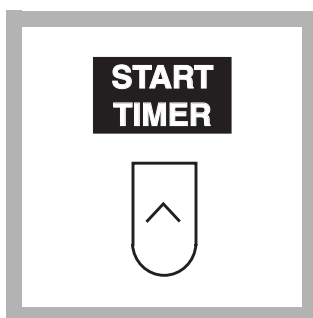


4. Add the contents of one Potassium 1 Reagent Pillow. Add the contents of one Potassium 2 Reagent Pillow. Stopper. Invert several times to mix.



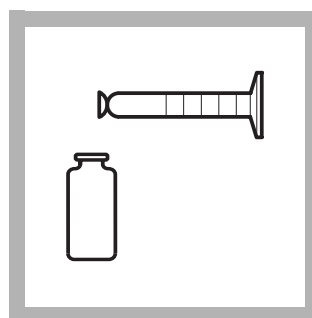
5. Add the contents of one Potassium 3 Reagent Pillow after the solution clears. Stopper. Shake for 30 seconds.

Note: A white turbidity will form if potassium is present.

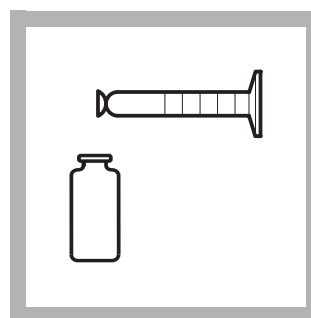


6. Press the soft key under **START TIMER**.

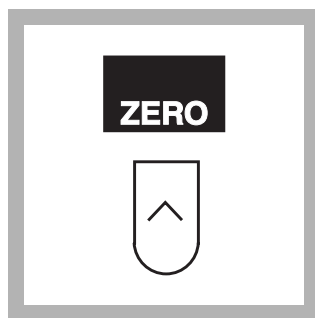
A 3-minute reaction period will begin.



7. Pour the solution from the cylinder into a 25-mL sample cell (the prepared sample).



8. When the timer beeps, fill the second sample cell (the blank) with 25 mL of sample. Place it into the cell holder. Close the light shield.

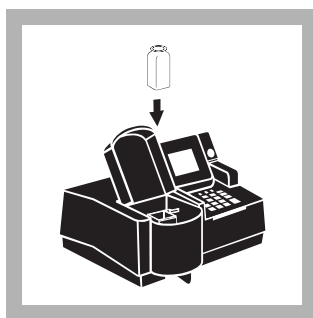


9. Press the soft key under **ZERO**.

The display will show:

0.0 mg/L K

Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



10. Within seven minutes after the timer beeps, place the prepared sample into the cell holder. Close the light shield. Results in mg/L potassium (or chosen units) will be displayed.

Note: Clean the cells with soap and a brush.

Interferences

The substances listed below have been tested and will not interfere at or below the levels stated. If these substances are present at higher levels, analysts need to conduct interference studies at the higher levels to determine if the substance interferes.

Interfering Substance	Interference Levels and Treatments
Ammonium Nitrogen	15 mg/L as N
Calcium	7000 mg/L as CaCO ₃
Chloride	15,000 mg/L
Magnesium	6000 mg/L as CaCO ₃

Sample Collection, Preservation and Storage

Collect samples in acid-washed plastic bottles. Adjust the pH to 2 or less with nitric acid (about 2 mL per liter). Preserved samples may be stored at least six months at room temperature. Adjust the pH to 4–5 with 5.0 N Sodium Hydroxide before analysis. Do not measure pH in the sample container with a pH electrode, as this will introduce potassium from the filling solution. Use pH paper or pour off sample and test pH in a separate beaker. Correct the test result for volume additions; see Section 1.2.2 *Correcting for Volume Additions*.

Accuracy Check

Standard Additions Method

- Leave the unspiked sample in the sample compartment. Verify that the units displayed are in mg/L. Select standard additions mode by pressing the soft keys under **OPTIONS, (MORE)** and then **STD ADD**.
- Press **ENTER** to accept the default sample volume (mL), 25.
- Press **ENTER** to accept the default standard concentration (mg/L), 250.
- Press the soft key under **ENTRY DONE**.
- Snap the neck off a Potassium Voluette Ampule Standard, 250-mg/L K.
- Use the TenSette Pipet to add 0.1 mL, 0.2 mL and 0.3 mL of standard, respectively to three 25-mL samples and mix each thoroughly.
- Analyze each standard addition sample as described above. Accept standard additions reading by pressing the soft key under **READ** each time. Each addition should reflect approximately 100% recovery.
- After completing the sequence, the display will show the extrapolated concentration value and the “best-fit” line through the standard additions data points, accounting for matrix interferences.
- See Section 1.4.1 *Standard Additions* for more information.

Calibration

Prepare calibration standards containing 1, 2, 3, 4, 5, 6, 7, and 8 mg/L potassium as follows:

- Into eight different 100-mL Class A volumetric flasks, pipet 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0 and 8.0 mL of the 100-mg/L Potassium Standard Solution using class A glassware or TenSette Pipet.

- b. Dilute to the mark with deionized water. Mix thoroughly.
- c. Use deionized water for the 0-mg/L potassium standard.

User Programming

- a. Start from the **MAIN MENU**. Press the soft key under **USER PROGRAM**.
- b. If you have not performed a potassium calibration before, press the soft key under **CREATE**. Key any available program number you wish to use for potassium testing. Press **ENTER**. Press the soft key under **COPY PROGRAM**. Select program number **3100** and press **ENTER**. If you already have a working potassium program, press the soft key under **EDIT**, select the program number and press **ENTER**.
- c. Press the down arrow until you highlight the parameter **Calib. table**. Press the soft key under **EDIT TABLE**. Press the up arrow to highlight the very first concentration in the list. Press the soft key under **EDIT ABS**. Press **CE** and then **ENTER** to erase the first, inaccurate, absorbance value. Repeat to erase all the absorbance values. Press the soft key under **ENTRY DONE**.

Note: An approximate calibration curve is preprogrammed within Program 3100. For improved accuracy, a new calibration should be performed with each new lot of reagents.

- d. Note which mg/L value is highlighted. Perform steps 3 through 7 of the potassium Tetraphenylborate Method on a potassium standard of the indicated concentration. Place a 25-mL sample cell containing only deionized water into the cell holder and close the lid. Press the soft key under **ZERO**. Place the prepared sample in the cell holder. Press the soft key under **READ** to accept the absorbance value. Repeat this step for each standard.
- e. In the **Curve fit:** display, press the soft key under **NEXT FORMULA** until **C = a + bA + CA² + dA³** is displayed. Press the soft key under **FORCE O:** once to **ON** is selected. Press **EXIT** until **Store Changes?** is displayed. Press the soft key under **YES**. The program is ready for use.

Note: Some variations of the calibration procedure are possible. See the DR/4000 Instrument Manual for details.

Summary of Method

Potassium in the sample reacts with sodium tetraphenylborate to form an insoluble white solid. The amount of turbidity produced is proportional to the potassium concentration.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to *Section 1*.

Pollution Prevention and Waste Management

The final samples are highly acidic. Neutralize to pH 6–9 and flush to drain for disposal. For more information on pollution prevention and waste management, refer to *Section 1*.

REQUIRED REAGENTS AND STANDARDS

Potassium Reagent Set (100 tests)	24591-00
Includes: (4) 14321-98, (4) 14322-18, (1) 14323-99	

Description	Quantity Required		Cat. No.
	per test	Unit	
Potassium 1 Reagent Powder Pillows	1 pillow	25/pkg	14321-98
Potassium 2 Reagent Powder Pillows	1 pillow	25/pkg	14322-98
Potassium 3 Reagent Powder Pillows	1 pillow	100/pkg	14323-99
Potassium Standard Solution, 100-mg/L.....	varies.....	500 mL.....	23517-49

REQUIRED EQUIPMENT AND SUPPLIES

Clippers, for opening powder pillows	1	each.....	968-00
Cylinder, mixing, graduated, 25-mL	1	each.....	1896-40
DR/4000 1-inch Cell Adapter	1	each.....	48190-00
Flask, volumetric, 100-mL, Class A	8	each.....	14574-42
Pipet, TenSette™, 1–10 mL	1	each.....	19700-10
Pipet Tips, for 19700-10 TenSette™ Pipet.....	varies.....	50/pkg.....	21997-96

OPTIONAL REAGENTS AND STANDARDS

Nitric Acid, ACS	500 mL.....	152-49
Nitric Acid Solution, 1:1	500 mL.....	2540-49
Potassium Standard Solution, 5-mg/L.....	500 mL.....	20583-49
Potassium Standard Solution, 100-mg/L.....	100 mL.....	22404-42
Potassium Standard Solution, 10-mL Voluette™ Ampule, 250-mg/L.....	16/pkg.....	14790-10
Sodium Hydroxide Standard Solution, 5.0 N.....	59 mL DB.....	2450-26
Water, deionized	4 liters.....	272-56

OPTION EQUIPMENT AND SUPPLIES

DR/4000 Carousel Module.....	each.....	48070-02
pH Paper, pH 1.0 to 11.0	5 rolls/pkg.....	391-33
pH Meter, <i>sension</i> ™1, portable	each.....	51700-00



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FAX: (970) 669-2932



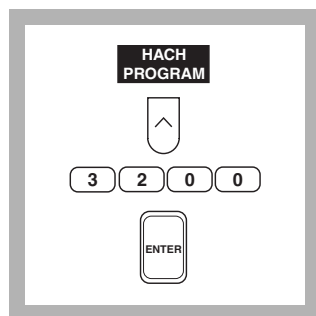
Method 8337

Direct Binary Complex Method

Powder Pillows

(0 to 5.00 mg/L as CTAB)

Scope and Application: For cooling tower and pool/spa water. The estimated detection limit for program number 3200 is 0.11 mg/L CTAB.



1. Press the soft key under **HACH PROGRAM**.

Select the stored program for quaternary ammonium compounds (QAC) by pressing **3200** with the numeric keys.

Press: **ENTER**

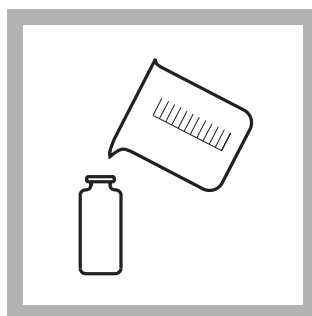
Note: If samples cannot be analyzed immediately, see *Sample Collection, Storage and Preservation* following these steps.

Note: The Flow Cell and Sipper Modules cannot be used with this procedure.

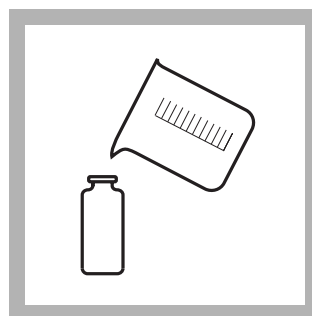


2. The display will show:
HACH PROGRAM: 3200 QAC

The wavelength (λ), **575 nm**, is automatically selected.



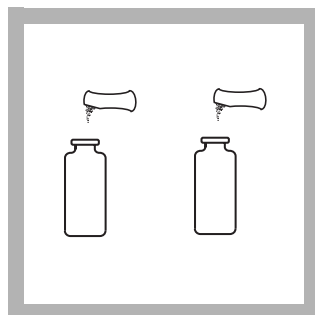
3. Fill a sample cell (the blank) with 25 mL of deionized water.



4. Fill another cell (the prepared sample) with 25 mL of sample.

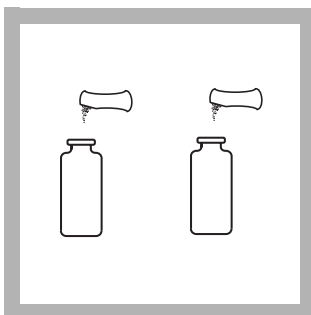
Note: For proof of accuracy, a 5.0-mg/L CTAB standard solution (see *Accuracy Check*) can be used in place of the sample.

QUATERNARY AMMONIUM COMPOUNDS, continued



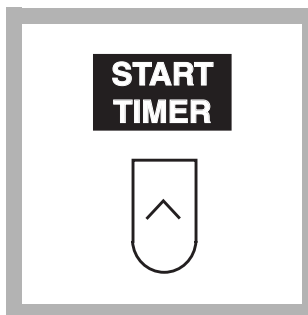
5. Add the contents of one Q.A.C. Reagent 1 Powder Pillow to each sample cell. Swirl (do not shake) the sample cells to dissolve the reagents.

Note: Shaking the sample cell causes air bubble turbidity that dissipates slowly, interfering with the test results.

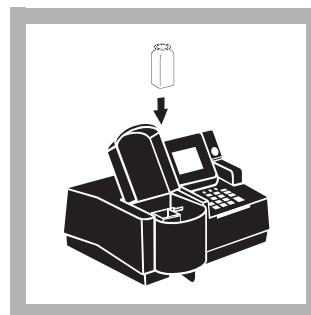


6. Add the contents of one Q.A.C. Reagent 2 Powder Pillow to each sample cell. Swirl (do not shake) the sample cells to dissolve the reagents.

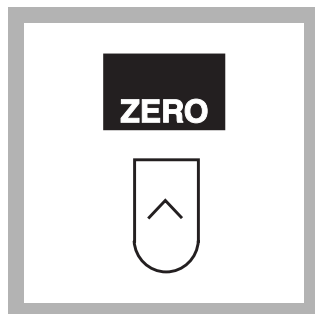
Note: A purple color will form if a quaternary ammonium compound is present.



7. Press the soft key under **START TIMER**. A 2-minute reaction period will begin.



8. When the timer beeps, place the blank into the cell holder. Close the light shield.



9. Press the soft key under **ZERO**.
The display will show:
0.00 mg/L CTAB



10. Place the prepared sample into the cell holder. Close the light shield. Results in mg/L (or chosen units) quaternary ammonium compounds as cetyl-trimethylammonium bromide will be displayed.

Interferences

Interference studies were conducted by preparing a CTAB standard solution of approximately 3 mg/L as well as a solution of the potential interference. The constituent was said to interfere when the resulting concentration changed by 10%.

Table 1 Interfering Substances and Suggested Treatments

Interfering Substance	Interference Levels and Treatments
Calcium (as CaCO ₃)	Positive interference above 1,350 mg/L
Chlorine, HOCl and OCl ⁻	Positive interference above 7 mg/L
Cyanuric acid	Negative interference above 70 mg/L
Igepal™ nonionic surfactant	Positive interference above 3 mg/L
Iodine, I ₃ ⁻	Positive interference above 3 mg/L
Iron, Fe ³⁺	Positive interference above 80 mg/L
Liquimine™ 14-P, filming amine	Positive interference above 1,825 mg/L
Magnesium, Mg ²⁺	Positive interference above 1,350 mg/L
Niaproof™ anionic surfactant	Negative interference above 11 mg/L
Polyacrylic acid	Negative interference above 16 mg/L
Sodium lauryl sulfate	Negative interference above 8 mg/L
Sodium polyphosphate	Positive interference above 1,325 mg/L
Tribenzylamine	Positive interference above 7 mg/L
Triton X-100™ nonionic surfactant	Positive interference above 4 mg/L
Urea	Positive interference above 8 mg/L
Highly buffered samples or extreme sample pH	May exceed the buffering capacity of the ampules or reagents and require sample pretreatment. Adjust the sample pH between 3 and 5 by using a pH meter or pH paper and adding dropwise an appropriate amount of acid or base such as 1.0 N Sulfuric Acid Standard Solution or 1.0 N Sodium Hydroxide Standard Solution. If significant volumes of acid or base are used, a volume correction should be made. See Section 1.2.2 <i>Correcting for Volume Additions</i> .

No Interferences	Highest Concentration Tested (mg/L)
Silica, SiO ₂	400
Potassium alum, AlKSO ₈	500
Sodium thiosulfate, Na ₂ S ₂ O ₃	30

After several samples have been analyzed, the sample cells may exhibit a build-up of a pink or purple color. A rinse with 1.0 N Sodium Hydroxide Solution followed by a Alconox™ detergent wash and deionized water rinse will eliminate the build-up when it occurs.

Sample Collection, Storage and Preservation

Collect samples in glass bottles that have been rinsed several times with sample before final sample filling. Do not use plastic containers as plastic adsorbs QACs.

Accuracy Check

Standard Additions Method

- a. Leave the unspiked sample in the sample compartment. Verify that the units displayed are in mg/L. Select standard additions mode by pressing the soft keys under **OPTIONS, (MORE)** and then **STD ADD**.
- b. Press **ENTER** to accept the default sample volume (mL), 25.0.
- c. Press **ENTER** to accept the default standard concentration (mg/L), 100.0.
- d. Press the soft key under **ENTRY DONE**.
- e. Open a Q.A.C. Standard Solution, 100-mg/L CTAB.
- f. Use the TenSette Pipet to add 0.1 mL, 0.2 mL and 0.3 mL of standard, respectively to three 25-mL samples and mix each thoroughly.
- g. Analyze each standard addition sample as described above. Accept the standard additions reading by pressing the soft key under **READ** each time. Each addition should reflect approximately 100% recovery.
- h. After completing the sequence, the display will show the extrapolated concentration value and the “best-fit” line through the standard additions data points, accounting for matrix interferences.
- i. See Section 1.4.1 *Standard Additions* for more information.

Standard Solution Method

Prepare a 5.0-mg/L CTAB standard solution by pipetting 5.0 mL of Q.A.C Standard, 100-mg/L as CTAB, into a 100-mL volumetric flask. Dilute to the mark with deionized water. Mix well. Prepare this solution daily. Perform the quaternary ammonium compound procedure as described above.

Method Performance

Precision

Standard: 5.00 mg/L CTAB

Program	95% Confidence Limits
3200	4.92–5.08 mg/L CTAB

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
3200	0.11 mg/L CTAB

For more information on derivation and use of Hach’s estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, appendix B, see Section 1.5.1.

Sensitivity

Program Number: 3200

Portion of Curve	Δ Abs	Δ Concentration
Entire Range	0.010	0.046 mg/L

See Section 1.5.3 *Sensitivity Explained* for more information.

Calibration Standard Preparation

To perform a quaternary ammonium compounds calibration using the Direct Binary Complex method, prepare calibration standards containing 1.00, 3.00, and 5.00 mg/L CTAB as follows:

- a. Into three different 100-mL Class A volumetric flasks, pipet 1.00, 3.00, and 5.00 mL of a 100-mg/L Q.A.C. Standard Solution (Cat. No 24153-42) using Class A glassware.
- b. Dilute to the mark with deionized water. Mix carefully to avoid foaming.
- c. Using the Direct Binary Complex method and the calibration procedure described in the *User-Entered Programs* section of the *DR/4000 Spectrophotometer Instrument Manual*, generate a calibration curve from the standards prepared above.

Summary of Method

The test method makes use of a colorimetric chemistry in which a quaternary ammonium compound reacts with an indicator to produce a color change from pale pink to vivid purple. The test is conducted in a stabilized, acid-buffered solution containing a masking agent to eliminate potential interferences. This test is applicable to the monitoring of QACs in swimming pools and cooling towers.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to *Section 1*.

Pollution Prevention and Waste Management

For information on pollution prevention and waste management, refer to *Section 1*.

QUATERNARY AMMONIUM COMPOUNDS, continued

REQUIRED REAGENTS AND STANDARDS

Quaternary Ammonium Compounds Reagent Set (100 tests)24592-00
Includes: (4) 24010-66, (8) 24012-68

Description	Quantity Required		Unit	Cat. No.
	per test			
Q.A.C. Reagent 1 Powder Pillows	2 pillows	50/pkg		24010-66
Q.A.C. Reagent 2 Powder Pillows	2 pillows	25/pkg		24012-68

REQUIRED EQUIPMENT AND SUPPLIES

Cylinder, graduated, 25-mL	1	each	508-40
Clippers, for opening powder pillows	1	each	968-00
DR/4000 1-Inch Cell Adapter	1	each	48190-00

OPTIONAL REAGENTS AND STANDARDS

Q.A.C. Standard Solution, 100-mg/L as CTAB	100 mL	24153-42
Sodium Hydroxide Standard Solution, 1.00 N.....	900 mL	1045-53
Sulfuric Acid Standard Solution, 1.0 N.....	50 mL SCDB	1270-26
Water, deionized	4 liters	272-56

OPTIONAL EQUIPMENT AND SUPPLIES

DR/4000 Carousel Module Kit	each	48070-02
Filter Paper, folded, 12.5-cm.....	100/pkg	1894-57
Flask, volumetric, Class A, 100-mL	each	14574-42
Funnel, poly, 65-mm	each	1083-67
Pipet Filler.....	each	12189-00
Pipet, volumetric, Class A, 1.00-mL	each	14515-35
Pipet, volumetric, Class A, 3.00-mL	each	14515-03
Pipet, volumetric, Class A, 5.00-mL	each	14515-37
Pipet, TenSette 0.1 to 1.0 mL	each	19700-01
Pipet Tips, for 19700-01 TenSette Pipet	50/pkg	21856-96



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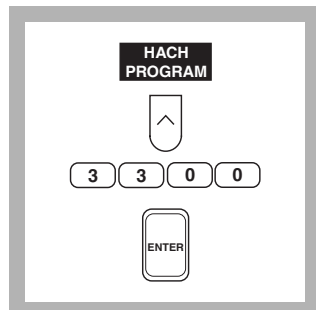
Method 8194

Diaminobenzidine Method*

(0 to 1.000 mg/L)

Scope and Application: For water and wastewater; distillation is required for determining total selenium. See the Distillation procedure following the Colorimetric procedure. The estimated detection limit for program number 3300 is 0.003 mg/L Se.

* Adapted from *Standard Methods for the Examination of Water and Wastewater*.



1. Press the soft key under **HACH PROGRAM**.

Select the stored program for selenium (Se) by pressing **3300** with the numeric keys.

Press: **ENTER**

Note: If samples cannot be analyzed immediately, see *Sample Collection, Storage and Preservation* following these steps. Adjust the pH of preserved samples before analysis.

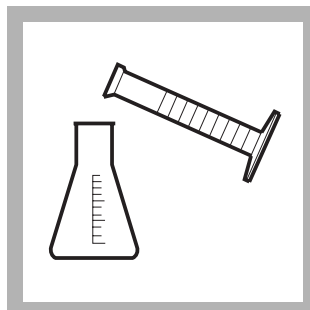
Note: The Flow Cell and Sipper Modules cannot be used with this procedure.



2. The display will show:
HACH PROGRAM: 3300 Selenium

The wavelength (λ), **420 nm**, is automatically selected.

Note: To determine total selenium, perform a distillation. See *Distillation*, after this procedure. Use this distillate in Step 4. See *Summary of Method* following these steps.

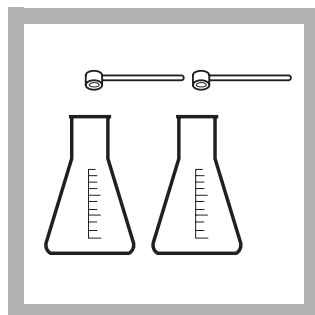


3. Measure 100 mL of deionized water into a 500-mL erlenmeyer flask (label the flask "blank").

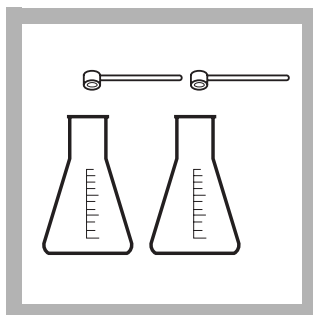


4. Measure 100 mL of sample into a second 500-mL erlenmeyer flask (label the flask "sample").

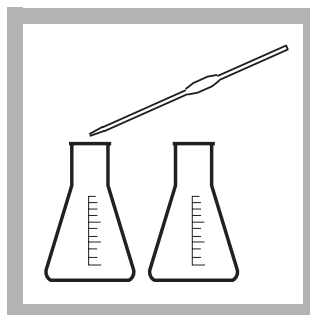
Note: For proof of accuracy, use a 0.5 mg/L selenium standard solution (see *Accuracy Check*) in place of the sample.



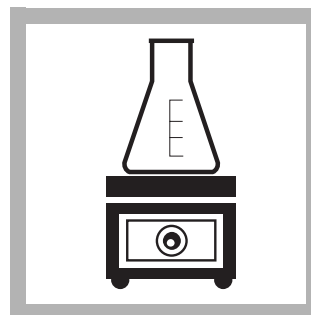
5. Add a 0.2-g scoop of TitrVer Hardness Reagent to each flask. Swirl to mix.



6. Add 0.05-g scoop of diaminobenzidine tetrahydrochloride to each flask. Swirl to mix.

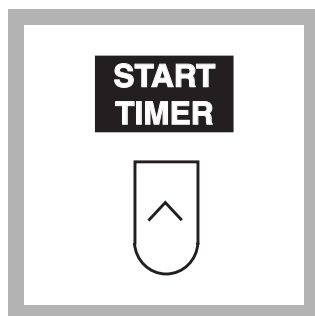


7. Add 5.0 mL of Buffer Solution, sulfate type, pH 2.0, to each flask. Swirl to mix.



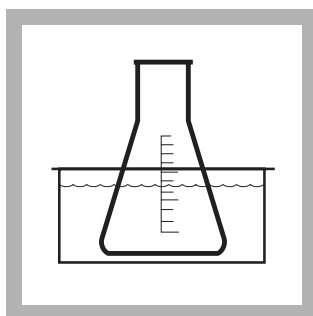
8. Heat each flask on a hot plate or over a flame, bringing the contents to a gentle boil.

Note: If the sample has been distilled as described under Distillation, omit the Buffer Solution. Adjust the pH of the sample distillate to 2.7 (± 0.2) using 5 N Sodium Hydroxide Standard Solution. Adjust the deionized water blank to the same pH value using 5.25 N Sulfuric Acid Standard Solution.



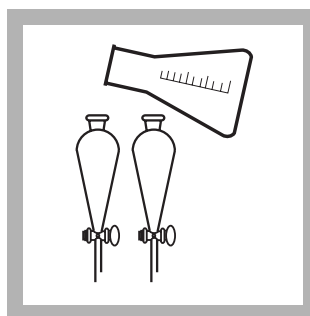
9. Press the soft key under **START TIMER**. A 5-minute reaction period will begin. Continue to boil the contents gently during this time period.

Note: A yellow color will develop if selenium is present.

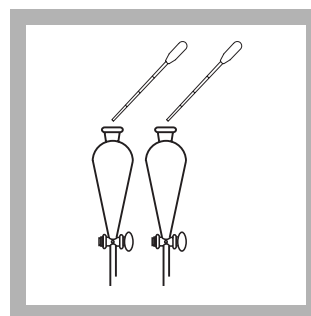


10. When the timer beeps, remove both flasks. Cool to room temperature using a water bath.

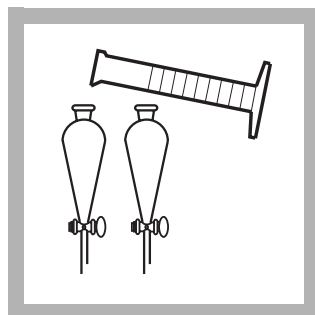
Note: Do not boil more than one minute after the timer beeps.



11. Transfer the contents of each flask to separate 250-mL separatory funnels. Label the funnels "blank" and "sample".

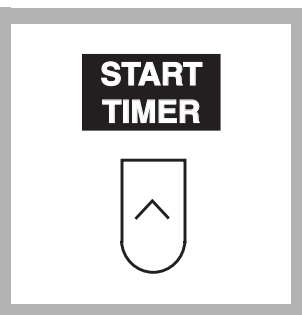


12. Add 2.0 mL of 12 N Potassium Hydroxide Standard Solution to each funnel using a calibrated 1.0-mL plastic dropper. Stopper. Shake each funnel to mix.

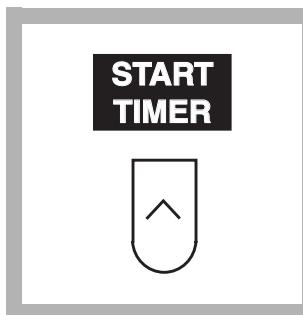


13. Add 30 mL of toluene to each funnel. Stopper. Swirl and invert each funnel, then open the stopcock to vent the funnel. Close the stopcock. Repeat twice with each funnel.

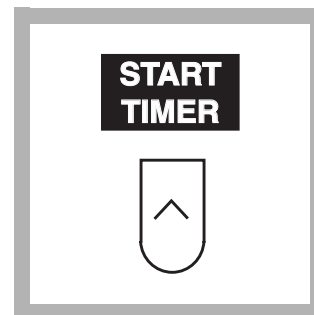
Note: Use toluene only with adequate ventilation.



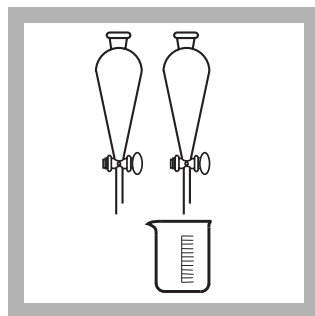
14. Press the soft key under **START TIMER**. A 30-second period will begin. Shake the funnel containing the *blank* vigorously for the 30-second period.



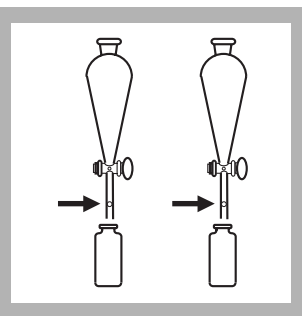
15. Press the soft key under **START TIMER**. A 30-second period will begin. Shake the funnel containing the *sample* vigorously for the 30-second period.



16. Press the soft key under **START TIMER**. A 4-minute reaction period will begin.



17. When the timer beeps, drain the lower water layer from each funnel and discard.



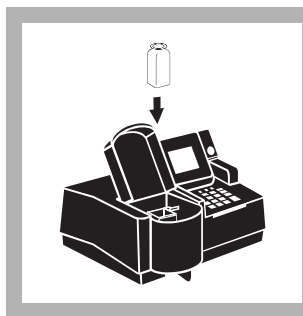
18. Insert a cotton plug into the delivery tube of each separatory funnel. Slowly drain the toluene into respective sample cells labeled “blank” and “sample”. Stopper the sample cells.

Note: Do not wait more than 5 minutes after the timer beeps before completing steps 19 through 21.

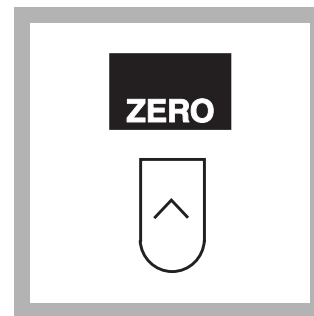
Note: Filtering the toluene through dry absorbent cotton will remove any water or suspended particles.

Note: The developed color is stable but should be measured as soon as possible.

Note: Glass stoppered sample cells can be used to prevent evaporation.



19. Place the blank into the cell holder. Close the light shield.

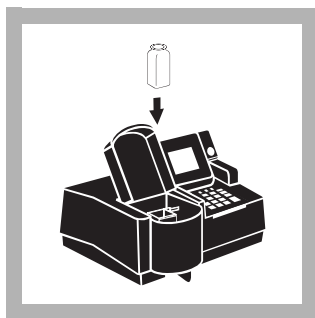


20. Press the soft key under **ZERO**.

The display will show:

0.000 mg/L Se

Note: For alternate concentration units press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



21. Place the prepared sample in the cell holder. Close the light shield. Results in mg/L selenium (or chosen units) will be displayed.

Note: Acetone is a suitable solvent for removing toluene from glassware.

Interferences

Table 1 Interfering Substances and Suggested Treatments

Interfering Substance	Interference Level and Treatment
Ferric iron	Up to 2.5 mg/L. Distill sample to eliminate interference.
Manganese	Will not interfere
Strong oxidizing agents (i.e., iodine, bromine or chlorine)	Can react with the indicator to give low results. Distill sample to eliminate interference.

There are no positive inorganic interferences with this method.

Sample Collection, Storage and Preservation

Collect samples in clean glass or plastic containers. Adjust the pH to 2 or less with nitric acid (about 1.5 mL per liter). Preserved samples can be stored for up to six months at room temperature. Correct the test result for volume additions; see Section 1.2.2 *Correcting for Volume Additions*.

Distillation

Always perform this procedure under a fume hood! This distillation involves the use of a strong acid and oxidizer at high temperatures. To avoid personal injury, observe all laboratory safety precautions when operating the distilling apparatus.

- Measure 500 mL of sample into a 1000-mL beaker.
- Add 1 mL of Methyl Orange Indicator Solution. Stir with a glass rod.
- Using a dropper, add 0.1 N Hydrochloric Acid Standard Solution dropwise until the solution becomes pink. Then add an additional 2 mL.
- Pipet 5.0 mL Calcium Chloride Solution. Mix well.

- e. Using a dropper, add 1 g/L Potassium Permanganate Standard Solution drop-wise until the solution is purple.
- f. Place the beaker on a hot plate. Evaporate the solution to approximately 250 mL. Periodically add 1 g/L Potassium Permanganate Solution to keep the solution purple.

Note: Any precipitate formed at this step is manganese dioxide and may be ignored.

- g. Cool the solution. While cooling, set up the distillation apparatus for the general purpose distillation as shown in the *Hach Distillation Manual*.
- h. Pour the treated sample solution into the distillation flask. Add a stirring bar to the flask.
- i. Pipet 5.0 mL of 0.1 N Sodium Hydroxide Standard Solution into the flask. Turn the stirrer power switch to ON. Set the stir control to 5.
- j. Turn on the water and adjust so a constant flow is maintained through the condenser. Set the heat control to 10.
- k. When only a few milliliters are left in the distillation flask, turn the power switch off. The distillate in the erlenmeyer flask may be discarded.
- l. Perform this step under a hood. When the flask has cooled, add 50 mL of 19.2 N Sulfuric Acid Standard Solution to the flask. Add the contents of one Potassium Bromide Powder Pillow to the flask.
- m. Fill a 250-mL beaker to the 75-mL mark with deionized water. Place it under the drip tube. Elevate the beaker with a laboratory jack so the tube extends below the level of the water.
- n. Add 1.0 mL of 30% Hydrogen Peroxide Solution to the flask. Turn the stir control to 5 and the heat control to 10. Cap the distillation flask.
- o. Heat the distillation flask until the yellow color is gone from the complete distillation apparatus, including the J-tube and condenser. Remove the beaker from under the drip tube.
- p. Turn off the heater switch. When the J-tube and condenser has cooled, rinse them with deionized water. Add the washings to the 250-mL beaker. Total volume in the beaker should be approximately 100 mL.
- q. Add the Phenol Solution drop-wise to the distilled sample to discharge the bromine color (a white precipitate of tribromophenol will form.)
- r. Allow the precipitate to settle. Using a dropper, collect about 5 mL of the clear, colorless distillate and transfer to a test tube.
- s. Test the solution for completeness of precipitation by adding 2 drops of Phenol Solution. If the solution becomes cloudy or white precipitate forms, residual bromine is still present (proceed to next step). If no cloudiness occurs, the sample is ready for analysis.
- t. Transfer the 5-mL aliquot back to the beaker and continue to add Phenol Solution until no turbidity is formed in subsequent 5-mL aliquots.
- u. Transfer the sample into a 500-mL volumetric flask. Rinse the beaker with deionized water and add to the flask.
- v. Dilute to volume with deionized water, stopper and mix well. The distillate is now ready for analysis.

Accuracy Check

Standard Additions Method

- a. Leave the unspiked sample in the sample compartment. Verify that the units displayed are in mg/L. Select standard additions mode by pressing the soft keys under **OPTIONS, (MORE)** and then **STD ADD**.
- b. Press **ENTER** to accept the default sample volume (mL), 100.00.
- c. Press **ENTER** to accept the default standard concentration (mg/L), 100.00.
- d. Press the soft key under **ENTRY DONE**.
- e. Prepare a 100-mg/L selenium standard solution by pipetting 10.0 mL of Selenium Standard Solution, 1000-mg/L, into a 100-mL volumetric flask. Dilute to volume with demineralized water and mix thoroughly.
- f. Use the TenSette Pipet to add 0.1, 0.2 mL and 0.3 mL of standard, respectively to three 100-mL samples and mix each thoroughly.
- g. Analyze each standard addition sample as described above. Accept the standard additions readings by pressing the soft key under **READ** each time. Each addition should reflect approximately 100% recovery.
- h. After completing the sequence, the display will show the extrapolated concentration value and the “best-fit” line through the standard additions data points, accounting for matrix interferences.
- i. See Section 1.4.1 *Standard Additions* for more information.

Standard Solution Method

Prepare a 100-mg/L selenium standard solution as described in *Standard Additions Method, step e*, above. Prepare a 0.5-mg/L selenium standard solution by pipetting 1.00 mL of the selenium standard solution, 100-mg/L, into a 200-mL volumetric flask. Dilute to volume with deionized water. Transfer 100 mL of the standard into a 500-mL erlenmeyer flask. Perform the test as described above.

Method Performance

Precision

Standard: 0.500 mg/L Se

Program	95% Confidence Limits
3300	0.497–0.503 mg/L Se

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
3300	0.003 mg/L Se

For more information on derivation and use of Hach’s estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, appendix B, see Section 1.5.1.

Sensitivity

Program Number: 3300

Portion of Curve	Δ Abs	Δ Concentration
Entire Range	0.010	0.0110 mg/L

See Section 1.5.3 *Sensitivity Explained* for more information.

