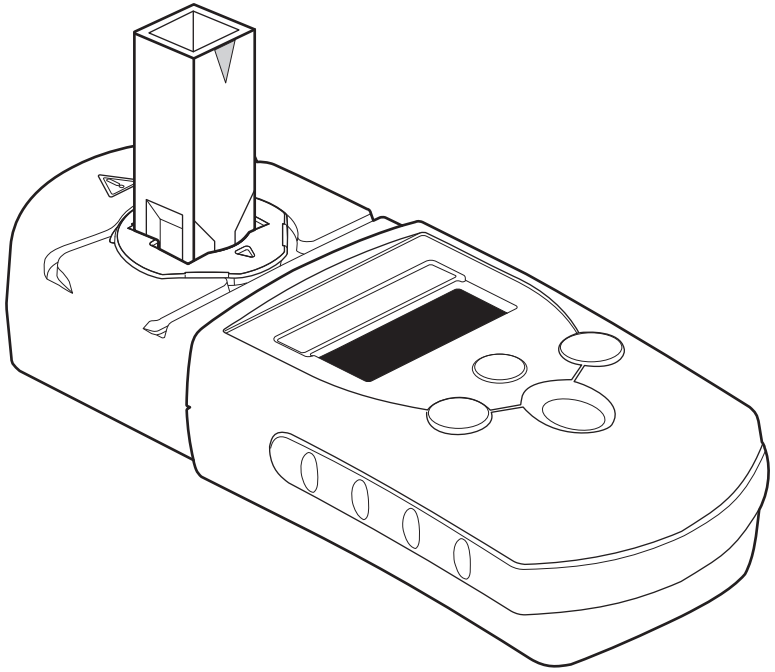
59574-88

Pocket Colorimeter™ II Test Kit

Immunoassay Instruction Manual

Alachlor, Atrazine and Metolachlor in Water
PCB in Soil and TPH in Soil and Water



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Pond In Pillow™
PourRite®
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Ratio™
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Alachlor, Atrazine, and Metolachlor in Water
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Table of Contents

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Safety Precautions

Please read this entire manual before unpacking, setting up, or operating this instrument. Pay particular attention to all danger and caution statements. Failure to do so could result in serious injury to the operator or damage to the equipment.

To ensure the protection provided by this equipment is not impaired, do not use or install this equipment in any manner other than that which is specified in this manual.

Laboratory Safety

As part of good laboratory practice, please familiarize yourself with the reagents used in these procedures. Read all product labels and the material safety data sheets (MSDS) before using them. It is always good practice to wear safety glasses when handling chemicals. Follow instructions carefully. Rinse thoroughly if contact occurs. If you have questions about reagents or procedures, please contact the manufacturer.

Use of Hazard Information

If multiple hazards exist, this manual will use the signal word (Danger, Caution, Note) corresponding to the greatest hazard.

DANGER
Indicates a potentially or imminently hazardous situation which, if not avoided, could result in death or serious injury.

CAUTION
Indicates a potentially hazardous situation that may result in minor or moderate injury.

NOTE
Information that requires special emphasis.

Precautionary Labels

Read all labels and tags attached to the instrument. Personal injury or damage to the instrument could occur if not observed.

 This symbol, if noted on the instrument, references the instruction manual for operational and/or safety information.

Specifications

Lamp: Light emitting diode (LED)

Detector: Silicon photodiode

Photometric Precision: ±0.0015 Absorbance

Filter bandwidth: 15 ±2 nm

Wavelength: 450 nm

Absorbance range: 0 to 2.50 Abs

Dimensions: 3.2 x 6.1 x 15.2 cm (1.25 x 2.4 x 6 inches)

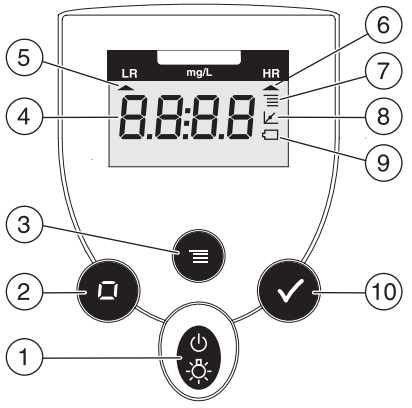
Weight: 0.2 kg (0.43 lbs)

Sample cells: 1 cm cuvette

Operating conditions: 0 to 50 °C (32 to 122 °F); 0 to 90% relative humidity (noncondensing)

Power supply: Four AAA alkaline batteries; approximate life is 2000 tests*

Instrument Keys and Display



Item	Description
1	**POWER** Key
2	**ZERO/SCROLL** Key
3	**MENU** Key
4	Numeric Display
5	Range Indicator
6	Range Indicator
7	Menu Indicator
8	Calibration Adjusted Indicator
9	Battery Low Indicator
10	**READ/ENTER** Key

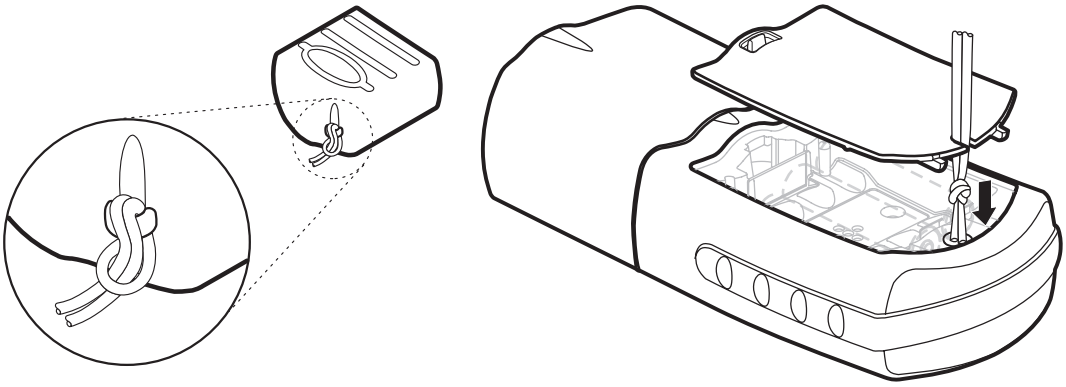
* Backlight usage will decrease battery life.

Instrument Cap Cord

The instrument cap for the Pocket Colorimeter™ II doubles as a light shield. Accurate measurements cannot be obtained unless the sample or blank is covered with the cap. Use the instrument cap cord to secure the cap to the body of the colorimeter and prevent loss of the cap. See Figure 1.

1. Loop the instrument cap cord through the ring on the cap.
2. Remove the battery compartment cover. Press the knotted end of the cord into the hole indicated by the arrow.
3. Slide the cord into the slot on the battery compartment cover. Snap the cover into place.

Figure 1 Attaching the Instrument Cap Cord



Operation

DANGER

Handling chemical samples, standards, and reagents can be dangerous. Review the necessary Material Safety Data Sheets and become familiar with all safety procedures before handling any chemicals.

DANGER

La manipulation des échantillons chimiques, étalons et réactifs peut être dangereuse. Lire les Fiches de Données de Sécurité des Produits (FDSP) et se familiariser avec toutes les procédures de sécurité avant de manipuler tous les produits chimiques.

PELIGRO

La manipulación de muestras químicas, estándares y reactivos puede ser peligrosa. Revise las fichas de seguridad de materiales y familiarícese con los procedimientos de seguridad antes de manipular productos químicos.

GEFAHR

Das Arbeiten mit chemischen Proben, Standards und Reagenzien ist mit Gefahren verbunden. Es wird dem Benutzer dieser Produkte empfohlen, sich vor der Arbeit mit sicheren Verfahrensweisen und dem richtigen Gebrauch der Chemikalien vertraut zu machen und alle entsprechenden Materialsicherheitsdatenblätter aufmerksam zu lesen.

PERIGO

A manipulação de amostras, padrões e reagentes químicos pode ser perigosa. Reveja a folha dos dados de segurança do material e familiarize-se com todos os procedimentos de segurança antes de manipular quaisquer produtos químicos.

PERICOLO

La manipolazione di campioni, standard e reattivi chimici può essere pericolosa. La preghiamo di prendere conoscenza delle Schede Techniche necessarie legate alla Sicurezza dei Materiali e di abituarsi con tutte le procedure di sicurezza prima di manipolare ogni prodotto chimico.

Data Sheet*

Location: _____

Date of Testing: _____

Operator: _____

Lot Number of Reagent Set: _____

Serial Number of Immunoassay Pocket Colorimeter: _____

CALIBRATORS

Calibrator Value	Absorbance	Comments

SAMPLES

Sample Number	Absorbance	Interpretation	Comments

* This page may be duplicated as needed.

Chapter 1 Procedure for Alachlor in Water

1.1 Overview

Alachlor is a widely-used herbicide for the control of grasses and broadleaf weeds. It is sold by several companies under a variety of trade names. The Maximum Contaminant Level for Alachlor in the United States has been set at 2 parts per billion (ppb) by the U.S. Environmental Protection Agency.

Samples, calibrators, and reagents are added to cuvettes coated with antibodies specific for Alachlor. The resultant color is measured with a Hach Pocket Colorimeter II. The concentration of Alachlor in a sample is determined by comparing the developed color intensity to that of an Alachlor calibrator. The Alachlor concentration is inversely proportional to the color development: the lighter the color, the higher the Alachlor concentration.

This method provides semi-quantitative screening based on thresholds for Alachlor in the following concentrations: 0.1 ppb and/or 0.5 ppb. Other concentrations of Alachlor in water can be tested by first diluting the sample with deionized water. (See Section Diluting Water Samples on page 1–17.)

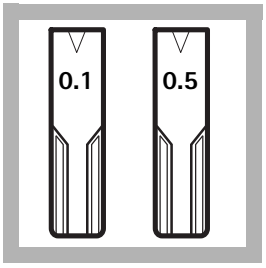
1.2 Testing Hints

1. **Read the entire procedure before starting.** Identify and have ready all the necessary reagents, cuvettes, and other apparatus before beginning the analysis.
2. **Timing is critical**; follow instructions carefully.
3. **A consistent technique when mixing the cuvettes is critical to this test.** Using the cuvette rack and mixing, as described in The 1-cm MicroCuvette Rack on page 2–3 , yields the best results. Cuvettes can be mixed individually, but test results may not be as consistent.
4. The test requires about 30 minutes for complete analysis. As many as 20 cuvettes (18 samples and 2 calibrators) can be run simultaneously.
5. Handle the cuvettes carefully. Scratches on the inside or outside may cause erroneous results. Gently 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.)
6. Antibody cuvettes and enzyme conjugate are manufactured in matched lots. Do not mix reagent lots.

7. Twenty Antibody Cuvettes are provided with each reagent set. One Antibody Cuvette will be used for each calibrator and each sample. Antibody Cuvettes are not reusable.
8. The Immunoassay Pocket Colorimeter provides a reading in absorbance units. This unit of measurement will allow you to compare your samples to the calibrators.
9. To avoid damaging the Color Developing Solution, do not expose it to direct sunlight.
10. 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.

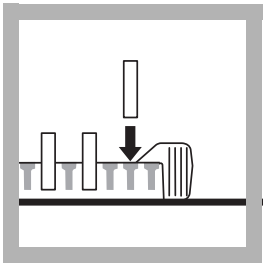
1.3 Procedure

Immunoassay

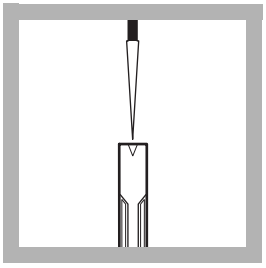


1. Label two Antibody Cuvettes: one for the 0.1 ppb Alachlor Calibrator, and the other for the 0.5 ppb Alachlor Calibrator. Label the required number of cuvettes with sample identification.

Note: *As many as 18 samples may be tested with each two calibrators.*



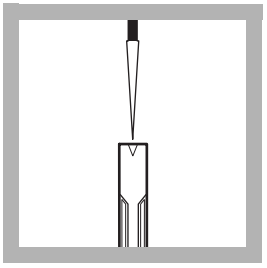
2. Place the cuvettes snugly into the rack.



3. Pipet 0.5 mL of the 0.1 ppb Alachlor Calibrator into the appropriate cuvette.

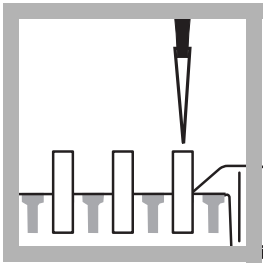
Pipet 0.5 mL of the 0.5 ppb Alachlor Calibrator into the second cuvette.

Note: *Use a new pipette tip for each cuvette.*



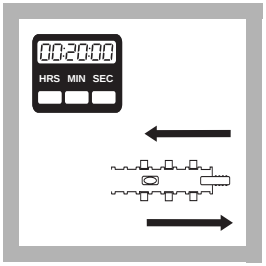
4. Pipet 0.5 mL of each sample to be tested into the appropriate cuvette.

Note: *Use a new pipette tip for each sample.*

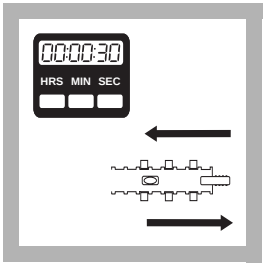


5. Using the Precision Pipettor, immediately pipet 0.5 mL of Alachlor Enzyme Conjugate into each cuvette.

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

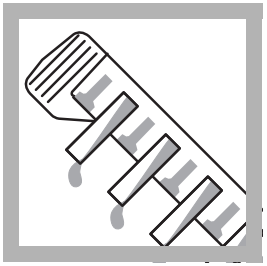


6. Begin a 20-minute reaction period and mix following the instructions on page 2–3.

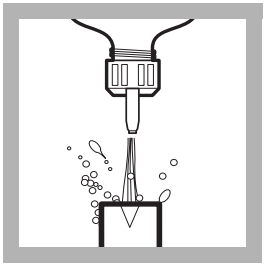


7. After 10 minutes, mix the contents of the rack a second time for a period of 30 seconds using the same technique.

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



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

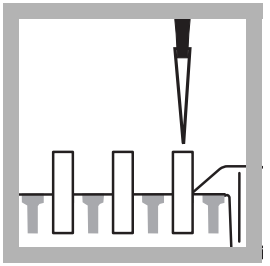


9. Wash each cuvette forcefully and thoroughly four times with deionized water. Empty the cuvettes into the waste container.

Note: *Ensure most of the water is drained from the cuvettes by turning the cuvettes upside down and gently tapping them on a paper towel to drain.*

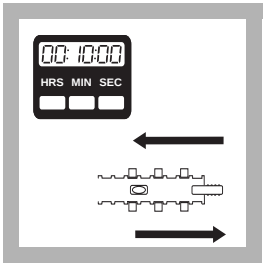
Color Development

Note: *Timing is critical; follow instructions carefully.*

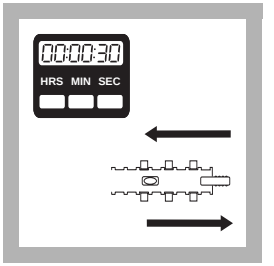


10. With the cuvettes still held snugly in the rack, pipet 0.5 mL of Color Developing Solution into each Antibody Cuvette.

Note: *Use a new pipette tip for each cuvette.*

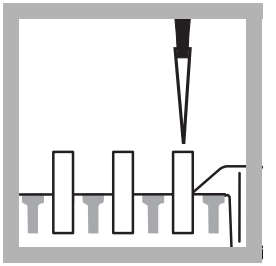


11. Begin a 10-minute reaction period and mix following the instructions on page 2–3.



12. 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.*



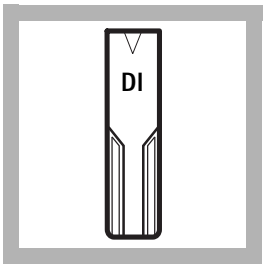
13. At the end of the 10-minute reaction period, pipette 0.5 mL of Stop Solution into each cuvette in the same order as the Color Developing Solution was added.

Slide the rack for 20 seconds using the technique described on page 2–3.

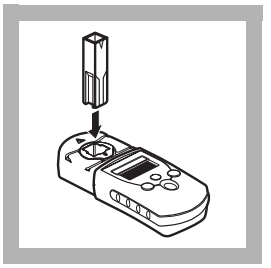
Note: *Blue solutions will turn yellow with the addition of the Stop Solution.*

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

Measuring the Color

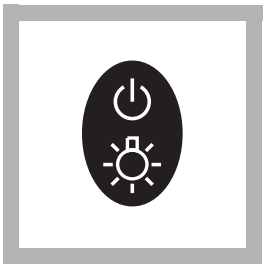


1. Label and fill a Zeroing Cuvette with deionized water. Wipe the outside of all the cuvettes with a tissue to remove water, smudges, and fingerprints.



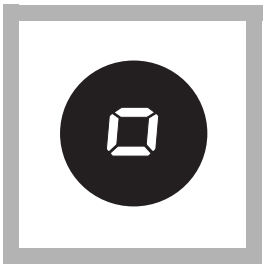
2. Insert the Zeroing Cuvette into the 1-cm micro-cuvette cell holder. Cover the zeroing cuvette with the instrument cap.

Note: *The arrow at the top of the cuvette should always face the display.*

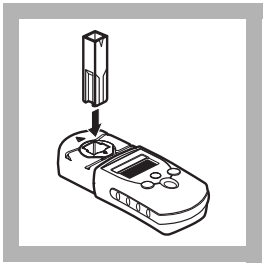


3. Press the **POWER** key to turn the meter on. The arrow can indicate either channel 1 or 2 for this method.

Note: *A user calibration should not be stored in the channel that you choose to use.*

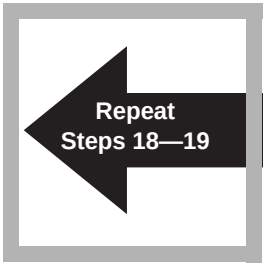


4. Press: **ZERO/SCROLL**. The instrument display will turn on and the display will show "---", followed by "0.000".



5. Remove the zeroing cuvette and insert the 0.1 ppb Alachlor calibrator into the cell holder. Cover the cuvette with the instrument cap.

6. Press: **READ/ENTER**. Record the absorbance value displayed.



7. Repeat step 5 and step 6 for all remaining calibrators and samples.

Record the absorbance value of each calibrator and sample. A Data Sheet (page 1–10) has been provided that may be photocopied and used for each set of tests.

1.4 Interpreting the Results

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

If the sample absorbance is...	Sample Alachlor Concentration is...
...less than calibrator absorbance	...greater than the calibrator value
...greater than calibrator absorbance	...less than the calibrator value

Example:

0.1 ppb Alachlor Calibrator: 0.450 Abs.

0.5 ppb Alachlor Calibrator: 0.230 Abs.

Sample #1: 0.150 Abs.

Sample #2: 0.350 Abs.

Sample #3: 0.550 Abs.

Sample #1 – Sample absorbance is less than the absorbance for both calibrators. Therefore the sample concentration is greater than 0.1 and greater than 0.5 ppb Alachlor.

Sample #2 – Sample absorbance is between the absorbance for the 0.1 and 0.5 ppb Alachlor calibrators. Therefore the concentration of Alachlor is between 0.1 and 0.5 ppb Alachlor.

Sample #3 – Sample absorbance is greater than the absorbance for both calibrators. Therefore the sample concentration is less than 0.5 ppb and less than 0.1 ppb Alachlor.

1.5 Storing and Handling Reagents

- Wear protective gloves and eyewear.
- When storing reagent sets for extended periods of time, keep them out of direct sunlight. Reagent shelf life can be extended by refrigerating the reagents and is strongly recommended.
- 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.

1.6 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:

Atrazine	Carbofuran	Carbendazim
Aldicarb	2, 4-D	
Diazoton	Chlorpyrifos	

1.7 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.