Calibration Standard Preparation

To perform a selenium calibration using the diaminobenzidine method, first prepare a 10 mg/L working standard by diluting 10 mL of 1000-mg/L Selenium Standard Solution (Cat. No. 22407-42) to 1000 mL using Class A glassware. Prepare calibration standards containing 0.050, 0.080, 0.500, and 1.000 mg/L selenium as follows:

- a. Into four different 1000 mL Class A volumetric flasks, pipet 5.00, 8.00, 50.00, and 100.00 mL of the 10-mg/L selenium working standard using Class A glassware.
- b. Dilute to the mark with deionized water. Mix thoroughly.
- c. Using the diaminobenzidine method and the calibration procedure described in the *User-Entered Programs* section of the *DR/4000 Spectrophotometer Instrument Manual*, generate a calibration curve from the standards prepared above.

Summary of Method

An EDTA masking agent is added to the sample to remove interferences such as iron prior to the test. The addition of a sulfate buffer adjusts the sample to the optimum pH of 1 to 2. Under these conditions, diaminobenzidine reacts with all selenium present as selenite (Se^{4+}) to give a yellow-colored piazselenol complex which is extracted and the color intensity measured colorimetrically. Selenium present as Se^{2-} and Se^{6+} is not detected unless the sample is distilled.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to *Section 1*.

Pollution Prevention and Waste Management

Toluene (F005) solutions are regulated as hazardous waste by the Federal RCRA. Do not pour these materials down the drain. Water saturated with toluene, toluene solutions, and the cotton plug used in the delivery tube of the separatory funnel should be collected for disposal with laboratory solvent wastes. See *Section 1* for more information on proper disposal of these materials.

REQUIRED REAGENTS AND STANDARDS

Selenium Reagent Set (100 tests).....	22442-00
Includes: (1) 452-49, (1) 7062-22, (2) 230-32, (1) 204-26, (1) 14470-17	

Description	Quantity Required		Cat. No.
	per test	Unit	
Buffer Solution, sulfate type, pH 2.0.....	10 mL	500 mL	452-49
Diaminobenzidine, tetrahydrochloride.....	0.1 g	5 g	7062-22
Potassium Hydroxide Standard Solution, 12 N.....	4 mL	100 mL	230-32
TitraVer Hardness Reagent, ACS	0.4 g	100 g	204-26
Toluene, ACS	60 mL	4 liters	14470-17
Water, deionized	100 mL	4 liters	272-56

REQUIRED EQUIPMENT AND SUPPLIES

Cotton Balls, absorbent	1	100/pkg	2572-01
Cylinder, graduated, 50-mL	1	each	508-41
Cylinder, graduated, 100-mL	1	each	508-42
DR/4000 1-Inch Cell Adapter	1	each	48190-00
Dropper, 0.5 & 1.0 mL marks, glass	1	5/pkg	14197-05
Dropper, 0.5 & 1.0 mL marks, plastic.....	1	20/pkg	21247-20
Flask, erlenmeyer, 500-mL.....	2	each	505-49
Funnel, separatory, 250-mL	2	each	520-46
Pipet, volumetric, 5-mL	1	each	515-37
Pipet Filler, safety bulb.....	1	each	14651-00
Ring, support, (3") 83-mm	1	each	580-00
Sample Cells, matched pair, 1-inch, glass, with stopper	2	pair	26126-02
Spoon, measuring, 0.05-g.....	1	each	492-00
Spoon, measuring, 0.2-g.....	1	each	638-00
Support, ring stand, (5 x 8") 127 x 203mm.....	1	each	563-00

Select one based on available voltage:

Hot Plate, 4" diameter, 120 VAC.....	1	each	12067-01
Hot Plate, 4" diameter, 240 VAC.....	1	each	12067-02

OPTIONAL REAGENTS AND STANDARDS

Description	Unit	Cat. No.
Acetone, ACS	500 mL	14429-49
Calcium Chloride Solution.....	1000 mL	428-53
Hydrochloric Acid Standard Solution, 0.1 N	1000 mL	14812-53
Hydrogen Peroxide, 30%, ACS.....	473 mL	144-11
Methyl Orange Indicator Solution, (0.50-g/L)	500 mL	148-49
Nitric Acid, ACS	500 mL	152-49
Phenol Solution, 30-g/L	29 mL	2112-20
Potassium Bromide Powder Pillows	100/pkg	14819-99
Potassium Permanganate Standard Solution, 1-g/L.....	100 mL	14164-42
Selenium Standard Solution, 1000-mg/L	100 mL	22407-42
Sodium Hydroxide Standard Solution, 0.100 N.....	1000 mL	191-53
Sodium Hydroxide Standard Solution, 5.0 N.....	100 mL MDB	2450-32
Sulfuric Acid Standard Solution, 5.25 N.....	100 mL MDB	2449-32
Sulfuric Acid Standard Solution, 19.2 N.....	500 mL	2038-49

OPTIONAL EQUIPMENT AND SUPPLIES

Beaker, 250-mL	each.....	500-46
Beaker, 1000-mL	each.....	500-53
Bottle, wash, 500-mL	each.....	620-11
Clippers, for opening powder pillows	each.....	968-00
Cylinder, graduated, 50-mL	each.....	508-41
Cylinder, graduated, 500-mL	each.....	508-49
Distillation Apparatus Set, general purpose	each.....	22653-00
Distillation Apparatus Heater, 115 VAC	each.....	22744-00
Distillation Apparatus Heater, 230 VAC	each.....	22744-02
DR/4000 Carousel Module Kit	each.....	48070-02
Dropper, 0.5- and 1-mL marks, glass	6/pkg.....	23185-06
Flask, volumetric, Class A, 1000-mL	each.....	14574-53
Jack, laboratory	each.....	22743-00
pH Meter, EC10, portable	each.....	50050-00
Pipet, serological, 10-mL	each.....	532-38
Pipet, TenSette, 0.1 to 1.0 mL	each.....	19700-01
Pipet Tips, for 19700-01 Pipet	50/pkg.....	21856-96
Pipet, volumetric, Class A, 1.00-mL	each.....	14515-35
Pipet, volumetric, Class A, 5.00-mL	each.....	14515-37
Pipet, volumetric, Class A, 8.00-mL	each.....	14515-08
Pipet, volumetric, Class A, 10.00-mL	each.....	14515-38
Pipet, volumetric, Class A, 50.00-mL	each.....	14515-41
Pipet, volumetric, Class A, 100.00-mL	each.....	14515-42
PourRite Ampule Breaker	each.....	24846-00
Rod, stirring, glass.....	3/pkg.....	1770-01
Stoppers, for cells, hollow, size #2.....	6/pkg.....	14480-01



FOR TECHNICAL ASSISTANCE, PRICE INFORMATION AND ORDERING:

In the U.S.A. – Call toll-free 800-227-4224

Outside the U.S.A. – Contact the HACH office or distributor serving you.

On the Worldwide Web – www.hach.com; E-mail – techhelp@hach.com

HACH COMPANY
WORLD HEADQUARTERS
Telephone: (970) 669-3050
FAX: (970) 669-2932



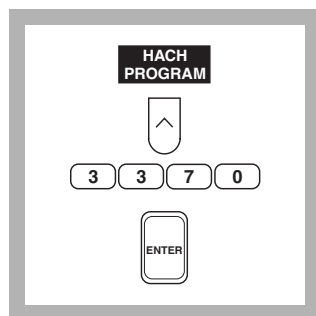
Method 8282

Heteropoly Blue Method*

ULR (0 to 1000.0 µg/L as SiO₂)

Scope and Application: For testing trace levels of soluble silica in pure and ultrapure water.
The estimated detection limit for program number 3370 is 1.0 µg/L.

* Adapted from *Standard Methods for the Examination of Water and Wastewater*.



1. Press the soft key under **HACH PROGRAM**.

Select the stored program for ultra low range silica by pressing **3370** with the numeric keys.

Press: **ENTER**

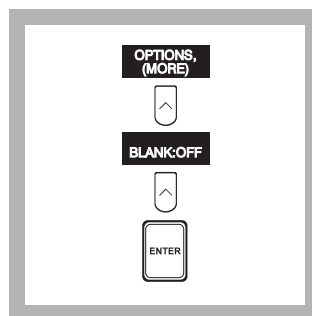
Note: If samples cannot be analyzed immediately, see *Sample Collection, Storage and Preservation* following these steps.

Note: The Flow Cell and Sipper Modules must be used with this procedure. See *Summary of Method*.



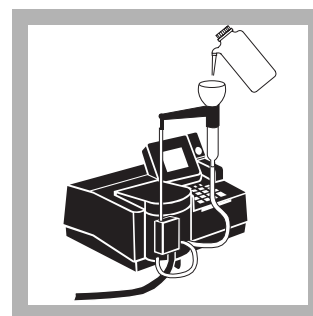
2. The display will show:
**HACH PROGRAM: 3370
Silica, ULR**

The wavelength (λ), **815 nm**, is automatically selected.



3. Account for the Molybdate 3 reagent blank by pressing the soft keys under **OPTIONS, (MORE)**, and then **BLANK:OFF**. Enter the reagent blank value found on the Molybdate 3 Reagent label and press **ENTER** until the read screen appears.

Note: Reagent blank values printed on analyzer reagent containers vary because the reagent dilutions vary according to the instrument. For this method, use the 1234D analyzer reagent blank value for a 3.78-liter volume of Molybdate 3 Reagent (Cat No. 1995-17). For a Series 5000, 2.9-liter volume of Molybdate 3 Reagent (Cat. No. 1995-03), multiply the reagent blank on the label by 1.09. For 100-mL (Cat No. 1995-32) and 1-liter (Cat. No. 1995-53) volumes, use the lab blank values on the bottle labels.

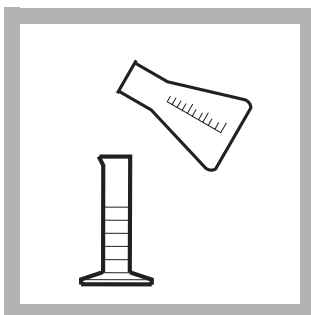


4. Insert the Flow Cell or Sipper Module and flush with 50 mL of low-silica deionized water.

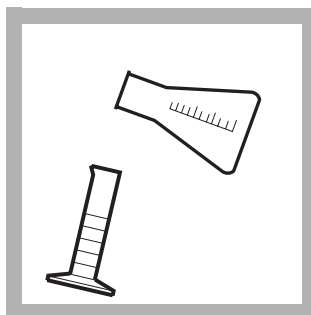
Note: Clean labware carefully; see more information in *Labware* following these steps.



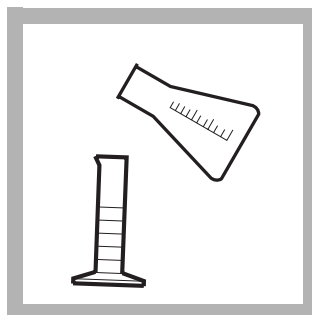
5. Fill two clean 250-mL Erlenmeyer flasks to overflowing with the sample to be tested.



6. Fill a clean 50-mL plastic graduated cylinder with sample from one of the flasks and then discard the cylinder contents.



7. Repeat the rinsing of the cylinder three times from the same sample flask, discarding each rinse.



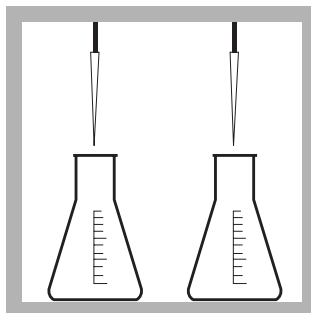
8. Fill this rinsed cylinder to the 50-mL mark with sample from the same flask, discarding any remaining sample in the flask.



9. Pour the contents of the 50-mL cylinder back into the original flask.

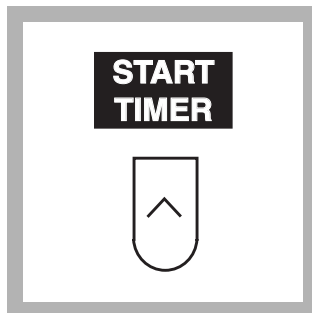


10. Repeat steps 6–10 for the second flask containing sample, then continue with Step 11.



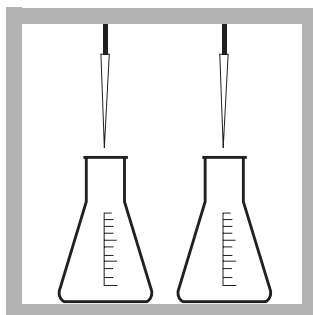
11. Using a TenSette Pipet, add 1.0 mL of Molybdate 3 Reagent to each flask. Swirl to mix.

***Note:** The TenSette Pipet is recommended for convenient reagent addition. An all-plastic 1.0-mL dropper is also available (See OPTIONAL EQUIPMENT AND SUPPLIES).*

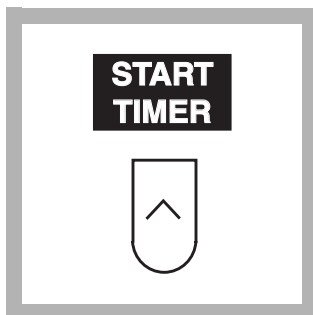


12. Press the soft key under **START TIMER**. A 4-minute reaction period will begin.

***Note:** Reaction time depends on sample temperature. The time given is for samples at 20 °C (68 °F). If the sample temperature is 10 °C (50 °F), wait 8 minutes. If the sample temperature is 30 °C (86 °F), wait 2 minutes.*

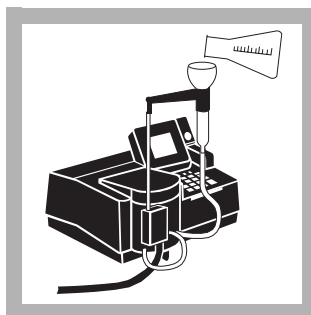


13. When the timer beeps, add 1.0 mL of Citric Acid F Reagent to each flask. Swirl to mix.

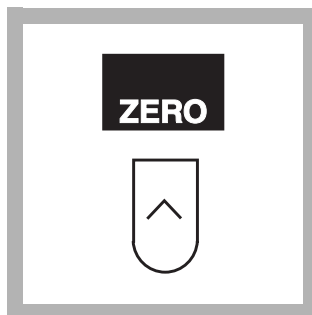


14. Press the soft key under **START TIMER**. A one-minute reaction period will begin. The destruction of possible phosphate interference occurs during this period.

Note: Reaction time depends on sample temperature. The time given is for samples at 20 °C (68 °F). If the sample temperature is 10 °C (50 °F), wait 2 minutes. If the sample temperature is 30 °C (86 °F), wait 30 seconds.



15. When the timer beeps, pour the contents of one flask into the Flow-Thru Cell or draw the contents into the Sipper Cell.



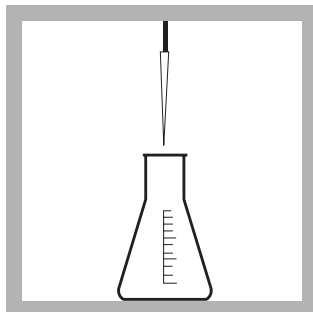
16. After the flow has stopped, press the soft key under **ZERO**.

The display will show:

-0.0 µg/L SiO₂

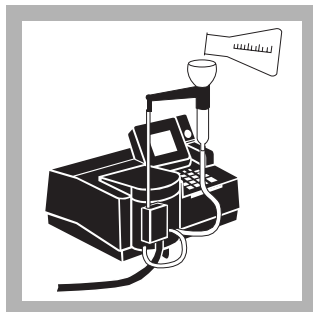
Note: If you are using a reagent blank correction, the display will show the correction.

Note: For alternate concentration units press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.

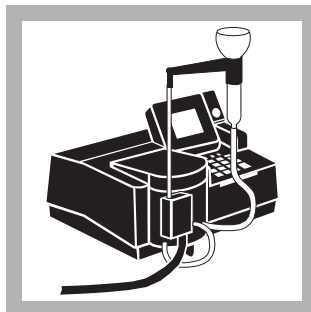


17. Add 1.0 mL of Amino Acid F Reagent to the remaining flask. Swirl to mix.

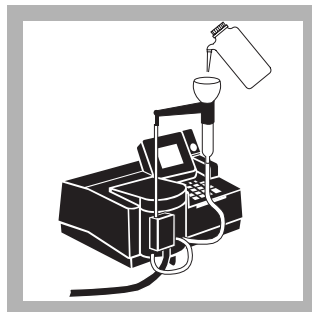
Note: A faint blue color will develop if silica is present.



18. Wait 15 seconds (but no more than 5 minutes) for color formation. Transfer the contents of the second flask into the Flow-Thru Cell or draw them into the Sipper Cell.



19. After the flow has stopped, results in µg/L SiO₂ (or chosen units) will be displayed.



20. Flush the Flow-Thru or Sipper Cell with at least 50 mL of deionized water immediately after use.

Interferences

Table 1 Interfering Substances and Suggested Treatments

Interfering Substance	Interference Levels and Treatments
Color	Eliminated by zeroing the instrument with the original sample (follow procedure)
Iron	Interferes at levels greater than 1 mg/L
pH (extreme)	Adjust pH to less than 7. See Section 1.3.1 <i>pH Interference</i> .
Phosphate (PO_4^{3-})	Interferes at levels greater than 50 mg/L PO_4^{3-}
Sulfides	Interfere at high levels
Turbidity	Eliminated by zeroing the instrument with the original sample (follow procedure)

Sample Collection, Storage and Preservation

Use only plastic containers with tight-fitting closures. Do not use glass containers; they will contaminate the sample with silica. Soak sampling containers with a solution made of one part Molybdate 3 Reagent to 50 parts of high quality deionized water of low silica concentration. Fill completely and let stand for several hours. Rinse thoroughly with low-level silica water, drain and close. Repeat this cleaning periodically.

Allow the sample stream to flow for 1–2 minutes before collection. Do not adjust the flow during the sampling period as this may introduce particulates. Rinse the container well with sample before collecting the portion for analysis. Analyze as soon as possible.

Reagent Preparation

Amino Acid F Reagent Solution is available in either 100-mL bottles or a package of 20 unit-dose ampules. The bottled reagent is stable for up to one year if the bottle is kept closed when not in use. The ampuled reagent is sealed under argon and is more stable with a shelf life greater than 1 year. Reduced sensitivity at high concentrations ($>1000 \mu\text{g/L}$) indicates reagent instability. Check the bottled reagent on a routine basis by performing an analysis on a 1-mg/L Silica Standard Solution (Cat. No. 1106-49). If the concentration is less than $950 \mu\text{g/L}$, use a fresh bottle of Amino Acid F Reagent Solution.

Prepare larger or smaller volumes of Amino Acid F Reagent by dissolving Amino Acid F Reagent Powder in Amino Acid F Reagent Solvent at a ratio of 11 grams per 100 mL. These reagents are available as the Amino Acid F Reagent Package listed under *OPTIONAL EQUIPMENT AND SUPPLIES*. This prepared solution has limited stability; test routinely with the 1-mg/L Silica Standard Solution as above.

If running a large number of samples, the variable volume dispensers are convenient (see *OPTIONAL EQUIPMENT AND SUPPLIES*). The dispensers are made of fluoropolymer plastic. Do not use a dispenser with glass bottles or glass parts; they will contaminate the reagent with silica.

Labware

All containers used in this test must be cleaned thoroughly to remove any traces of silica. Use plastic containers for all analysis and storage because glass can contaminate the sample with silica. Small bottles or flasks with screw caps work well.

Clean containers by normal means (do not use phosphate detergents), then rinse with high quality deionized water of low-level silica concentration. Soak for 10 minutes with a 1:50 dilution of Molybdate 3 Reagent in low-level silica water. Rinse repeatedly with either low-level silica water or the sample before use. Keep containers tightly closed when not in use. Fill the Flow-Thru or Sipper Cell with this same mixture of Molybdate 3 and water, and let stand for several minutes before use. Rinse with low-level silica water.

Cleaning the Flow-Thru or Sipper Cell

The Flow-Thru or Sipper Cell may accumulate a buildup of colored products, especially if the reacted solutions are allowed to stand in the cell for long periods after measurement. Remove the color by rinsing with a 1:5 dilution of ammonium hydroxide, followed by several deionized water rinses. Cover the Flow-Thru Cell when it is not in use.

Accuracy Check

Standard Additions Method

- a. Leave the unspiked sample in the sample compartment. Verify that the units displayed are in $\mu\text{g/L}$. Select standard additions mode by pressing the soft keys under **OPTIONS, (MORE)** and then **STD ADD**.
- b. Press **ENTER** to accept the default sample volume (mL), 50.0.
- c. Use either a 1-mg/L or 10-mg/L (1000 or 10,000 $\mu\text{g/L}$) Silica Standard Solution. When prompted for the standard concentration, enter the silica concentration that will be used (either 1000- or 10,000- $\mu\text{g/L}$). Press **ENTER**.
- d. Press the soft key under **ENTRY DONE**.
- e. Use the TenSette Pipet to add 0.1 mL, 0.2 mL and 0.3 mL of standard, respectively, to three 50-mL samples and mix each thoroughly.
- f. Analyze each standard addition sample as described above. Accept the standard additions reading by pressing the soft key under **READ** each time. Each addition should reflect approximately 100% recovery.
- g. After completing the sequence, the display will show the extrapolated concentration value and the “best-fit” line through the standard additions data points, accounting for matrix interferences.
- h. See Section 1.4.1 *Standard Additions* for more information.

Method Performance

Precision

Standard: 500 µg/L silica

Program	95% Confidence Limits
3370	498.2–501.8 µg/L silica

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
3370	1.0 µg/L silica

For more information on derivation and use of Hach's estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, Appendix B, see Section 1.5.1.

Sensitivity

Program Number: 3370

Portion of Curve	ΔAbs	ΔConcentration
Entire Range	0.010	11.84 µg/L

See Section 1.5.3 *Sensitivity Explained* for more information.

Summary of Method

A number of modifications are necessary to adapt the Low Range Silica method for analyzing trace levels in the Ultra Low Range method. It is absolutely necessary to use the one-inch Flow-Thru or Sipper Cell with liquid reagents. The Flow-Thru or Sipper Cell increases the reproducibility of the optics and reduces the instability of the readings that result from moveable sample cells. Liquid reagents ensure reproducible readings and lower blank values by eliminating slight turbidity that may remain when using powdered reagents. In addition, the liquid reagents are directly used with Hach process analyzers for continuous silica measurement.

Silica and phosphate in the sample react with molybdate ion under acidic conditions to form yellow silicomolybdic acid complexes and phosphomolybdic acid complexes. Addition of citric acid destroys the phosphate complexes. Amino Acid F Reagent is then added to reduce the yellow silicomolybdic acid to an intense blue color, which is proportional to the silica concentration.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to *Section 1*.

Pollution Prevention and Waste Management

For information on pollution prevention and waste management, refer to *Section 1*.

REQUIRED REAGENTS AND STANDARDS

	Cat. No.
ULR Silica Reagent Set (using Amino Acid F solution, 100 tests)	25535-00
Includes: (2) 1995-32, (2) 22542-32, (1) 23864-42	
ULR Silica Reagent Set (using Amino Acid F ampules, 40 tests)	25814-00
Includes: (1) 1995-32, (1) 22542-32, (2) 23864-20	

Description	Quantity Required per test	Unit	Cat. No.
Amino Acid F Reagent Solution	1.0 mL	100 mL	23864-42
<i>or</i>			
Amino Acid F Reagent Solution, 1.2-mL Ampules	1 each	20/pkg	23864-20
Citric Acid Reagent Solution	2 mL	500 mL	22542-49
Molybdate 3 Reagent Solution	2.0 mL	1 L	1995-53

REQUIRED EQUIPMENT AND SUPPLIES

Cylinder, graduated, 50-mL, poly	1	each	1081-41
DR/4000 Flow Cell Module Kit, 1-inch	1	each	48070-04
<i>or</i>			
DR/4000 Flow Cell Module Kit, 1-cm	1	each	48070-05
<i>or</i>			
DR/4000 Sipper Module Kit, 1-inch		each	48090-03
Flask, Erlenmeyer, 250-mL, PMP, w/cap	2	each	20898-46
Pipet, TenSette, 0.1- to 1.0-mL	1	each	19700-01
Pipet Tips, for 19700-01 Pipet	5	50/pkg	21856-96

OPTIONAL REAGENTS AND STANDARDS

Amino Acid F Reagent Package, includes:		23531-03
Amino Acid F Reagent Powder	308 g	23532-55
Amino Acid F Dilution Solvent	2.7 liters	23530-03
Ammonium Hydroxide, ACS	500 mL	106-49
Citric Acid F Reagent Solution	100 mL MDB	22542-32
Molybdate 3 Reagent Solution	2.9 L	1995-03
Molybdate 3 Reagent Solution	3.78 L	1995-17
Molybdate 3 Reagent Solution	100 mL	1995-32
Silica Standard Solution, 1 mg/L SiO ₂	500 mL	1106-49
Silica Standard Solution, 10 mg/L SiO ₂	500 mL	1403-49

OPTIONAL EQUIPMENT AND SUPPLIES

Beaker, polypropylene, 100-mL	each	1080-42
Bottle, 1000-mL, for use w/ variable volume dispenser	6/pkg	7137-54
Dispenser, variable volume, 1.0- to 5.0-mL	each	23121-37
Dropper, 0.5 & 1.0 mL marks, plastic	10/pkg	21247-10
Flask, Erlenmeyer, 250-mL, PMP w/ cap	4/pkg	20898-76
Pipet Tips, for 19700-01 TenSette Pipet	1000/pkg	21856-28
PourRite Ampul Breaker	each	24846-00
<i>Standard Methods for the Examination of Water and Wastewater</i> , 18th edition	each	22708-00
Thermometer, -10 to 110 °C	each	1877-01



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Method 8186

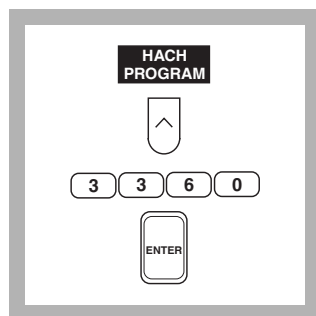
Heteropoly Blue Method*

LR (0 to 1.600 mg/L as SiO₂)

Scope and Application: For water and seawater

The estimated detection limit for program number 3360 is 0.01 mg/L SiO₂.

* Adapted from *Standard Methods for the Examination of Water and Wastewater*.



1. Press the soft key under **HACH PROGRAM**.

Select the stored program number for low range silica by pressing **3360** with the numeric keys.

Press: **ENTER**

Note: If samples cannot be analyzed immediately, see *Sample Collection, Storage and Preservation* following these steps.

Note: The Flow Cell and Sipper Modules are recommended for this procedure. Use a 25-mL sample and reagents with the Flow Cell Module.



2. The display will show: **HACH PROGRAM: 3360 Silica, LR**

The wavelength (λ), **815 nm**, is automatically selected.

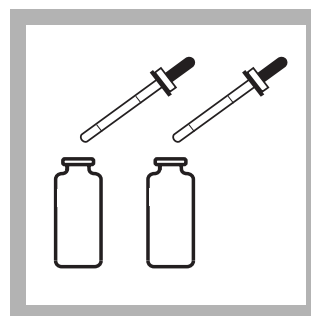
Note: For best results, determine a reagent blank for each new lot of reagent as follows. Prepare a reagent blank by repeating steps 3 through 12, using low silica deionized water as the sample. Zero the instrument on deionized water by pressing the soft key under **ZERO**. Insert the reagent blank and the blank value will be displayed. Correct for the reagent blank by pressing the soft keys under **OPTIONS, (MORE)**, and then **BLANK:OFF**. Enter the reagent blank value and press **ENTER**. Repeat for each new lot of reagent.



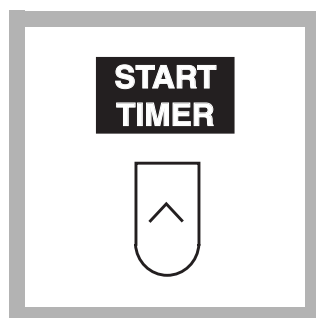
3. Fill two sample cells with 10 mL of sample.

Note: For proof of accuracy, use a 0.50-mg/L Silica Standard Solution in place of the sample (see *OPTIONAL REAGENTS AND STANDARDS*).

Note: For turbid samples, see the *Interferences* section following these steps.



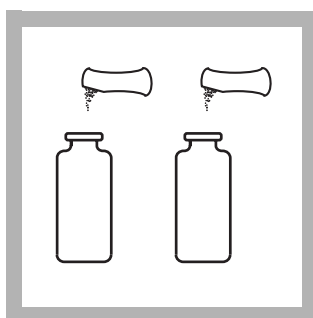
4. Add the 0.5 mL of Molybdate 3 Reagent to each sample cell. Swirl to mix.



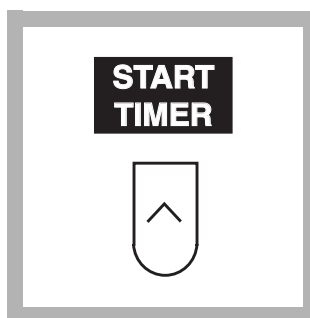
5. Press the soft key under **START TIMER**.

A 4-minute reaction period will begin.

Note: Reaction time depends on sample temperature. The time given is for samples at 20 °C (68 °F). If the sample temperature is 10 °C (50 °F), wait 8 minutes. If the sample temperature is 30 °C (86 °F), wait 2 minutes.



6. When the timer beeps, add the contents of one Citric Acid Reagent Powder Pillow to each sample cell. Swirl to mix.



7. Press the soft key under **START TIMER**.

A one-minute reaction period will begin. The destruction of possible phosphate interference occurs during this period.

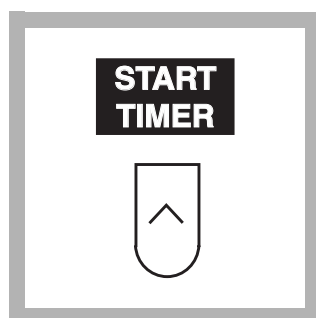
Note: Reaction time depends on sample temperature. The time given is for samples at 20 °C (68 °F). If the sample temperature is 10 °C (50 °F), wait 2 minutes. If the sample temperature is 30 °C (86 °F), wait 30 seconds.



8. When the timer beeps, add the contents of one Amino Acid F Reagent Powder Pillow to one of the sample cells. Swirl to mix. This is the prepared sample.

Note: The sample cell without the Amino Acid F Reagent is the blank.

Note: If testing for very low levels of silica, use the ultra low range silica method.

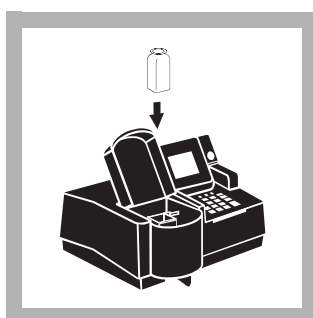


9. Press the soft key under **START TIMER**.

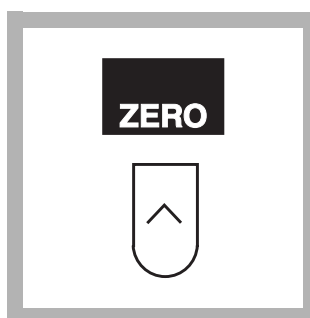
A 2-minute reaction period will begin.

Note: A blue color will develop if silica is present.

Note: Wiping the cells with a damp cloth, followed by a dry one removes fingerprints and other marks that may affect measurements.



10. When the timer beeps, place the blank in the cell holder. Close the light shield.



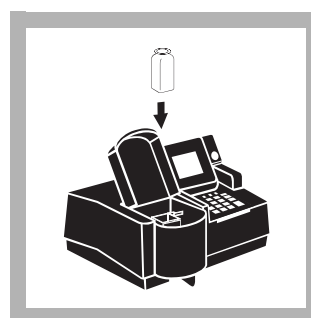
11. Press the soft key **ZERO**.

The display will show:

0.00 mg/L SiO₂

Note: If you are using a reagent blank correction, the display will show the correction.

Note: For alternate concentration units press the soft keys under **OPTIONS**, **(MORE)**, then **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



12. Place the prepared sample in the cell holder. Close the light shield. Results in mg/L SiO₂ (or chosen units) will be displayed.

Note: The results can be expressed as silicon (Si). Press the soft keys under **OPTIONS**, **(MORE)**, then **FORM** to scroll through the options. Press **ENTER** to return to the read screen.

Interferences

Table 1 Interfering Substances and Suggested Treatments

Interfering Substance	Interference Levels and Treatments
Color	Eliminated by zeroing the instrument with the original sample
Iron	Large amounts interfere
Phosphate	Does not interfere at levels less than 50 mg/L PO ₄ . At 60 mg/L PO ₄ , an interference of -2% occurs. At 75 mg/L PO ₄ the interference is -11%.
Slow reacting forms of silica	Occasionally a sample contains silica which reacts very slowly with molybdate. The nature of these "molybdate-unreactive" forms is not known. A pretreatment with sodium bicarbonate, then sulfuric acid will make these forms reactive to molybdate. The pretreatment is given in <i>Standard Methods for the Examination of Water and Wastewater</i> under Silica-Digestion with Sodium Bicarbonate. A longer reaction time with the sample and the molybdate and acid reagents (before adding citric acid) may help in lieu of the bicarbonate pretreatment.
Sulfides	Interfere at all levels
Turbidity	Eliminated by zeroing the instrument with the original sample

Sample Collection, Storage and Preservation

Collect samples in clean plastic bottles. Analyze samples as soon as possible after collection. If prompt analysis is not possible, store samples for up to 28 days by cooling to 4 °C (39 °F) or below. Warm samples to room temperature before analysis.

Accuracy Check

Standard Additions Method

- a. Leave the unspiked sample in the sample compartment. Verify that the units displayed are in mg/L. Select standard additions mode by pressing the soft keys under **OPTIONS, (MORE)** and then **STD ADD**.
- b. Press **ENTER** to accept the default sample volume (mL), 10.0.
- c. Press **25.00** and then press **ENTER** to accept the standard concentration (mg/L), 25.
- d. Press the soft key under **ENTRY DONE**.
- e. Open a 25-mg/L Silica Standard Solution bottle.
- f. Use the TenSette Pipet to add 0.2 mL, 0.4 mL and 0.6 mL of standard, respectively to three 10-mL samples and mix each thoroughly.
- g. Analyze each standard addition sample as described above. Accept the standard additions reading by pressing the soft key under **READ** each time. Each addition should reflect approximately 100% recovery.
- h. After completing the sequence, the display will show the extrapolated concentration value and the "best-fit" line through the standard additions data points, accounting for matrix interferences.
- i. See Section 1.4.1 *Standard Additions* for more information.

Standard Solution Method

Use the 1.00-mg/L SiO₂ Standard Solution listed under Optional Reagents and Standards in place of the sample. Perform the silica procedure as described above.

To adjust the calibration curve using the reading obtained with the 1.00 mg/L Standard Solution, press the soft keys under **OPTIONS, (MORE)** then **STD:OFF**. Press **ENTER** to accept the displayed concentration, the value of which depends on the selected units. If an alternate concentration is used, enter the actual concentration and press **ENTER** to return to the read screen. See Section 1.5.5 *Adjusting the Standard Curve* for more information.

Method Performance

Precision

Standard: 1.00 mg/L SiO₂

Program	95% Confidence Limits
3360	0.950–1.050 mg/L SiO ₂

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
3360	0.01 mg/L SiO ₂

For more information on derivation and use of Hach's estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, appendix B, see Section 1.5.1.

Sensitivity

Program Number: 3360

Portion of Curve	ΔAbs	ΔConcentration
0.010 Abs	0.010	0.012 mg/L
0.80 mg/L	0.010	0.011 mg/L
1.44 mg/L	0.010	0.011 mg/L

See Section 1.5.3 *Sensitivity Explained* for more information.

Calibration Standard Preparation

Preparing silica standards is difficult. Standards are easily contaminated and should be made by a trained chemist.

To perform a silica calibration using the Heteropoly Blue method, prepare calibration standards containing 0.20, 0.50, 0.80, 1.20 and 1.60 mg/L silica as follows:

- a. Into five different 100-mL volumetric flasks, pipet 2.00, 5.00, 8.00, 12.00 and 16.00 mL of a 10.00-mg/L Silica Standard Solution using Class A glassware.

- b. Dilute each flask to volume with ultra-low silica deionized water. Stopper and invert several times to mix.
- c. Using the Heteropoly Blue method and the calibration procedure described in the *User-Entered Programs* section of the *DR/4000 Spectrophotometer Instrument Manual*, generate a calibration curve from the standards prepared above.

Summary of Method

Silica and phosphate in the sample react with molybdate ion under acidic conditions to form yellow silicomolybdic acid complexes and phosphomolybdic acid complexes. Addition of citric acid destroys the phosphate complexes. An Amino Acid is then added to reduce the yellow silicomolybdic acid to an intense blue color, which is proportional to the silica concentration.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to *Section 1*.

Pollution Prevention and Waste Management

For information on pollution prevention and waste management, refer to *Section 1*.