1.8 Sample Collection and Storage

Collect samples in a clean glass bottle. Do not pre-rinse the bottle with the sample. If there is greater than 0.5 ppm chlorine in the sample, dechlorinate with sodium thiosulfate (Cat. No. 323-32), using 1 drop per 25 mL of 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.

1.9 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.

Required Reagents

Description	Unit	Cat. No.
Reagent Set, Alachlor*	20 cuvettes	28130-00
Deionized water	500 mL	272-48

Required Apparatus

Battery, alkaline AAA 1.5 volts	4/pkg	46743-00
Caps, flip spout	2/pkg	25818-02
Instruction Manual, Immunoassay Pocket Colorimeter	each	59574-88
Marker, laboratory	each	20920-00
Pipettor, fixed volume, 0.5–mL	each	27641-00
Tips, for pipettor 27641-00	100/pkg	27642-00
Pocket Colorimeter, Immunoassay	each	59530-66
Rack, for 1-cm MicroCuvettes	each	48799-00
Talking Timer, Sper Scientific	each	27644-00
Wipes, disposable	box	20970-00

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

Chapter 2 Procedure for PCB in Soil

2.1 Overview

Soil samples are collected and PCB is extracted. Sample extracts, calibrators, and reagents are added to cuvettes coated with antibodies that are specific for PCB. Color develops and is then measured with a Pocket Colorimeter II. The test requires about 20 to 30 minutes for complete analysis. As many as 10 cuvettes can be run simultaneously.

The concentration of PCB in a sample is determined by comparing the developed color intensity to that of a PCB calibrator. The PCB concentration is inversely proportional to the color development: the lighter the color, the higher the PCB concentration. The Immunoassay Pocket Colorimeter provides a reading in terms of absorbance. This unit of measurement will allow you to compare your samples to the calibrators.

This method provides semi-quantitative screening based on thresholds for PCB in the following concentrations: 1 ppm, 5 ppm, 10 ppm and/or 50 ppm as Aroclor 1248.

2.2 Testing Hints

1. **Read the entire procedure before starting.** Identify and have ready all the necessary reagents, cuvettes, and other apparatus before beginning the analysis.
2. **Timing is critical**; follow instructions carefully.
3. **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 The 1-cm MicroCuvette Rack on page 2–3. Cuvettes can be mixed individually, but test results may not be as consistent.
4. Handle the cuvettes carefully. Scratches on the inside or outside may cause erroneous results. 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.)
5. Antibody cuvettes and enzyme conjugate are made in matched lots. Do not mix reagent lots.
6. Twenty Antibody Cuvettes are provided with each reagent set. One Antibody Cuvette will be used for each calibrator and each sample. Antibody Cuvettes are not reusable.
7. To avoid damaging the Color Developing Solution, do not expose it to direct sunlight.

8. 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:

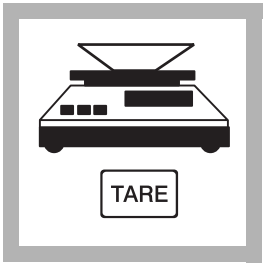
Range	Quantity of calibrator and sample extract used
1 ppm and 5 ppm	50 µL
10 ppm and 50 ppm	10 µL

9. 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.
10. 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.
11. 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.

2.3 Procedure for Soil Extraction

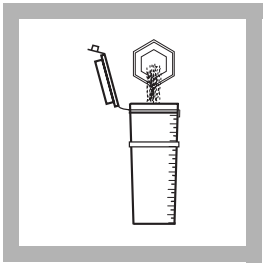
The Soil Extractant contains methyl alcohol which is poisonous and flammable. Before using this and other reagents, read the Material Safety Data Sheet (MSDS) for proper use of protective equipment and other safety information.

Note: *The manufacturer recommends wearing protective gloves for this procedure.*

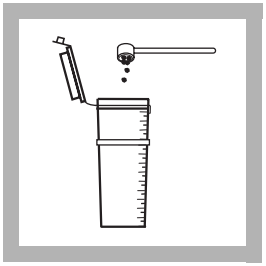


1. Place a plastic weighing boat on the AccuLab® balance. Zero or tare the balance.

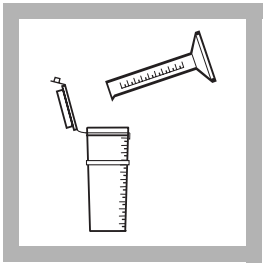
Note: *Refer to the instructions for operating the AccuLab balance.*



2. Weigh out 5.0 g of soil in the plastic weighing boat. Carefully pour the soil into the extraction vial.

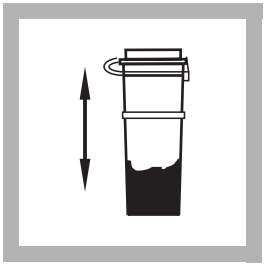


3. Use the 5 g scoop to add one scoop of sodium sulfate to the extraction vial.



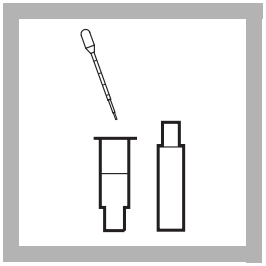
4. Use the graduated cylinder to transfer 10 mL of soil extractant into an extraction vial.

Note: *Read Testing Hints on page 1—19, before performing the test.*



5. Cap the extraction vial tightly and shake vigorously for one minute.

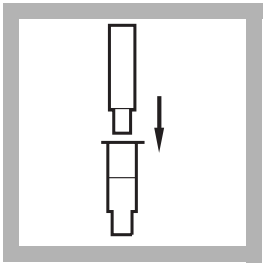
6. Allow to settle for at least one minute. Gently open the extraction vial.



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.

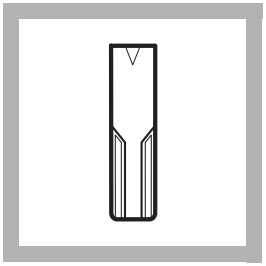
Use the resultant filtrate for the immunoassy in Procedure for Soil Extracts on page 1—22.

Note: *It may be necessary to place the filtration assembly on a table and press down on the plunger.*

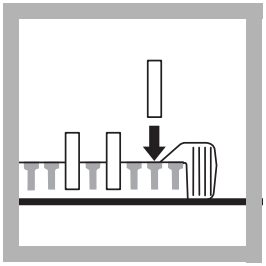
2.4 Procedure for Soil Extracts

Note: *Read Testing Hints on page 1–19 before proceeding.*

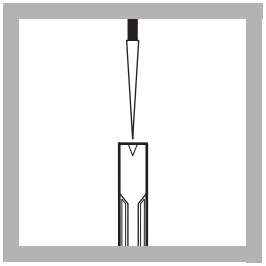
Immunoassay



1. Label an Antibody Cuvette for each Calibrator to be used and for each sample to be tested.

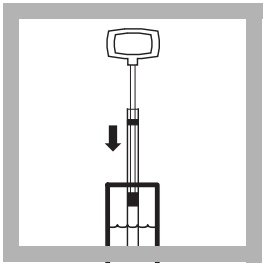


2. Place the cuvettes into the rack snugly.



3. Use the Precision Pipettor to pipet 0.5 mL of Diluent Solution into each Calibrator cuvette.

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

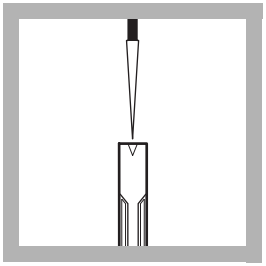


4. Have the necessary apparatus ready as the next three steps must be done without delays.

Use a WireTrol™ pipet to transfer the appropriate volume of Calibrator and sample extract into each cuvette.

Note: *When testing at the 1 ppm and/or 5 ppm levels use 50 µL of calibrator and sample. When testing at the 10 ppm and/or 50 ppm levels, use 10 µL of calibrator and sample.*

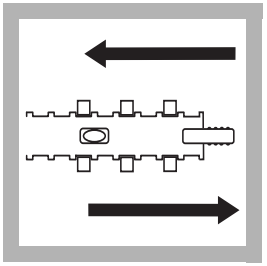
Note: *Use a separate capillary tube for each solution.*



5. Using the Precision Pipettor, immediately pipet 0.5 mL of PCB Enzyme Conjugate into each cuvette.

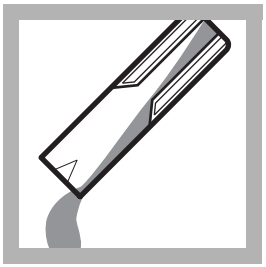
Note: *The same pipette tip can be used repeatedly for this step.*

6. Begin a 10-minute reaction time and proceed immediately to the next step.

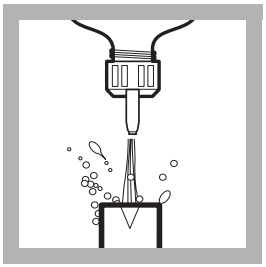


7. Mix the contents of the cuvettes for 30 seconds using the mixing technique described on page 2–3.

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



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

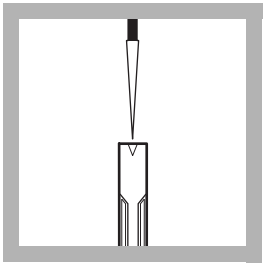


10. Wash each cuvette forcefully and thoroughly four times with deionized water. Empty the cuvettes into the waste container.

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*1



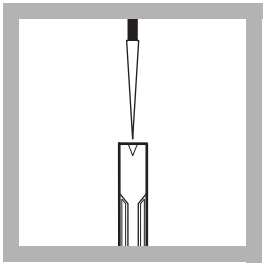
11. With the cuvettes still held snugly in the rack, pipet 0.5 mL of Color Developing Solution into each Antibody Cuvette.

Note: *Use a new pipette tip for each cuvette.*

12. Begin a 5-minute reaction period.

After 2.5 minutes have passed, slide the rack for 20 seconds using the technique described on page 2–3.

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



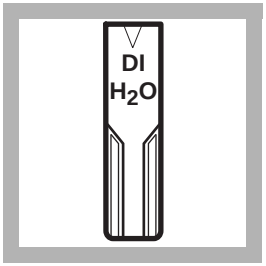
13. At the end of the 5-minute reaction period, pipette 0.5 mL of Stop Solution into each cuvette in the same order as the Color Developing Solution was added.

Slide the rack for 20 seconds using the technique described on page 2–3.

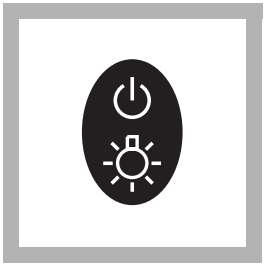
Note: *Blue solutions will turn yellow with the addition of the Stop Solution.*

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

Measuring the Color

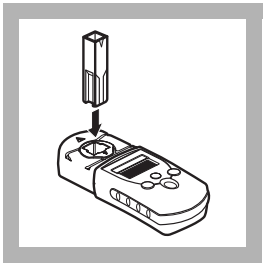


1. Label and fill a Zeroing Cuvette with deionized water. Wipe the outside of all the cuvettes with a tissue to remove water, smudges, and fingerprints.



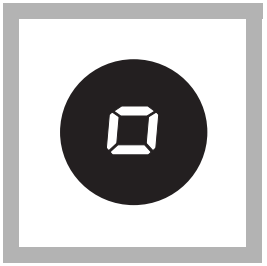
2. Press the **POWER** key to turn the meter on. The arrow can indicate either channel 1 or 2 for this method.

Note: *A user calibration should not be stored in the channel that you choose to use.*

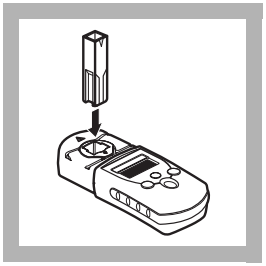


3. Insert the Zeroing Cuvette into the 1-cm micro-cuvette cell holder. Cover the zeroing cuvette with the instrument cap.

Note: *The arrow at the top of the cuvette should always face forward.*



4. Press: **ZERO/SCROLL**. The instrument display will turn on and the display will show "---", followed by "0.000".



5. Remove the zeroing cuvette and insert the first PCB calibrator into the cell holder. Cover the cuvette with the instrument cap.

6. Press: **READ/ENTER**. Record the absorbance value displayed. Hold the adapter in place when removing the cuvette.

7. Repeat step 5 and step 6 for all remaining calibrators, and samples.

Record the absorbance value of each calibrator and sample. A Data Sheet (page 1–10) has been provided that may be photocopied and used for each set of tests.

2.5 Interpreting the Results

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

If the sample absorbance is...	Sample PCB Concentration is...
...less than calibrator absorbance	...greater than the calibrator value
...greater than calibrator absorbance	...less than the calibrator value

Example of Count Values

1 ppm PCB Calibrator: 0.450 Abs.

5 ppm PCB Calibrator: 0.230 Abs.

Sample #1: 0.150 Abs.

Sample #2: 0.350 Abs.

Sample #3: 0.550 Abs.

Interpretation

Sample #1 – Sample absorbance is less than the absorbance for both calibrators. Therefore the sample concentration is greater than 1 ppm and greater than 5 ppm as Aroclor 1248.

Sample #2 – Sample absorbance is between the absorbance for the 1 ppm and the 5 ppm PCB calibrators. Therefore the concentration of PCB is between 1 ppm and 5 ppm PCB as Aroclor 1248.

Sample #3 – Sample absorbance is greater than the absorbance for both calibrators. Therefore the sample concentration is less than 5 ppm and less than 1 ppm PCB as Aroclor 1248.

2.6 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.

2.7 Sensitivity

The PCB immunoassay cannot differentiate between the various Aroclors, but it detects their presence in differing degrees.

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-dicholorophenyl	pentachlorophenol	1,4-dichlorobenzene
2,4,5-trichlorphenyl	1,2-dichlorobenzene	1,2,4-trichlorobenzene

2.8 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.

2.9 Summary of Method

Hach immunoassay tests use antigen/antibody reactions to test for specific organic compounds in water and soil. PCB-specific antibodies, attached to the walls of plastic cuvettes, 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.

Required Reagents

Description	Unit	Cat. No.
Reagent Set, PCB in Soil*	20 cuvettes	27735-00

Required Apparatus

Battery, alkaline AAA 1.5 volts	4/pkg	46743-00
Caps, flip spout	2/pkg	25818-02
Instrument Manual, Immunoassay Pocket Colorimeter	each	59574-88
Marker, laboratory	each	20920-00
Pipettor, fixed volume, 0.5–mL	each	27641-00
Tips, for pipettor 27641-00	100/pkg	27642-00
Pocket Colorimeter, Immunoassay	each	59530-66
Rack, for 1-cm Micro Cuvettes	each	48799-00
Talking Timer, Sper Scientific	each	27644-00
Wipes, disposable	box	20970-00
For Soil Extraction only:		
Soil Extraction Kit	each	27751-00
Includes:		
Balance, Acculab Pocket Pro 150B	each	27969-00
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
Soil Scoop, 5 g, 4.25 cc	each	26572-05
Weighing Boat, 8.9 cm square	20/pkg	21790-20
Soil Extraction Refill Kit (resupplies the consumables for 27751-00)	each	27752-00

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

Chapter 3 Procedure for Metolachlor in Water

3.1 Overview

Metolachlor is a widely-used herbicide for the control of grasses and broadleaf weeds. It is sold by several companies under a variety of trade names.

Samples, calibrators, and reagents are added to cuvettes coated with antibodies specific for Metolachlor. The resultant color is measured with a Pocket Colorimeter II. The concentration of Metolachlor in a sample is determined by comparing the developed color intensity to that of a Metolachlor calibrator. The Metolachlor concentration is inversely proportional to the color development: the lighter the color, the higher the Metolachlor concentration.

This method provides semi-quantitative screening based on thresholds for Metolachlor in the following concentrations: 0.5 ppb and/or 2.0 ppb. Other concentrations of Metolachlor in water can be tested by first diluting the sample with deionized water. (See Summary of Method on page 1–35.)

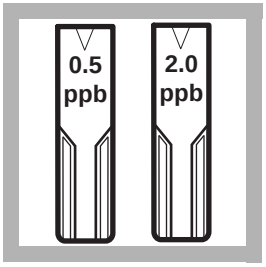
3.2 Testing Hints

1. **Read the entire procedure before starting.** Identify and have ready all the necessary reagents, cuvettes, and other apparatus before beginning the analysis.
2. **Timing is critical**; follow instructions carefully.
3. **A consistent technique when mixing the cuvettes is critical to this test.** Using the cuvette rack and mixing as described in The 1-cm MicroCuvette Rack on page 2–3, yields the best results. Cuvettes can be mixed individually, but test results may not be as consistent.
4. The test requires about 30 minutes for complete analysis. As many as 20 cuvettes (18 samples and 2 calibrators) can be run simultaneously.
5. Handle the cuvettes carefully. Scratches on the inside or outside may cause erroneous results. Gently 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.)
6. Antibody cuvettes and enzyme conjugate are made in matched lots. Do not mix reagent lots.

7. Twenty Antibody Cuvettes are provided with each reagent set. One Antibody Cuvette will be used for each calibrator and each sample. Antibody Cuvettes are not reusable.
8. The Immunoassay Pocket Colorimeter provides a reading in terms of absorbance. This unit of measurement will allow you to compare your samples to the calibrators.
9. To avoid damaging the Color Developing Solution, do not expose it to direct sunlight.
10. 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.

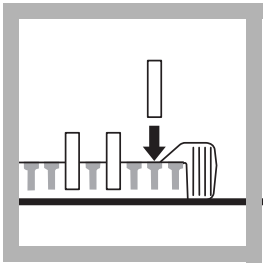
3.3 Procedure

Immunoassay

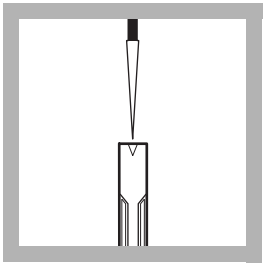


1. Label two Antibody Cuvettes: one for the 0.5 ppb Metolachlor Calibrator, and the other for the 2.0 ppb Metolachlor Calibrator. Label the required number of cuvettes with sample identification.

Note: *As many as 18 samples may be tested with each two calibrators.*



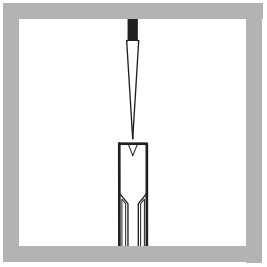
2. Place the cuvettes snugly into the rack.



3. Pipet 0.5 mL of the 0.5 ppb Metolachlor Calibrator into the appropriate cuvette.

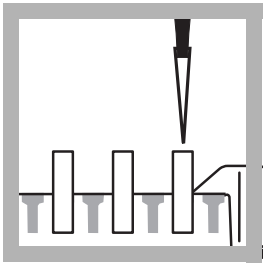
Pipet 0.5 mL of the 2.0 ppb Metolachlor Calibrator into the second cuvette.

Note: *Use a new pipette tip for each cuvette.*



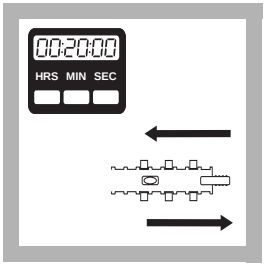
4. Pipet 0.5 mL of each sample to be tested into the appropriate cuvette.