REQUIRED REAGENTS AND STANDARDS

	Cat. No.
Low Range Silica Reagent Set (100 tests)	24593-00
Includes: (1) 22540-69, (1) 21062-69 (2) 1995-26, (1) 1117-02	

Description	Quantity Required per test	Unit	Cat. No.
Amino Acid F Reagent Powder Pillows (for 10-mL sample)	1 pillow	100/pkg	22540-69
Citric Acid Powder Pillows	2 pillows	100/pkg	21062-69
Molybdate 3 Reagent Solution	1.0 mL	50 mL	1995-26

REQUIRED EQUIPMENT AND SUPPLIES

DR/4000 1-Inch Cell Adapter	1	each	48190-00
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OPTIONAL REAGENTS AND STANDARDS

Amino Acid F Reagent Powder	410 g	22833-55
Silica Standard Solution, 0.5-mg/L SiO ₂	3.78 liter	21008-17
Silica Standard Solution, 1-mg/L SiO ₂	500 mL	1106-49
Silica Standard Solution, 10-mg/L SiO ₂	500 mL	1403-49
Silica Standard Solution, 50-mg/L SiO ₂	200 mL	1117-29
Silica Standard Solution, 25-mg/L as SiO ₂	236 mL	21225-31
Sodium Bicarbonate	454 g	776-01
Sodium Hydroxide Standard Solution, 1.00 N	900 mL	1045-53
Sulfuric Acid Standard Solution, 1.0 N	1000 mL	1270-53

OPTIONAL EQUIPMENT AND SUPPLIES

Description	Unit	Cat. No.
Bottle, 118 mL, polyethylene, oblong	6/pkg	23184-06
DR/4000 Carousel Module Kit	each	48070-02
DR/4000 Flow Cell Module Kit, 1-inch	each	48070-04
DR/4000 Flow Cell Module Kit, 1-cm	each	48070-05
DR/4000 Sipper Module Kit, 1-inch	each	48090-03
Dropper, 0.5- & 1.0-mL marks, glass	6/pkg	23185-06
Flask, volumetric, Class A, 100-mL	each	14574-42
Flask, volumetric, Class A, 100-mL	6/pkg	14574-72
Flask, volumetric, Class A, 250-mL	each	14574-66
Pipet, Mohr, serological, 2-mL, poly	each	2106-36
Pipet, TenSette, 0.1 to 1.0 mL	each	19700-01
Pipet Tips, for 19700-01 Pipet	50/pkg	21856-96
Pipet, volumetric, Class A, 1.00-mL	each	14515-35
Pipet, volumetric, Class A, 2.00-mL	each	14515-36
Pipet, volumetric, Class A, 3.00-mL	each	14515-03
Pipet, volumetric, Class A, 4.00-mL	each	14515-04
Pipet, volumetric, Class A, 5.00-mL	each	14515-37
Pipet, volumetric, Class A, 6.00-mL	each	14515-06
Pipet, volumetric, Class A, 8.00-mL	each	14515-08
Pipet Filler, safety bulb	each	14651-00
<i>Standard Methods for the Examination of Water and Wastewater</i> , 18th edition	each	22708-00
Thermometer, -10 to 110 °C	each	1877-01



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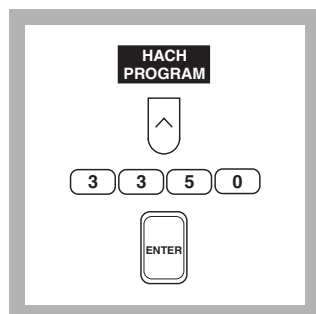
Method 8185

Silicomolybdate Method

HR (0 to 100.0 mg/L)

Scope and Application: For water and seawater.

The estimated detection limit for program number 3350 is 0.3 mg/L as SiO₂.



- 1.** Press the soft key under **HACH PROGRAM**.

Select the stored program number for high range silica by pressing **3350** with the numeric keys.

Press: **ENTER**

Note: If samples cannot be analyzed immediately, see *Sample Collection, Storage and Preservation* following these steps.

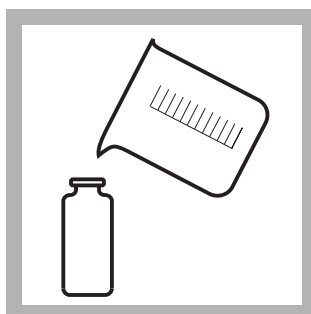
Note: The Flow Cell and Sipper Modules can be used with this procedure. Use a 25-mL sample and reagents with the Flow Cell Module.



- 2.** The display will show: **HACH PROGRAM: 3350 Silica, HR**

The wavelength (λ), **452 nm**, is automatically selected.

Note: For best results, determine a reagent blank for each new lot of reagent as follows. Prepare a reagent blank by repeating steps 3 through 12, using ultra low silica deionized water as the sample. Zero the instrument on deionized water by pressing the soft key under **ZERO**. Insert the reagent blank and the blank value will be displayed. Correct for the reagent blank by pressing the soft keys under **OPTIONS, (MORE)**, and then **BLANK:OFF**. Enter the reagent blank value and press **ENTER**. Repeat for each new lot of reagent.



- 3.** Fill a sample cell with 10 mL of sample.

Note: For proof of accuracy, use a 50-mg/L Silica Standard Solution in place of the sample (see *OPTIONAL REAGENTS AND STANDARDS*).

Note: Sample temperature should be 15–25 °C (59–77 °F).

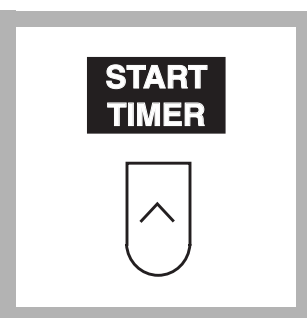


- 4.** Add the contents of one Molybdate Reagent Powder Pillow for High Range Silica to the sample cell (the prepared sample). Swirl to mix.



5. Add the contents of one Acid Reagent Powder Pillow for High Range Silica. Swirl to mix.

Note: A yellow color will develop if silica or phosphorus is present.



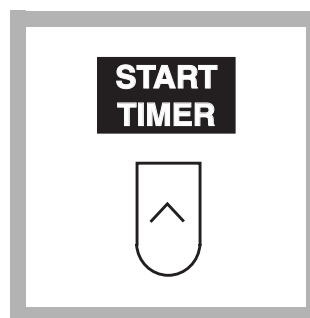
6. Press the soft key under **START TIMER**.

A 10-minute reaction period will begin.



7. When the timer beeps, add the contents of one Citric Acid Powder Pillow to the sample cell. Swirl to mix.

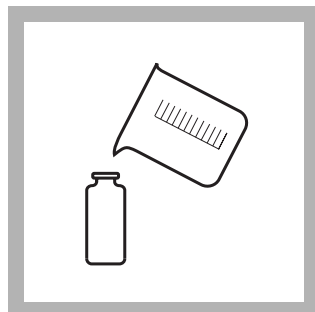
Note: Any yellow color due to phosphorus is removed in this step.



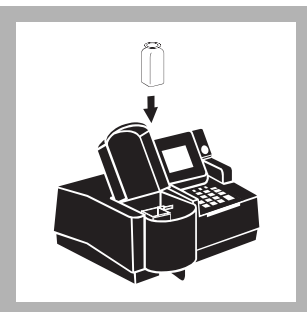
8. Press the soft key under **START TIMER**.

A 2-minute reaction period will begin.

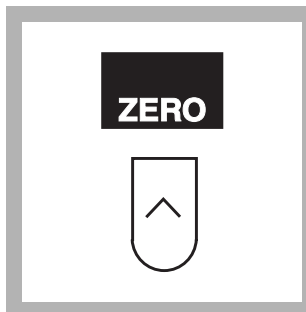
Note: Perform steps 9-12 within 3 minutes after the timer beeps.



9. When the timer beeps, fill a second sample cell with 10 mL of the original sample (the blank).



10. Place the blank in the cell holder. Close the light shield.



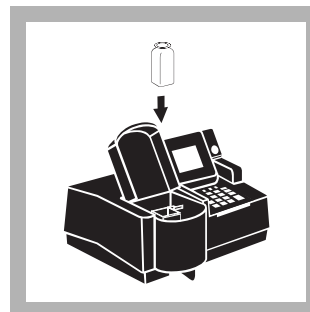
11. Press the soft key under **ZERO**.

The display will show:

0.0 mg/L SiO₂

Note: If you are using a reagent blank correction, the display will show the correction.

Note: For alternate concentration units press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



12. Place the prepared sample in the cell holder. Close the light shield. Results in mg/L silica (SiO₂) (or chosen units) will be displayed.

Note: The results can be expressed as silicon (Si). Press the soft keys under **OPTIONS**, then **FORM**: to scroll through the options. Press **ENTER** to return to the read screen.

Interferences

Table 1 Interfering Substances and Suggested Treatments

Interfering Substance	Interference Levels and Treatments
Color	Eliminated by zeroing the instrument with the original sample.
Iron	High levels of Fe^{2+} and Fe^{3+} interfere.
Phosphate	Does not interfere below 50 mg/L PO_4^{3-} . At 60 mg/L PO_4^{3-} , a negative 2% interference occurs. At 75 mg/L PO_4^{3-} the interference is negative 11%.
Sulfides (S^{2-})	High levels interfere.
Turbidity	Eliminated by zeroing the instrument with the original sample.

Occasionally a sample contains silica which reacts very slowly with molybdate. The nature of these “molybdate-unreactive” forms is not known. A pretreatment with sodium bicarbonate, then sulfuric acid will make these forms reactive to molybdate. The pretreatment is given in *Standard Methods for the Examination of Water and Wastewater* under Silica-Digestion with Sodium Bicarbonate. A longer reaction time with the sample and the molybdate and acid reagents (before adding citric acid) may help in lieu of the bicarbonate treatment.

Sample Collection, Storage and Preservation

Collect samples in clean plastic bottles (glass is not recommended). Analyze sample as soon as possible after collection. If prompt analysis is not possible, store samples at 4 °C (39 °F) for up to 7 days. Warm samples to room temperature before analyzing.

Accuracy Check

Standard Additions Method

- Leave the unspiked sample in the sample compartment. Verify that the units displayed are in mg/L. Select standard additions mode by pressing the soft keys under **OPTIONS, (MORE)** and then **STD ADD**.
- Press **ENTER** to accept the default sample volume (mL), 10.0.
- Press **ENTER** to accept the default standard concentration (mg/L), 1000.
- Press the soft key under **ENTRY DONE**.
- Open a 1000-mg/L Silica Standard Solution.
- Use the TenSette Pipet to add 0.1 mL, 0.3 mL, and 0.5 mL of standard, respectively to three 10-mL samples and mix each thoroughly.
- Analyze each standard addition sample as described above. Accept the standard additions reading by pressing the soft key under **READ** each time. Each addition should reflect approximately 100% recovery.
- After completing the sequence, the display will show the extrapolated concentration value and the “best-fit” line through the standard additions data points, accounting for matrix interferences.
- See Section 1.4.1 *Standard Additions* for more information.

Standard Solution Method

To check test accuracy, use the 50-mg/L Silica Standard Solution listed under *OPTIONAL REAGENTS AND STANDARDS*. Analyze according to the HR Silica procedure described above using deionized water as the blank.

To adjust the calibration curve using the reading obtained with the 50.0-mg/L Standard Solution, press the soft keys under **OPTIONS, MORE** then **STD: OFF**. Press **ENTER** to accept the displayed concentration, the value of which depends on the selected units. If an alternate concentration is used, enter the actual concentration and press **ENTER** to return to the read screen. See Section 1.5.5 *Adjusting the Standard Curve* for more information.

Method Performance

Precision

Standard: 50.0 mg/L SiO₂

Program	95% Confidence Limits
3350	49.7–50.3 mg/L SiO ₂

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
3350	0.3 mg/L SiO ₂

For more information on derivation and use of Hach's estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by 40 CFR part 136, appendix B, see Section 1.5.1.

Sensitivity

Program Number: 3350

Portion of Curve	ΔAbs	ΔConcentration
0.010 Abs	0.010	1.006 mg/L
50.0 mg/L	0.010	1.036 mg/L
90.0 mg/L	0.010	1.060 mg/L

See Section 1.5.3 *Sensitivity Explained* for more information.

Summary of Method

Silica and phosphate in the sample react with molybdate ion under acidic conditions to form yellow silicomolybdic acid complexes and phosphomolybdic acid complexes. Addition of citric acid destroys the phosphate complexes. Silica is then determined by measuring the remaining yellow color.

Calibration Standard Preparation

To perform a silica calibration using the Silicomolybdate method, prepare calibration standards containing 5.0, 10.0, 20.0, 50.0, 80.0, and 100.0 mg/L silica (SiO_2) as follows:

- a. Into each of six different 100-mL Class A volumetric flasks, pipet 0.50, 1.00, 2.00, 5.00, 8.00, and 10.00 mL of a 1000-mg/L Silica Standard Solution. Only use Class A pipets.
- b. Dilute each flask to volume with ultra-low silica deionized water. Stopper each flask and then invert several times to mix.
- c. Using the Silicomolybdate method and the calibration procedure described in the *User-Entered Programs* section of the *DR/4000 Spectrophotometer Instrument Manual*, generate a calibration curve from the standards prepared above.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to *Section 1*.

Pollution Prevention and Waste Management

For information on pollution prevention and waste management, refer to *Section 1*.

REQUIRED REAGENTS AND STANDARDS

	Cat. No.
High Range Silica Reagent Set for 10-mL samples (100 tests)	24296-00
Includes: (1) 21074-69, (1) 21062-69, (1) 21073-69	

Description	Quantity Required per test	Unit	Cat. No.
Acid Reagent Powder Pillows for High Range Silica	1	100/pkg	21074-69
Citric Acid Powder Pillows	1	100/pkg	21062-69
Molybdate Reagent Powder Pillows for High Range Silica	1	100/pkg	21073-69
Water, deionized	10 mL	4 liters	272-56

REQUIRED EQUIPMENT AND SUPPLIES

Clippers, for opening powder pillows	1	each	968-00
DR/4000 1-Inch Cell Adapter	1	each	48190-00

OPTIONAL REAGENTS AND STANDARDS

Silica Standard Solution, 10-mg/L	500 mL	1403-49
Silica Standard Solution, 50-mg/L	200 mL	1117-29
Silica Standard Solution, 1000-mg/L	500 mL	194-49
Sodium Bicarbonate	454 g	776-01
Sulfuric Acid Standard Solution, 1.000 N	100 mL MDB	1270-32

OPTIONAL EQUIPMENT AND SUPPLIES

Description	Unit	Cat. No.
Clippers, shears, 7¼-inch	each.....	23694-00
DR/4000 Carousel Module Kit	each.....	48070-02
DR/4000 Flow Cell Module Kit, 1-inch.....	each.....	48070-04
DR/4000 Flow Cell Module Kit, 1-cm.....	each.....	48070-05
DR/4000 Sipper Module Kit, 1-inch.....	each.....	48090-03
Pipet, TenSette, 0.1 to 1.0 mL	each.....	19700-01
Pipet Tips, for 19700-01 Pipet	50/pkg.....	21856-96
Pipet, volumetric, Class A, 0.50-mL	each.....	14515-34
Pipet, volumetric, Class A, 1.00-mL	each.....	14515-35
Pipet, volumetric, Class A, 2.00-mL	each.....	14515-36
Pipet, volumetric, Class A, 5.00-mL	each.....	14515-37
Pipet, volumetric, Class A, 8.00-mL	each.....	14515-08
Pipet, volumetric, Class A, 10.00-mL	each.....	14515-38
<i>Standard Methods for the Examination of Water and Wastewater</i> , 18th edition.....	each.....	22708-00
Thermometer, -10 to 110 °C.....	each.....	1877-01

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FAX: (970) 669-2932



Method 8120

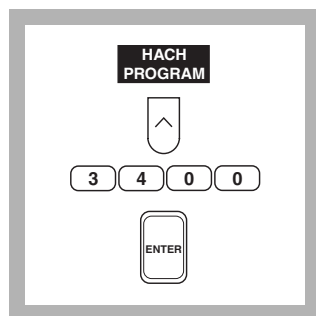
Colorimetric Method

Powder Pillows

(0 to 0.700 mg/L)

Scope and Application: For water and wastewater; digestion is required for determining silver in samples with interferences. See the *DIGESTION* procedure following the colorimetric method.

The estimated detection limit for program number 3400 is 0.006 mg/L.



- 1.** Press the soft key under **HACH PROGRAM**.

Select the stored program number for silver by pressing **3400** with the numeric keys.

Press: **ENTER**

Note: If samples cannot be analyzed immediately, see *Sample Collection, Storage and Preservation* following these steps. Adjust the pH of preserved samples before analysis.

Note: The Flow Cell and Sipper Modules can be used with this procedure if rinsed well with water between the sample and blank.

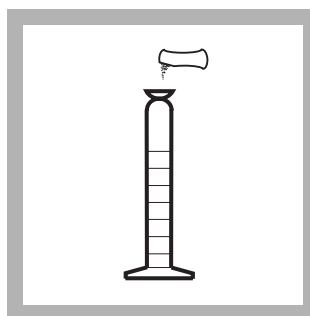


- 2.** The display will show:

HACH PROGRAM: 3400 Silver

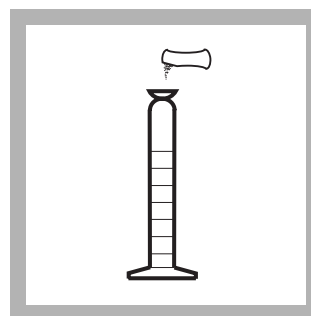
The wavelength (λ), **560 nm**, is automatically selected.

Note: For best results, determine a reagent blank for each new lot of reagent as follows. Prepare a reagent blank by repeating steps 3 through 11 using deionized water as the sample. Zero the instrument on deionized water by pressing the soft key under **ZERO**. Insert the reagent blank and the blank value will be displayed. Correct for the reagent blank by pressing the soft keys under **OPTIONS, (MORE)**, and then **BLANK:OFF**. Enter the reagent blank value and press **ENTER**. Repeat for each new lot of reagent.



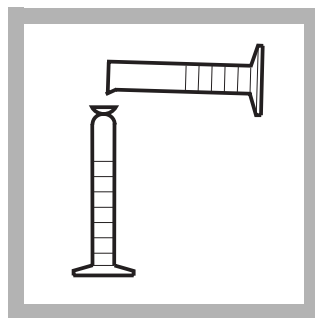
- 3.** Add the contents of one Silver 1 Powder Pillow to a **dry** 50-mL graduated mixing cylinder.

Note: If the Silver 1 Powder becomes wet at this point, the powder will not dissolve completely, which will inhibit color development.



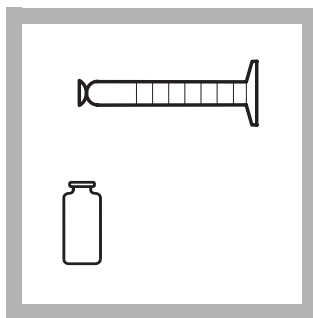
- 4.** Add the contents of one Silver 2 Reagent Solution Pillow to each the cylinder. Swirl to completely wet the powder.

Note: If clumps of dry powder are present when the sample is poured in, the powder will not dissolve completely. This will inhibit color formation.



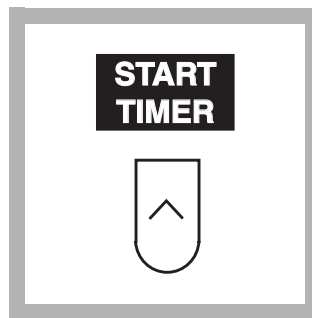
5. Using a 50-mL graduated cylinder, add 50 mL of sample to the 50-mL graduated mixing cylinder from Step 4. Stopper. Invert repeatedly for one minute.

Note: For proof of accuracy, use a 0.50 mg/L silver standard solution in place of the sample (see Accuracy Check).

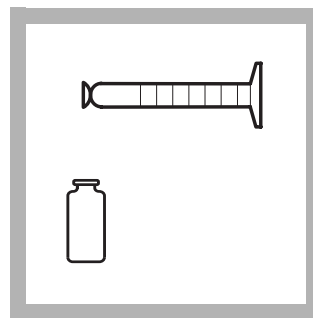


6. Fill a cell to the 10-mL mark with the mixture (the blank). Add the contents of one Sodium Thiosulfate Powder Pillow. Swirl for 30 seconds to mix.

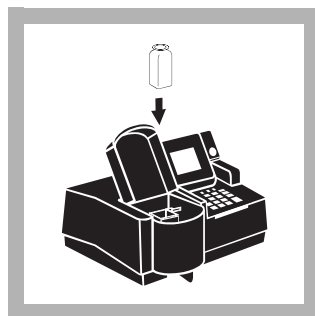
Note: It is important to generate a blank for each sample.



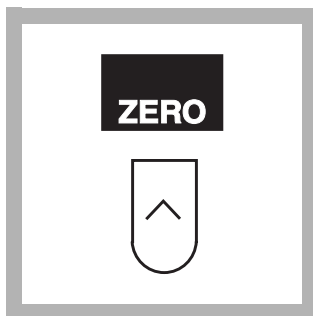
7. Press the soft key under **START TIMER**. A 2-minute reaction period will begin.



8. Fill a second cell to the 10-mL mark from the remaining portion of the mixture (the prepared sample).



9. When the timer beeps, place the blank in the cell holder. Close the light shield.



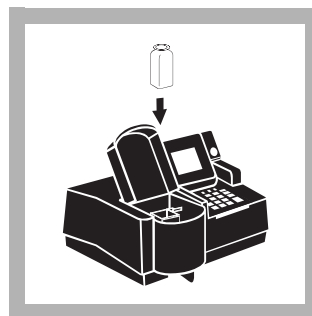
10. Press the soft key under **ZERO**.

The display will show:

0.000 mg/L Ag

Note: If you are using a reagent blank correction, the display will show the correction.

Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



11. Place the prepared sample in the cell holder. Close the light shield. Results in mg/L silver (or chosen units) will be displayed.

Note: Rinse the cells carefully between samples to avoid development of a film on the cell walls.

Interferences

Interference studies were conducted by preparing a known silver solution (about 0.4 mg/L) and the potential interfering ion. The ion was said to interfere when the resulting concentration changed by $\pm 10\%$.

Table 1 Interfering Substances and Suggested Treatments

Interfering Substance	Interference Levels and Treatments
Aluminum	Negative interference above 30 mg/L
Ammonia	Negative interference above 750 mg/L
Cadmium	Negative interference above 15 mg/L
Calcium	Positive interference above 600 mg/L
Chloride	Negative interference above 19 mg/L
Chromium ⁶⁺	Negative interference above 90 mg/L
Copper	Negative interference above 7 mg/L
Iron	Negative interference above 30 mg/L
Lead	Negative interference above 13 mg/L
Manganese	Negative interference above 19 mg/L
Magnesium	Positive interference above 2000 mg/L
Mercury	Positive interference above 2 mg/L
Nickel	Negative interference above 19 mg/L
Zinc	Negative interference above 70 mg/L

Sample Collection, Storage and Preservation

Collect samples in acid-cleaned plastic bottles. Using pH paper, adjust the pH to 2 or less with concentrated nitric acid (about 2 mL/liter). Store preserved samples at room temperature for up to 6 months. If the sample contains particulates or only dissolved metal content is being determined, filter through a 0.45 mm filter at collection. After filtration, adjust the pH to 2 or less as described above.

Before analysis, adjust the pH to 9–10 with 5.0 N Sodium Hydroxide. Do not use a pH meter because of silver contamination from the electrode. Correct for volume additions; see Section 1.2.2 *Correcting for Volume Additions*.

Accuracy Check

Standard Additions Method

- Leave the unspiked sample in the sample compartment. Verify that the units displayed are in mg/L. Select standard additions mode by pressing the soft keys under **OPTIONS, (MORE)** and then **STD ADD**.
- Press **ENTER** to accept the default sample volume (mL), 50.
- Press **ENTER** to accept the default standard concentration (mg/L), 50.000.
- Press the soft key under **ENTRY DONE**.
- Add 5.00 mL of 1000 mg/L Silver Standard Solution to a 100-mL volumetric Class A flask. Dilute to volume with deionized water. This is a 50.0 mg/L standard solution.
- Use the TenSette Pipet to add 0.1 mL, 0.2 mL and 0.3 mL of standard, respectively to three 50-mL samples and mix each thoroughly.

- g. Analyze each standard addition sample as described above. Accept the standard additions reading by pressing the soft key under **READ** each time. Each addition should reflect approximately 100% recovery.
- h. After completing the sequence, the display will show the extrapolated concentration value and the “best-fit” line through the standard additions data points, accounting for matrix interferences.
- i. See Section 1.4.1 *Standard Additions* for more information.

Standard Solution Method

Prepare a 0.5 mg/L silver standard solution by pipetting 0.50 mL of Silver Standard Solution, 1000-mg/L, into a 1000-mL volumetric flask using a Class A volumetric pipet. Dilute to the mark with deionized water. Prepare this solution daily. Perform the silver procedure as described above.

To adjust the calibration curve using the reading obtained with the 0.5-mg/L silver standard solution, press the soft keys under **OPTIONS, MORE** then **STD: OFF**. Press **ENTER** to accept the displayed concentration, the value of which depends on the selected units. If an alternate concentration is used, enter the actual concentration and press **ENTER** to return to the read screen. See Section 1.5.5 *Adjusting the Standard Curve* for more information.

Method Performance**Precision**

Standard: 0.500 mg/L Ag

Program	95% Confidence Limits
3400	0.497–0.503 mg/L Ag

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
3400	0.006 mg/L Ag

For more information on derivation and use of Hach’s estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, appendix B, see Section 1.5.1.

Sensitivity

Program Number: 3400

Portion of Curve	Δ Abs	Δ Concentration
Entire Range	0.010	0.0048 mg/L

See Section 1.5.3 *Sensitivity Explained* for more information.

Digestion

This digestion is for samples containing organic matter, thiosulfate or cyanide. Possible sources for these compounds are wastewater, silver electroplating baths and silver strike solutions. Digestion should be done with a Digesdahl Digestion Apparatus.

Warning: Always wear safety glasses and use a safety shield, or operate the Digesdahl within a closed fume hood. Follow the additional safety precautions in the Digesdahl Digestion Apparatus Manual.

Caution: Poisonous hydrogen cyanide gas is generated during this digestion. Use a fume hood.

- a. Add an appropriate size sample to the 100-mL digestion flask for use with the Digesdahl. Add several boiling chips to prevent bumping.

Note: Appropriate sample size is determined experimentally. The final sample concentration (after dilution to 100 mL) should be 0–0.6 mg/L. Larger dilutions may be necessary for electroplating baths and silver strike solutions. Do not exceed the maximum sample volume of 25 mL. Several 25-mL aliquots may be digested in succession to concentrate a very dilute sample.

- b. Turn on the water aspirator and make sure there is suction in the fractionating head.
- c. Add 3 mL of concentrated sulfuric acid to the sample in the volumetric flask. Immediately place the head on the digestion flask. Never use less than 3 mL of acid.
- d. Place the digestion flask on the heater. Turn the temperature dial to 440 °C (825 °F).
- e. After the sample begins to char or the sulfuric acid reflux line becomes visible, wait 3–5 minutes.
- f. Visually confirm the presence of acid in the flask before adding hydrogen peroxide!
- g. Add 10 mL of 50% hydrogen peroxide to the sample via the capillary funnel in the fractionating head.
- h. After the hydrogen peroxide has boiled off, heat the sample until heavy white sulfuric acid fumes are present. Continue heating and reduce the sample volume to near dryness. Do not let the sample go completely dry at any time.

Note: If the sample goes to dryness, turn the Digesdahl off and cool completely. Add water to flask before handling. Repeat digestion from the beginning.

Note: If only thiosulfate is present in the sample, proceed to Step I.

- i. Add another 3 mL of sulfuric acid via the capillary funnel.
- j. Add another 5 mL of hydrogen peroxide. Check the solution for digestion completion. If digestion is not complete, continue adding hydrogen peroxide in 5 to 10 mL portions. Several portions may be necessary.

Note: Digestion is complete when the digestate is colorless or the color of the digestate does not change upon addition of hydrogen peroxide. Also, a completely digested sample will not foam.

- k. After digestion is complete and all the hydrogen peroxide is boiled off, reduce the volume of the digestate to near dryness. Do not allow the sample to become completely dry. Remove the flask from the heater. Cool to room temperature.
- l. Slowly add about 25 mL of deionized water to the cooled flask.

- m. Add 2 drops of 1 g/L Phenolphthalein Indicator Solution. Add 2 drops of 1-g/L Thymolphthalein Indicator Solution.
- n. Using sodium hydroxide, adjust the pH of the solution to 9–10. The solution will be pink in this pH range.

Note: A purple color indicates a pH greater than 10. If this occurs, add a drop of sulfuric acid and 2 drops of each indicator; repeat pH adjustment. Initially, use 50% sodium hydroxide, then 1 N Sodium Hydroxide as the end point is approached.

- o. Filter turbid digestates. Quantitatively transfer the filtrate (or unfiltered sample) to a clean 100-mL volumetric flask. Dilute to the mark with deionized water. The sample is ready for analysis.

Summary of Method

Silver ions in basic solution react with cation 2B to form a green to brown to red-purple complex. The sodium thiosulfate acts as a decolorizing agent for the blank. The Silver 1 and Silver 2 reagents contain the buffer, indicator and masking agents. Organic extractions are not necessary and this method does not have as many interferences as the traditional dithizone method.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to *Section 1*.

Pollution Prevention and Waste Management

For information on pollution prevention and waste management, refer to *Section 1*.

REQUIRED REAGENTS AND STANDARDS

	Cat. No.
Silver Reagent Set (50 tests)	22966-00
Includes: (1) 22935-66, (1) 22936-66, (1) 22937-66	

Description	Quantity Required		Cat. No.
	per test	Unit	
Silver 1 Reagent Powder Pillow	1 pillow	50/pkg	22935-66
Silver 2 Reagent Solution Pillow	1 pillow	50/pkg	22936-66
Sodium Thiosulfate Powder Pillow	1 pillow	50/pkg	22937-66

REQUIRED EQUIPMENT AND SUPPLIES

Boiling Chips, silicon carbide	2-3	500 g	20557-34
Clippers, for opening powder pillows	1	each	968-00
Cylinder, graduated, 50-mL	1	each	21179-41
Cylinder, graduated, mixing, 50-mL	1	each	1896-41
DR/4000 1-Inch Cell Adapter	1	each	48190-00

OPTIONAL REAGENTS AND STANDARDS

Hydrogen Peroxide, 50%	490 mL	21196-49
Phenolphthalein Indicator Solution, 1-g/L	15 mL SCDB	1897-36
Silver Standard Solution, 1000-mg/L Ag	100 mL	14613-42
Sodium Hydroxide Solution, 1.00 N	100 mL MDB	1045-32
Sodium Hydroxide Solution, 5.0 N	100 mL MDB	2450-32
Sodium Hydroxide Solution, 50%	473 mL	2180-11
Sulfuric Acid, ACS, concentrated	2.5 L	979-09
Thymolphthalein Indicator Solution, 1-g/L	15 mL SCDB	21853-36
Water, deionized	4 liters	272-56

OPTIONAL EQUIPMENT AND SUPPLIES

Digesdahl Digestion Apparatus, 115 VAC, 50/60 Hz	each	23130-20
Digesdahl Digestion Apparatus, 230 VAC, 50/60 Hz	each	23130-21
DR/4000 Carousel Module Kit	each	48070-02
DR/4000 Flow Cell Module Kit, 1-inch	each	48070-04
DR/4000 Flow Cell Module Kit, 1-cm	each	48070-05
DR/4000 Sipper Module Kit, 1-inch	each	48090-03
Flask, volumetric, Class A, 100-mL	each	14574-42
Flask, volumetric, Class A, 1000-mL	each	14574-53
Pipet, serological, 10-mL	each	532-38
Pipet, TenSette, 0.1 to 1.0 mL	each	19700-01
Pipet, TenSette, 1.0 to 10.0 mL	each	19700-10
Pipet Tips, for 19700-01 Pipet	50/pkg	21856-96
Pipet Tips, for 19700-10 Pipet	50/pkg	21997-96
Pipet, volumetric, Class A, 0.50-mL	each	14515-34
Pipet, volumetric, Class A, 5.00-mL	each	14515-37
Pipet, Filler, safety bulb	each	14651-00
Safety Glasses	each	18421-00
Safety Shield, for Digesdahl	each	20974-00



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✓ Method 8051

SulfaVer 4 Method*

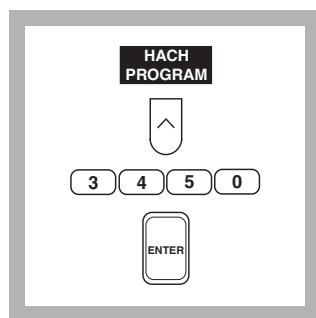
Powder Pillows or AccuVac® Ampuls

(0 to 70.0 mg/L)

Scope and Application: For water, wastewater and seawater. USEPA accepted for reporting for wastewater analyses.

* Adapted from *Standard Methods for the Examination of Water and Wastewater*. Procedure is equivalent to USEPA method 375.4 for wastewater.

Using Powder Pillows



1. Press the soft key under **HACH PROGRAM**.

Select the stored program for sulfate (SO_4^{2-}) by pressing **3450** with the numeric keys.

Press: **ENTER**

Note: If samples cannot be analyzed immediately, see *Sample Collection, Storage and Preservation* following these steps.

Note: The Flow Cell and Sipper Cell Modules cannot be used with this procedure.

Note: For best results, perform a new calibration for each lot of reagent. See *Calibration Standard Preparation* following these steps.

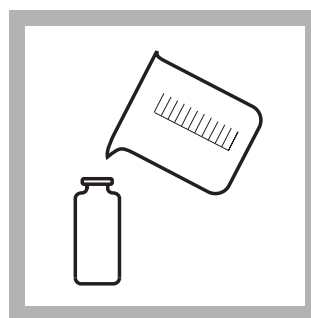


2. The display will show: **HACH PROGRAM: 3450 Sulfate**

The wavelength (λ), **450 nm**, is automatically selected.

Note: For best results, determine a reagent blank for each new lot of reagent as follows. Prepare a reagent blank by repeating steps 3 through 10, using deionized water as the sample. Zero the instrument on deionized water by pressing the soft key under **ZERO**. Insert the reagent blank and the blank value will be displayed. Correct for the reagent blank by pressing the soft keys under **OPTIONS, (MORE)**, and then **BLANK:OFF**. Enter the reagent blank value and press **ENTER**. Repeat for each new lot of reagent.

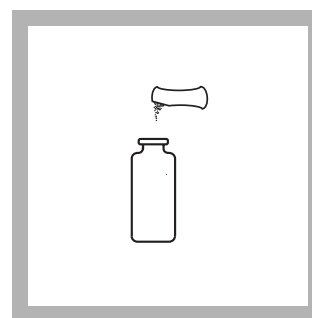
Note: You must adjust the standard curve for each new lot of reagent. See *Standard Curve Adjustment* following these steps.



3. Fill a clean sample cell with 25 mL of sample.

Note: Filter highly turbid or colored samples. Use filtered sample in this step and in Step 6. Use labware listed under **OPTIONAL EQUIPMENT AND SUPPLIES**.

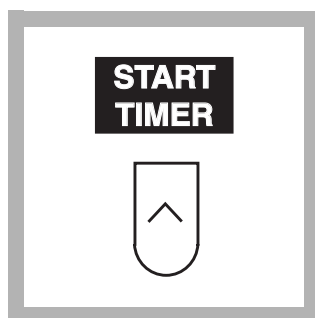
Note: For proof of accuracy, use a 70.0 mg/L sulfate standard solution (see *Standard Curve Adjustment*) in place of the sample.



4. Add the contents of one SulfaVer 4 Reagent Powder Pillow to the sample cell (the prepared sample). Swirl to mix.

Note: A white turbidity will develop if sulfate is present.

Note: Accuracy is not affected by undissolved powder which has settled.



5. Press the soft key under **START TIMER**.

A 5-minute reaction period will begin.

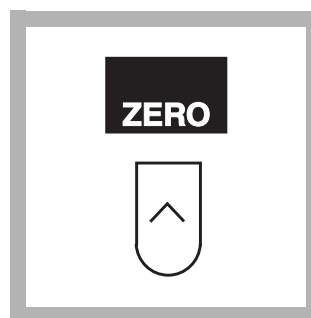
Note: Allow cell to stand undisturbed.



6. Fill a second sample cell with 25 mL of sample (the blank).



7. When the timer beeps, place the blank in the cell holder. Close the light shield.



8. Press the soft key under **ZERO**.

The display will show:

0.0 mg/L SO₄²⁻

Note: If you are using a reagent blank correction, the display will show the correction.

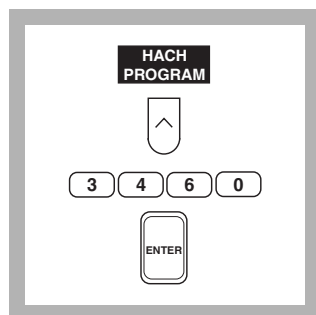
Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



9. Within five minutes after the timer beeps, place the prepared sample into the cell holder. Close the light shield. Results in mg/L sulfate (or chosen units) will be displayed.

Note: Clean the sample cells with soap and a brush.

Using AccuVac Ampuls



- 1.** Press the soft key under **HACH PROGRAM**.

Select the stored program for sulfate (SO_4^{2-}) by pressing **3460** with the numeric keys.

Press: **ENTER**

Note: If samples cannot be analyzed immediately, see *Sample Collection, Storage and Preservation* following these steps.

Note: The Flow Cell and Sipper Cell Modules cannot be used with this procedure.

Note: For best results, perform a new calibration for each lot of reagent. See *Calibration Standard Preparation* following these steps.



- 2.** The display will show:
HACH PROGRAM: 3460 Sulfate, AV

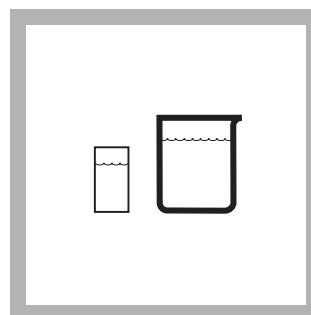
The wavelength (λ), **450 nm**, is automatically selected.

Note: You must determine a reagent blank for each new lot of reagent as follows. Prepare a reagent blank by repeating steps 3 through 10, using deionized water as the sample. Zero the instrument on deionized water by pressing the soft key under **ZERO**. Insert the reagent blank and the blank value will be displayed. Correct for the reagent blank by pressing the soft keys under **OPTIONS, (MORE)**, and then **BLANK:OFF**. Enter the reagent blank value and press **ENTER**. Repeat for each new lot of reagent.