Note: *Use a new pipette tip for each sample.*

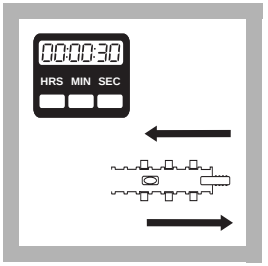


5. Using the Precision Pipettor, immediately pipet 0.5 mL of Metolachlor Enzyme Conjugate into each cuvette.

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

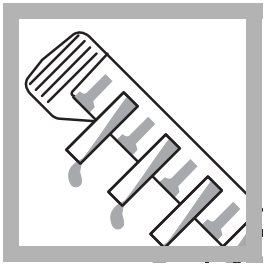


6. Begin a 20-minute reaction period and mix following the instructions on page 2–3.

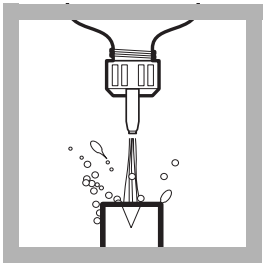


7. After 10 minutes, mix the contents of the rack a second time for a period of 30 seconds using the same technique.

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



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

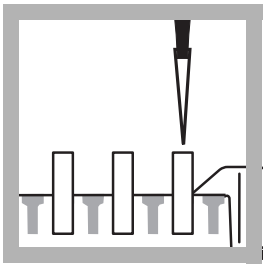


9. Wash each cuvette forcefully and thoroughly four times with deionized water. Empty the cuvettes into the waste container.

Note: *Ensure most of the water is drained from the cuvettes by turning the cuvettes upside down and gently tapping them on a paper towel to drain.*

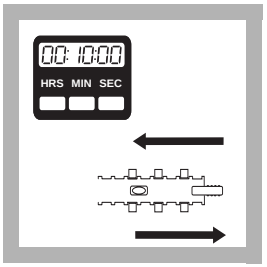
Color Development

Note: *Timing is critical; follow instructions carefully.*

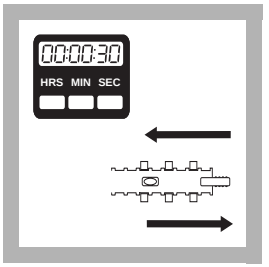


10. With the cuvettes still held snugly in the rack, pipet 0.5 mL of Color Developing Solution into each Antibody Cuvette.

Note: *Use a new pipette tip for each cuvette.*

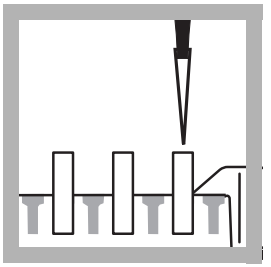


11. Begin a 10-minute reaction period and mix following the instructions on page 2–3.



12. 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.*



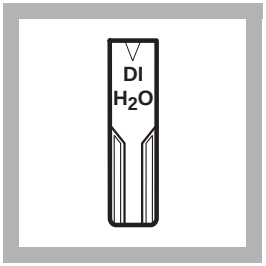
13. At the end of the 10-minute reaction period, pipette 0.5 mL of Stop Solution into each cuvette in the same order as the Color Developing Solution was added.

Slide the rack for 20 seconds using the technique described on page 2–3.

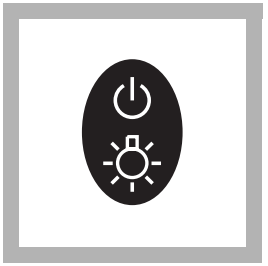
Note: *Blue solutions will turn yellow with the addition of the Stop Solution.*

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

Measuring the Color

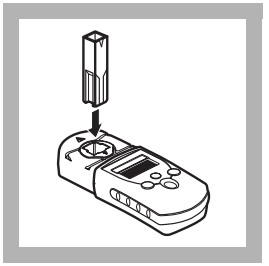


14. Label and fill a Zeroing Cuvette with deionized water. Wipe the outside of all the cuvettes with a tissue to remove water, smudges, and fingerprints.



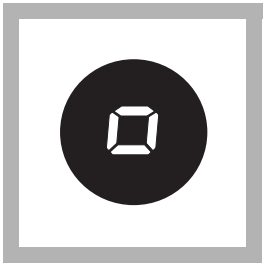
15. Press the **POWER** key to turn the meter on. The arrow can indicate either channel 1 or 2 for this method.

Note: *A user calibration should not be stored in the channel that you choose to use.*

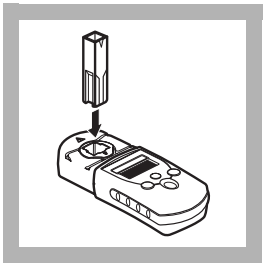


16. Insert the Zeroing Cuvette into the 1-cm micro-cuvette cell holder. Cover the zeroing cuvette with the instrument cap.

Note: *The arrow at the top of the cuvette should always face the display.*

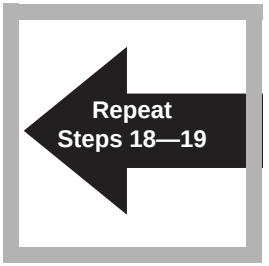


17. Press: **ZERO/SCROLL**. The instrument display will turn on and the display will show "---", followed by "0.000".



18. Remove the zeroing cuvette and insert the 0.5 ppb Metolachlor calibrator into the cell holder. Cover the cuvette with the instrument cap.

19. Press: **READ/ENTER**. Record the absorbance value displayed.



20. Repeat step 18 and step 19 for all remaining calibrators and samples.

Record the absorbance value of each calibrator and sample. A Data Sheet (page 1–10) has been provided that may be photocopied and used for each set of tests.

3.4 Interpreting the Results

There is an inverse relationship between the concentration of Metolachlor and the absorbance. In other words, the higher the absorbance value, the lower the concentration of Metolachlor.

If the sample absorbance is...	Sample Metolachlor Concentration is...
...less than calibrator absorbance	...greater than the calibrator value
...greater than calibrator absorbance	...less than the calibrator value

Example:

0.5 ppb Metolachlor Calibrator: 0.450 Abs.

2.0 ppb Metolachlor Calibrator: 0.230 Abs.

Sample #1: 0.150 Abs.

Sample #2: 0.350 Abs.

Sample #3: 0.550 Abs.

Sample #1 – Sample absorbance is less than the absorbance for both calibrators. Therefore the sample concentration is greater than 0.5 and greater than 2.0 ppb Metolachlor.

Sample #2 – Sample absorbance is between the absorbencies the 0.5 and 2.0 ppb Metolachlor calibrators. Therefore the concentration of Metolachlor is between 0.5 and 2.0 ppb Metolachlor.

Sample #3 – Sample absorbance is greater than the absorbance for both calibrators. Therefore the sample concentration is less than 2.0 ppb and less than 0.5 ppb Metolachlor.

3.5 Storing and Handling Reagents

- Wear protective gloves and eyewear.
- When storing reagent sets for extended periods of time, keep them out of direct sunlight. Reagent shelf life can be extended by refrigerating the reagents and is strongly recommended.
- 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.

3.6 Sensitivity

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

Compound	Concentration to give a positive response of 0.5 ppb Metolachlor	Concentration to give a positive response of 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	Chlorpyirfos	

3.7 Sample Collection and Storage

Collect samples in a clean glass bottle. Do not pre-rinse the bottle with the sample. If there is greater than 0.5 ppm chlorine in the sample, dechlorinate with sodium thiosulfate (Cat. No. 323-32), using 1 drop per 25 mL of 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.

3.8 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
Deionized water	500 mL	272-48

Required Apparatus

Battery, alkaline AAA 1.5 volts	4/pkg	46743-00
Caps, flip spout	2/pkg	25818-02
Instruction Manual, Immunoassay Pocket Colorimeter	each	59574-88
Marker, laboratory	each	20920-00
Pipettor, fixed volume, 0.5–mL	each	27641-00
Tips, for pipettor 27641-00	100/pkg	27642-00
Pocket Colorimeter, Immunoassay	each	59530-66
Rack, for 1-cm MicroCuvettes	each	48799-00
Talking Timer, Sper Scientific	each	27644-00
Wipes, disposable	box	20970-00

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

Chapter 4 Procedure for Atrazine in Water

4.1 Overview

Atrazine is a widely-used herbicide for the control of broadleaf weeds. It is sold by several companies under a variety of trade names. Atrazine is stable in water and is a regulated substance. The Maximum Contaminant Level for Atrazine in the United States has been set at 3 parts per billion (ppb) by the U.S. Environmental Protection Agency.

Samples, calibrators, and reagents are added to cuvettes coated with antibodies specific for Atrazine. The resultant color is measured with a Pocket Colorimeter II. The concentration of Atrazine in a sample is determined by comparing the developed color intensity to that of an Atrazine calibrator. The Atrazine concentration is inversely proportional to the color development: the lighter the color, the higher the Atrazine concentration.

This method provides semi-quantitative screening based on thresholds for Atrazine in the following concentrations: 0.1 ppb, 0.5 ppb, and/or 3.0 ppb.

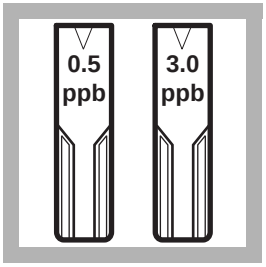
4.2 Testing Hints

1. **Read the entire procedure before starting.** Identify and have ready all the necessary reagents, cuvettes, and other apparatus before beginning the analysis.
2. **Timing is critical**; follow instructions carefully.
3. **A consistent technique when mixing the cuvettes is critical to this test.** Using the cuvette rack and mixing, as described in The 1-cm MicroCuvette Rack on page 2–3 , yields the best results. Cuvettes can be mixed individually, but test results may not be as consistent.
4. The test requires about 30 minutes for complete analysis. As many as 20 cuvettes (18 samples and 2 calibrators) can be run simultaneously.
5. Handle the cuvettes carefully. Scratches on the inside or outside may cause erroneous results. Gently 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.)
6. Antibody cuvettes and enzyme conjugate are made in matched lots. Do not mix reagent lots.

7. Cuvettes should fit snugly into the cuvette rack. Do not force the cuvettes into the rack as they may be difficult to remove and the contents may spill.
8. 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. (See Measuring the Color on page 1–42.)
9. Five Zeroing Cuvettes are provided with each reagent set. The Zeroing Cuvettes can be re-used.
10. 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.
11. The Immunoassay Pocket Colorimeter provides a reading in absorbance. This unit of measurement will allow you to compare your samples to the calibrators.
12. To avoid damaging the Color Developing Solution, do not expose it to direct sunlight.
13. For European applications, use the 0.1 ppb and 0.5 ppb Atrazine calibrators rather than the 0.5 ppb and 3.0 ppb calibrators.

4.3 Procedure

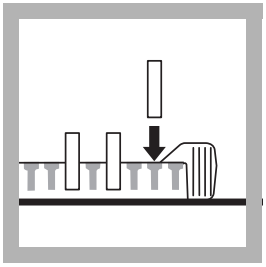
Immunoassay



1. Label two Antibody Cuvettes: one for the 0.5 ppb Atrazine Calibrator, and the other for the 3.0 ppb Atrazine Calibrator. Label the required number of cuvettes with sample identification.

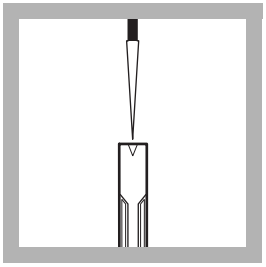
Note: *As many as 18 samples may be tested with each two calibrators.*

Note: *For European applications, use 0.1 ppb and 0.5 ppb calibrators.*



2. Place the cuvettes into the rack snugly.

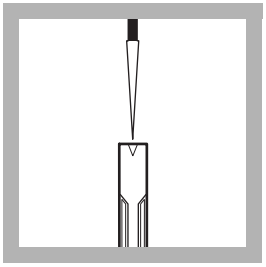
Note: *Cuvettes should remain in the rack when it is inverted and tapped gently.*



3. Pipet 0.5 mL of the 0.5 ppb Atrazine Calibrator into the appropriate cuvette.

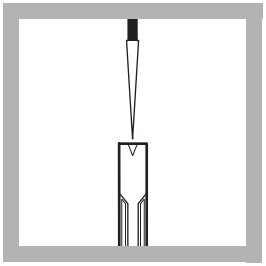
Pipet 0.5 mL of the 3.0 ppb Atrazine Calibrator into the second cuvette.

Note: *Use a new pipette tip for each cuvette.*



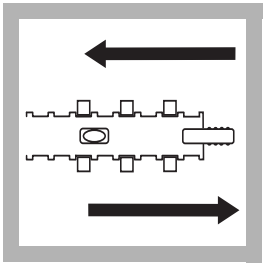
4. Pipet 0.5 mL of each sample to be tested into a cuvette.

Note: *Use a new pipette tip for each sample.*



5. Immediately pipet 0.5 mL of Atrazine Enzyme Conjugate into each cuvette.

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



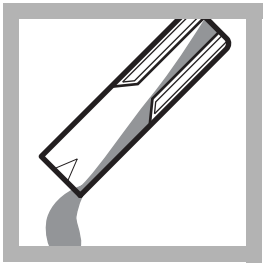
6. Set the rack on a hard, flat surface that is at least twice the length of the rack.

Holding one end of the rack, slide it back and forth along its long axis for 30 seconds to mix. The rack should move through a distance equal to its length in each direction.

7. Begin a 20-minute reaction time.

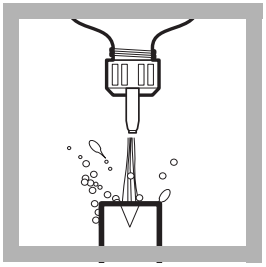
When 10 minutes have passed, shake the rack for 20 seconds as described in step 6

Note: *Time this step carefully.*



8. After the 20-minute period, discard the contents of all the cuvettes. Use an appropriate waste container.

Note: *If all the cuvettes are snugly fitted into the rack, they may be emptied simultaneously by inverting the rack over the waste container.*



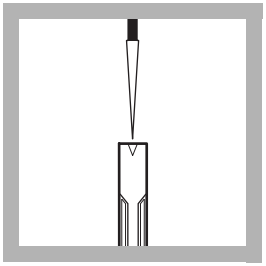
9. Wash each cuvette forcefully and thoroughly four times with deionized water. Empty the cuvettes into the waste container.

Note: *Ensure most of the water is drained from the cuvettes by turning the cuvettes upside down and gently tapping them on a paper towel to drain.*

Note: *Rinsing may be done with the cuvettes still in the rack.*

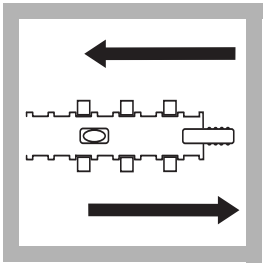
Color Development

Note: *Timing is critical; follow instructions carefully*



10. 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.*



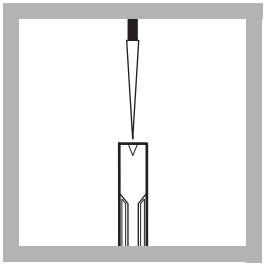
11. Set the rack on a hard, flat surface that is at least twice the length of the rack.

Holding one end of the rack, slide it back and forth along its long axis for 30 seconds to mix. The rack should move through a distance equal to its length in each direction.

12. Begin a 10-minute reaction period.

After five minutes have passed, slide the rack for 20 seconds using the technique described in step 11.

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



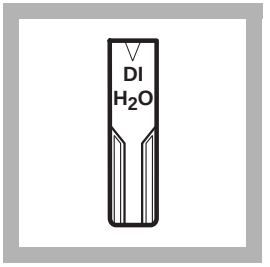
13. At the end of the 10-minute reaction period, pipette 0.5 mL of Stop Solution into each cuvette in the same order as the Color Developing Solution was added.

Slide the rack for 20 seconds using the technique described in step 11.

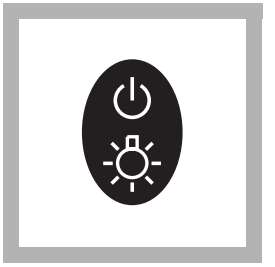
Note: *Blue solutions will turn yellow with the addition of the Stop Solution.*

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

Measuring the Color

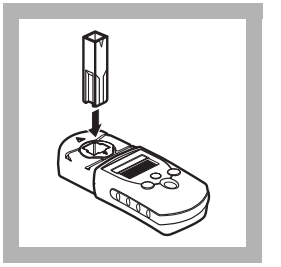


14. Label and fill a Zeroing Cuvette with deionized water. Wipe the outside of all the cuvettes with a tissue to remove water, smudges, and fingerprints.



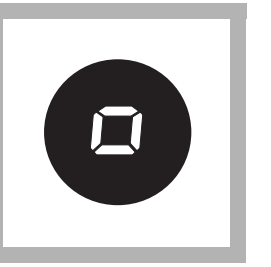
15. Press the **POWER** key to turn the meter on. The arrow can indicate either channel 1 or 2 for this method.

Note: *A user calibration should not be stored in the channel that you choose to use.*

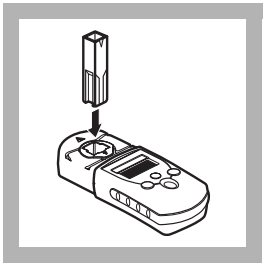


16. Insert the Zeroing Cuvette into the 1-cm micro-cuvette cell holder. Cover the zeroing cuvette with the instrument cap.

Note: *The arrow at the top of the cuvette should always face the key pad.*

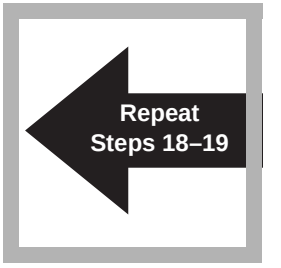


17. Press: **ZERO/SCROLL**. The instrument display will turn on and the display will show "---", followed by "0.000".

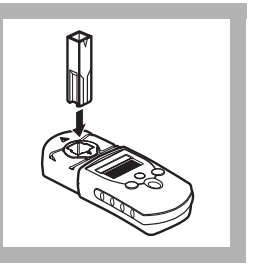


18. Remove the zeroing cuvette and insert the 0.5 ppb Atrazine calibrator into the cell holder. Cover the cuvette with the instrument cap.

19. Press: **READ/ENTER**. Record the absorbance value displayed.



20. Repeat step 18 and step 19 for the remaining calibrator.



21. Insert the first sample cuvette into the cell holder. Cover the cuvette with the instrument cap.

22. Press: **READ/ENTER**. Record the absorbance value displayed.

23. Repeat step 21 and *step* 22 for all of the remaining samples.

4.4 Interpreting the Results

There is an inverse relationship between the concentration of Atrazine and the absorbance. In other words, the higher the count value, the lower the concentration of Atrazine.

If the sample absorbance is...	Sample Atrazine Concentration is...
...less than calibrator absorbance	...greater than the calibrator value
...greater than calibrator absorbance	...less than the calibrator value

Example:

0.5 ppb Atrazine Calibrator: 0.450 Abs.

3.0 ppb Atrazine Calibrator: 0.230 Abs.

Sample #1: 0.150 Abs

Sample #2: 0.350 Abs

Sample #3: 0.550 Abs

Sample #1 – Sample absorbance is less than the absorbance for both calibrators. Therefore the sample concentration is greater than 0.5 and greater than 3.0 ppb Atrazine.

Sample #2 – Sample absorbance is between the absorbance for the 0.5 and 3.0 ppb Atrazine calibrators. Therefore the concentration of Atrazine is between 0.5 and 3.0 ppb Atrazine.

Sample #3 – Sample absorbance is greater than the absorbance for both calibrators. Therefore the sample concentration is less than 3.0 ppb and less than 0.5 ppb Atrazine.

4.5 Storing and Handling Reagents

- Wear protective gloves and eye wear.
- When storing reagent sets for extended periods of time, keep them out of direct sunlight. Reagent shelf life can be extended by refrigerating the reagents and is strongly recommended.
- 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.