Note: You must adjust the standard curve for each new lot of reagent. See *Standard Curve Adjustment* following these steps.



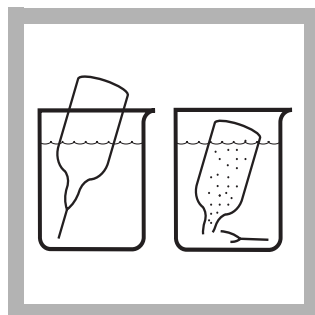
- 3.** Insert the 1-inch Cell Adapter into the sample cell module by sliding it under the thumb screw and into the alignment grooves. Fasten with the thumb screw.



- 4.** Fill a Zeroing Vial with at least 10 mL of sample (the blank). Collect at least 40 mL of sample in a 50-mL beaker.

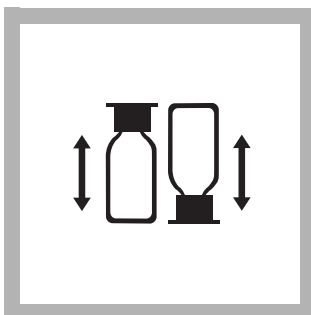
Note: Filter highly turbid or colored samples. Use filtered sample in this step and in Step 5. Use labware listed under **OPTIONAL EQUIPMENT AND SUPPLIES**.

Note: For proof of accuracy, use a 70.0 mg/L sulfate standard solution (see *Standard Curve Adjustment*) in place of the sample.



5. Fill a SulfaVer 4 Sulfate AccuVac Ampul with sample (the prepared sample).

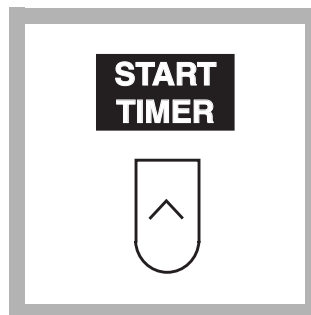
Note: Keep tip immersed until the ampul fills completely.



6. Quickly invert the ampul several times to mix. Wipe off any liquid or fingerprints.

Note: A white turbidity will develop if sulfate is present.

Note: Accuracy is not affected by undissolved powder which has settled.



7. Press the soft key under **START TIMER**.

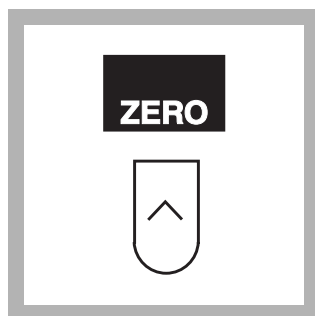
A 5-minute reaction period will begin.

Note: Allow cell to stand undisturbed.

Note: Read samples within five minutes after the timer beeps.



8. When the timer beeps, place the blank in the cell holder. Close the light shield.



9. Press the soft key under **ZERO**.

The display will show:

0.0 mg/L SO₄²⁻

Note: If you are using a reagent blank correction, the display will show the correction.

Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



10. Within five minutes after the timer beeps, place the prepared sample into the cell holder. Close the light shield. Results in mg/L sulfate (or chosen units) will be displayed.

Interferences

Table 1 Interfering Substances and Suggested Treatments

Interfering Substance	Interference Levels and Treatments
Calcium	Greater than 20,000 mg/L as CaCO ₃
Chloride	Greater than 40,000 mg/L as Cl
Magnesium	Greater than 10,000 mg/L as CaCO ₃
Silica	Greater than 500 mg/L as SiO ₂

Sample Collection, Storage and Preservation

Collect samples in clean plastic or glass bottles. Samples may be stored up to 7 days by cooling to 4 °C (39 °F) or lower. Warm to room temperature before analysis.

Accuracy Check

Standard Additions Method

- Leave the unspiked sample in the sample compartment. Verify that the units displayed are in mg/L. Select standard additions mode by pressing the soft keys under **OPTIONS, (MORE)** and then **STD ADD**.
- Press **ENTER** to accept the default sample volume (mL), 25.
- Press **ENTER** to accept the default standard concentration (mg/L), 1000.
- Press the soft key under **ENTRY DONE**.
- Snap the neck off a Sulfate 2-mL Ampule Standard, 1000-mg/L sulfate.
- Use the TenSette Pipet to add 0.1 mL, 0.2 mL and 0.3 mL of standard, respectively to three 25-mL samples and mix each thoroughly (for AccuVac Ampuls, use 50-mL beakers).
- Analyze each standard addition sample as described above. Accept the standard additions reading by pressing the soft key under **READ** each time. Each addition should reflect approximately 100% recovery.
- After completing the sequence, the display will show the extrapolated concentration value and the “best-fit” line through the standard additions data points, accounting for matrix interferences.
- See Section 1.4.1 *Standard Additions* for more information.

Standard Curve Adjustment

Using Class A glassware, prepare a 70-mg/L sulfate standard solution by pipetting 7 mL of Sulfate Standard Solution, 1000-mg/L, into a 100-mL volumetric flask. Dilute to the mark with deionized water. Prepare this solution daily. Perform the SulfaVer procedure as described above.

To adjust the calibration curve using the reading obtained with the 70-mg/L standard solution, press the soft keys under **OPTIONS, MORE** then **STD: OFF**. Press **ENTER** to accept the displayed concentration, the value of which depends on the selected units. If an alternate concentration is used, enter the actual concentration and press **ENTER** to return to the read screen. See Section 1.5.5 *Adjusting the Standard Curve* for more information.

Calibration Standard Preparation

To perform a sulfate calibration using the SulfaVer method, use Class A glassware to prepare calibration standards containing 10, 20, 30, 40, 50, 60 and 70 mg/L SO_4^{2-} as follows:

- a. Into seven different 100-mL Class A volumetric flasks, pipet 1, 2, 3, 4, 5, 6, and 7 mL of the 1000-mg/L Sulfate Standard Solution using Class A glassware.
- b. Dilute to the mark with deionized water. Mix thoroughly.
- c. Using the SulfaVer method and the calibration procedure described in the *User-Entered Programs* section of the *DR/4000 Spectrophotometer Instrument Manual*, generate a calibration curve from the standards prepared above.

Summary of Method

Sulfate ions in the sample react with barium in the SulfaVer 4 and form a precipitate of barium sulfate. The amount of turbidity formed is proportional to the sulfate concentration. The SulfaVer 4 also contains a stabilizing agent to hold the precipitate in suspension.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to *Section 1*.

Pollution Prevention and Waste Management

SulfaVer 4 contains barium chloride. The final solution will contain barium chloride (D005) at a concentration regulated as a hazardous waste by the Federal RCRA.

See *Section 1* for more information on proper disposal of these materials.

REQUIRED REAGENTS AND STANDARDS

Description	Quantity Required per test	Unit	Cat. No.
SulfaVer 4 Reagent Powder Pillows.....	1	100/pkg	12065-99
<i>or</i>			
SulfaVer 4 Sulfate Reagent AccuVac Ampuls	1	25/pkg	25090-25

REQUIRED EQUIPMENT AND SUPPLIES

DR/4000 1-inch Cell Adapter	1	each	48190-00
DR/4000 AccuVac Ampul Adapter.....	1	each	48187-00
Sample Cell, 10-mL with cap (zeroing vial).....	1	each	21228-00

OPTIONAL REAGENTS AND STANDARDS

Sulfate Standard Solution, 50-mg/L.....	500 mL		2578-49
Sulfate Standard Solution, 1000-mg/L.....	500 mL		21757-49
Sulfate Standard Solution, 2-mL PourRite Ampul, 1000-mg/L.....	20/pkg		21757-20
Water, deionized	4 liters		272-56

OPTIONAL EQUIPMENT AND SUPPLIES

AccuVac Snapper Kit	each		24052-00
Beaker, 50-mL.....	each		500-41
Brush, test tube	each		690-00
DR/4000 Carousel Module.....	each		48070-02
Filter Paper, folded 12.5-cm.....	100/pkg		1894-57
Flask, volumetric, 50-mL, Class A	each		14574-41
Funnel, poly, 65-mm	each		1083-67
Pipet, volumetric, Class A, 1.00-mL	each		14515-35
Pipet, volumetric, Class A, 2.00-mL	each		14515-36
Pipet, volumetric, Class A, 3.00-mL	each		14515-03
Pipet, volumetric, Class A, 4.00-mL	each		14515-04
Pipet, volumetric, Class A, 5.00-mL	each		14515-37
Pipet, volumetric, Class A, 6.00-mL	each		14515-06
Pipet, volumetric, Class A, 7.00-mL	each		14515-07
Pipet Filler, safety bulb.....	each		14651-00
Pipet, TenSette, 0.1 to 1.0 mL	each		19700-01
Pipet Tips, for 19700-01 TenSette Pipet	50/pkg		21856-96
PourRite Ampule Breaker Kit	each		24846-00



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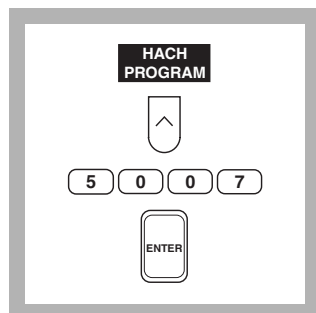


Turbidimetric Method

UniCell™ Vials

LR (40 to 150 mg/L SO₄²⁻)

Scope and Application: For wastewater, raw water, and process control.

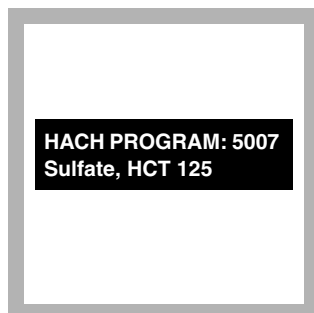


- 1.** Press the soft key under **HACH PROGRAM**.

Select the stored program for UniCell sulfate by pressing **5007** with the numeric keys.

Press: **ENTER**

Note: If samples cannot be analyzed immediately, see *Sample Collection, Storage, and Preservation* following these steps

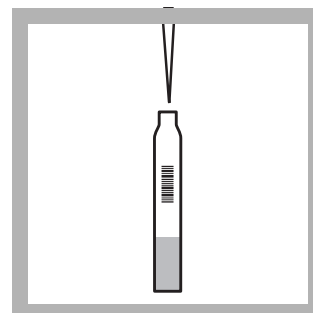


- 2.** The display will show:
HACH PROGRAM: 5007 Sulfate, HCT 125

The wavelength (λ), **430 nm**, is automatically selected.

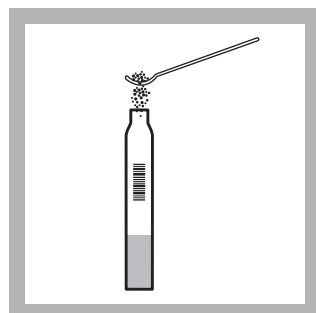


- 3.** Insert the Test Tube Adapter into the sample cell module by sliding it under the thumb screw and into the alignment grooves. Fasten with the thumb screw.



- 4.** Pipet 5 mL of sample into the sample vial.

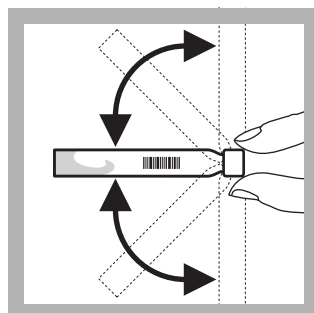
Note: Filter highly turbid or colored samples.



- 5.** Use the small black spoon (included) to add 1 spoonful of Barium Chloride A (HCT 125 A) to the sample vial.

Note: A white turbidity will develop if sulfate is present.

Note: Accuracy is not affected by undissolved powder which has settled.

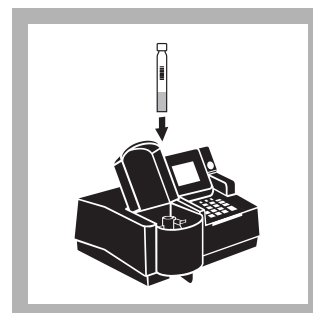


- 6.** Immediately begin mixing the sample by inverting repeatedly for two minutes.

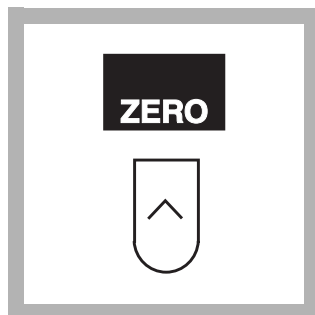


- 7.** Clean the outside of the zero vial (white cap) with a towel.

Note: Wiping with a damp towel, followed by a dry one, will remove fingerprints or other marks.



- 8.** Place the zero vial in the cell holder. Close the light shield.

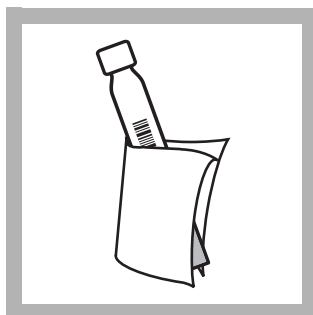


9. Press the soft key under **ZERO**.

The display will show:

0 mg/L SO₄²⁻

Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



10. Clean the outside of the sample vial with a towel.

Note: Wiping with a damp towel, followed by a dry one, will remove fingerprints or other marks.



11. After the timer beeps, place the sample into the cell holder. Close the light shield. Results in mg/L sulfate will be displayed.

Sample Collection, Storage, and Preservation

Analyze samples within 3 hours after collection for best results. Samples may be stored up to 28 days by cooling to 4 °C (39 °F) or lower. Warm to room temperature before analysis.

Accuracy Check

Standard Solution Method

Use a 100-mg/L Sulfate Standard Solution listed under **OPTIONAL REAGENTS AND STANDARDS**. Perform the sulfate procedure as described.

Standard Additions Method

- a. Leave the unspiked sample in the sample compartment. Verify that the units displayed are in mg/L. Select standard additions mode by pressing the soft keys under **OPTIONS, (MORE)** and then **STD ADD**.
- b. Press **ENTER** to accept the default sample volume (mL), 25.0.
- c. Press **ENTER** to accept the default standard concentration (mg/L), 2500.
- d. Press the soft key under **ENTRY DONE**.
- e. Snap the neck off a Sulfate 10-mL Ampule Standard, 2500 mg/L sulfate.
- f. Use a pipet to add 0.1 mL, 0.2 mL and 0.3 mL of standard (2500-mg/L SO₄²⁻), respectively, to three 25-mL samples in 25-mL mixing cylinders. Mix each sample thoroughly.
- g. Analyze each standard addition sample as described above. Accept the standard additions reading by pressing the soft key under **READ** each time. Each addition should reflect approximately 100% recovery.

- h. After completing the sequence, the display will show the extrapolated concentration value and the “best-fit” line through the standard additions data points, accounting for matrix interferences.
- i. See Section 1.4.1 *Standard Additions* for more information.

Standard Curve Adjustment

Using Class A glassware, prepare a 100-mg/L sulfate standard solution by pipetting 4 mL of Sulfate Standard Solution, 2500-mg/L, into a 100-mL volumetric flask. Dilute to the mark with deionized water. Prepare this solution daily. Perform the procedure as described above.

To adjust the calibration curve using the reading obtained with the 100-mg/L standard solution, press the soft keys under **OPTIONS, MORE** then **STD: OFF**. Press **ENTER** to accept the displayed concentration, the value of which depends on the selected units. If an alternate concentration is used, enter the actual concentration and press **ENTER** to return to the read screen. See Section 1.5.5 *Adjusting the Standard Curve* for more information

Method Performance

Precision

Standard: 100 mg/L SO_4^{2-}

Program	95% Confidence Limits
5007	85–115 mg/L SO_4^{2-}

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
5007	40 mg/L SO_4^{2-}

For more information on derivation and use of Hach’s estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by 40 CFR part 136, appendix B, see Section 1.5.1.

Sensitivity

Program Number: 5007

Portion of Curve	ΔAbs	$\Delta\text{Concentration}$
40 mg/L SO_4^{2-}	0.010	1 mg/L SO_4^{2-}
150 mg/L SO_4^{2-}	0.010	2 mg/L SO_4^{2-}

See Section 1.5.3 *Sensitivity Explained* for more information.

Summary of Method

Sulfate ions in the sample react with barium chloride in aqueous solution to form a precipitate of barium sulfate. The resulting turbidity is measured photometrically.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to *Section 1*.

Barium Chloride A —(HCT 125 A) contains barium chloride.

Pollution Prevention and Waste Management

The UniCell reagent Barium Chloride A (HCT 125 A) contains barium chloride. The final solution will contain barium chloride (D005) at a concentration regulated as a hazardous waste by the Federal RCRA.

See *Section 1* for more information on proper disposal of these materials.

REQUIRED REAGENTS AND STANDARDS

Description	Unit	Cat. No.
Sulfate SO ₄ , UniCell HCT 125	23/pkg	HCT 125

OPTIONAL REAGENTS AND STANDARDS

Description	Quantity Required per test	Unit	Cat. No.
Sulfate Standard Solution, 100-mg/L as SO ₄	1	500 mL.....	891-49
Sulfate Standard Solution, 2500-mg/L as SO ₄	1	500 mL.....	14252-49

OPTIONAL EQUIPMENT AND SUPPLIES

Cylinder, graduated, 25-mL	3	each.....	20886-40
Flask, volumetric, 100-mL, Class A	1	each.....	14574-42
Pipettor, Jencons, 100–1000 µL	1	each.....	27949-00
Replacement tips for 27949-00	400/pkg		27950-00
Pipettor, Jencons, 1–5 mL	1	each.....	27951-00
Replacement tips for 27951-00	100/pkg		27952-00



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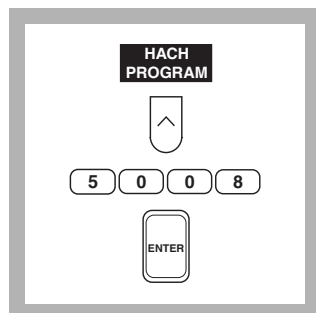


Turbidimetric Method

UniCell™ Vials

HR (150 to 900 mg/L SO₄²⁻)

Scope and Application: For wastewater, raw water, and process control.



- 1.** Press the soft key under **HACH PROGRAM**.

Select the stored program for UniCell Sulfate by pressing **5008** with the numeric keys.

Press: **ENTER**

Note: If samples cannot be analyzed immediately, see *Sample Collection, Storage, and Preservation* following these steps.



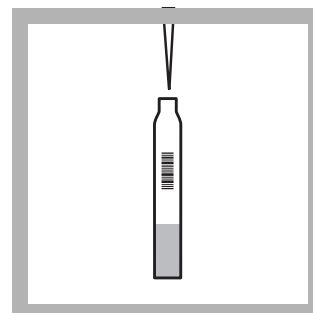
- 2.** The display will show:
HACH PROGRAM: 5008 Sulfate, HCT 126

The wavelength (λ), **880 nm**, is automatically selected.

Note: For best results, adjust the standard curve for each new lot of reagent. See *Standard Curve Adjustment* following these steps.

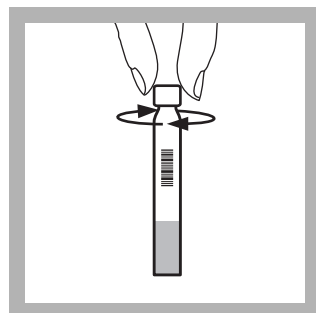


- 3.** Insert the Test Tube Adapter into the sample cell module by sliding it under the thumb screw and into the alignment grooves. Fasten with the thumb screw.



- 4.** Pipet 2 mL of sample into a sample vial.

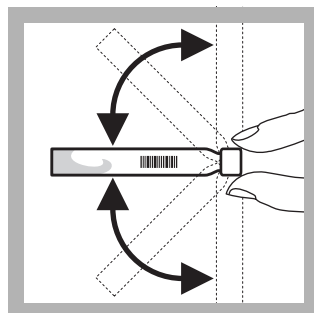
Note: Filter highly turbid or colored samples.



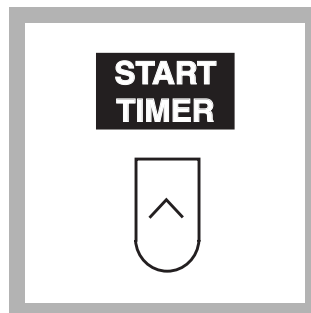
- 5.** Use the small black spoon (included) to add 1 spoonful of Barium Chloride A (HCT 126 A) to the sample vial.

Note: A white turbidity will develop if sulfate is present.

Note: Accuracy is not affected by undissolved powder which has settled.

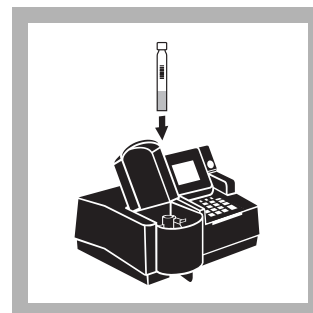


- 6.** Immediately begin mixing the sample by inverting repeatedly for one minute.

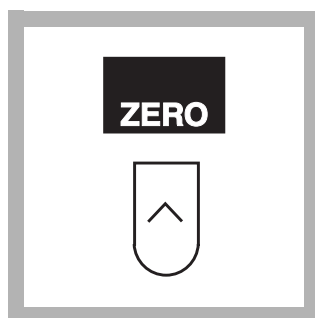


- 7.** Press the soft key under **START TIMER**. A 30-second reaction period will begin.

Note: Allow the vial to stand undisturbed.



- 8.** When the timer beeps, place the zero vial (white cap) in the cell holder. Close the light shield.



9. Press the soft key under **ZERO**.

The display will show:

0 mg/L SO₄²⁻

Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



10. After the timer beeps, place the sample into the cell holder. Close the light shield. Results in mg/L sulfate will be displayed.

Interferences

The ions listed in the following table have been individually tested up to the given concentrations. Cumulative effects and the influence of other ions have not been evaluated.

Ion	No interference up to:
Na ⁺ , K ⁺	2000 mg/L
Ca ²⁺ , NO ₃ ⁻ , Cl ⁻	1000 mg/L
Cd ²⁺ , Cr ³⁺ , Cu ²⁺ , Fe ²⁺ , Fe ³⁺ , Mg ²⁺ , Mn ²⁺ , NH ₄ ⁺ , Ni ²⁺ , Si ²⁺ , Sn ²⁺ , Zn ²⁺	500 mg/L
Al ³⁺ , Pb ²⁺ , Hg ²⁺ , PO ₄ ³⁻ , CO ₃ ²⁻ , I ⁻ , CN ⁻ , NO ₂ ⁻	50 mg/L
Cr ⁶⁺	20 mg/L
Ag ⁺	2.5 mg/L

Sample Collection, Storage, and Preservation

Analyze samples within 3 hours after collection for best results. Samples may be stored up to 28 days by cooling to 4 °C (39 °F) or lower. Warm to room temperature before analysis.

Accuracy Check

Standard Solution Method

Prepare a 500-mg/L SO₄ standard solution by pipetting 20 mL of 2500-mg/L SO₄ standard solution into a 100-mL volumetric flask. Dilute to the mark with deionized water. Stopper and invert to mix. Perform the sulfate procedure as described.

Standard Additions Method

- a. Leave the unspiked sample in the sample compartment. Verify that the units displayed are in mg/L. Select standard additions mode by pressing the soft keys under **OPTIONS, (MORE)** and then **STD ADD**.
- b. Press **ENTER** to accept the default sample volume (mL), 25.
- c. Press **ENTER** to accept the default standard concentration (mg/L), 2500.
- d. Press the soft key under **ENTRY DONE**.
- e. Snap the neck off a Sulfate 10-mL Ampule Standard, 2500-mg/L sulfate.
- f. Use a pipet to add 0.2 mL, 0.4 mL and 0.6 mL of standard, respectively to three 25-mL samples in 25-mL mixing cylinders. Mix each sample thoroughly.
- g. Analyze each standard addition sample as described above. Accept the standard additions reading by pressing the soft key under **READ** each time. Each addition should reflect approximately 100% recovery.
- h. After completing the sequence, the display will show the extrapolated concentration value and the “best-fit” line through the standard additions data points, accounting for matrix interferences.
- i. See Section 1.4.1 *Standard Additions* for more information.

Standard Curve Adjustment

Using Class A glassware, prepare a 500-mg/L sulfate standard solution by pipetting 20 mL of Sulfate Standard Solution, 2500-mg/L, into a 100-mL volumetric flask. Dilute to the mark with deionized water. Prepare this solution daily. Perform the procedure as described above.

To adjust the calibration curve using the reading obtained with the 500-mg/L standard solution, press the soft keys under **OPTIONS, MORE** then **STD: OFF**. Press **ENTER** to accept the displayed concentration, the value of which depends on the selected units. If an alternate concentration is used, enter the actual concentration and press **ENTER** to return to the read screen. See Section 1.5.5 *Adjusting the Standard Curve* for more information.

Method Performance

Precision

Standard: 500 mg/L SO_4^{2-}

Program	95% Confidence Limits
5008	450–550 mg/L SO_4^{2-}

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
5008	150 mg/L SO_4^{2-}

For more information on derivation and use of Hach’s estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by 40 CFR part 136, appendix B, see Section 1.5.1.

SULFATE, continued

Sensitivity

Program Number: 5008

Portion of Curve	Δ Abs	Δ Concentration
170 mg/L SO_4^{3-}	0.010	7 mg/L SO_4^{3-}
900 mg/L SO_4^{3-}	0.010	9 mg/L SO_4^{3-}

See Section 1.5.3 *Sensitivity Explained* for more information.

Summary of Method

Sulfate ions in the sample react with barium chloride in aqueous solution to form a precipitate of barium sulfate. The resulting turbidity is measured photometrically.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to *Section 1*.

Barium Chloride A —(HCT 126 A) contains barium chloride.

Pollution Prevention and Waste Management

The UniCell reagent Barium Chloride A (HCT 126 A) contains barium chloride. The final solution will contain barium chloride (D005) at a concentration regulated as a hazardous waste by the Federal RCRA.

See *Section 1* for more information on proper disposal of these materials.

REQUIRED REAGENTS AND STANDARDS

Description	Unit	Cat. No.
Sulfate SO_4 , UniCell™ HCT 126	23/pkg.....	HCT 126

OPTIONAL REAGENTS AND STANDARDS

Description	Quantity Required per test	Unit	Cat. No.
Sulfate Standard Solution, 2500-mg/L.....		500 mL.....	14252-49

OPTIONAL EQUIPMENT AND SUPPLIES

Cylinder, graduated, 25-mL	1	each.....	20886-40
Flask, volumetric, 100-mL, Class A	1	each.....	14574-42
Pipettor, Jencons, 100–1000 μL	1	each.....	27949-00
Replacement tips for 27949-00		400/pkg.....	27950-00
Pipettor, Jencons, 1–5 mL	1	each.....	27951-00
Replacement tips for 27951-00		100/pkg.....	27952-00



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DR/4000 PROCEDURE

SULFIDE

✓ Method 8131

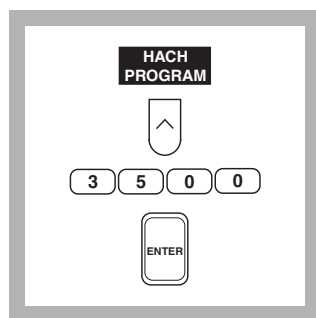
Methylene Blue Method*

(0 to 800 µg/L)

Scope and Application: For testing total sulfides, H_2S , HS^- and certain metal sulfides in groundwater, wastewater brines and seawater; USEPA accepted for reporting wastewater analysis**

* Adapted from *Standard Methods for the Examination of Water and Wastewater*.

** Procedure is equivalent to USEPA method 376.2 and Standard Method 4500-S²⁻-D for wastewater.



1. Press the soft key under **HACH PROGRAM**.

Select the stored program number for sulfide (S^{2-}) by pressing **3500** with the numeric keys.

Press: **ENTER**

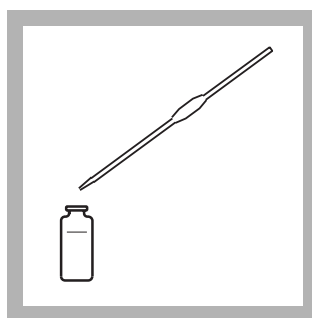
Note: The Flow Cell and Sipper Modules can be used with this procedure.

Note: Samples must be analyzed immediately and cannot be preserved for later analysis. Avoid excessive agitation of samples.



2. The display will show: **HACH PROGRAM: 3500 Sulfide**

The wavelength (λ), **665 nm**, is automatically selected.



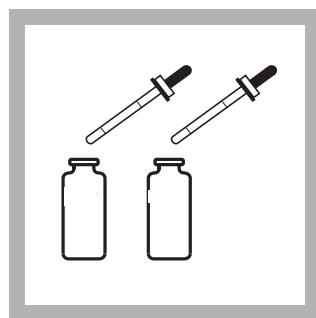
3. Measure 25 mL of sample into a sample cell. This will be the prepared sample.

Note: For turbid samples, see *Interferences* (following these steps) for pretreatment instructions.

Note: Excessive agitation will cause loss of sulfide. Use a pipet to minimize sulfide loss.

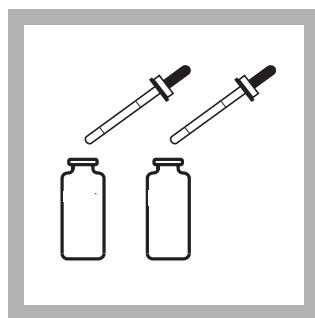


4. Measure 25 mL of deionized water into a second sample cell (the blank).



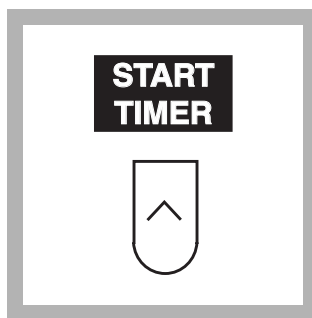
5. Add 1.0 mL of Sulfide 1 Reagent to each cell. Swirl to mix.

Note: Use the calibrated 1-mL dropper.

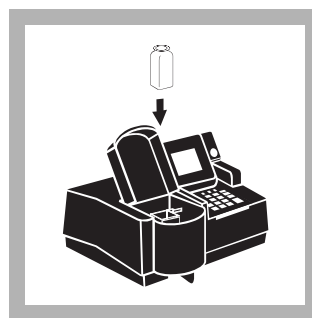


6. Add 1.0 mL of Sulfide 2 Reagent to each cell. Immediately swirl to mix.

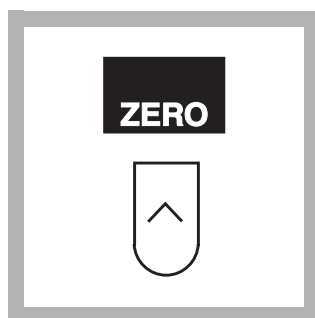
Note: A pink color will develop, then the solution will turn blue if sulfide is present.



7. Press the soft key under **START TIMER**. A 5-minute reaction period will begin.



8. When the timer beeps, place the blank in the cell holder. Close the light shield.



9. Press the soft key under **ZERO**.

The display will show:

0 µg/L S²⁻

Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



10. Place the prepared sample in the cell holder. Close the light shield. Results in µg/L sulfide (or chosen units) will be displayed.

Note: Some sulfide loss may occur if dilution is necessary.

Interferences

Table 1 Interfering Substances and Suggested Treatments

Interfering Substance	Interference Levels and Treatments
Strong reducing substances (sulfite, thiosulfate and hydrosulfite)	Interfere by reducing the blue color or preventing its development
Sulfide, high levels	High concentrations of sulfide may inhibit full color development and require sample dilution. Some sulfide loss may occur when the sample is diluted.
Turbidity	For turbid samples, prepare a sulfide-free blank as follows. Use it in place of the deionized water blank in the procedure. <ol style="list-style-type: none">1. Measure 25 mL of sample into a 50-mL erlenmeyer flask.2. Add Bromine Water dropwise with constant swirling until a permanent yellow color just appears.3. Add Phenol Solution dropwise until the yellow color just disappears. Use this solution in place of deionized water in step 4.

Sample Collection, Storage and Preservation

Collect samples in clean plastic or glass bottles. Fill completely and cap tightly. Avoid excessive agitation or prolonged exposure to air. Analyze samples immediately.

Method Performance

Precision

Standard: 400 µg/L S²⁻

Program	95% Confidence Limits
3500	399–401 µg/L S ²⁻

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
3500	2 µg/L S ²⁻

For more information on derivation and use of Hach's estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, appendix B, see Section 1.5.1.

Sensitivity

Program Number: 3500

Portion of Curve	ΔAbs	ΔConcentration
Entire Range	0.010	4.9 µg/L

See Section 1.5.3 *Sensitivity Explained* for more information.

Determining Soluble Sulfides

Determine soluble sulfides by centrifuging the sample in completely filled, capped tubes and analyzing the supernatant. Insoluble sulfides are then estimated by subtracting the soluble sulfide concentration from the total sulfide result.

Summary of Method

Hydrogen sulfide and acid-soluble metal sulfides react with N,N-dimethyl-p-phenylenediamine sulfate to form methylene blue. The intensity of the blue color is proportional to the sulfide concentration.

High sulfide levels in oil field waters may be determined after proper dilution.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to Section 1.

Pollution Prevention and Waste Management

Sulfide 2 reagent contains potassium dichromate. The final solution will contain hexavalent chromium (D007) at a concentration regulated as a hazardous waste by Federal RCRA. Please see Section 1 for further information on proper disposal of these materials.

SULFIDE, continued

REQUIRED REAGENTS AND STANDARDS

	Cat. No.
Sulfide Reagent Set (100 tests)	22445-00
Includes: (2) 1816-32, (2) 1817-32	

Description	Quantity Required		Unit	Cat. No.
	per test			
Sulfide 1 Reagent	2 mL	100	mL MDB	1816-32
Sulfide 2 Reagent	2 mL	100	mL MDB	1817-32
Water, deionized	25 mL	4	liters	272-56

REQUIRED EQUIPMENT AND SUPPLIES

Cylinder, graduated, 25-mL	1	each	508-40
<i>or</i>			
Pipet, volumetric, Class A, 25-mL	1	each	14515-40
DR/4000 1-Inch Cell Adapter	1	each	48190-00
Pipet Filler, safety bulb	1	each	14651-00

OPTIONAL REAGENTS AND STANDARDS

Bromine Water, 30-g/L	29 mL	2211-20
Phenol Solution, 30-g/L	29 mL	2112-20

OPTIONAL EQUIPMENT AND SUPPLIES

DR/4000 Carousel Module Kit	each	48070-02
DR/4000 Flow Cell Module Kit, 1-inch	each	48070-04
DR/4000 Flow Cell Module Kit, 1-cm	each	48070-05
DR/4000 Sipper Module Kit, 1-inch	each	48090-03
Dropper, for 1-oz. bottle	each	2258-00
Flask, Erlenmeyer, 50-mL	each	505-41



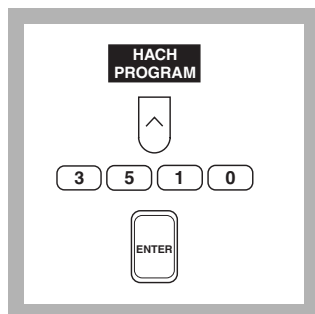
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Scope and Application: For boiler water, foodstuffs

* Reagent sets for this method are only available in Europe.



1. Press the soft key under **HACH PROGRAM**.

Select the stored program for Sulfite (HPT 430) by pressing **3510** with the numeric keys.

Press: **ENTER**

Note: If samples cannot be analyzed immediately, see *Sample Collection, Storage and Preservation* following these steps.

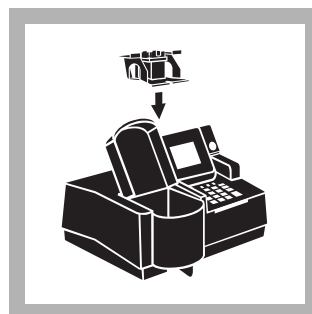
Note: The Flow Cell and Sipper Cell Modules cannot be used with this procedure.



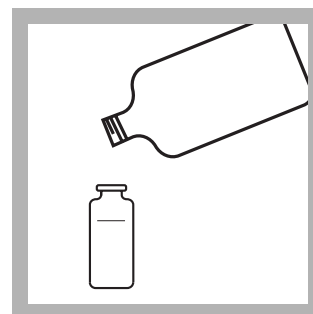
2. The display will show:
HACH PROGRAM: 3510 Sulfite HPT 430

The wavelength (λ), **435 nm**, is automatically selected.

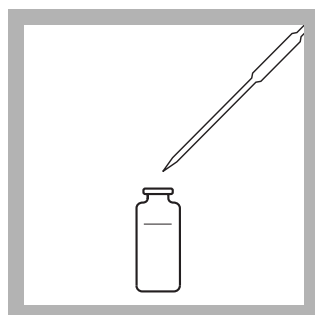
Note: For best results, perform a new calibration for each lot of reagent. See *Accuracy Check* following these steps.



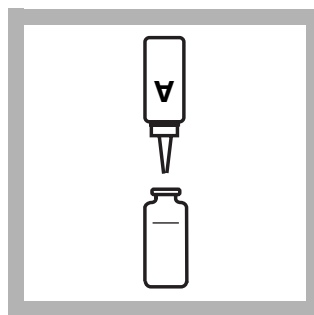
3. Place the DR/4000 1-inch Cell Adapter into the sample cell module by sliding it under the thumb screw and into the alignment grooves. Fasten with the thumb screw.



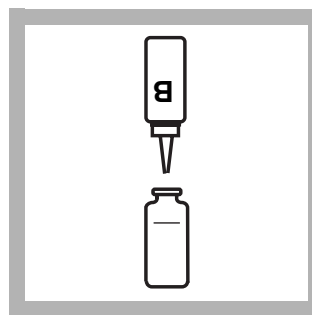
4. Fill a clean sample cell with 10 mL of deionized water. Cap the cell. This is the blank.



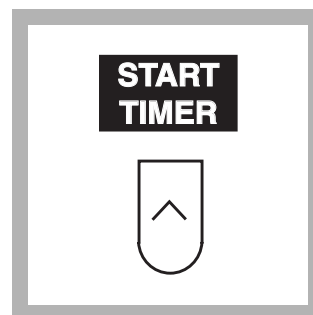
5. Pipet 10 mL of sample into a second sample cell.



6. Add 5 drops of Sulfite Reagent A (HPT 430 A). Swirl to mix.



7. Add 2 drops of Sulfite Reagent B (HPT 430 B). Swirl to mix.



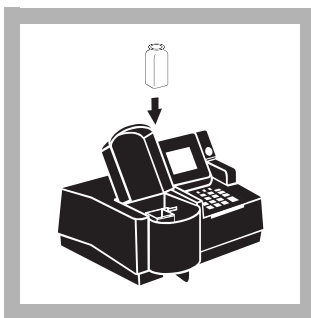
8. Press the soft key under **START TIMER**.

A 3-minute reaction period will begin.

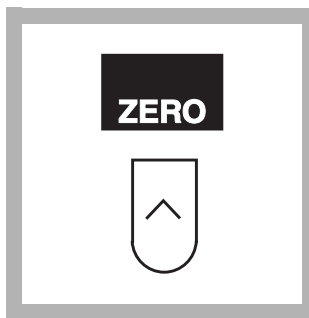
Note: Allow cell to stand undisturbed.



9. When the timer beeps, wipe the cells with a damp towel, followed by a dry one, to remove fingerprints and other marks.



10. Place the blank cell into the cell holder. Close the light shield.



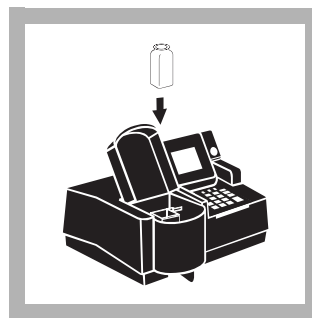
11. Press the soft key under **ZERO**.

The display will show:

0.00 mg/L SO₃²⁻

Note: If you are using a reagent blank correction, the display will show the correction.

Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



12. Place the prepared sample into the cell holder. Close the light shield. Results in mg/L Sulfite (or chosen units) will be displayed.

Note: Clean the sample cells with soap and a brush.

Interferences

A sulfide concentration greater than 5.0 mg/L in the sample gives results with a high bias.

Sample Collection, Storage and Preservation

Collect samples in clean plastic or glass bottles. Samples must be analyzed immediately. Warm to 15–25 °C (59–77°F) before analysis.

Accuracy Check

Standard Additions Method

- Leave the unspiked sample in the sample compartment. Verify that the units displayed are in mg/L. Select standard additions mode by pressing the soft keys under **OPTIONS**, **(MORE)** and then **STD ADD**.
- Press **ENTER** to accept the default sample volume (mL), 25.
- Press **ENTER** to accept the default standard concentration (mg/L), 15.
- Press the soft key under **ENTRY DONE**.
- Use a pipet to add 1.0 mL, 2.0 mL and 3.0 mL of standard, respectively, into three 25-mL samples and mix each thoroughly.
- Analyze each standard addition sample as described above. Accept the standard additions reading by pressing the soft key under **READ** each time. Each addition should reflect approximately 100% recovery.

- g. After completing the sequence, the display will show the extrapolated concentration value and the “best-fit” line through the standard additions data points, accounting for matrix interferences.
- h. See Section 1.4.1 *Standard Additions* for more information.

Standard Curve Adjustment

Using Class A glassware, prepare a 3.0-mg/L sulfite standard solution by pipetting 20 mL of 15-mg/L Sulfite Standard Solution into a 100-mL volumetric flask. Dilute to the mark with deionized water. Prepare this solution daily. Perform the Colorimetric Sulfite procedure as described above.

To adjust the calibration curve using the reading obtained with the 3.0-mg/L standard solution, press the soft keys under **OPTIONS, (MORE)** then **STD: OFF**. Press **ENTER** to accept the displayed concentration, the value of which depends on the selected units. If an alternate concentration is used, enter the actual concentration and press **ENTER** to return to the read screen. See Section 1.5.5 *Adjusting the Standard Curve* for more information.

Summary of Method

The reagents react with sulfites to form a yellow complex.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to *Section 1*.

REQUIRED REAGENTS AND STANDARDS

Description	Unit	Cat. No.
Sulfite Colorimetric Reagent Set*	100/pkg	HPT 430
Includes:		
Sulfite Reagent A	28 mL	HPT 430 A
Sulfite Reagent B	8.7 mL	HPT 430 B

REQUIRED EQUIPMENT AND SUPPLIES

DR/4000 1-inch Cell Adapter	each	48190-00
Sample Cells, 1-inch, matched pair	2/pkg	26126-02

OPTIONAL REAGENTS AND STANDARDS

Sulfite Standard Solution, 15 mg/L	500 mL	24084-49
Water, deionized	4 liters	272-56

OPTIONAL EQUIPMENT AND SUPPLIES

DR/4000 Carousel Module	each	48070-02
Flask, volumetric, 100-mL, Class A	each	14574-42
Pipet, TenSette, 0.1 to 1.0 mL	each	19700-01
Pipet Tips, for 19700-01 TenSette Pipet	50/pkg	21856-96

* Available in Europe only



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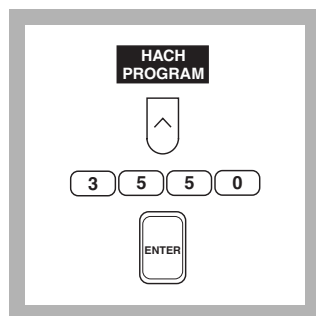
Method 8193

Tyrosine Method*

(0 to 9.00 mg/L)

Scope and Application: For water, wastewater and boiler water.
The estimated detection limit for program number 3550 is 0.09 mg/L.

* Adapted from Kloster, M.B., *Journal American Water Works Association*, Vol. 66, No. 1, p. 44 (1974)



1. Press the soft key under **HACH PROGRAM**.

Select the stored program for tannin and lignin by pressing **3550** with the numeric keys.

Press: **ENTER**

Note: If samples cannot be analyzed immediately, see *Sample Collection, Storage and Preservation* following these steps.

Note: The Flow Cell and Sipper Modules can be used with this procedure.

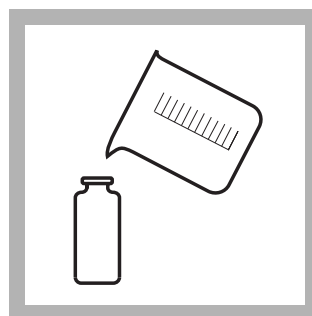


2. The display will show:
**HACH PROGRAM: 3550
Tannin and Lignin**

The wavelength (λ), **700 nm**, is automatically selected.



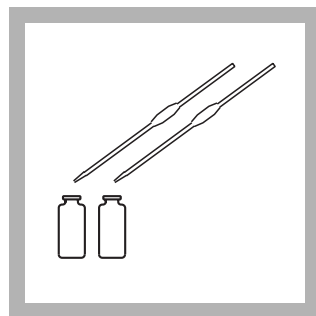
3. Fill a clean sample cell to the 25-mL mark with deionized water (the blank).



4. Fill a clean sample cell to the 25-mL mark with sample (the prepared sample).

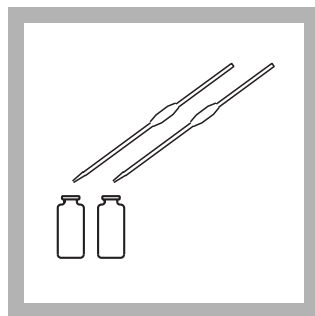
Note: Filter turbid samples and report results as mg/L soluble tannic acid.

Note: For proof of accuracy, use a 2.0-mg/L tannic acid solution in place of the sample (see *Accuracy Check*).



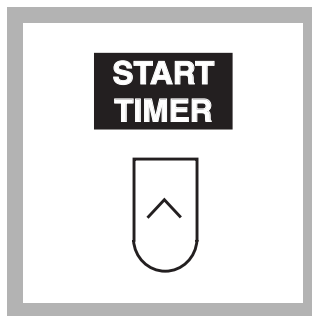
5. Pipet 0.5 mL of TanniVer 3 Tannin-Lignin Reagent into each cell. Swirl to mix.

Note: For best results, use a volumetric pipet to add the TanniVer 3 Reagent.

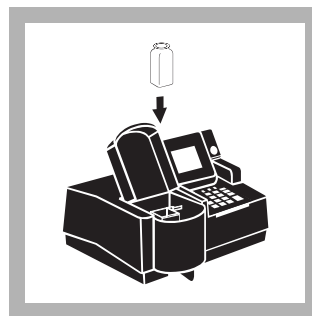


6. Pipet 5.0 mL of Sodium Carbonate Solution into each cell. Swirl to mix.

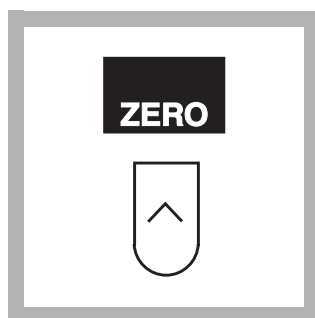
Note: A blue color will develop if tannins and/or lignins are present.



7. Press the soft key under **START TIMER**. A 25-minute reaction period will begin.



8. When the timer beeps, place the blank in the cell holder. Close the light shield.



9. Press the soft key under **ZERO**.

The display will show:

0 mg/L Tannin

Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



10. Place the prepared sample into the cell holder. Close the light shield. Results in mg/L (or chosen units) tannin will be displayed.

Interferences

Table 1 Interfering Substances and Suggested Treatments for Powder Pillows

Interfering Substance	Interference Levels and Treatments
Ferrous iron	Causes a positive interference. Two mg/L of ferrous iron produces a color equivalent to about 1 mg/L of tannic acid. To eliminate interference of ferrous iron up to 20 mg/L, add one 0.2-g scoop of Sodium Pyrophosphate to the sample before testing.
Sulfite	Interference is eliminated by adding 1 mL of formaldehyde to the sample before testing the sample.

Sample Collection, Storage and Preservation

Collect samples in clean plastic or glass bottles.

Accuracy Check

Standard Solution Method

Prepare a 200-mg/L tannic acid standard solution by dissolving 0.200 grams of tannic acid in deionized water and diluting to 1000 mL. Prepare this solution monthly. Prepare a 2.0-mg/L tannic acid standard by diluting 10.00 mL of the stock solution to 1000 mL with deionized water. Prepare this standard daily. Perform the tannin and lignin test on the standard solution as described above.

Method Performance

Precision

Standard: 2.00 mg/L tannic acid

Program	95% Confidence Limits
3550	1.95–2.05 mg/L tannin

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
3550	0.09 mg/L tannin

For more information on derivation and use of Hach's estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by 40 CFR part 136, appendix B, see Section 1.5.1.

Sensitivity

Program Number: 3550

Portion of Curve	Δ Abs	Δ Concentration
Entire Range	0.010	0.066 mg/L

See Section 1.5.3 *Sensitivity Explained* for more information.

Calibration Standard Preparation

To perform a tannin and lignin calibration using the Tyrosine Method, prepare calibration standards containing 3.0, 6.0, and 9.0 mg/L as follows:

- a. Prepare a 200-mg/L tannic acid standard solution. Dissolve 0.200 grams of tannic acid into deionized water in a 1-liter Class A volumetric flask. Dilute to volume with deionized water. Stopper and invert several times to mix. Prepare this solution monthly.
- b. Into three different 200-mL volumetric flasks, pipet 3.00, 6.00, and 9.00 mL of the 200-mg/L tannic acid standard solution using Class A glassware. Prepare these standards daily.
- c. Dilute to the mark with deionized water. Stopper and invert several times to mix thoroughly.
- d. Using the Tyrosine method and the calibration procedure described in the *User-Entered Programs* section of the *DR/4000 Spectrophotometer Instrument Manual*, generate a calibration curve from the standards prepared above.

Summary of Method

This test measures all hydroxylated aromatic compounds, including tannin, lignin, phenol and cresol. This method produces a blue color proportional to the amount of these compounds present in the sample. The results are reported as total tannin and lignin expressed as mg/L tannic acid.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to *Section 1*.

Pollution Prevention and Waste Management

For information on pollution prevention and waste management, refer to *Section 1*.

REQUIRED REAGENTS AND STANDARDS

	Cat. No.
Tannin and Lignin Reagent Set (up to 100 tests)	22446-00
Includes: (2) 675-49, (1) 2560-32	

Description	Quantity Required		Cat. No.
	per test	Unit	
Sodium Carbonate Solution	10 mL	500 mL	675-49
TanniVer 3 Tannin-Lignin Reagent	1 mL	100 mL	2560-32
Water, deionized	25 mL	4 liters	272-56

REQUIRED EQUIPMENT AND SUPPLIES

DR/4000 1-Inch Cell Adapter	1	each	48190-00
Pipet, Filler, safety bulb	1	each	14651-00
Pipet, volumetric, Class A, 5.0-mL	1	each	14515-37
Pipet, volumetric, Class A, 0.5-mL	1	each	14515-34

OPTIONAL REAGENTS AND STANDARDS

Formaldehyde, ACS	100 mL	MDB	2059-32
Sodium Pyrophosphate, ACS	50 g		784-25
Tannic Acid	113 g		791-14

OPTIONAL EQUIPMENT AND SUPPLIES

Balance, analytical, Mettler, 110/220 VAC		each	22310-00
Cylinder, graduated, 25-mL		each	508-40
DR/4000 Carousel Module Kit		each	48070-02
DR/4000 Flow Cell Module Kit, 1-inch		each	48070-04
DR/4000 Flow Cell Module Kit, 1-cm		each	48070-05
DR/4000 Sipper Module Kit, 1-inch		each	48090-03
Filter Paper, folded, 12.5-cm	100/pkg		1894-57
Flask, volumetric, Class A, 200-mL		each	14574-45
Flask, volumetric, Class A, 1000-mL		each	14574-53
Funnel, poly, 65-mm		each	1083-67
Pipet, TenSette, 0.1- to 1.0-mL		each	19700-01
Pipet Tips, for 19700-01 Pipet	50/pkg		21856-96
Pipet, volumetric, Class A, 3.00-mL		each	14515-03
Pipet, volumetric, Class A, 6.00-mL		each	14515-06
Pipet, volumetric, Class A, 9.00-mL		each	14515-09
Pipet, volumetric, Class A, 10.00-mL		each	14515-38
Spoon, measuring, 0.2-g		each	638-00



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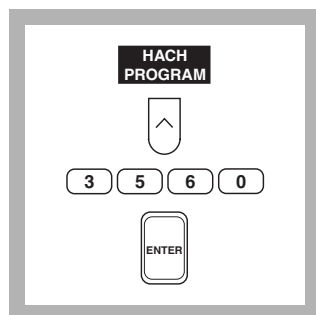


Method 10132

THM Plus™ Water Bath Method

Scope and Application: For screening THMs in drinking water.

(0–200 ppb as Chloroform)



1. Press the soft key under **HACH PROGRAM**. Select the stored program number for Trihalomethane (THM) Plus by pressing 3560 on the numeric keys.

Press **ENTER**.

Note: For the most precise results, use matched cells. See page 6.



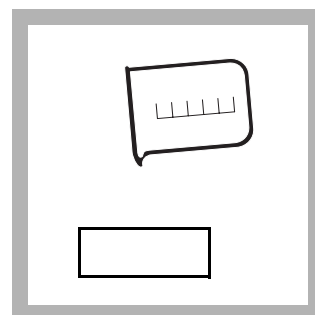
2. The display will show: **HACH PROGRAM: 3560 THM Plus**

The wavelength (λ), 515 nm, is automatically selected.



3. Prepare a hot water bath by adding 500 mL of water to an evaporating dish. Put the dish on a hot plate and turn heater on high.

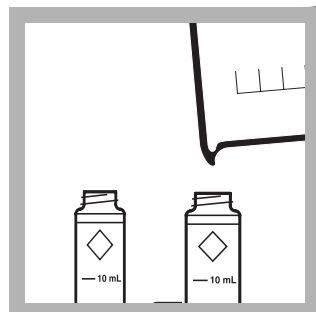
Note: If analyzing more than four samples, use 450 mL of water.



4. Prepare a cooling bath by adding 500 mL of cold (18–25 °C) tap water to a second evaporating dish.

Note: Maintain the water temperature between 18 and 25 °C.

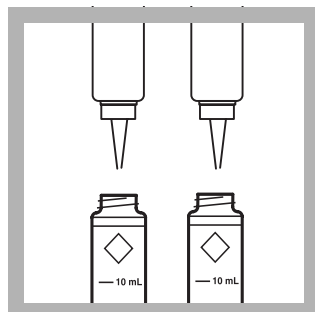
Note: If analyzing more than four samples, use 450 mL of water.



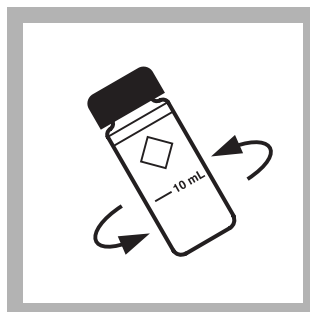
5. Fill two sample cells to the 10 mL mark with sample. Label one **sample** and the other **blank**.

Note: Perform steps 5 through 9 **rapidly** so as not to lose volatile THMs from the sample. If you are testing more than one sample, complete steps 5 through 9 for one sample before going on to the next.

Note: If dispensing sample with a pipette, the pipette must dispense quickly without causing aeration or back pressure.

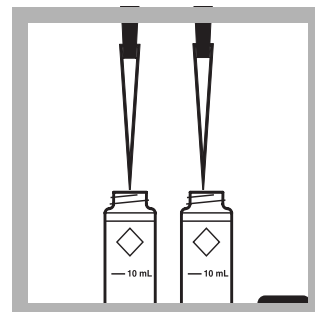


6. Add three drops of THM Plus Reagent 1 to each cell.



7. Cap tightly and mix gently by swirling each cell three times.

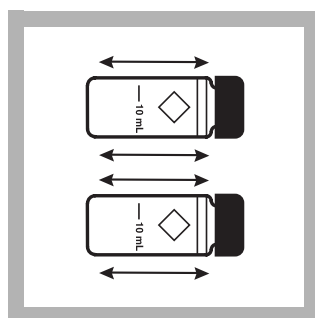
Note: Vigorous shaking can cause loss of THMs.



8. Use a TenSette® pipette to add 3 mL of THM Plus Reagent 2 to each cell.

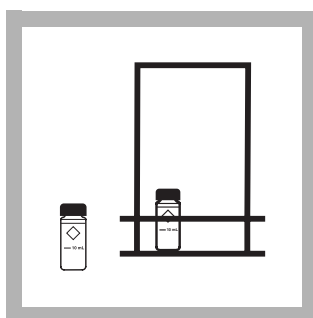
Note: The liquid is viscous and a small amount may remain in the tip after dispensing. This will not affect the results.

Note: The THM Plus Reagent 2 must be at room temperature before use.



9. Cap tightly and mix by shaking ten times.

Note: Thorough mixing ensures that all of the THM goes into the liquid and does not accumulate in the head space.

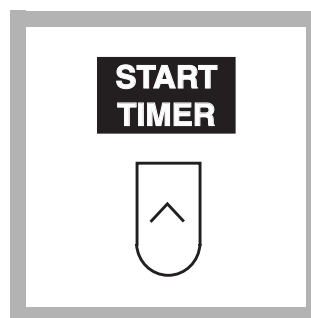


10. Place the sample cell in the cell holder assembly. Set the blank aside.

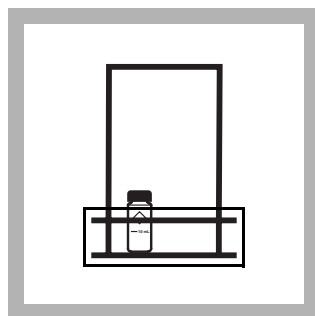


11. Place the basket in the hot-water bath when the water is boiling rapidly.

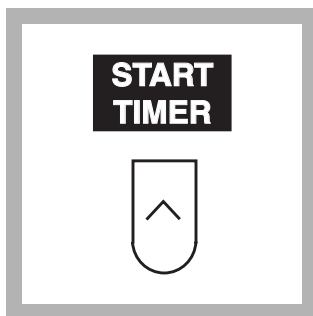
Note: Do not allow water to rise above the white line near the top of the sample cells.



12. Press: **START TIMER 1** to begin a five-minute reaction period.

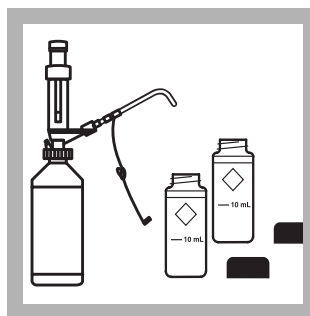


13. At the end of the reaction period, remove the basket and sample cell from the hot-water bath and place in the cooling bath.



14. Press: **START TIMER 2**. Cool for three minutes.

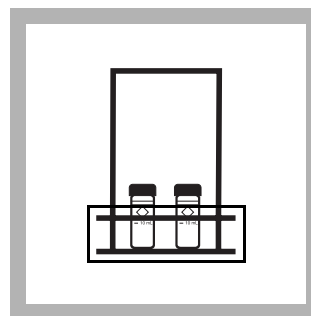
At the end of the cooling period, remove the cell from the cooling bath.



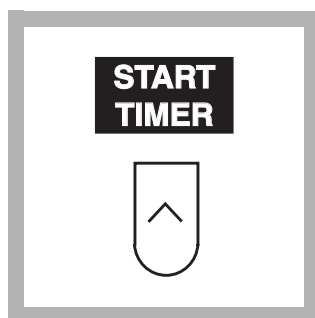
15. Use the Repipet Jr. to add 1 mL of THM Plus Reagent 3 to the sample cell and to the blank. Swirl to mix.

Note: The sample and blank will become warm.

Note: The liquid is viscous and may not be entirely dispensed if measured using any other pipetting method.



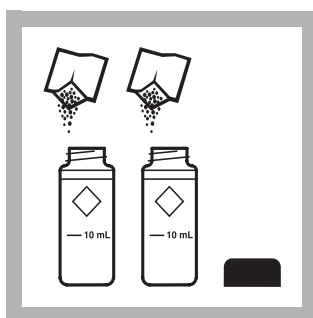
16. Replace the cooling water with fresh, cold tap water. Place the basket containing the sample and blank cells into the cooling bath.



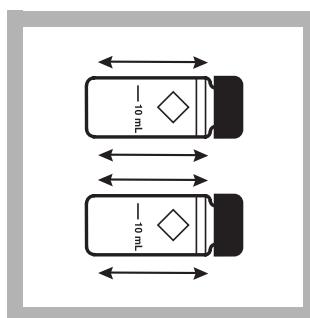
17. Press: **START TIMER 3** to begin a three-minute cooling time.

At the end of the cooling period, remove the cells from the cooling bath.

Note: At the end of the cooling time, the temperature of the sample should be between 15 and 25 °C .

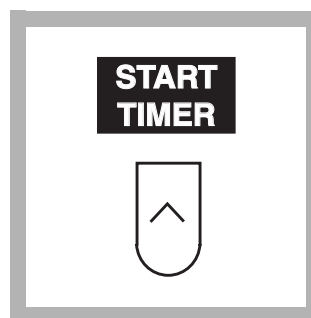


18. Add one THM Plus Reagent 4 Powder Pillow each to the sample cell and to the blank.

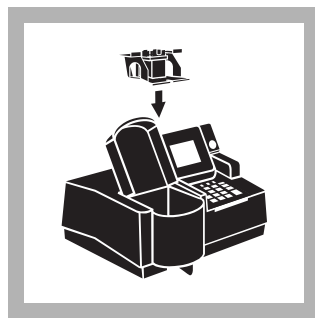


19. Cap each cell tightly and mix by shaking ten times.

Note: All the powder should dissolve.



20. Press **START TIMER 4** to begin a 15-minute color development time.



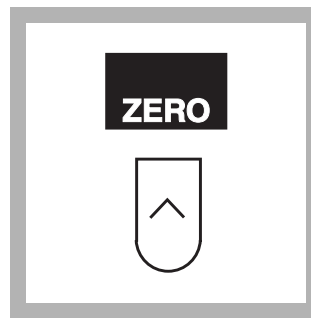
21. While the color is developing, insert the AccuVac® Ampul Adapter into instrument.



22. Wipe the reagent blank with a damp towel, followed by a dry one, to remove fingerprints or other marks.



23. At the end of the 15 minutes, place the blank into the cell holder and close the light shield.



24. Press the soft key under **ZERO**.

The display will show:

0 ppb CHCl₃

Note: For alternate concentration units, press the soft key under **OPTIONS**, and then the soft key under **UNITS**. Scroll through the available options. Press **ENTER** to return to the Read screen.



25. Wipe the sample cell with a damp towel, followed by a dry one, to remove fingerprints or other marks.



26. Place the prepared sample into the cell holder. Close the lid. Results will be displayed in ppb chloroform.

Sampling and Storage

Collect samples in 40-mL glass bottles sealed with Teflon®-lined septa caps. Use Cat. No. 27940-05 or equivalent for best results. Fill the bottles slowly to overflowing so that no air is included with the sample. Seal the bottles tightly and invert to check that no air has been trapped.

Because trihalomethane compounds (THMs) are extremely volatile, immediate analysis yields the greatest accuracy. If the samples cannot be analyzed immediately, cool samples to 4 °C. This will slow the formation of any additional THM compounds in chlorinated samples. Store the preserved samples at 4 °C in an atmosphere free of organic vapors. Samples should not be held more than 14 days. Allow the samples to equilibrate to 15–20 °C before analyzing.

Accuracy Check

Standard Additions Method

Prepare the standard additions sample at the same time as the unspiked water sample. Snap the neck off a THM Standard Ampule, 10 ppm as chloroform. Using a Wiretrol™ Pipet (Cat. No. 25689-05), add 0.050 mL of the standard to 10 mL of water sample. Immerse the tip of the pipet below the surface of the water sample and dispense the aliquot of chloroform standard. Cap the sample cell immediately and swirl three times to mix. Prepare the sample and the spiked sample according to the procedure *steps 6–26*.

- a. Leave the unspiked sample in the sample compartment after completing *step 26*. Verify that the units displayed are in ppb. Select standard additions mode by pressing the soft keys under **OPTIONS, (MORE)** and the **STD ADD**.
- b. Press **ENTER** to accept the default sample volume (mL), 10.0.
- c. Use the keypad to enter **10000**, then press **ENTER** to accept the standard concentration (ppb) 10,000.
- d. Press the soft key under **ENTRY DONE**.

- e. Read the standard additions sample prepared above. Accept the standard additions reading by pressing the soft key under **READ**. The addition should reflect 80–120% recovery.
- f. See Section 1.4.1 *Standard Additions* for more information.



Chloroform is extremely volatile! Do not shake it when mixing.

Standard Solutions Method

Prepare a 99 ppb chloroform standard by pipetting 10.0 mL of organic-free water into a sample cell. Snap the neck off a THM Standard Ampule, 10 ppm as chloroform. Using a Wiretrol Pipette (Cat. No. 25689-05), transfer 0.100 mL of the chloroform standard into the organic-free water. Immerse the end of the pipet tip under the water to dispense the chloroform. Cap the sample cell immediately and swirl three times to mix. Immediately perform *steps 6–25* of the procedure. Do not make up the standard in advance. Use the standard immediately upon preparation.

Method Performance

Precision

Standard: 60 ppb CHCl_3

Program	95% Confidence Limits
3560	56–64 ppm CHCl_3

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
3560	6 ppb CHCl_3

For more information on derivation and use of Hach's estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, appendix B, see Section 1.5.1.

Sensitivity

Program Number: 3560

Portion of Curve	ΔAbs	$\Delta\text{Concentration}$
Entire Range	0.010	21 ppb as CHCl_3

See Section 1.5.3, *Sensitivity Explained* for more information.

Sample Cell Matching

The THM Plus method requires that the 1" sample cells be optically matched for best performance. Although sample cells supplied by Hach Company are distortion-free, nicks and scratches from handling, fingerprints, and other foreign material on the glass surfaces may cause an optical mismatch between two sample cells and introduce error into the test results. This type of error may be avoided by optically matching the sample cells and following the cell precaution statements listed in the procedure.

Procedure:

1. Turn on your instrument and select the THM Plus method. Select the wavelength indicated in the procedure if your instrument has not automatically done so.
2. Change the instrument to the absorbance mode.
3. Pour at least 10 mL of deionized water into each of the samples cells to be matched.
4. Place one of the sample cells into the cell holder. Note and mark the orientation of the cell in the cell holder. Close the light shield. (Sample cells should be carefully wiped with a lint free cloth to remove any fingerprints or other foreign matter on the outside of the cell.)
5. Press: **ZERO**. The display will show: **0.000 Abs**
6. Place the next sample cell into the cell holder. Close the light shield.
7. Wait for the absorbance value to stabilize and record the value.
8. Turn the cell 180 degrees and repeat *steps 6–7*. Try to achieve an absorbance value within ± 0.001 Abs of the first cell. Note the orientation of the sample cell in the cell holder. This will allow the cells to be oriented consistently in the cell holder.

Reagent Storage

Refrigerate THM Plus Reagent 2 for maximum stability. Long-term exposure to temperatures above 35 °C may cause reagent degradation.

Interferences

The substances in the following table have been tested and found not to interfere up to the indicated levels (in ppm):

Interferences which have no effect up to the maximum level tested

Interference	Maximum Level tested
Chlorine	<10 ppm
Copper	<1000 ppm
Hardness, Ca	<1000 ppm as CaCO ₃ May have some turbidity until Reagent 3 is added
Hardness, Mg	<4000 ppm as CaCO ₃ May have some turbidity until Reagent 3 is added
Iron	<10 ppm
Lead	<2 ppm
Mercury	<10 ppm
Monochloramine	<20 ppm
Nickel	<10ppm
Sodium Bisulfite	<100 ppm
EDTA	Interferes negatively at all levels

Additional disinfection by-products which react

Compound	Effect
1,1,1-trichloro-2-propanone	Interferes positively
1,1,1-trichloroacetonitrile	Interferes positively
Chloral hydrate	Interferes positively
Dibromochloroacetic acid	Interferes positively
Dichlorobromoacetic acid	Interferes positively
Tribromoacetic acid	Interferes positively
Trichloroacetic acid	Interferes positively

Summary of Method

The THM Plus method reacts with the trihalogenated disinfection by-products formed as the result of the disinfection of drinking water with chlorine in the presence of naturally occurring organic materials. These disinfection by-products (DBPs) may be produced in the treatment plant or the distribution system as long as the water is in contact with free chlorine residual. The formation of the DBPs is influenced by chlorine contact time, chlorine dose and residual, temperature, pH, precursor concentration, and bromide concentration.

The predominant DBPs formed by the chlorination of drinking water are the trihalomethanes or THMs. The four trihalogenated compounds that form are chloroform, bromoform, dichlorobromomethane, and dibromochloromethane. These four compounds comprise the Total Trihalomethanes (TTHMs) group which is regulated under the Safe Drinking Water Act. The combined concentration of the TTHMs, reported as chloroform, is regulated to be 100 ppb or less in drinking water samples. Other DBPs that may be present and react under the conditions of the THM Plus method are listed in Interferences.

In the THM Plus method, THM compounds present in the sample react with N, N,-diethylnicotinamide under heated alkaline conditions to form a dialdehyde intermediate. The sample is then cooled and acidified to pH 2.5. The dialdehyde intermediate formed is then reacted with 7-naphthylamine-1,3 disulfonic acid to form a colored Schiff base which absorbs at 515 nm. The color formed is directly

TRIHALOMETHANES, continued

proportional to the total amount of THM compounds present in the sample. The results are reported as ppb chloroform.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used.

REQUIRED REAGENTS

Reagent Set (50 tests).....27908-00
Includes: (1) 27539-29, (1) 27540-48, (1) 27541-42, (1) 27566-99

Description	Quantity Required		Cat. No.
	Per Test	Unit	
THM Plus Reagent 1	6 drops	15 mL/bottle.....	27539-29
THM Plus Reagent 2	6 mL	330 mL/bottle.....	27540-48
THM Plus Reagent 3	2 mL	110 mL/bottle.....	27541-42
THM Plus Reagent 4	2 pillows	100 pillows.....	27566-99

REQUIRED APPARATUS

Beaker, 600-mL	each.....	500-52
Cell Holder Assembly, TTHM	1	each.....47880-00
Evaporating Dish, 125 mm x 65 mm	2	each.....27647-00
Hot Plate, 7 x 7 in., 120 VAC	1	each.....23441-00
Hot Plate, 7 x 7 in., 240 VAC	1	each.....23441-02
Repipetter, 1 mL.....	1	each.....21113-02
Pipet, Tensette, 1–10 mL.....	1	each.....19700-10
Pipet tips, 1–10 mL (for 19700-10).....	50/pkg.....	25589-96
Sample cells, 10-mL, w/caps.....	2	6/box.....24276-06
Wipers, Disposable, KimWipes	280/pkg.....	20970-00

OPTIONAL REAGENTS

Chloroform, 10-ppm ampoule.....	each.....	27567-07
Water, Reagent, Organic-free	500 mL.....	26415-49

OPTIONAL APPARATUS

Flask, volumetric, 100 mL, class A.....	each.....	14574-42
Pipet, filler, safety bulb	each.....	14651-00
Pipet, volumetric, class A, 10 mL	each.....	14515-38
Pipettes, Wiretrol™, 50–100 µL	250/pkg.....	25689-05
Timer, 5-channel, 1 sec. to 100 hrs.	each.....	26304-00
Vials, glass, 40-mL, with Septa cap	5/pkg.....	27940-05



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HACH COMPANY
WORLD HEADQUARTERS
Telephone: (970) 669-3050
FAX: (970) 669-2932



Method 10140

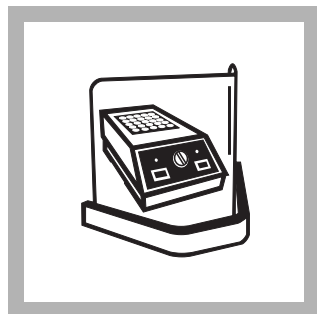
THM Reactor Method*

Powder Pillows

(0–200 ppb as Chloroform)

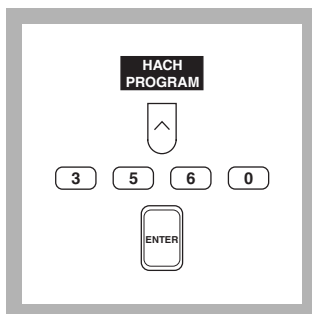
Scope and Application: For drinking water.

* Patent Pending



1. Place the reactor in a fume hood and place a plastic shield in front of the reactor.

Turn on the COD Reactor. Preheat to 100 °C.



2. Press the soft key under **HACH PROGRAM**. Select the stored program number for Trihalomethane (THM) Plus by pressing **3560** on the numeric keys.

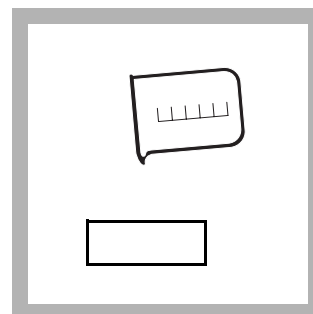
Press **ENTER**.

Note: For the most precise results, use matched cells. See Sample Cell Matching on page 5.



3. The display will show:
**HACH PROGRAM:
3560 THM Plus**

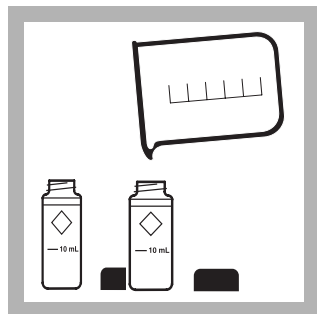
The wavelength (λ), 515 nm, is automatically selected.



4. Prepare a cooling bath by adding 500 mL of cold (18–25 °C) tap water to an evaporating dish.

Note: Maintain the water temperature between 18 and 25 °C.

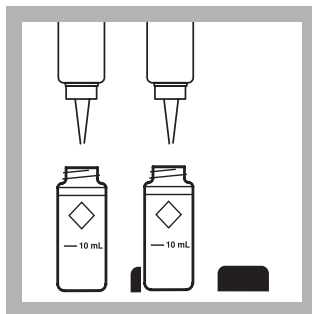
Note: If analyzing more than four samples, use 450 mL of water.



5. Fill two sample cells to the 10 mL mark with sample. Label one **sample** and the other **blank**.

Note: Perform steps 5 through 9 **rapidly** so as not to lose volatile THMs from the sample. If you are testing more than one sample, complete steps 5 through 9 for one sample before going on to the next.

Note: If dispensing sample with a pipette, the pipette must dispense quickly without causing aeration or back pressure.