4.6 Sensitivity

The Atrazine immunoassay test cannot differentiate between the various triazines 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 result at 3 ppb (in ppb)
Ametryne	1
Atrazine	3
Atrazine, de-ethylated	115
Atrazine, de-isopropyl	714
Cyanazine	460
Cyromazine	1200
Prometon	8
Prometryne	0.7
Propazine	2.3
Simetryne	5.4
Simazine	37
Terbuthylazine	91
Terbutryne	8.3

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

Alachlor	Carbofuran	Metaolachlor
Aldicarb	Diaminoatrazine	2,4-D
Carbendazim	Melamine	

4.7 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.

4.8 Summary of Method

The 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

Battery, alkaline AAA 1.5 volts	4/pkg	46743-00
Caps, flip spout	2/pkg	25818-02
Instruction Manual, Immunoassay Pocket Colorimeter	each	59574-88
Marker, laboratory	each	20920-00
Pipettor, fixed volume, 0.5–mL	each	27641-00
Tips, for pipettor 27641-00	100/pkg	27642-00
Pocket Colorimeter, Immunoassay	each	59530-66
Rack, for 1-cm Micro Cuvettes	each	48799-00
Talking Timer, Sper Scientific	each	27644-00
Wipes, disposable	box	20970-00

Optional Reagents

Reagent Set, Atrazine	100 cuvettes	27627-10

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

Chapter 5 Procedure for TPH in Soil and Water

5.1 Overview

This TPH test can be used for both soil and water testing. When testing soil, purchase the Soil Extraction Kit (see page 1–58) and perform the Procedure for Soil Extraction on page 1–48. When testing water samples only, proceed directly with the Immunoassay Procedure for Soil Extracts and Water Samples on page 1–50.

Soil samples or water samples are collected. If testing soils, first extract the TPH using the procedure in *Chapter* 5.3. Water samples or soil extracts, calibrators, and reagents are added to cuvettes that are coated with antibodies specific for TPH. Color develops and is then measured with a Hach Pocket Colorimeter. The test requires about 20 to 30 minutes for complete analysis. As many as 10 cuvettes can be run simultaneously.

The concentration of TPH in a sample is determined by comparing the developed color intensity to that of a TPH calibrator. The TPH concentration is inversely proportional to the color development: the lighter the color, the higher the TPH concentration. The Immunoassay Pocket Colorimeter provides a reading in terms of absorbance. This unit of measurement will allow you to compare your samples to the calibrators.

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

Higher concentrations in water can be tested by first diluting the sample with deionized water (see Diluting Water Samples on page 1–56). Test for other TPH compounds (i.e., gasoline) by using the conversion factors given in Table 1 and Table 2 on page 1–56.

5.2 Tips and Techniques

1. **Read the entire procedure before starting.** Identify and have ready all the necessary reagents, cuvettes, and other apparatus before beginning the analysis.
2. **Timing is critical**; follow instructions carefully.
3. **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 The 1-cm

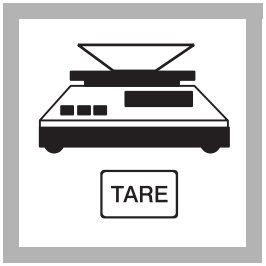
MicroCuvette Rack on page 2–3. Cuvettes can be mixed individually, but test results may not be as consistent.

4. 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.)
5. Antibody cuvettes and enzyme conjugate are made in matched lots. Do not mix reagent lots.
6. 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.
7. To avoid damaging the Color Developing Solution, do not expose it to direct sunlight.
8. 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.
9. If the soil sample contains more than 20% moisture, it must be dried before analysis. Please contact Technical Support (see page 2–20) for further information about soil drying techniques.

5.3 Procedure for Soil Extraction

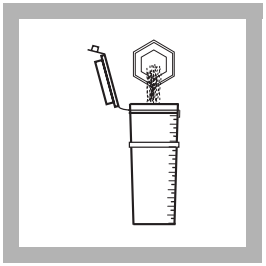
The Soil Extractant contains methyl alcohol which is poisonous and flammable. Before using this and other reagents, read the Material Safety Data Sheet (MSDS) for proper use of protective equipment and other safety information.

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



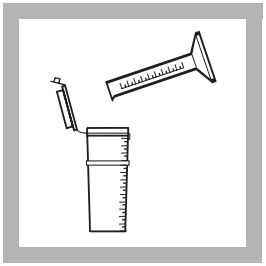
1. Place a plastic weighing boat on the AccuLab® balance. Zero, or tare, the balance.

Note: *Refer to the operating Instructions for AccuLab Balance.*

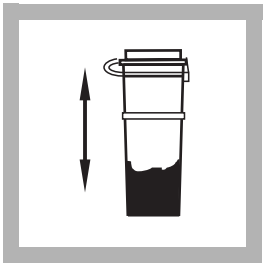


2. Weigh out 10 g of soil in the plastic weighing boat. Carefully pour the soil into an extraction vial.

3. Use the 5 g scoop to add one scoop of sodium sulfate to the extraction vial.

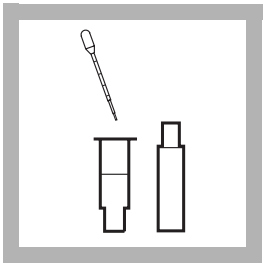


4. Use the graduated cylinder to transfer 10 mL of Soil Extractant into the extraction vial.



5. Cap the extraction vial tightly and shake vigorously for one minute.

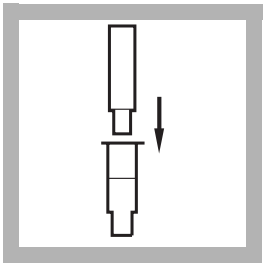
6. Allow to settle for at least one minute. Carefully open the extraction vial.



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*

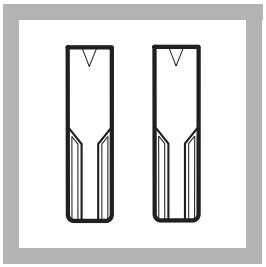


8. Insert the filtration plunger into the filtration barrel. Press firmly on the plunger until the sample extract is forced upward into the center of the plunger. Use the resultant filtrate for the immunoassay in the Immunoassay Procedure for Soil Extracts and Water Samples on page 1–50.

Note: *You may need to place the filtration assembly on a table and press down on the plunger.*

5.4 Immunoassay Procedure for Soil Extracts and Water Samples

Immunoassay

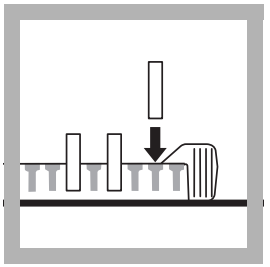


9. Label an Antibody Cuvette for each calibrator and each sample to be tested.

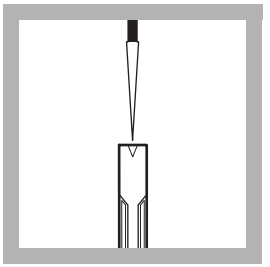
To select the proper calibrators, see Table 1 on page 1–55.

Note: *As many as 10 cuvettes may be tested at one time and may comprise any combination of samples and calibrators.*

Note: *See example below.*

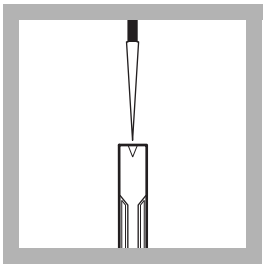


10. Place the cuvettes into the rack snugly.



11. Use the Precision Pipettor to pipet 0.5 mL of Diluent Solution into each Calibrator cuvette.

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



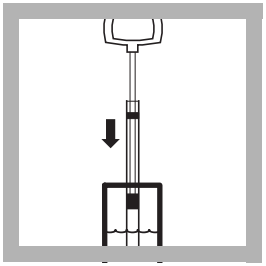
12. **If testing soil:** Pipet 0.5 mL of *Diluent Solution* into each sample cuvette.

If testing water: Pipet 0.5 mL of each *water sample* into the appropriate cuvette.

Note: *Use a new pipette tip for each water sample.*

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.

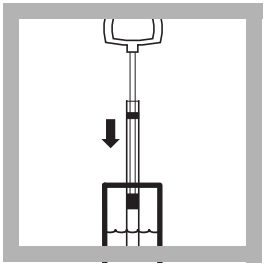


13. Have the necessary apparatus at hand as the next three steps must be done without delays.

Use a WireTrol™ pipet to transfer 50 µL of each calibrator to be used into the calibrator cuvettes.

Mix the cuvettes after each addition.

Note: *Use a separate capillary tube for each solution.*

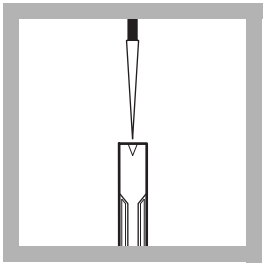


14. **If testing soil:** Use a WireTrol pipet to transfer 50 µL of the filtered extract from step 8 into the appropriately labeled cuvette.

Mix 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

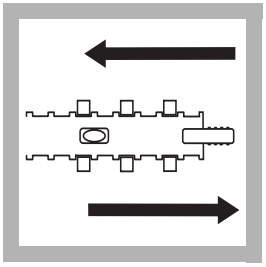
Note: *Use a separate capillary tube for each solution.*



15. Using the Precision Pipettor, immediately pipet 0.5 mL of TPH Enzyme Conjugate into each calibrator and sample cuvette.

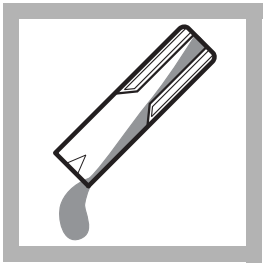
Note: *The same pipette tip can be used repeatedly for this step.*

16. Begin a 10-minute reaction time and proceed immediately to the next step.

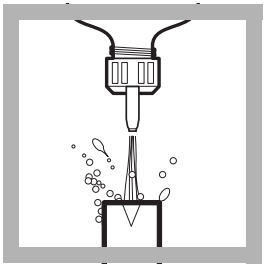


17. Mix the contents of the cuvettes for 30 seconds using the technique described on page 1–16.

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



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



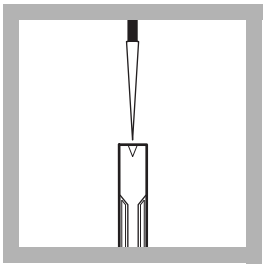
20. Wash each cuvette forcefully and thoroughly four times with deionized water. Empty the rinse water into the waste container.