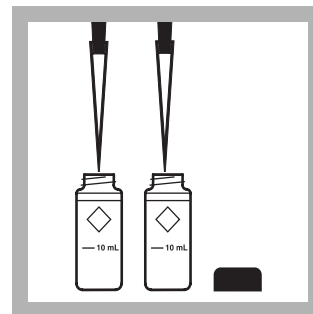


6. Add three drops of THM Plus Reagent 1 to each cell.



7. Cap tightly and mix gently by swirling each cell three times.

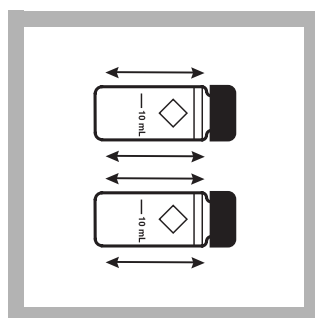
Note: Vigorous shaking can cause loss of THMs.



8. Use a TenSette® pipette to add 3 mL of THM Plus Reagent 2 to each cell.

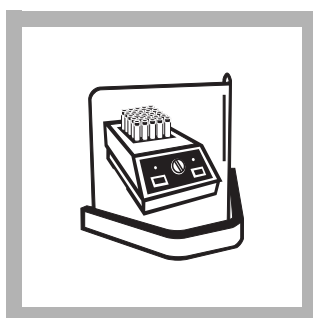
Note: The liquid is viscous and a small amount may remain in the tip after dispensing. This will not affect the results.

Note: The THM Plus Reagent 2 must be at room temperature before use.

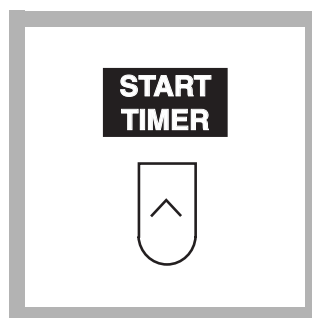


9. Cap tightly and mix by shaking ten times.

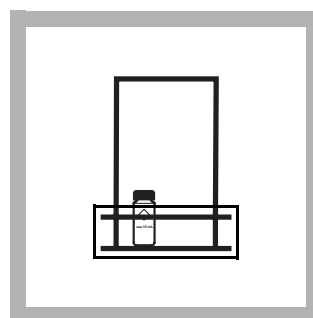
Note: Thorough mixing ensures that all of the THM goes into the liquid and does not accumulate in the head space.



10. Place the sample cell in the THM reactor at 100 °C. Set the blank aside.



11. Press **800 START TIMER** to begin an eight-minute reaction period.

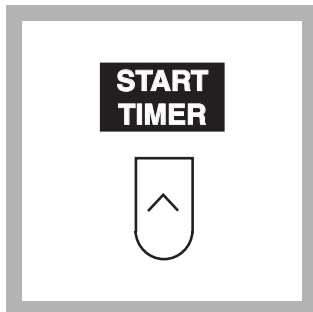


12. At the end of the reaction period, remove the cell from the reactor and place in the cell holder assembly. Place the assembly in a cooling bath.

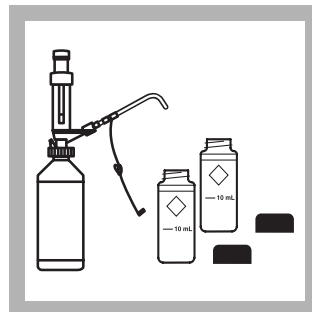


13. Press: **NEXT TIMER** twice.

Note: Pressing **NEXT TIMER** twice skips Timer 1, which is used for a water bath digestion.



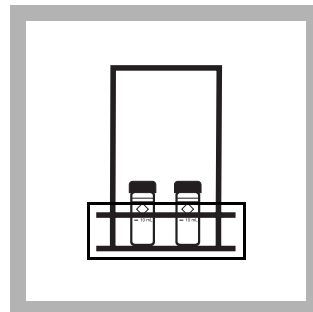
14. Press: **START TIMER 2**. Cool for three minutes. At the end of the cooling period, remove the cell from the cooling bath.



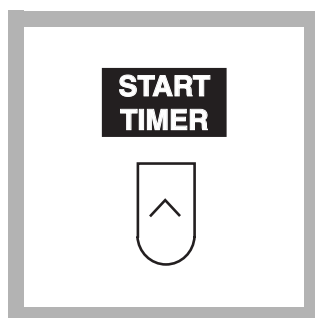
15. Use the Repipet Jr. to add 1 mL of THM Plus Reagent 3 to the sample cell and to the blank. Swirl to mix.

Note: The sample will become warm

Note: The liquid is viscous and may not be entirely dispensed if measured using any other pipetting method.



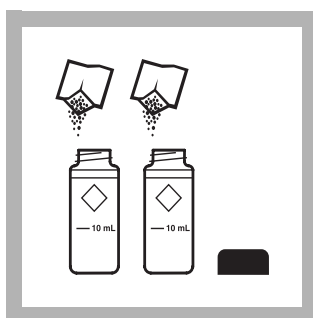
16. Replace the cooling water with fresh, cold tap water. Place the assembly containing the sample and blank cells into the cooling bath.



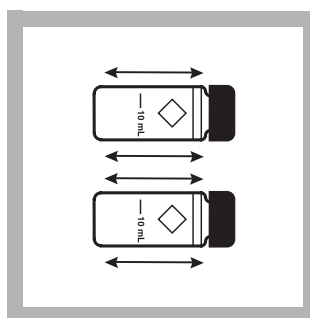
17. Press: **START TIMER 3** to begin a three-minute cooling time.

At the end of the cooling period, remove the cells from the cooling bath.

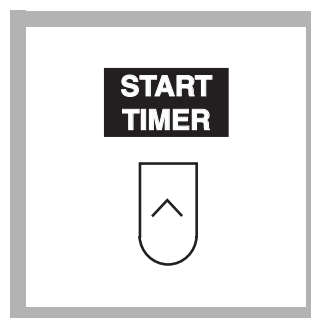
Note: At the end of the cooling time, the sample temperature should be between 15 and 25 °C.



18. Add one THM Plus Reagent 4 Powder Pillow each to the sample cell and to the blank.



19. Cap each cell tightly and shake to dissolve.



20. Press **START TIMER 4** to begin a 15-minute color development time.



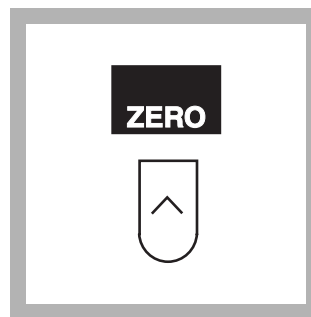
21. While the color is developing, insert the AccuVac® Ampul Adapter into instrument.



22. Wipe the reagent blank with a damp towel, followed by a dry one, to remove fingerprints or other marks.



23. At the end of the 15 minutes, place the blank into the cell holder and close the light shield.



24. Press the soft key under **ZERO**.

The display will show:

0 ppb CHCl₃

Note: For alternate concentration units, press the soft key under **OPTIONS**, and then the soft key under **UNITS**. Scroll through the available options. Press **ENTER** to return to the Read screen.



25. Wipe the sample cell with a damp towel, followed by a dry one, to remove fingerprints or other marks.



26. Place the prepared sample into the cell holder. Close the lid. Results will be displayed in ppb chloroform.

Sampling and Storage

Collect samples in 40-mL glass bottles sealed with Teflon®-lined septa caps. Use Cat. No. 27940-05 or equivalent for best results. Fill the bottles slowly to overflowing so that no air is included with the sample. Seal the bottles tightly and invert to check that no air has been trapped.

Because trihalomethane compounds (THMs) are extremely volatile, immediate analysis yields the greatest accuracy. If the samples cannot be analyzed immediately, cool samples to 4 °C. This will slow the formation of any additional THM compounds in chlorinated samples. Store the preserved samples at 4 °C in an atmosphere free of organic vapors. Samples should not be held more than 48 hours. Allow the samples to equilibrate to room temperature before analyzing.

Ascorbic acid cannot be used as a preservative with the THM Plus method. Sodium Thiosulfate may be used as a preservative in samples containing hardness of 100 mg/L or less as CaCO₃.

Accuracy Check

Standard Additions Method

Prepare the standard additions sample at the same time as the unspiked water sample. Snap the neck off a THM Standard Ampule, 10 ppm as chloroform. Using a Wiretrol™ Pipet (Cat. No. 25689-05), add 0.050 mL of the standard to 10 mL of water sample. Immerse the tip of the pipet below the surface of the water sample and dispense the aliquot of chloroform standard. Cap the sample cell immediately and swirl three times to mix. Prepare the sample and the spiked sample according to the procedure steps 6–26.

- a. Leave the unspiked sample in the sample compartment after completing step 26. Verify that the units displayed are in ppb. Select standard additions mode by pressing the soft keys under **OPTIONS, (MORE)** and the **STD ADD**.
- b. Press **ENTER** to accept the default sample volume (mL), 10.0.
- c. Use the keypad to enter **10000**. Press **ENTER**.
- d. Press the soft key under **ENTRY DONE**.

- e. Read the standard additions sample prepared above. Accept the standard additions reading by pressing the soft key under **READ**. The addition should reflect 80–120% recovery. To view % Recovery, press the soft key under **EDIT TABLE**.

See the *Procedures Manual* for more information.

Standard Solutions Method



Chloroform is extremely volatile! Do not shake it when mixing.

Prepare a 99 ppb chloroform standard by pipetting 10.0 mL of organic-free water into a sample cell. Snap the neck off a THM Standard Ampule, 10 ppm as chloroform. Using a Wiretrol Pipette (Cat. No. 25689-05), transfer 0.100 mL of the chloroform standard into the organic-free water. Immerse the end of the pipet tip under the water to dispense the chloroform. Cap the sample cell immediately and swirl three times to mix. Immediately perform steps 6–25 of the procedure. Do not make up the standard in advance. Use the standard immediately upon preparation.

Method Performance

Precision

Standard: 60 ppb CHCl₃

Program	95% Confidence Limits
3560	56–64 ppm CHCl ₃

For more information on determining precision data and method detection limits, refer to the *Procedures Manual*.

Estimated Detection Limit

Program	EDL
3560	6 ppb CHCl ₃

For more information on the derivation and use of Hach's estimated detection limit, see the *Procedures Manual*. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, appendix B, see the *Procedures Manual*.

Sensitivity

Portion of Curve	ΔAbs	ΔConcentration
Entire Range	0.010	21 ppb as CHCl ₃

See the *Procedures Manual* for more information.

Sample Cell Matching

The THM Plus method requires that the 1" sample cells be optically matched for best performance. Although sample cells supplied by Hach Company are distortion-free, nicks and scratches from handling, fingerprints, and other foreign material on the glass surfaces may cause an optical mismatch between two sample cells and introduce error into the test results. This type of error may be avoided by optically matching the sample cells and following the cell precaution statements listed in the procedure.

Procedure:

1. Turn on your instrument and select the THM Plus method. Select the wavelength indicated in the procedure if your instrument has not automatically done so.
2. Change the instrument to the absorbance mode.
3. Pour at least 10 mL of deionized water into each of the samples cells to be matched.
4. Place one of the sample cells into the cell holder. Note and mark the orientation of the cell in the cell holder. Close the light shield. (Sample cells should be carefully wiped with a lint free cloth to remove any fingerprints or other foreign matter on the outside of the cell.)
5. Press: **ZERO**. The display will show: **0.000 Abs**
6. Place the next sample cell into the cell holder. Close the light shield.
7. Wait for the absorbance value to stabilize. Record the value.
8. Turn the cell 180 degrees and repeat *steps 6–7*. Try to achieve an absorbance value within ± 0.001 Abs of the first cell. Note the orientation of the sample cell in the cell holder. This will allow the cells to be oriented consistently in the cell holder.

Reagent Storage

Refrigerate THM Plus Reagent 2 for maximum stability. Long-term exposure to temperatures above 35 °C may cause reagent degradation.

Interferences

The substances in the following table have been tested and found not to interfere up to the indicated levels (in ppm):

Interferences that have no effect up to the maximum level tested

Interference	Maximum Level tested
Chlorine	<10 ppm
Copper	<1000 ppm
Hardness, Ca	<1000 ppm as CaCO ₃ May have some turbidity until Reagent 3 is added
Hardness, Mg	<4000 ppm as CaCO ₃ May have some turbidity until Reagent 3 is added
Iron	<10 ppm
Lead	<2 ppm
Mercury	<10 ppm
Monochloramine	<20 ppm
Nickel	<10ppm
Sodium Bisulfite	<100 ppm
EDTA	Interferes negatively at all levels

Additional disinfection by-products that react

Compound	Effect
1,1,1-trichloro-2-propanone	Interferes positively
1,1,1-trichloroacetonitrile	Interferes positively
Chloral hydrate	Interferes positively
Dibromochloroacetic acid	Interferes positively
Dichlorobromoacetic acid	Interferes positively
Tribromoacetic acid	Interferes positively
Trichloroacetic acid	Interferes positively

Summary of Method

The THM Plus method reacts with the trihalogenated disinfection by-products formed as the result of the disinfection of drinking water with chlorine in the presence of naturally occurring organic materials. These disinfection by-products (DBPs) may be produced in the treatment plant or the distribution system as long as the water is in contact with free chlorine residual. The formation of the DBPs is influenced by chlorine contact time, chlorine dose and residual, temperature, pH, precursor concentration, and bromide concentration.

The predominant DBPs formed by the chlorination of drinking water are the trihalomethanes or THMs. The four trihalogenated compounds that form are chloroform, bromoform, dichlorobromomethane, and dibromochloromethane. These four compounds comprise the Total Trihalomethanes (TTHMs) group which is regulated under the Safe Drinking Water Act. The combined concentration of the TTHMs, reported as chloroform, is regulated to be 100 ppb or less in drinking water samples. Other DBPs that may be present and react under the conditions of the THM Plus method are listed in Interferences.

In the THM Plus method, THM compounds present in the sample react with N, N,-diethylnicotinamide under heated alkaline conditions to form a dialdehyde intermediate. The sample is then cooled and acidified to pH 2.5. The dialdehyde intermediate formed is then reacted with 7-naphthylamine-1,3 disulfonic acid to form a colored Schiff base which absorbs at 515 nm. The color formed is directly proportional to the total amount of THM compounds present in the sample. The results are reported as ppb chloroform.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the Material Safety Data Sheet for information specific to the reagents used.

THM Plus™ TRIHALOMETHANES, continued

REQUIRED REAGENTS

Reagent Set (50 tests)	27908-00
Includes: (1) 27539-29, (1) 27540-48, (1) 27541-42, (1) 27566-99	

Description	Quantity Required		Cat. No.
	Per Test	Unit	
THM Plus Reagent 1	6 drops	30 mL/bottle	27539-29
THM Plus Reagent 2	6 mL	330 mL/bottle	27540-48
THM Plus Reagent 3	2 mL	100 mL/bottle	27541-42
THM Plus Reagent 4	2 pillows	100 pillows	27566-99

REQUIRED APPARATUS

Beaker, 600-mL	each	500-52
Cell Holder Assembly, TTHM	1 each	47880-00
Evaporating Dish, 125 mm x 65 mm	1 each	27647-00
Repipet Jr., 1-mL	1 each	21113-02
Pipet, Tensette, 1–10 mL	1 each	19700-10
Pipet tips, 1–10 mL (for 19700-10)	50/pkg	25589-96
Sample cells, 10 mL, w/caps	2 6/box	24276-06
THM Reactor, Model 49100, 115 VAC	each	49100-00
THM Reactor, Model 49100, 230 VAC	each	49100-02
Wipers, Disposable, KimWipes	280/pkg	20970-00

OPTIONAL REAGENTS

THM Standard Ampules, 10-ppm as Chloroform	7/pkg	27567-07
Water, Reagent, Organic-free	500 mL	26415-49

OPTIONAL APPARATUS

Flask, volumetric, 100-mL, class A	each	14574-42
Pipet, filler, safety bulb	each	14651-00
Pipet, volumetric, class A, 10-mL	each	14515-38
Pipettes, Wiretrol™, 50–100 µL	250/pkg	25689-05
Vials, glass, 40-mL, with Septa cap	5/pkg	27940-05



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FAX: (970) 669-2932



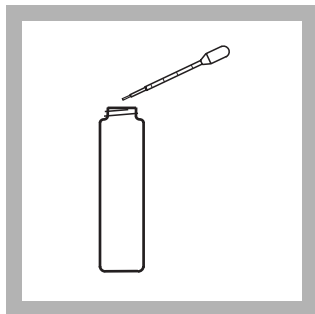
Method 10017

ToxTrak Method

(0 to 100 % Inhibition)

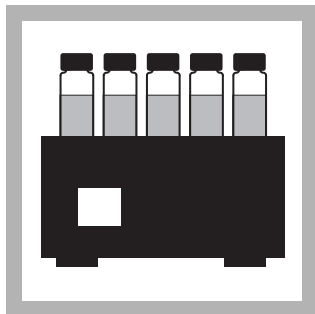
Scope and Application: For water and wastewater

Inoculum Development Using Indigenous Biomass



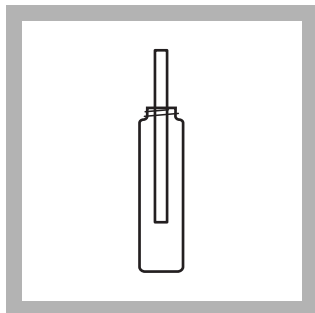
Using Indigenous Biomass

1. Using one of the dropper pipets provided, add 1.0 mL of source culture to a Tryptic Soy Broth Tube.

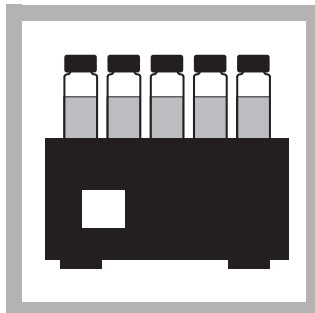


2. Incubate until the vial contents are visibly turbid (turbidity indicates bacterial growth).

Inoculum Development Using Aqua QC-Stiks™

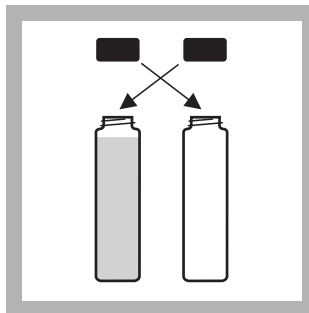


1. Inoculate a Lauryl Tryptose broth tube with an *E. coli* Aqua QC-Stik according to the instructions that come with the stick.



2. Incubate the Lauryl Tryptose Broth Tube until the medium is visibly turbid. Turbidity will develop much faster if incubation is done at 35 °C instead of room temperature. At 35 °C, 12 hours is usually sufficient.

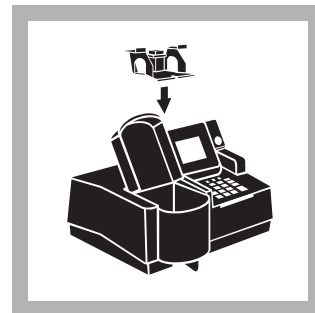
Note: If toxicity tests will be run on consecutive days, inoculum may be kept several days at room temperature.



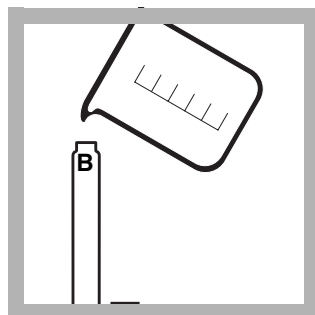
3. Inoculate a new Lauryl Tryptose Broth Tube by first inverting the tube and switching the caps of the two tubes. Then invert the new tube. After incubation, this new vial may be used in subsequent tests.

Note: In this way, several medium vials may be inoculated from a single initial tube.

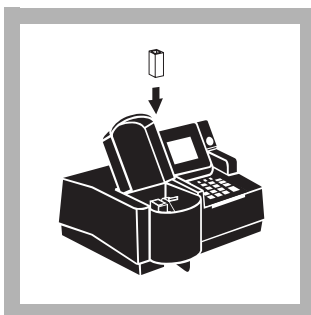
Note: Cultures 10 to 72 hours old give best results.



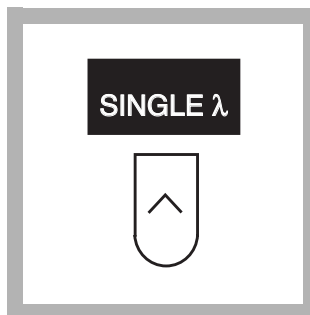
4. Insert the COD sample cell adapter into the sample cell compartment.



5. Fill a Test 'N' Tube sample cell with deionized water. This is the blank.



6. Place the blank into the cell adapter. Close the light shield.



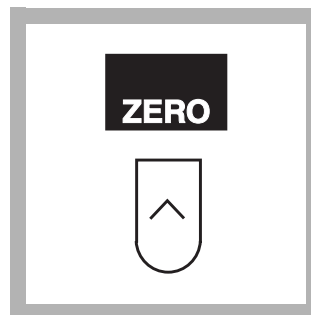
7. Press the soft key under **SINGLE λ**.

Press the soft key under **GO TO λ**.

Select 603 nm by pressing the numeric keys **6 0 3**.

Press: **ENTER**

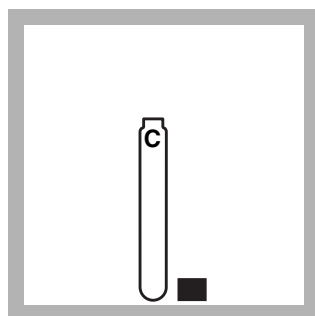
Note: The Flow Cell and Sipper Modules cannot be used with this procedure.



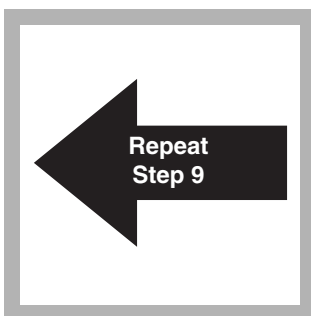
8. Press the soft key under **ZERO**.

The display will show:

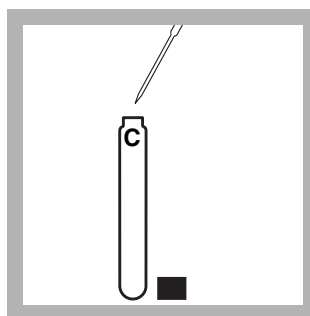
0.000 ABS



9. Label a cell "Control." Open one ToxTrak Reagent Powder Pillow and add the contents to the empty reaction cell.

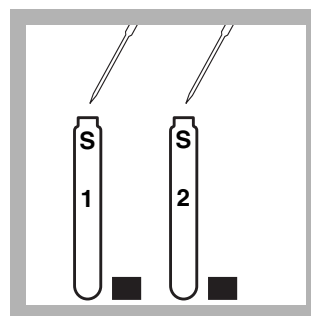


10. For each sample or dilution, repeat Step 9. Label each cell clearly.



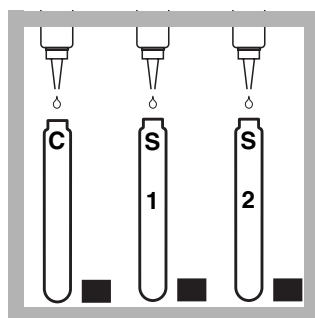
11. Add 5.0 mL of deionized water to the Control cell.

Note: For a Control cell, use deionized water that is free of toxicity, or use another water source that represents baseline toxicity.



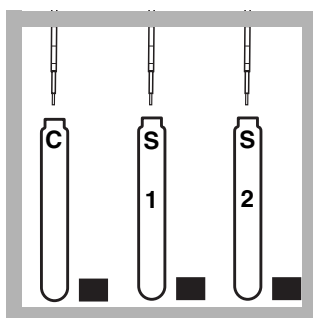
12. Add 5.0 mL of sample (or dilutions) to each sample cell.

Note: To determine the approximate threshold level of toxicity for a sample, dilute 1 mL of sample to 10 mL with deionized water and run the test. Continue to make serial 1/10 dilutions of the sample until a level is reached which gives 0% Inhibition in final calculation.

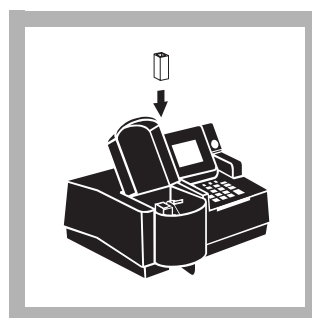


13. Add 2 drops of Accelerator Solution to each tube. Cap and shake to mix.

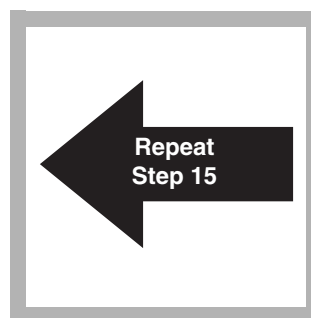
Note: Shaking serves to fully oxygenate the samples, assuring that oxygen concentration is not a factor in determining respiration rate.



14. Add 0.5 mL of inoculum (previously prepared) to each tube. Cap and invert to mix.



15. Place the Control into the adapter. Close the light shield. Record the absorbance.

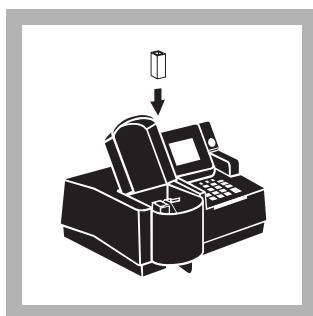


16. Repeat Step 15 for all samples and dilutions. Be sure to record each absorbance.

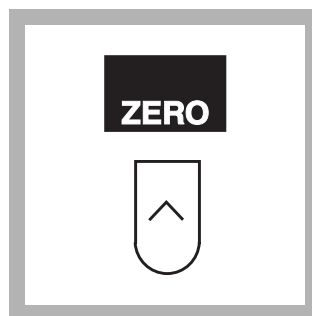


17. Allow the solutions in the tubes to react until the absorbance of the Control decreases 0.60 ± 0.10 abs. This takes 45–75 minutes. Invert occasionally.

Note: Reaction time varies according to temperature, age of the culture, bacteria concentrations, etc.

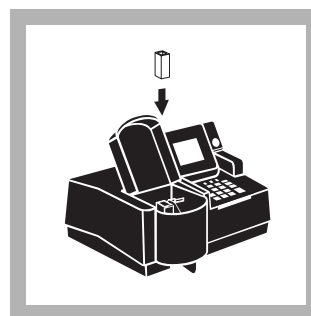


18. After the absorbance of the Control has decreased to 0.60 ± 0.10 abs., place the Blank into the adapter and close the light shield.

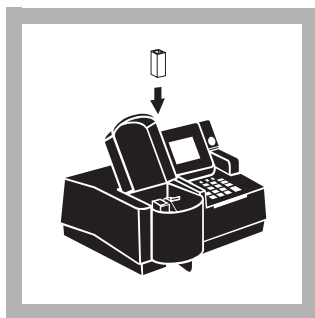


19. Press the soft key under **ZERO**.

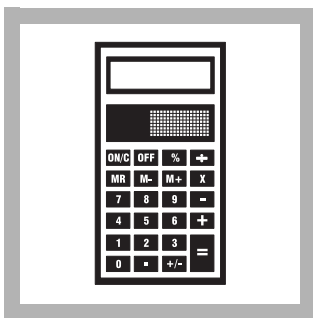
The display will show:
0.000 ABS



20. Place the Control into the adapter and close the light shield. Record the absorbance value of the Control.



21. Place each sample or dilution into the adapter and close the light shield. Record each absorbance value.



22. Calculate the % Inhibition as follows:

$$\%I = [1 - \Delta A_{\text{sample}} \div \Delta A_{\text{control}}] \times 100$$

Where

ΔA = Initial absorbance value – Final absorbance value

Note: Some toxins increase respiration and will give a negative % Inhibition on all respiration-based toxicity tests. After repeated testing, samples that give a % Inhibition in step 18 that is more negative than -10% should be considered toxic.

Interpreting Results

The % Inhibition results obtained are only a relative measurement. They do not represent a true quantitative measurement of toxic concentration. The % Inhibition does not necessarily increase in direct proportion to the concentration of toxins. To determine the minimum inhibition concentration of a toxin, it is possible to make tenfold dilutions of the sample and determine the % Inhibition for the dilutions until the sample is diluted sufficiently so that no inhibition is observed. This is the No Observed Effect Concentration (NOEC).

Due to the many variables involved in the test, the limits of detection are on the order of 10% Inhibition. This would correlate to the Lowest Observed Effect Concentration (LOEC). If a sample shows less than 10% Inhibition, repeat the test. After several repetitions, look at the series of data to determine the likelihood of toxicity. Results below 10% are not reliable, but can be used to surmise some presence of toxicity if they are consistent. See examples below:

Data Points: % Inhibition	Conclusion
7%, 9%, 5%, 8%, 5%	May be slightly toxic
7%, -4%, -5%, 5%, 1%	Most likely not toxic
-7%, -9%, -5%, -8%, -5%	May be slightly toxic

Some toxins will increase respiration and will give a negative % Inhibition on this and all other respiration-based toxicity tests. After repeated testing, samples that always give a % Inhibition that is more negative than -10% should be considered toxic.

Disposal of Test Cultures

Dispose of active bacterial cultures grown during incubation by using one of these methods:

1. Autoclave used test containers at 121 °C for 15 minutes at 15 pounds of pressure. Once the containers are sterile, pour the contents down the drain with running water. The reaction tubes may be washed and re-used.
2. Sterilize test containers by using a 1:10 dilution of commercial laundry bleach. Pour the test container contents and test containers into the bleach solution. Allow 10–15 minutes of contact time with the bleach solution. Then pour the liquid down the drain and wash the reaction tubes for reuse.

Summary of Method

This method is based on the reduction of resazurin, a redox-active dye, by bacterial respiration. When it is reduced, resazurin changes color from blue to pink. Toxic substances can inhibit the rate of resazurin reduction. A chemical accelerant has been added to shorten the reaction time.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to *Section 1*.

Pollution Prevention and Waste Management

For information on pollution prevention and waste management, refer to *Section 1*.

TOXICITY, continued

REQUIRED REAGENTS AND STANDARDS

	Cat. No.
ToxTrak Reagent Set (25 tests)	25972-00
Includes all consumable reagents and apparatus used in the test	

Description	Quantity Required		Unit	Cat. No.
	per test			
ToxTrak Reagent Powder Pillows	2 pillows	50/pkg		25607-66
ToxTrak Accelerator Solution	4 drops	15 mL	SCDB	25608-36
Tryptic Soy Broth Tubes	1	15/pkg		22777-00
Water, deionized	varies	200 mL		272-29
Aqua QC-Stiks, <i>Escherichia coli</i>	1	3 cultures		27063-03

REQUIRED EQUIPMENT AND SUPPLIES

Clippers, to open powder pillows	each	936-00
Dropper, 0.5- and 1.0-mL marks	20/pkg	21247-20
DR/4000 1-cm Adapter, square	each	48584-00
Forceps, flat square tip	each	14537-00
Pipet, volumetric, class A, 5.00 mL	each	14515-37
Pipet Filler, safety bulb	each	14651-00
Sample Cell, 9.5 x 1 x 1 cm, with cap	10/pkg	26275-10

OPTIONAL REAGENTS AND STANDARDS

Culture Set (Bactrol Disks and Lauryl Tryptose Broth Tubes)	25 cultures	25978-00
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OPTIONAL EQUIPMENT AND SUPPLIES

Alcohol Burner, 60-mL	each	20877-60
Bunsen Burner, propane	each	21627-00
Germicidal Cloth	50/pkg	24632-00
Incubator, Dri-Bath, 25-well, 115/230 VAC	each	45900-00
Incubator, Dri-Bath, 25-well, 115/230 VAC, with European power cord	each	45900-02
Test Tube Rack, 13-mm, 6 x 15 rows	each	24979-00



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Method 10050

Immunoassay Method

(10 and 100 ppm TPH thresholds*)

Scope and Application: *Soil and water*

* Test is semi-quantitative. Results are expressed as greater or less than the threshold value used.

This TPH test can be used for both soil and water testing. When testing soil, purchase the necessary items (see *Required Apparatus*) and perform the *Soil Extraction Procedure*. When testing water samples only, proceed directly with *Immunoassay Procedure for Soil Extracts and Water Samples*. The test requires about 20 to 30 minutes for complete analysis. As many as 10 cuvettes can be run simultaneously.

Tips and Techniques

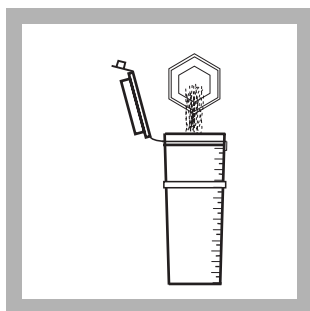
- **Read the entire procedure before starting.** Identify and have ready all the necessary reagents, cuvettes, and other apparatus before beginning the analysis.
- **Timing is critical;** follow instructions carefully.
- **A consistent technique when mixing the cuvettes is critical to this test.** The best results come from using the cuvette rack and mixing as described in *Using the 1-cm MicroCuvette Rack*. Cuvettes can be mixed individually, but test results may not be as consistent.
- Handle the cuvettes carefully. Scratches on the inside or outside may cause erroneous results. Carefully clean the outside of the cuvettes with a clean absorbent cloth or tissue before placing them into the instrument. (Kimwipe® tissues are provided with the kit.)
- Antibody cuvettes and enzyme conjugate are made in matched lots. Do not mix reagent lots.
- Twenty Antibody Cuvettes are provided with each reagent set. One Antibody Cuvette will be used for each calibrator or sample. Antibody Cuvettes are not reusable.
- To avoid damaging the Color Developing Solution, do not expose it to direct sunlight.
- Store the reagents at 4 °C when they are not in use. Allow the reagents to reach room temperature before using them in an analysis. Actual testing may be done at temperatures ranging from 1 °C to 38 °C.
- The Soil Extractant contains methyl alcohol which is poisonous and flammable. Before using this and other reagents, read the Material Safety Data Sheet (MSDS) for proper use of protective equipment and other safety information.

Note: *Hach Company recommends wearing protective nitrile gloves for this procedure.*

Soil Extraction Procedure



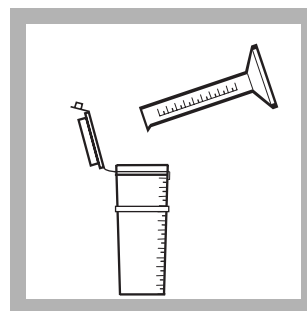
1. Weigh out 10 g of soil in the plastic weighing boat.



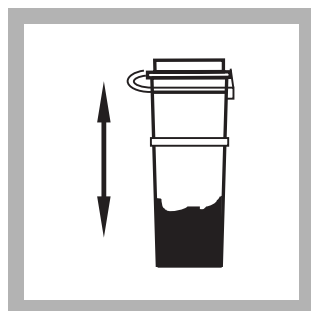
2. Carefully pour the soil into an extraction vial.



3. Use the 5-gram scoop to add one scoop of sodium sulfate to the extraction vial.



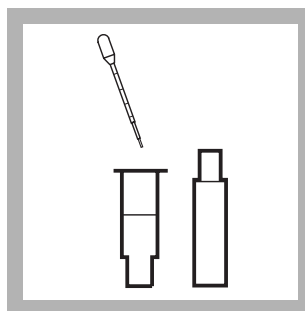
4. Use the graduated cylinder to transfer 10 mL of Soil Extractant into the extraction vial.



5. Cap the extraction vial tightly and shake vigorously for one minute.



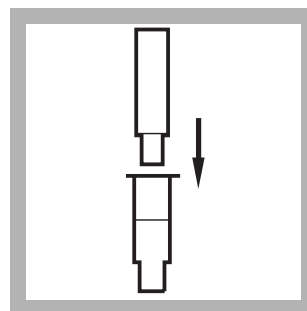
6. Allow to settle for at least one minute. Carefully open the extraction vial.



7. Using the disposable bulb pipet, withdraw 1.0–1.5 mL from the liquid layer at the top of the extraction vial.

Transfer it into the filtration barrel (the bottom part of the filtering assembly into which the plunger inserts).

Note: Do not use more than 1.5 mL. The bulb is marked in 0.25-mL increments

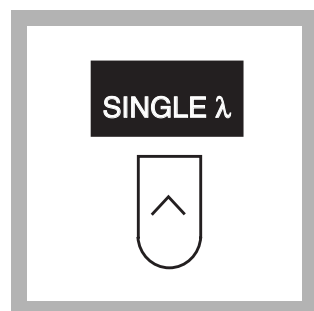


8. Insert the filtration plunger into the filtration barrel. Press firmly on the plunger until the sample extract is forced upward into the center of the plunger.

Use the resultant filtrate for the immunoassay procedure.

Note: It may be necessary to place the filtration assembly on a table and press down on the plunger.

Immunoassay Procedure for Soil Extracts and Water Samples



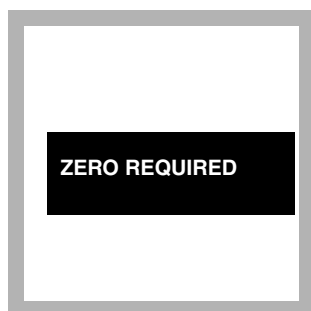
1. Press the soft key under **SINGLE λ**.

Press the soft key under **GO TO λ**.

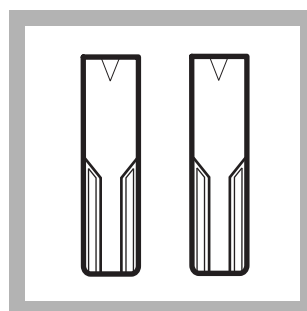
Select 450 nm by pressing the numeric keys **450**.

Press: **ENTER**

Note: The Flow Cell and Sipper Modules cannot be used with this procedure.



2. The display will show:
ZERO REQUIRED

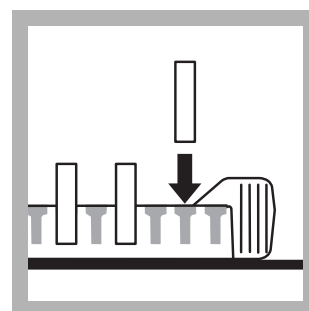


3. Label an Antibody Cuvette for each calibrator and each sample to be tested.

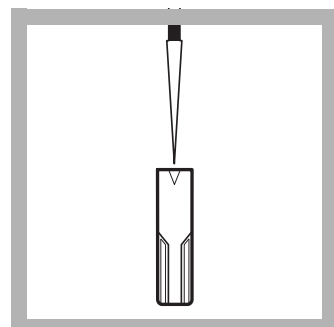
To select the proper calibrators, see *Table 1* or *Table 2*.

Note: As many as 10 cuvettes may be tested at one time and may be any combination of samples and calibrators.

Note: See example below.



4. Place the cuvettes into the rack snugly.

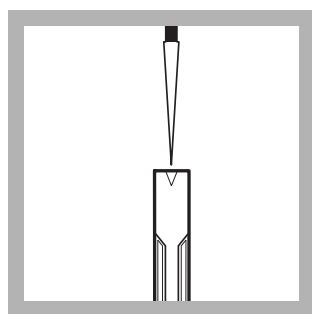


5. Pipet 0.5 mL of *Diluent Solution* into each Calibrator cuvette.

Note: The same pipet tip can be used repeatedly for this step.

EXAMPLE

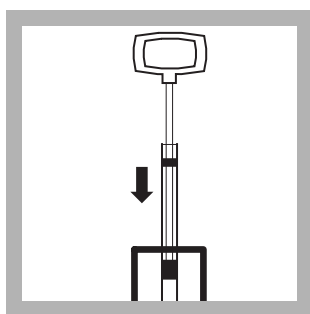
To test samples against the 50 ppm and 100 ppm diesel fuel calibrators, label one Antibody Cuvette “50” and a second cuvette “100.” Then label an Antibody Cuvette for each of up to eight samples to be tested. In this example, there is room for eight samples; samples plus calibrators cannot exceed 10. Using more calibrators will reduce the number of samples that can be run at the same time.



6. If testing soil: Pipet 0.5 mL of *Diluent Solution* into each sample cuvette.

If testing water: Pipet 0.5 mL of each *water sample* into the appropriate cuvette.

Note: Use a new pipet tip for each water sample.

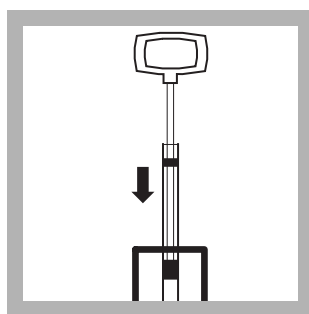


7. Have the necessary apparatus at hand for the next four steps as they must be done without delay.

Use a Wiretrol® pipet to transfer 50 µL of each calibrator to be used into the calibrator cuvettes.

Mix the contents of the cuvettes after each addition.

Note: Use a separate capillary tube for each solution.

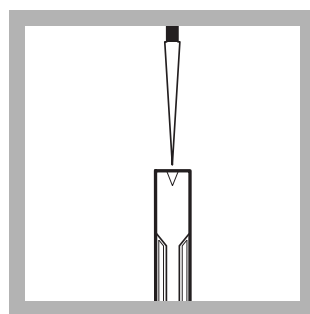


8. If testing soil: Use a Wiretrol pipet to transfer 50 µL of the filtered extract from *step 8* of the *Soil Extraction Procedure* into the appropriately labeled cuvette.

Note: Use a separate capillary tube for each solution.

Mix the contents of the cuvettes after the addition of each sample.

If testing water: Use a Wiretrol pipet to transfer 50 µL of methanol into each sample cuvette



9. Immediately pipet 0.5 mL of TPH Enzyme Conjugate into each calibrator and sample cuvette.

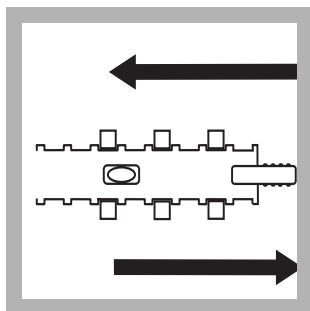
Note: The same pipet tip can be used repeatedly for this step.



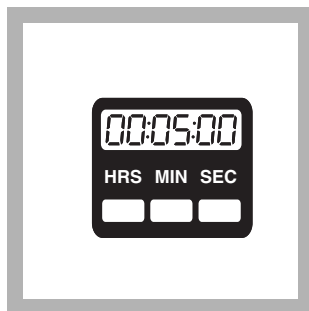
10. Key 1000.

Press the soft key under **START TIMER**.

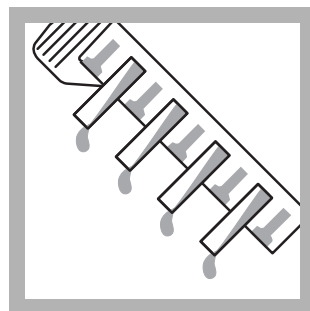
A 10-minute reaction time will begin. Proceed immediately to the next step.



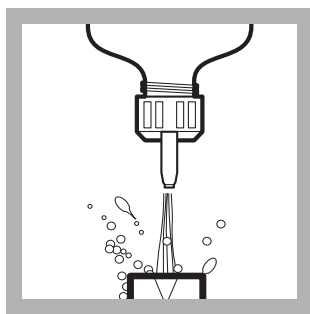
11. Mix the contents of the cuvettes for 30 seconds using the technique described in *Using the 1-cm MicroCuvette Rack*.



12. After 5 minutes, mix the contents of the rack a second time for a period of 30 seconds using the same technique.



13. At the end of the 10-minute period, discard the contents of all the cuvettes into an appropriate waste container.

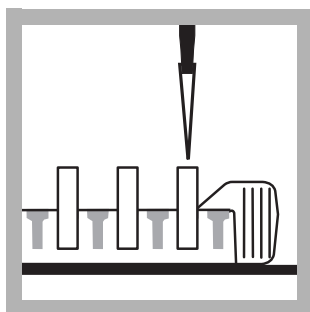


14. Wash each cuvette forcefully and thoroughly four times with deionized water. Empty the rinse water into the waste container.

Note: Make sure that most of the water is drained from the cuvettes by turning the cuvettes upside down and tapping them lightly on a paper towel.

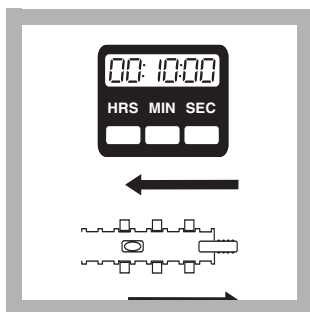
Color Development

Note: Timing is critical; follow instructions carefully



15. With the cuvettes still held snugly in the rack, pipet 0.5 mL of Color Developing Solution into each Antibody Cuvette.

Note: Use a new pipet tip for each cuvette.



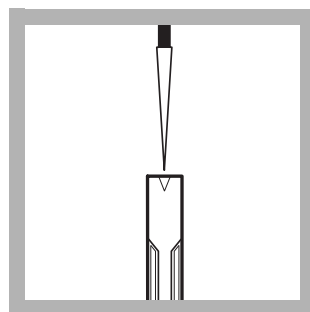
16. Key **1000**. Press the soft key under **START TIMER**.

A 10-minute reaction period will begin. Mix for 30 seconds following the instructions in *Using the 1-cm MicroCuvette Rack*.



17. After 5 minutes, mix the contents of the rack a second time for a period of 30 seconds using the same technique.

Note: Solutions will turn blue in some or all of the cuvettes.

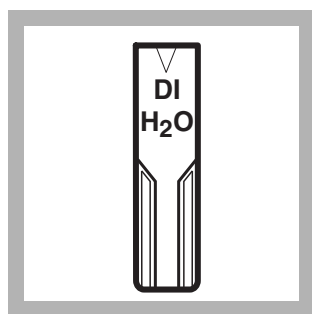


18. At the end of the 10-minute reaction period, pipet 0.5 mL of Stop Solution into each cuvette in the same order as the Color Developing Solution was added in step 15.

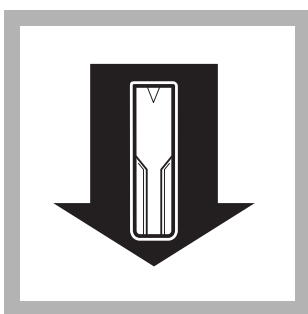
Slide the rack for 20 seconds using the technique described in *Using the 1-cm MicroCuvette Rack*.

Note: Blue solutions will turn yellow with the addition of the Stop Solution.

Note: The same pipet tip can be used repeatedly for this step.

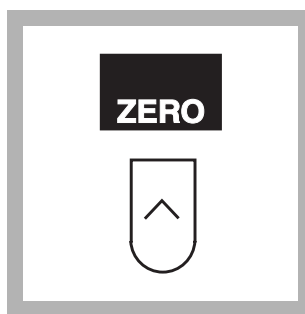


19. Label and fill a Zeroing Cuvette with deionized water. Wipe the outside of all the cuvettes with a tissue to remove water, smudges, and fingerprints.



20. Place the filled zeroing cuvette into the cell holder with the arrow pointing left.

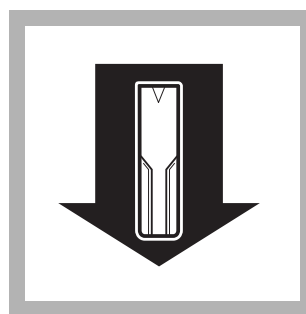
Orient the arrow in the same direction for all cuvettes.



21. Touch **Zero**.

The display will show:

0.000 Abs



22. Place the first calibrator into the cell holder. Read the results.

The display will give an absorbance reading. Record the results for each calibrator and sample.



23. Repeat *step 22* for all remaining calibrators and samples.

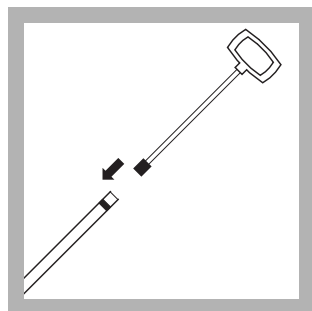
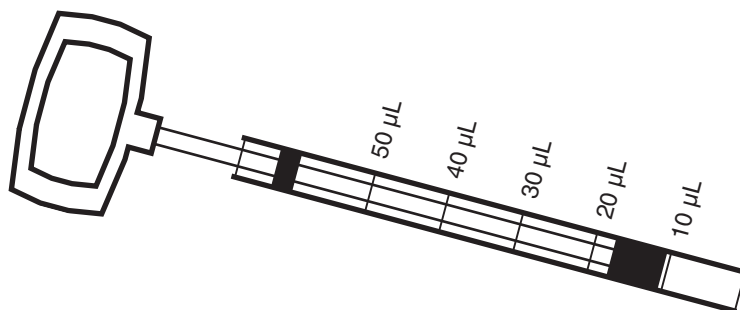
See *Interpreting and Reporting Results* for help with interpretation of results.

Using the Wiretrol® Pipet

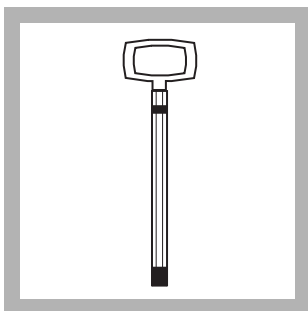
The Wiretrol Pipet can accurately measure small quantities of liquids. It consists of two parts: a Teflon®-tipped plunger and a calibrated capillary tube. Use *Figure 1* to determine the quantity measured at each line on the capillary tube.

The plunger can be re-used; the capillary tubes must be discarded after one use.

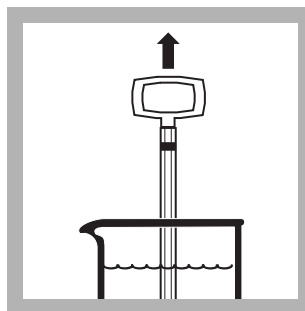
Figure 1 Wiretrol Pipet



1. Wet the orange Teflon® tip of the Wiretrol plunger in the sample, and carefully insert it into the end of the capillary tube with the colored band on it.

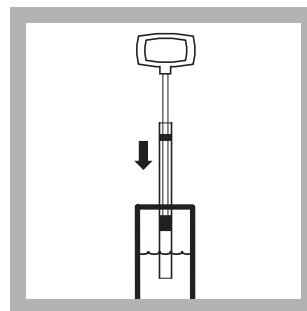


2. Push the tip to the other end of the capillary tube until it barely extends beyond the end of the capillary tube.



3. Submerge the capillary tube below the surface of the liquid to be collected. Slowly and smoothly draw the Wiretrol plunger up until the bottom of the plunger tip reaches the appropriate volume line.

Note: Touch the end of the tube to the side of the vessel to release drops on the capillary tube tip.

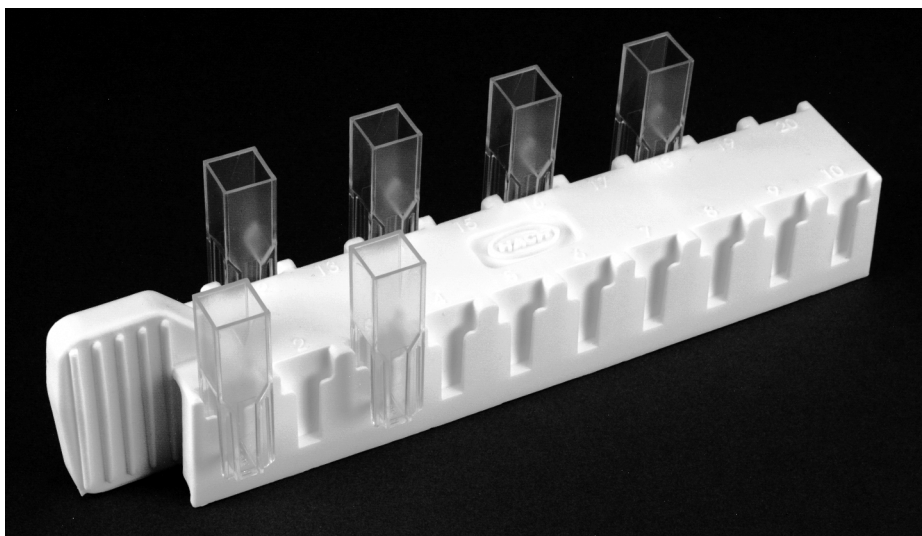


4. To discharge the pipet, place the tip of the capillary tube below the surface of the solution and push the Wiretrol plunger down in one smooth motion. Change capillary tubes for each calibrator and sample.

Using the 1-cm MicroCuvette Rack

This rack (see *Figure 2*) has been designed specifically to aid in achieving precise and accurate results when using the immunoassay technique to analyze several samples at the same time.

Figure 2 **The 1-cm MicroCuvette Rack**



Loading the Rack — The cuvette rack is designed so that it may be inverted with the cuvettes in place. Identify each cuvette with a sample or calibrator number and place all the cuvettes in the rack before beginning the procedure. Fit the cuvettes snugly into the rack, but do not force them or they may be difficult to remove and their contents may spill. The cuvettes should remain in place when the rack is inverted and tapped lightly.

Mixing — Set the rack on a hard, flat surface that is at least twice the length of the rack. Hold the rack by one end and vigorously slide it back and forth along its long axis for 30 seconds. The rack should move through a distance equal to its own length in each direction.

Interpreting and Reporting Results

There is an inverse relationship between the concentration of TPH and the reading. In other words, the higher the reading, the lower the concentration of TPH.

If the sample reading is...	the sample TPH Concentration is...
...less than calibrator reading	...greater than the calibrator concentration
...greater than calibrator reading	...less than the calibrator concentration

Example

Readings:

TPH Calibrator #1: **0.480 Abs**

TPH Calibrator #2: **0.360 Abs**

Sample #1: **0.200 Abs**

Sample #2: **0.400 Abs**

Sample #3: **0.550 Abs**

Interpretation

Interpretation for a soil sample:

Sample #1 — Sample reading is less than the readings for both calibrators. Therefore the sample concentration of TPH is greater than both 20 ppm and 50 ppm diesel fuel.

Sample #2 — Sample reading is between the readings for the TPH calibrators. Therefore the sample concentration of TPH is between 20 ppm and 50 ppm diesel fuel.

Sample #3 — Sample reading is greater than the readings for both calibrators. Therefore the sample concentration of TPH is less than both 20 ppm and 50 ppm diesel fuel.

Interpretation for a water sample:

Sample #1 — Sample reading is less than the readings for both calibrators. Therefore the sample concentration of TPH is greater than both 2 ppm and 5 ppm diesel fuel.

Sample #2 — Sample reading is between the readings for the TPH calibrators. Therefore the sample concentration of TPH is between 2 ppm and 5 ppm diesel fuel.

Sample #3 — Sample reading is greater than the readings for both calibrators. Therefore the sample concentration of TPH is less than both 2 ppm and 5 ppm diesel fuel.

Storing and Handling Reagents

- Wear protective gloves and eyewear.
- When storing reagent sets for extended periods of time, keep them out of direct sunlight. Store reagents at a temperature of 4 °C when not in use.
- Keep the foil pouch containing the TPH Antibody Cuvettes sealed when not in use.
- If Stop Solution comes in contact with the eyes, wash thoroughly for 15 minutes with cold water and seek medical help *immediately*.

Sensitivity

The antibodies used in the TPH Test Kit react with a variety of compounds found in petroleum fuels; however, each TPH calibrator has been formulated to represent a specific concentration of diesel fuel. To use the calibrators for other TPH compounds, see *Table 1* or *Table 2* to select the proper TPH calibrator for the compound, sample, and range you want to test.

Example:

To use the TPH calibrators for gasoline, find “Gasoline” in the first column of *Table 1* or *Table 2*. Read across the column to find the ppm represented by each calibrator. For gasoline, calibrator #1 = 15 ppm, calibrator #2 = 35 ppm, and so forth.

Table 1 Various TPHs in Soil

Compound	TPH calibrator #1	TPH calibrator #2	TPH calibrator #3	TPH calibrator #4
	ppm			
Diesel	20	50	100	200
Gasoline	15	35	70	140
Kerosene	35	75	140	250
Benzene	20	45	85	160
Toluene	15	30	50	90
Ethylbenzene	5	15	35	75
m-Xylene	9	20	35	70
o-Xylene	10	20	40	80
p-Xylene	3	5	9	16
BTEX	5	15	25	45

Table 2 Various TPHs in Water

Compound	TPH calibrator #1	TPH calibrator #2	TPH calibrator #3	TPH calibrator #4*
	ppm			
Diesel	2	5	10	20
Gasoline	1.5	3.5	7	14
Kerosene	3.5	7.5	14	25
Benzene	2	4.5	8.5	16
Toluene	1.5	3	5	9
Ethylbenzene	0.5	1.5	3.5	7.5
m-Xylene	0.9	2	3.5	7
o-Xylene	1	2	4	8
p-Xylene	0.3	0.5	0.9	16
BTEX	0.5	1.5	2.5	4.5

* To test concentrations in water higher than those covered by the calibrators, dilute the original sample as described below.

Diluting Water Samples

Higher concentrations in water can be tested by first diluting the sample with deionized water (see *Sensitivity*). Test for other TPH compounds (i.e., gasoline) by using the conversion factors given in *Table 1* and *Table 2*. Dilute the sample to 50 mL with deionized water in a graduated cylinder.
(See *Reagent Set, TPH 20 cuvettes 27743-00.*)

Choose the mL of sample from *Table 3*. Use the multiplier value for the chosen quantity to multiply the value from *Table 2*, above.

Table 3

mL Sample	Multiplier
0.5	100
1.0	50
2.0	25
5.0	10
10.0	5
25.0	2

For example: If a 0.5-mL water sample is diluted to 50 mL and tested, the calibrator levels for diesel fuel in water would represent 200, 500, 1000, and 2000 ppm respectively.

Sample Collection and Storage

Analyze the samples as soon as possible after collection. If the samples must be stored, collect them in glass or Teflon containers that have been washed with soap and water and rinsed with methanol. The container should be capped with a Teflon-lined cap. If a Teflon cap is not available, aluminum foil rinsed in methanol may be used as a substitute cap liner.

When collecting water samples, fill the container completely (no head space) and cover the container with a tightly-sealed lid immediately after collection.

For Soil: Store the samples at 4 °C for no longer than 14 days.

For Water: Chill the sample in an ice bath or refrigerator to limit the loss of volatile compounds. Store samples no longer than 24 hours.

Interferences

Interfering Substance	Interference Levels and Treatments
Chlorine in water samples	Interfere above 2 ppm Remove with sodium thiosulfate

Summary of Method

This method provides semi-quantitative screening based on thresholds for TPH as diesel fuel in the following concentrations:

Soil	20, 50, 100, 200 ppm as diesel fuel
Water	2, 5, 10, 20 ppm as diesel fuel

Hach immunoassay tests use antigen/antibody reactions to test for specific organic compounds in water and soil. Antibodies specific for TPH are attached to the walls of plastic cuvettes. They selectively bind and remove TPH from complex sample matrices. A prepared sample and a reagent containing enzyme-conjugate molecules (analyte molecules attached to molecules of an enzyme) are added to the Antibody Cuvettes. During incubation, enzyme-conjugate molecules and TPH compete for binding sites on the antibodies. Samples with higher levels of analyte will have more antibody sites occupied by TPH and fewer antibody sites occupied by the enzyme-conjugate molecules.

After incubation, the sample and unbound enzyme conjugate are washed from the cuvette and a color-development reagent is added. The enzyme in the conjugate catalyzes the development of color. Therefore, there is an inverse relationship between color intensity and the amount of TPH in the sample. The resulting color is then compared with a calibrator to determine whether the TPH concentration in the sample is greater or less than the threshold levels. The TPH concentration is inversely proportional to the color development: the lighter the color, the higher the TPH concentration.

Required Reagents

Description	Unit	Cat. No.
Reagent Set, TPH *	20 cuvettes	27743-00
Deionized water	500 mL	272-48

Required Apparatus

Adapter, 1-cm MicroCell	each	48588-00
Caps, flip spout	2/pkg	25818-02
Marker, laboratory	each	20920-00
TenSette®, Pipet, 0.1- to 1.0-mL	each	19700-01
Tips, for pipettor 19700-01	1000/pkg	21856-28
Rack, for 1-cm Micro Cuvettes	each	48799-00
Wipes, disposable	box	20970-00

For Soil Extraction only:

Soil Scoop, 5-g, 4.25-cc	each	26572-05
Soil Extraction Refill Kit	each	27752-00

Includes:

Dropper, LDPE, 0.5 and 1.0-mL	20/pkg	21247-20
Filter and Barrel Assembly	20/pkg	25676-20
Sodium Sulfate, anhydrous	250 g	7099-14
Soil Extractant Solution	200 mL	25677-29
Soil Sample Container	20/pkg	25929-20
Weighing Boat, 8.9-cm square	20/pkg	21790-20

* Immunoassay components are manufactured for Hach Company by Beacon Analytical Systems, Inc.



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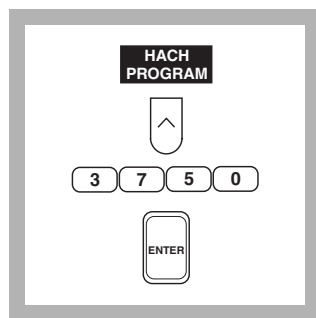
Method 10047

Attenuated Radiation Method (Direct Reading)

(0 to 5000 Formazin Attenuation Units*)

Scope and Application: For testing turbidity in water, wastewater, estuary water, seawater and industrial process water. Results may not be used for compliance reporting. The estimated detection limit for program number 3750 is 14 Formazin Attenuation Units (FAUs).

* A Formazin Attenuation Unit (FAU) is equivalent to a Nephelometric Turbidity Unit (NTU).



1. Press the soft key under **HACH PROGRAM**.

Select the stored program number for turbidity in FAUs by pressing **3750** with the numeric keys.

Press: **ENTER**

Note: If samples cannot be analyzed immediately, see Sample Collection, Preservation and Storage following these steps.

Note: The Flow Cell and Sipper Modules cannot be used with this procedure.

Note: Results are given in FAU (Formazin Attenuation Units), not Nephelometric Turbidity Units. An FAU is equivalent to a NTU when measuring formazin. They are not necessarily equivalent when measuring samples or other types of standards.



2. The display will show: **HACH PROGRAM: 3750 Turbidity, Absorb**

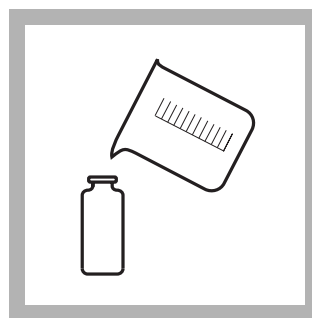
The wavelength (λ), **860 nm**, is automatically selected.



3. Use a set of matched sample cells. Fill one of the clean stoppered sample cells to the 10-mL mark with deionized water (the blank). Stopper.

Note: For highly colored samples, filter a portion of the sample and use it in place of the deionized water. See **OPTIONAL EQUIPMENT AND SUPPLIES** for labware.

Note: For colored samples, see the Interferences section.



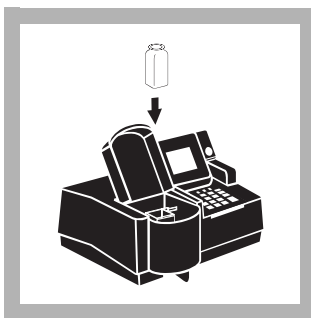
4. Rinse the other matched sample cell with sample. Then fill the sample cell to the 10-mL mark with sample. Stopper (this is the prepared sample).



5. Wipe the sides of both sample cells using a clean, soft cloth.

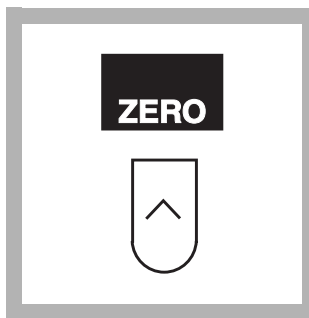
Note: Handle the sample cells by grasping the top of the cell.

Note: Apply a small amount of silicone oil to the outside of the sample cells. This minimizes the effects of surface defects on the measurement.



6. Place the blank into the cell holder. Close the light shield.

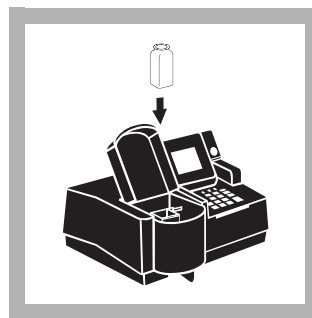
Note: Avoid disturbing the liquid in the sample cell.



7. Press the soft key under **ZERO**.

The display will show:

0 FAU



8. Gently invert the prepared sample several times. Immediately place it into the cell holder. Close the light shield. Results in FAU turbidity will be displayed.

Note: Do not shake the sample. Shaking causes air bubbles, which will cause falsely high turbidity readings.

Interferences

Interfering Substance	Interference Levels and Treatments
Air bubbles	Interfere at all levels. Degass the sample using the optional Degassing Kit or Ultrasonic Bath listed under <i>REQUIRED EQUIPMENT AND SUPPLIES</i> at the end of this procedure.
Color	Interferes if the color absorbs light at 860 nm
Temperature extremes	May interfere by changing the turbidity of the sample. Analyze samples as soon as possible after collection. Analyze at the same temperature as the original sample.

Sample Collection, Preservation and Storage

Collect samples in glass bottles. Analyze as soon as possible after collection. Changes in temperature can affect the constituents in the sample, which can influence turbidity readings. If immediate analysis is not possible, store the sample at 4 °C (39 °F) for up to 48 hours. Before analysis, warm the sample to room temperature or, preferably, to the temperature of the sample when it was collected.

Accuracy Check

Standard Solution Method

Prepare a 1000-FAU standard solution by pipetting 25.00 mL of a 4000-NTU Formazin Stock Solution into a 100-mL Class A volumetric flask. Dilute to the mark with deionized water. Stopper and invert several times to mix. Prepare this solution monthly. Perform the turbidity procedure as described above. The result should be 1000 FAU.

To adjust the calibration curve using the reading obtained with the 1000-FAU standard solution, press the soft keys under **OPTIONS, MORE** then **STD: OFF**. Press **ENTER** to accept the displayed concentration, the value of which depends on the selected units. If an alternate concentration is used, enter the actual concentration and press **ENTER** to return to the read screen. See Section 1.5.5 *Adjusting the Standard Curve* for more information.

Method Performance

Precision

Standard 1000 FAU

Program	95% Confidence Limits
3750	976–1024 FAU

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
3750	14 FAU

For more information on derivation and use of Hach's estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by 40 CFR part 136, appendix B, see Section 1.5.1.

Sensitivity

Program Number: 3750

Portion of Curve	Δ Abs	Δ Concentration
0.010 Abs	0.010	6.1 FAU
2500 FAU	0.010	24.5 FAU
4500 FAU	0.010	51.0 FAU

See Section 1.5.3 *Sensitivity Explained* for more information.

Calibration Standard Preparation

To perform a turbidity calibration using the attenuated radiation procedure, prepare turbidity calibration standards containing 40, 200, 1000, 2000, and 4000 FAU as follows:

- a. Pipet 1.00, 5.00, 25.00, and 50.00 mL of well mixed 4000-NTU Formazin Stock Solution into four different 100-mL Class A volumetric flasks, using Class A pipets.
- b. Immediately dilute each flask to volume with turbidity free deionized water. Stopper and invert each flask to mix. (The 4000-NTU standard is not diluted).
- c. Using the Attenuated Radiation method, and the calibration procedure described in the *User-Entered Programs* section of the *DR/4000 Spectrophotometer Instrument Manual*, generate a calibration curve from the standards prepared above.

Summary Of Method

This test measures turbidity, which is an optical property of the sample that results from the scattering and absorption of light by particles in the sample. The amount of turbidity measured depends on the size, shape, color and refractive properties of the particles.

TURBIDITY, continued

Formazin standards are used for calibration and readings are taken using Formazin Attenuation Units (FAU). A 400-NTU Formazin stock standard is also defined as 400 FAU. The optical measurement method for FAUs is very different than the NTU method. Color interference is minimized by taking measurements at 860 nm.

This test cannot be used for USEPA reporting purposes, but it may be used for daily in-plant monitoring and is best suited for measuring turbidity levels greater than 20 NTU. A turbidimeter should be used for accurately monitoring low levels of turbidity and for reporting purposes.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to *Section 1*.

Pollution Prevention and Waste Management

For information on pollution prevention and waste management, refer to *Section 1*.

REQUIRED REAGENTS AND STANDARDS

Description	Quantity Required		Cat. No.
	Per Test	Unit	
Water, deionized	10 mL	4 L	272-56

REQUIRED EQUIPMENT AND SUPPLIES

DR/4000 1-Inch Cell Adapter	1	each	48190-00
Sample Cells, 10 mL, 1-inch, matched pair, with stoppers	2	2/pkg	20950-00

OPTIONAL EQUIPMENT AND SUPPLIES

Bath, ultrasonic		each	24895-00
Beaker, 50-mL		each	500-41
DR/4000 Carousel Module		each	48070-02
Flask, volumetric, Class A, 100- mL		each	14574-42
Filter, membrane, 47-mm	200/pkg		13530-01
Filter Disks, 0.2-µm	10/pkg		23238-10
Filter Holder, magnetic		each	13529-00
Filter Paper, glass fiber, 47-mm	100/pkg		2530-00
Formazin Stock Solution, 4000-NTU	500 mL		2461-49
Oiling Cloth, for applying silicone oil		each	47076-00
Pipet, TenSette®, 0.1- to 1.0-mL		each	19700-01
Pipet Tips, for 19700-01 TenSette Pipet	50/pkg		21856-96
Pipet, volumetric, Class A, 1.00-mL		each	14515-35
Pipet, volumetric, Class A, 5.00-mL		each	14515-37
Pipet, volumetric, Class A, 25.00-mL		each	14515-40
Pipet, volumetric, Class A, 50.00-mL		each	14515-41
Pump, vacuum, hand-operated		each	14283-00
Sample Degassing Kit		each	43975-00
Sample Degassing and Filtration Kit		each	43975-10
Silicone Oil	15 mL DB		1269-36



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Method 8196

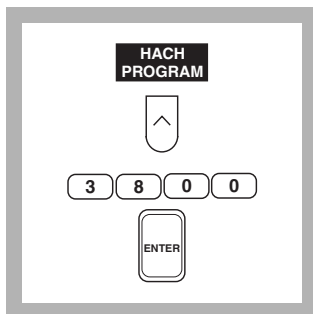
Esterification Method*

(0 to 2800 mg/L)

Scope and Application: For digester sludges.

The estimated detection limit for program number 3800 is 12 mg/L as acetic acid (HOAC).

* Adapted from *The Analyst*, 87, 949 (1962)



1. Press the soft key under **HACH PROGRAM**.

Select the stored program number for volatile acids by pressing **3 8 0 0** with the numeric keys.

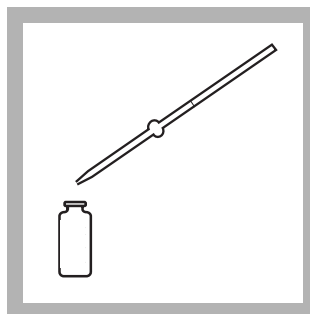
Press: **ENTER**

Note: If samples cannot be analyzed immediately, see *Sample Collection, Preservation and Storage* following these steps.

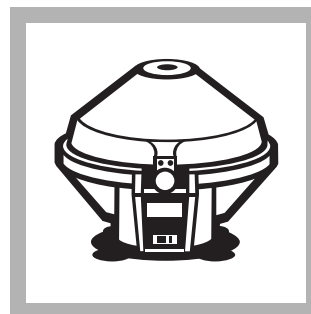


2. The display will show: **HACH PROGRAM: 3800 Volatile Acids**

The wavelength (λ), **495 nm**, is automatically selected.

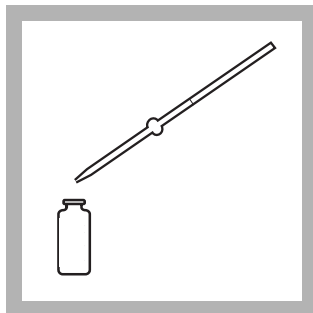


3. Pipet 0.5 mL of deionized water into a dry 25-mL sample cell (the blank).



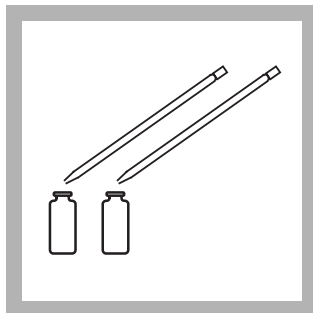
4. Filter or centrifuge 25 mL of sample using the labware listed under **REQUIRED EQUIPMENT AND SUPPLIES**.

Note: Centrifuging is faster than filtration.

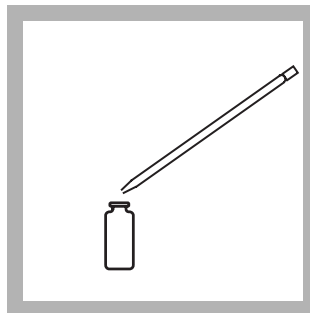


5. Pipet 0.5 mL of the filtrate or supernatant into another dry 25-mL sample cell (the prepared sample).

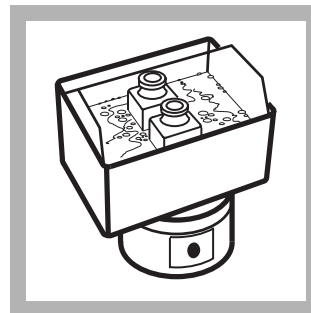
Note: For proof of accuracy, use 0.5 mL of a 500-mg/L volatile acid standard solution in place of the sample (see *Accuracy Check*).



6. Pipet 1.5 mL of ethylene glycol into each sample cell. Swirl to mix.

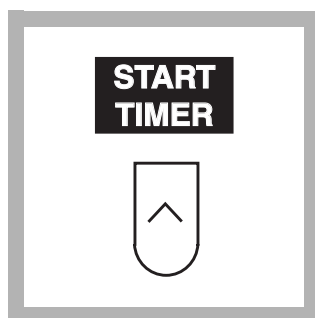


7. Pipet 0.2 mL of 19.2 N Sulfuric Acid Standard Solution into each cell. Swirl to mix.



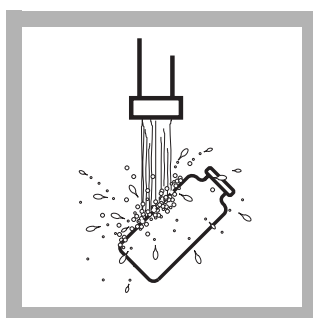
8. Place both cells into a boiling water bath.

Note: You may boil the sample cells in a 600-mL beaker.

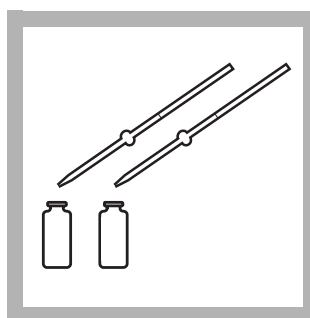


9. Press the soft key under **START TIMER**.

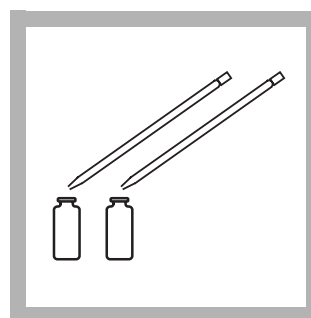
Note: A 3-minute reaction period will begin.



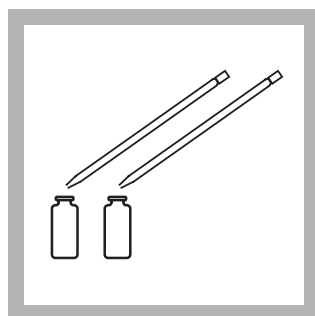
10. When the timer beeps, cool the solutions to 25 °C (until the cell feels cold) with running tap water.



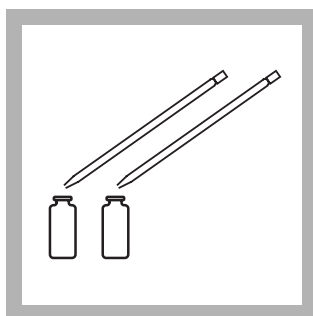
11. Using a pipet filler, pipet 0.5 mL of Hydroxylamine Hydrochloride Solution into each cell. Swirl to mix.



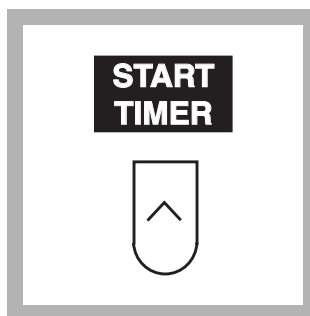
12. Using a pipet filler, pipet 2.0 mL of 4.5 N Sodium Hydroxide Standard Solution into each cell. Swirl to mix.



13. Add 10 mL of Ferric Chloride Sulfuric Acid Solution to each cell. Swirl to mix.

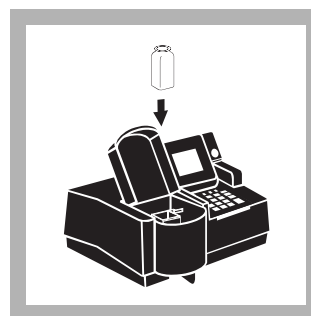


14. Add 10 mL of deionized water into each cell. Swirl to mix.

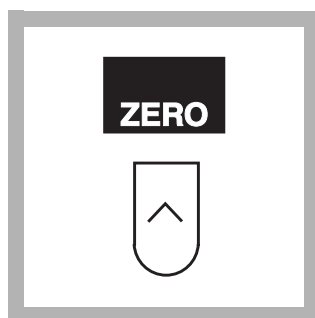


15. Immediately press the soft key under **START TIMER**. Another 3-minute reaction period will begin.

Note: During this 3-minute reaction period, complete Steps 16-17.



16. Blot each sample cell dry. Immediately place the blank in the cell holder. Close the light shield.

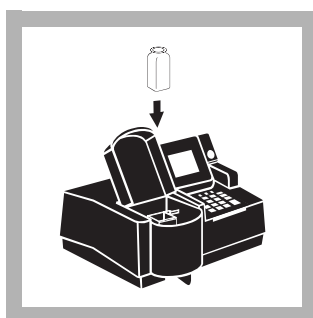


17. Press the soft key under **ZERO**.

The display will show:

0 mg/L HOAC

Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



18. When the timer beeps, immediately place the prepared sample in the cell holder. Close the light shield. Results in mg/L volatile acids as acetic acid will be displayed.

Sample Collection, Preservation and Storage

Collect samples in clean plastic or glass bottles. Analyze as soon as possible after collection. Samples can be stored for up to 24 hours by cooling to 4 °C (39 °F) or below. Warm samples to room temperature before analysis.

Accuracy Check

Standard Additions Method

- Leave the unspiked sample in the sample compartment. Verify that the units displayed are in mg/L. Select standard additions mode by pressing the soft keys under **OPTIONS**, **(MORE)** and then **STD ADD**.
- Press **ENTER** to accept the default sample volume (mL), 25.
- Press **ENTER** to accept the default standard concentration (mg/L), 62,500.
- Press the soft key under **ENTRY DONE**.
- Snap the neck off a Volatile Acid Voluette® Ampule Standard, 62,500 mg/L as acetic acid.
- Use the TenSette® Pipet to add 0.1 mL, 0.2 mL, and 0.3 mL of standard, respectively, to three 25-mL samples and mix each thoroughly.
- Analyze each standard addition sample as described above. Accept the standard additions reading by pressing the soft key under **READ** each time. Each addition should reflect approximately 100% recovery.
- After completing the sequence, the display will show the extrapolated concentration value and the “best-fit” line through the standard additions data points, accounting for matrix interferences.
- See Section 1.4.1 *Standard Additions* for more information.

Standard Solution Method

Prepare a 500-mg/L volatile acid standard solution by pipetting 4.00 mL of a 62,500-mg/L Volatile Acid Standard Solution into a 500-mL Class A volumetric flask. Dilute to volume with deionized water. Prepare this solution daily. Perform the esterification procedure as described above.

To adjust the calibration curve using the reading obtained with the 500-mg/L standard solution, press the soft keys under **OPTIONS, MORE** then **STD: OFF**. Press **ENTER** to accept the displayed concentration, the value of which depends on the selected units. If an alternate concentration is used, enter the actual concentration and press enter to return to the read screen. See Section 1.5.5 *Adjusting the Standard Curve* for more information.

Method Performance

Precision

Standard 500 mg/L as acetic acid (HOAC)

Program	95% Confidence Limits
3800	492–507 mg/L HOAC

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
3800	12 mg/L as HOAC

For more information on derivation and use of Hach's estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by 40 CFR part 136, appendix B, see Section 1.5.1.

Sensitivity

Program Number: 3800

Portion of Curve	Δ Abs	Δ Concentration
Entire Range	0.010	23.4 mg/L

See Section 1.5.3 *Sensitivity Explained* for more information.

Calibration Standard Preparation

To perform a volatile acids calibration using the esterification method, use the 62,500-mg/L Volatile Acids Standard Solution (Cat. No. 14270-10) listed under optional reagents and standards. Prepare standards containing 313, 625, 938, 1250, 1563, 2188, and 2500 mg/L volatile acids as follows:

- a. Pipet 1.00, 2.00, 3.00, 4.00, 5.00, 7.00, and 8.00 mL of the 62,500-mg/L Volatile Acids Standard Solution into seven different 200-mL Class A volumetric flasks, using Class A glassware.
- b. Dilute to the mark with deionized water and mix thoroughly.
- c. Using the esterification method and the calibration procedure described in the *User-Entered Programs* section of the *DR/4000 Spectrophotometer Instrument Manual*, generate a calibration curve from the standards prepared above.

Summary of Method

The volatile acid test is designed specifically for determining volatile acids in digester sludges. The method is based on esterification of the carboxylic acids present in the sample and subsequent determination of the esters by the ferric hydroxamate reaction. All volatile acids present are reported as their equivalent mg/L as acetic acid.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to *Section 1*.

Pollution Prevention and Waste Management

For information on pollution prevention and waste management, refer to *Section 1*.

REQUIRED REAGENTS AND STANDARDS

	Cat. No.
Volatile Acids Reagent Set (90 tests)	22447-00
Includes: (1) 2039-53, (2) 2042-53, (1) 818-42, (1) 2040-53, (1) 2038-32	

Description	Quantity Required		Cat. No.
	Per Test	Unit	
Ethylene Glycol	3 mL	1000 mL	2039-53
Ferric Chloride-Sulfuric Acid Solution	20 mL	1000 mL	2042-53
Hydroxylamine Hydrochloride Solution, 100-g/L	1 mL	100 mL	818-42
Sodium Hydroxide Standard Solution, 4.5 N	4 mL	1000 mL	2040-53
Sulfuric Acid Standard Solution, 19.2 N	0.4 mL	100 mL MDB	2038-32
Water, deionized	20.5 mL	4 L	272-56

REQUIRED EQUIPMENT AND SUPPLIES

Cylinder, graduated, 10-mL	1	each	508-38
DR/4000 1-Inch Cell Adapter	1	each	48190-00
Filter Paper, folded, 12.5-cm	1	100/pkg	1894-57
Finger Cots	2	2/pkg	14647-02
Flask, Erlenmeyer, 50-mL	1	each	505-41

VOLATILE ACIDS, continued

REQUIRED EQUIPMENT AND SUPPLIES((continued)

Funnel, poly, 65-mm	1	each.....	1083-67
Hot Plate, 4" micro, 120 VAC	1	each.....	12067-01
Hot Plate, 4" micro, 240 VAC	1	each.....	12067-02
Pipet, Filler, safety bulb.....	1	each.....	14651-00
Pipet, TenSette®, 1.0- to 10.0-mL	1	each.....	19700-10
Pipet Tips, for 19700-10 Pipet	2	50/pkg.....	21997-96
Pipet, serological, 2-mL	1	each.....	532-36
Pipet, volumetric, Class A, 0.50-mL	1	each.....	14515-34
Pipet, volumetric, Class A, 10.00-mL	1	each.....	14515-38
Water Bath and Rack.....	1	each.....	1955-55

OPTIONAL REAGENTS AND STANDARDS

Description	Unit	Cat. No.
Volatile Acids Standard Solution, 10-mL Voluette® Ampule, 62,500 mg/L as HOAC16/pkg		14270-10
Volatile Acids Standard Solution, 1000-mg/L as HOAC	100 mL.....	14205-42

OPTIONAL EQUIPMENT AND SUPPLIES

Ampule Breaker Kit	each.....	21968-00
Aspirator, Nalgene® vacuum pump	each.....	2131-00
Bottle, wash, 500-mL	each.....	620-11
Beaker, 600-mL	each.....	500-52
Centrifuge, Spinette, 115 VAC, 60 Hz	each.....	22413-00
Centrifuge Tubes, 15-mL	10/pkg.....	22787-39
Centrifuge Tube Caps.....	20/pkg.....	25852-20
Cylinder, graduated, mixing, 25-mL	each.....	1896-40
Cylinder, graduated, 100-mL	each.....	508-42
Cylinder, graduated, 250-mL	each.....	508-46
DR/4000 Carousel Module Kit	each.....	48070-02
DR/4000 Flow Cell Module Kit, 1-inch.....	each.....	48070-04
DR/4000 Flow Cell Module Kit, 1-cm.....	each.....	48070-05
DR/4000 Sipper Module Kit, 1-inch	each.....	48090-03
Filter Paper, 9-cm dia.	100/pkg.....	506-55
Flask, filtering, 500-mL	each.....	546-49
Flask, volumetric, Class A, 200-mL	each.....	14574-45
Funnel, Buchner	each.....	550-87
Pipet, TenSette, 0.1- to 1.0-mL	each.....	19700-01
Pipet Tips, for 19700-01 Pipet	50/pkg.....	21856-96
Pipet, serological, 5-mL	each.....	532-37
Pipet, volumetric, Class A, 1.00-mL	each.....	14515-35
Pipet, volumetric, Class A, 2.00-mL	each.....	14515-36
Pipet, volumetric, Class A, 3.00-mL	each.....	14515-03
Pipet, volumetric, Class A, 4.00-mL	each.....	14515-04
Pipet, volumetric, Class A, 5.00-mL	each.....	14515-37
Pipet, volumetric, Class A, 7.00-mL	each.....	14515-07
Pipet, volumetric, Class A, 8.00-mL	each.....	14515-08
Test Tube Holder	each.....	634-00
Tubing, rubber	3.6 m (12 ft).....	560-18
Tweezers, balance weight, plastic	each.....	14282-00

* Nalgene is a registered trademark of Nalge Nunc International



FOR TECHNICAL ASSISTANCE, PRICE INFORMATION AND ORDERING:
In the U.S.A. – Call toll-free 800-227-4224
Outside the U.S.A. – Contact the HACH office or distributor serving you.
On the Worldwide Web – www.hach.com; E-mail – techhelp@hach.com

HACH COMPANY
WORLD HEADQUARTERS
Telephone: (970) 669-3050
FAX: (970) 669-2932



✓ Method 8009

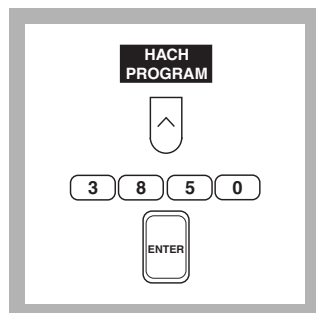
Zincon Method*

(0 to 3.000 mg/L)

Scope and Application: For water and wastewater. Digestion is required for determining total zinc; see the Digestion section following this procedure. USEPA Approved** for wastewater analyses. The estimated detection limit for program number 3850 is 0.009 mg/L Zn.

* Adapted from *Standard Methods for the Examination of Water and Wastewater*.

** *Federal Register*, 45 (105) 36166 (May 29, 1980)



1. Press the soft key under **HACH PROGRAM**.

Select the stored program number for zinc (Zn) by pressing **3850** with the numeric keys.

Press: **ENTER**

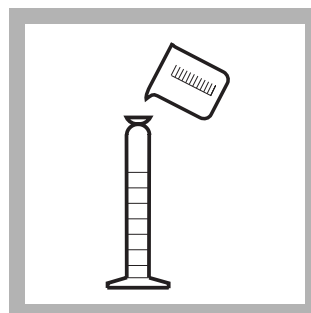
Note: If samples cannot be analyzed immediately, see *Sample Collection, Preservation and Storage* following these steps. Adjust pH of preserved samples before analysis.

Note: The Flow Cell and Sipper Modules cannot be used with this procedure.



2. The display will show: **HACH PROGRAM: 3850 Zinc**

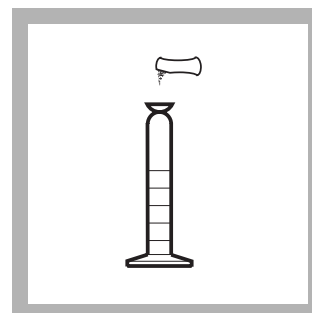
The wavelength (λ), **620 nm**, is automatically selected.



3. Fill a 25-mL graduated mixing cylinder with 20 mL of sample.

Note: Use only glass-stoppered cylinders in this procedure. Rinse with 1:1 hydrochloric acid and deionized water before use.

Note: For proof of accuracy, use a 0.5-mg/L zinc standard solution in place of the sample (see *Accuracy Check*).

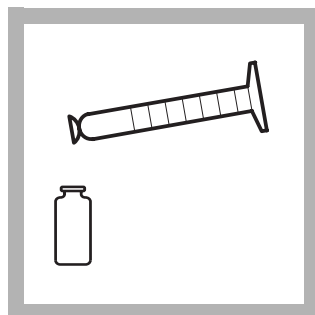


4. Add the contents of one Zincon 5 Reagent Powder Pillow to the cylinder. Stopper. Invert several times to dissolve the powder completely.

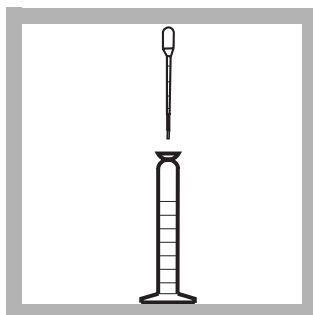
Caution! The reagent in this step contains cyanide and is very poisonous if taken internally or if the fumes are inhaled. Do not add to an acidic sample (<pH 4).

Note: Inconsistent readings may result for low zinc concentrations if all the particles are not dissolved.

Note: The sample should be orange. If the color is brown or blue, dilute the sample and repeat the test. Either the zinc concentration is too high or an interfering metal is present.

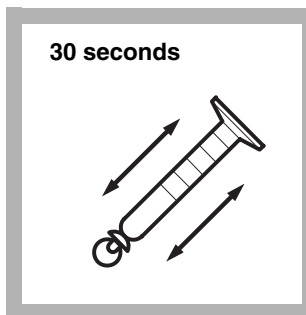


5. Pour 10 mL of the solution into a sample cell (the blank).



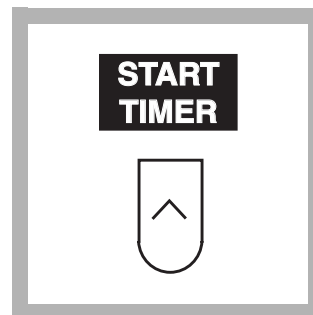
6. Add 0.5 mL of cyclohexanone to the remaining solution in the graduated mixing cylinder.

Note: Use a plastic dropper. Rubber bulbs may contaminate the cyclohexanone.



7. Press the soft key under **START TIMER**. During this time period, stopper the cylinder and shake vigorously for 30 seconds (this is the prepared sample).

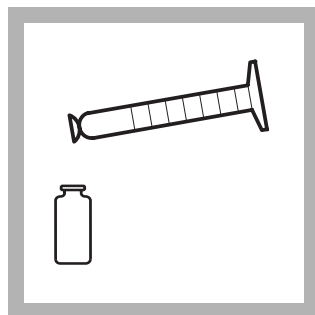
Note: The sample will be reddish-orange, brown, or blue depending on the zinc concentration.



8. Press the soft key under **START TIMER**.

A 3-minute reaction period will begin.

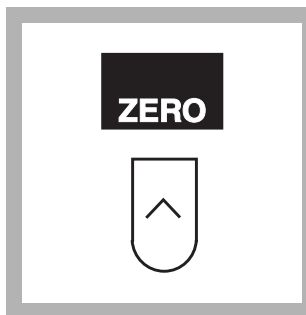
Note: During this time period, complete Step 9.



9. During the reaction period, pour the solution from the cylinder into a sample cell (the prepared sample).



10. When the timer beeps, place the blank in the cell holder. Close the light shield.

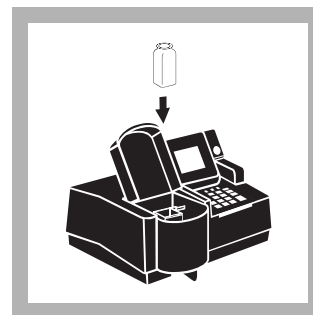


11. Press the soft key under **ZERO**.

The display will show:

0.000 mg/L Zn

Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



12. Place the prepared sample in the cell holder. Close the light shield. Results in mg/L zinc (or chosen units) will be displayed.

Interferences

The following substances may interfere when present in concentrations exceeding those listed:

Interfering Substance	Interference Levels and Treatments
Aluminum	Greater than 6 mg/L
Cadmium	Greater than 0.5 mg/L
Copper	Greater than 5 mg/L
Iron (ferric)	Greater than 7 mg/L
Manganese	Greater than 5 mg/L
Nickel	Greater than 5mg/L
Organic Material	Large amounts may interfere.
Highly buffered or extreme sample pH	May exceed the buffering capacity of the reagents and require sample pretreatment. Adjust pH to 4–5.

Sample Collection, Preservation and Storage

Collect samples in acid-cleaned plastic or glass bottles. If prompt analysis is impossible, preserve the sample by adjusting to pH 2 or less with nitric acid (about 2 mL per liter). Preserved samples may be stored up to six months at room temperature.

Before analysis, adjust the pH to 4–5 with 5.0 N sodium hydroxide. Do not exceed pH 5 as zinc may precipitate. Correct the test result for volume additions; see Section 1.2.2 *Correcting for Volume Additions*.

Accuracy Check

Standard Additions Method

- Leave the unspiked sample in the sample compartment. Verify that the units displayed are in mg/L. Select standard additions mode by pressing the soft keys under **OPTIONS, (MORE)** and then **STD ADD**.
- Press **ENTER** to accept the default sample volume (mL), 50.
- Press **ENTER** to accept the default standard concentration (mg/L), 25.
- Press the soft key under **ENTRY DONE**.
- Snap the neck off a Zinc Voluette® Ampule Standard, 25-mg/L Zn.
- Use the TenSette® Pipet to add 0.1 mL, 0.2 mL, and 0.3 mL of standard, respectively to three 50-mL samples and mix each thoroughly.
- Analyze each standard addition sample as described above. Accept the standard additions reading by pressing the soft key under **READ** each time. Each addition should reflect approximately 100% recovery.
- After completing the sequence, the display will show the extrapolated concentration value and the “best-fit” line through the standard additions data points, accounting for matrix interferences.
- See Section 1.4.1 *Standard Additions* for more information.

Standard Solution Method

Using Class A glassware, prepare a 0.50-mg/L zinc standard solution by pipetting 5.00 mL of Zinc Standard Solution, 100-mg/L, into a 1000-mL volumetric flask. Dilute to the mark with deionized water. Prepare this solution daily. Perform the Zincon procedure as described above.

To adjust the calibration curve using the reading obtained with the 0.50-mg/L Zinc standard solution, press the soft keys under **OPTIONS, MORE** then **STD: OFF**. Press **ENTER** to accept the displayed concentration, the value of which depends on the selected units. If an alternate concentration is used, enter the actual concentration and press **ENTER** to return to the read screen. See Section 1.5.5 *Adjusting the Standard Curve* for more information.

Digestion

Digestion is required if total zinc is being determined. The following is not the USEPA digestion (see Section 2.1.1 *USEPA Mild Digestion with Hot Plate for Metals Analysis Only* for more information).

- a. If nitric acid has not been added to the sample previously, add 5 mL of concentrated nitric acid to one liter of sample (use a glass serological pipet and pipet filler). If the sample was acidified at collection, add 3 mL of nitric acid to one liter of sample.
- b. Transfer 100 mL of acidified sample to a 250-mL Erlenmeyer flask.
- c. Add 5 mL of 1:1 hydrochloric acid.
- d. Heat sample on a hot plate for 15 minutes at 95 °C. Make sure the sample does not boil.
- e. Filter cooled sample through a membrane filter and adjust the volume to 100 mL with deionized water.
- f. Adjust the pH to 4–5 with 5.0 N sodium hydroxide before analysis (see *Sample Collection, Preservation and Storage* for instructions).

Method Performance

Precision

Standard 0.500 mg/L Zn

Program	95% Confidence Limits
3850	0.495–0.505 mg/L Zn

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
3850	0.009 mg/L Zn

For more information on derivation and use of Hach's estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by 40 CFR part 136, appendix B, see Section 1.5.1.

Sensitivity

Program Number: 3850

Portion of Curve	Δ Abs	Δ Concentration
Entire Range	0.010	0.0132 mg/L

See Section 1.5.3 *Sensitivity Explained* for more information.

Calibration Standard Preparation

To perform a zinc calibration using the Zincon method, prepare calibration standards containing 0.20, 0.50, 1.00, 1.50, 2.00, 2.50, and 3.00 mg/L zinc as follows:

- a. Prepare a 10.00-mg/L Zinc stock working standard. Pipet 10.00 mL of a 1000-mg/L Zinc Standard Solution into a 1-liter Class A volumetric flask using a 10.00-mL Class A volumetric pipet. Dilute this flask to volume with deionized water. Stopper and invert several times to mix.
- b. Pipet 2.00, 5.00, 10.00, 15.00, 20.00, 25.00, and 30.00 mL of the 10.00-mg/L Zinc Stock Working Standard into seven different 100-mL class A volumetric flasks, respectively.
- c. Dilute each flask to volume with deionized water. Stopper each flask and then invert several times to mix.
- d. Using the Zincon method and the calibration procedure described in the *User-Entered Programs* section of the *DR/4000 Spectrophotometer Instrument Manual*, generate a calibration curve from the standard prepared above.

Summary of Method

Zinc and other metals in the sample are complexed with cyanide. Adding cyclohexanone causes a selective release of zinc. The zinc then reacts with 2-carboxy-2'-hydroxy-5'-sulfoformazyl benzene (zincon) indicator to form a blue-colored species. The blue color is masked by the brown color from the excess indicator. The intensity of the blue color is proportional to the amount of zinc present.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to *Section 1*.

Pollution Prevention and Waste Management

ZincoVer 5 reagent contains potassium cyanide. Cyanide solutions are regulated as hazardous wastes by the Federal RCRA. Cyanide should be collected for disposal as a reactive (D003) waste. Be sure that cyanide solutions are stored in a caustic solution with pH >11 to prevent release of hydrogen cyanide gas. See *Section 1* for further information on proper disposal of these materials.

REQUIRED REAGENTS AND STANDARDS

	Cat. No.
Zinc Reagent Set, 20-mL sample size (100 tests = 100 samples + 100 blanks)	24293-00
Includes: (1) 14033-32, (1) 21066-69	

Description	Quantity Required		Cat. No.
	Per Test	Unit	
Cyclohexanone	1 mL	100 mL MDB	14033-32
ZincoVer 5 Reagent Powder Pillows	1 pillow	100/pkg	21066-69

REQUIRED EQUIPMENT AND SUPPLIES

Cylinder, graduated, mixing, 25-mL	1	each	20886-40
DR/4000 1-Inch Cell Adapter	1	each	48190-00

OPTIONAL REAGENTS AND STANDARDS

Bleach, household	1 gal.....	obtain locally
Hydrochloric Acid Solution, 1:1 (6.0 N).....	500 mL.....	884-49
Nitric Acid Solution, 1:1	500 mL.....	2540-49
Nitric Acid, ACS	500 mL.....	152-49
Sodium Hydroxide Standard Solution, 5.0 N.....	1 L.....	2450-53
Sodium Hydroxide Standard Solution, 5.0 N.....	59 mL DB.....	2450-26
Sodium Hydroxide Solution, 50%	500 mL.....	2180-49
Water, deionized.....	4 L.....	272-56
Zinc Standard Solution, 100-mg/L.....	100 mL.....	2378-42
Zinc Standard Solution, 1000-mg/L.....	100 mL.....	14177-42
Zinc Standard Solution, 2-mL PourRite® Ampule, 25-mg/L as Zn.....	20/pkg.....	14246-20
Zinc Standard Solution, 10-mL Voluette® Ampule, 25-mg/L Zn	16/pkg.....	14246-10

OPTIONAL EQUIPMENT AND SUPPLIES

Description	Unit	Cat. No.
Ampule Breaker Kit	each.....	21968-00
Aspirator, Nalgene vacuum pump	each.....	2131-00
Beaker, 1000-mL	each.....	500-53
Cylinder, graduated, 100-mL	each.....	508-42
Dropper 0.5- and 1.0-mL marks.....	10/pkg.....	21247-10
Filter Discs, glass, 47-mm.....	100/pkg.....	2530-00
Filter Holder, 47-mm.....	each.....	2340-00
Flask, Erlenmeyer, 250-mL.....	each.....	505-46
Flask, volumetric, Class A, 100-mL	each.....	14574-42
Flask, volumetric, Class A, 100-mL	6/pkg.....	14574-72
Flask, volumetric, Class A, 1000- mL	each.....	14574-53
Hot Plate, 3 ½" diameter, 120 VAC.....	each.....	12067-01
Hot Plate, 3 ½" diameter, 240 VAC.....	each.....	12067-02
pH Paper, pH 1.0 to 11.0	5 rolls/pkg.....	391-33
pH Meter, <i>sensio</i> TM 1, portable	each.....	50050-00
Pipet Filler, safety bulb.....	each.....	14651-00
Pipet, TenSette, 0.1- to 1.0-mL	each.....	19700-01
Pipet Tips, for 19700-01 Pipet	50/pkg.....	21856-96
Pipet, serological, 2-mL	each.....	532-36
Pipet, volumetric, Class A, 0.50-mL	each.....	14515-34
Pipet, volumetric, Class A, 2.00-mL	each.....	14515-36
Pipet, volumetric, Class A, 5.00-mL	each.....	14515-37
Pipet, volumetric, Class A, 10.00-mL	each.....	14515-38
Pipet, volumetric, Class A, 15.00-mL	each.....	14515-39
Pipet, volumetric, Class A, 20.00-mL	each.....	14515-20
Pipet, volumetric, Class A, 25.00-mL	each.....	14515-40
PourRite Ampule Breaker	each.....	24846-00



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On the Worldwide Web – www.hach.com; E-mail – techhelp@hach.com

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WORLD HEADQUARTERS
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FAX: (970) 669-2932

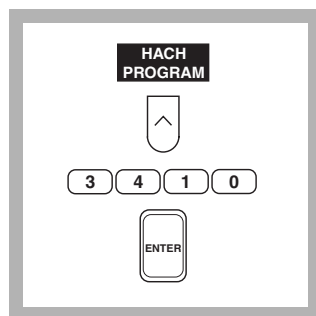


UniCell™ Vials

(0 to 6.00 mg/L Zn)

Scope and Application: For waste water, drinking water, surface water, raw water and process control. The estimated detection limit for program number 3410 is 0.10 mg/L Free Zn.

* Reagent sets for this method are only available in Europe.



1. Press the soft key under **HACH PROGRAM**.

Select the stored program for UniCell Zinc by pressing **3410** with the numeric keys.

Press: **ENTER**

Note: If samples cannot be analyzed immediately, see Sample Collection, Storage and Preservation following these steps. Adjust pH of preserved samples before analysis.

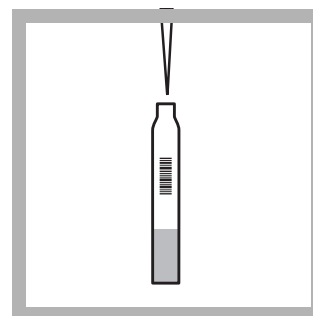


2. The display will show:
HACH PROGRAM: 3410
Zinc, HCT 170

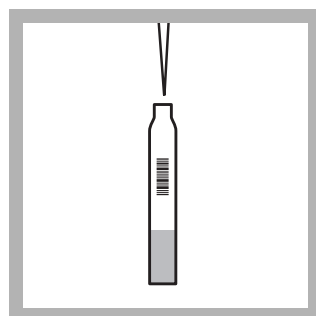
The wavelength (λ), **490 nm**, is automatically selected.



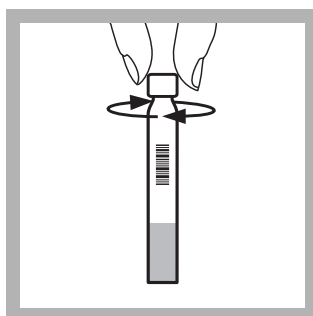
3. Insert the Test Tube Adapter into the sample cell module by sliding it under the thumb screw and into the alignment grooves. Fasten with the thumb screw.



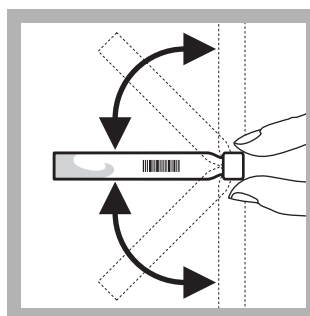
4. Pipet 4.0 mL of sample into a sample vial.



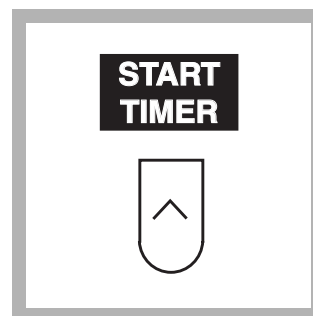
5. Pipet 0.4 mL of Demasking Agent A (HCT 170 A) into the sample vial.



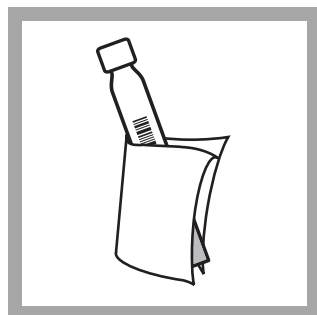
6. Immediately cap the sample vial with the **orange** UniCap B (HCT 170 B).



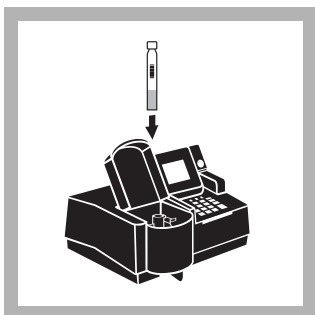
7. Invert the sample vial repeatedly until the reagent in the cap is completely dissolved.



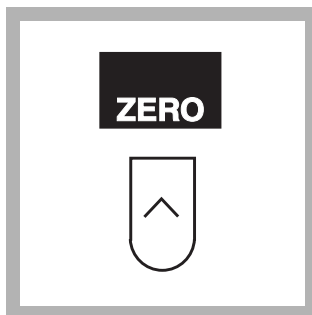
8. Press the soft key under **START TIMER**. A 3-minute reaction period will begin.



9. Wipe the zero vial (**white** cap) and the sample vial with a damp cloth followed by a dry one.



10. Place the zero vial into the cell holder. Close the light shield.

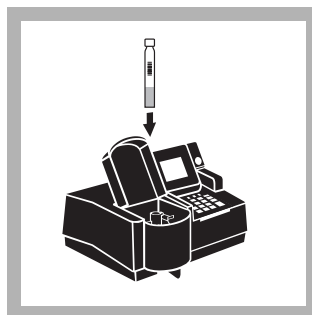


11. Press the soft key under **ZERO**.

The display will show:

0.00 mg/L Free Zn

Note: If the sample was pretreated with the the Metal Prep Set (HCT 200), press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Select **Tot Zn** and press **ENTER** to return to the read screen.



12. Place the prepared sample into the cell holder. Close the light shield. Results in mg/L zinc will be displayed.

Interferences

The ions listed in the following table have been individually tested up to the given concentrations. Cumulative effects and the influence of other ions have not been evaluated.

Ion	No interference above:
Cl ⁻ , Ca ²⁺	1000 mg/L
Mg ²⁺	500 mg/L
Fe ²⁺ , Fe ³⁺ , Sn ²⁺ , Ni ²⁺ , Cu ²⁺ , Cr ³⁺ , Cr ⁶⁺ , CO ₃ ²⁻	50 mg/L
Co ²⁺	20 mg/L
Pb ²⁺	5 mg/L

Total zinc including undissolved zinc and complexed zinc can only be determined after digesting with the Metal Prep Set (HCT 200). (Total zinc measuring range is 0.12–7.20 mg/L.)

Sample Collection, Storage and Preservation

Collect samples in acid-cleaned or plastic containers. No acid addition is necessary if analyzing the samples immediately. To preserve samples, adjust the pH to 2 or less with concentrated nitric acid (about 2 mL per liter). Preserved samples may be stored up to six months at room temperature. If reporting only dissolved free iron, filter sample immediately after collection and before adding nitric acid.

Before analysis, adjust the pH to between 3 and 5 with 5.0 N Sodium Hydroxide Standard Solution. Correct the test results for volume additions.

Accuracy Check

Standard Additions Method

- a. Leave the unspiked sample in the sample cell compartment. Verify that the units displayed are in mg/L. Select standard additions mode by pressing the soft keys under **OPTIONS, (MORE)** and then **STD ADD**.
- b. Press **ENTER** to accept the default sample volume (mL), 100.0.
- c. Press **ENTER** to accept the default standard concentration (mg/L), 1000.0.
- d. Press the soft key under **ENTRY DONE**.
- e. Use a pipet to add 0.1 mL, 0.2 mL and 0.3 mL of standard, respectively, to three 100-mL samples and mix each thoroughly.
- f. Analyze each standard addition sample as described above. Accept the standard additions readings by pressing the soft key under **READ** each time. Each addition should reflect approximately 100% recovery.
- g. After completing the sequence, the display will show the extrapolated concentration value and the “best-fit” line through the standard additions data points, accounting for matrix interferences.

See Section 1.4.1 *Standard Additions* for more information.

Standard Solution Method

Prepare a 2.0-mg/L Zn standard solution by pipetting 0.2 mL of 1000-mg/L Zn into a 100-mL volumetric flask. Dilute to the mark with deionized water. Stopper and invert to mix. Prepare this solution daily. Perform the zinc procedure as described.

To adjust the calibration curve using the reading obtained with the 0.20-mg/L standard solution press the soft keys under **OPTIONS, (MORE)** and then **STD:OFF**. Press **ENTER** to accept the default concentration, the value of which will depend on the selected units. If an alternate concentration is used, enter the actual concentration and press **ENTER** to return to the read screen. See Section 1.5.5 *Adjusting the Standard Curve* for more information.

Method Performance

Precision

Standard: 0.30 mg/L Free Zn

Program	95% Confidence Limits
3410	1.63–2.37 mg/L Free Zn

For more information on determining precision data and method detection limits, refer to Section 1.5.

Estimated Detection Limit

Program	EDL
3410	0.10 mg/L Free Zn

For more information on derivation and use of Hach's estimated detection limit, see Section 1.5.2. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, appendix B, see Section 1.5.1.

Sensitivity

Program Number: 3410

Portion of Curve	Δ Abs	Δ Concentration
1.00	0.010	0.085 mg/L
3.00	0.010	0.090 mg/L
5.00	0.010	0.095 mg/L

See Section 1.5.3 *Sensitivity Explained* for more information.

Summary of Method

Zinc ions form a water soluble orange-red complex with 4-(2-pyridylazo)-resorcinol (PAR) at pH 5.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to Section 1.

REQUIRED REAGENTS AND STANDARDS

Description	Unit	Cat. No.
Zinc - Zn, UniCell™ HCT 170*	23/pkg	HCT 170

OPTIONAL REAGENTS

Sulfuric Acid Standard Solution, 1.00 N	1 L	1270-53
Nitric Acid Solution, 1:1	500 mL	2540-49
Sodium Hydroxide, 5 N	1 L	2450-53
Zinc Standard Solution, 1000-mg/L as Zn	100 mL	14177-42

OPTIONAL EQUIPMENT AND SUPPLIES

Graduated cylinder, mixing, 100-mL	each	20886-42
Flask, volumetric, 100-mL	each	14574-42
Pipettor, (Jencons) 1–5 mL	each	27951-00
Replacement tips for 27951-00	pkg/100	27952-00
Pipettor, (Jencons) 100–1000 μ L	each	27949-00
Replacement tips for 27949-00	pkg/400	27950-00
pH Paper	pkg/100	26013-00

* Available in Europe only



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