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.*

Note: *Read Tips and Techniques on page 1–47 before proceeding.*

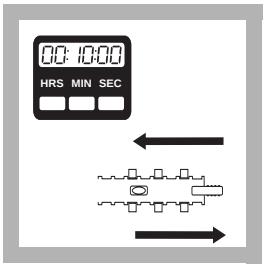
Color Development

Note: *Timing is critical; follow instructions carefully.*



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

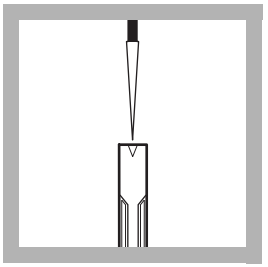
Note: *Use a new pipette tip for each cuvette.*



22. Begin a 10-minute reaction period and mix following the instructions on page 1–16.

23. 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.*



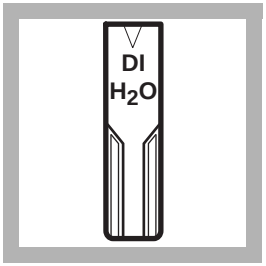
24. At the end of the 10-minute reaction period, pipette 0.5 mL of Stop Solution into each cuvette in the same order as the Color Developing Solution was added in step 21.

Slide the rack for 20 seconds using the technique described on page *1–16*.

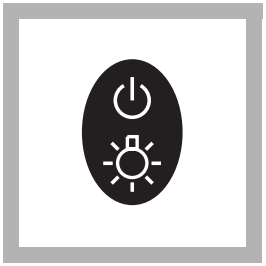
Note: *Blue solutions will turn yellow with the addition of the Stop Solution.*

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

Measuring the Color

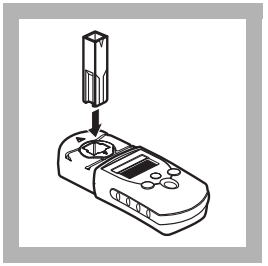


25. Label and fill a Zeroing Cuvette with deionized water. Wipe the outside of all the cuvettes with a tissue to remove water, smudges, and fingerprints.



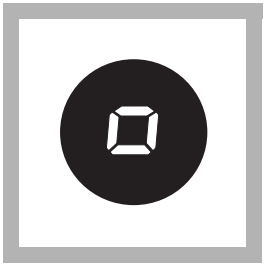
26. Press the **POWER** key to turn the meter on. The arrow can indicate either channel 1 or 2 for this method.

Note: *A user calibration should not be stored in the channel that you choose to use.*

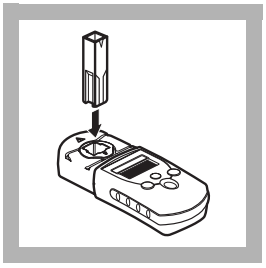


27. Insert the Zeroing Cuvette into the adapter. Cover with the instrument cap.

Note: *The arrow at the top of the cuvette should always face forward.*

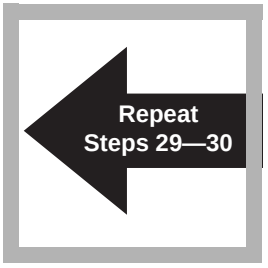


28. Press: **ZERO/SCROLL**. The instrument display will turn on and the display will show "---", followed by "0.000".



29. Remove the zeroing cuvette and insert the first TPH calibrator into the cell holder. Cover the cuvette with the instrument cap.

30. Press: **READ/ENTER**. Record the absorbance value displayed. Hold the adapter in place when removing the cuvette.



31. Repeat step 29 and step 30 for all remaining calibrators and samples.

Record the absorbance value of each calibrator and sample. A Data Sheet (page 1–10) has been provided that may be photocopied and used for each set of tests.

5.5 Interpreting the Results

There is an inverse relationship between the concentration of TPH and the absorbance value. In other words, the higher the absorbance value, the lower the concentration of TPH.

If the sample absorbance is...	the sample TPH Concentration is...
...less than calibrator absorbance	...greater than the calibrator value
...greater than calibrator absorbance	...less than the calibrator value

Example of Count Values

TPH Calibrator #1: 0.800 Abs.

TPH Calibrator #2: 0.600 Abs.

Sample #1: 0.150 Abs.

Sample #2: 0.700 Abs.

Sample #3: 0.950 Abs.

Interpretation

Interpretation for a soil sample:

Sample #1 – Sample absorbance is less than the absorbance for both calibrators. Therefore the sample concentration of TPH is greater than both 20 ppm and 50 ppm diesel fuel.

Sample #2 – Sample absorbance is between the absorbance for the TPH calibrators. Therefore the sample concentration of TPH is between 20 ppm and 50 ppm diesel fuel.

Sample #3 – Sample absorbance is greater than the absorbance 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 absorbance is less than the absorbance for both calibrators. Therefore the sample concentration of TPH is greater than both 2 ppm and 5 ppm diesel fuel.

Sample #2 – Sample absorbance is between the absorbance for the TPH calibrators. Therefore the sample concentration of TPH is between 2 ppm and 5 ppm diesel fuel.

Sample #3 – Sample absorbance is greater than the absorbance for both calibrators. Therefore the sample

* See Diluting Water Samples on page 1–56 for instructions on how to correct for sample dilutions.

concentration of TPH is less than both 2 ppm and 5 ppm diesel fuel.

5.6 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 eyes, wash thoroughly for 15 minutes with cold water and seek immediate medical help.

5.7 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 to select the proper TPH calibrator for the compound, sample, and range you want to test. To test concentrations in water higher than those covered by the calibrators, dilute the original sample as described in Diluting Water Samples on page 1–56.

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

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

Table 1 (Continued)

Compound	TPH calibrator #1	TPH calibrator #2	TPH calibrator #3	TPH calibrator #4
	ppm			
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

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

5.7.1 Diluting Water Samples

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 undiluted sample from Table 3. Use the multiplier value for the chosen quantity to multiply the value from Table 1 or Table 2, above.

Table 3

mL Sample	Multiplier	mL Sample	Multiplier
0.5	100	5.0	10
1.0	50	10.0	5
2.0	25	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.

5.8 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.

5.9 Summary of Method

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.

Required Reagents

Description	Unit	Cat. No.
Reagent Set, TPH *	20 cuvettes	27743-00
Deionized water	500 mL	272-48

Required Apparatus

Battery, alkaline AAA, 1.5 volts	4/pkg	46743-00
Caps, flip spout	2/pkg	25818-02
Instruction Manual, Immunoassay Pocket Colorimeter	each	59574-88
Marker, laboratory	each	20920-00
Pipettor, fixed volume, 0.5-mL	each	27641-00
Tips, for pipettor 27641-00	100/pkg	27642-00
Pocket Colorimeter™, Immunoassay	each	59530-66
Rack, for 1-cm Micro Cuvettes	each	48799-00
Talking Timer, Sper Scientific	each	27644-00
Wipes, disposable	box	20970-00

For Soil Extraction only:

Soil Extraction Kit	each	27751-00
Includes:		
Balance, Acculab Pocket Pro 150B	each	27969-00
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
Soil Scoop, 5 g, 4.25 cc	each	26572-05
Weighing Boat, 8.9 cm square	20/pkg	21790-20
Soil Extraction Refill Kit (resupplies the consumables for 27751-00)	each	27752-00

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

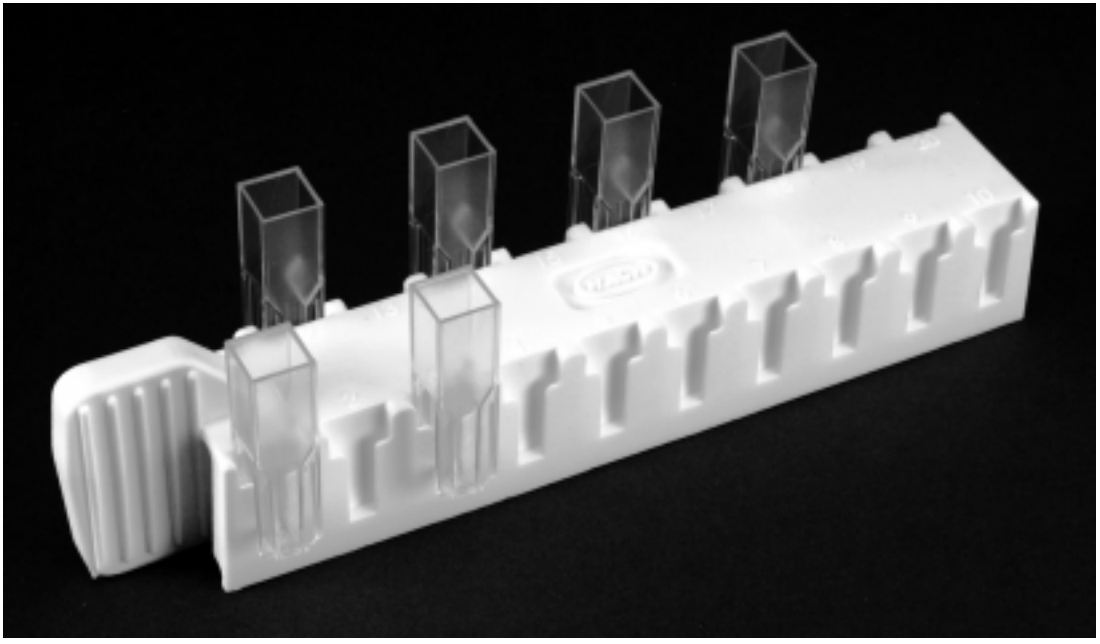
Section 2 Instrument Manual

Accessories

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

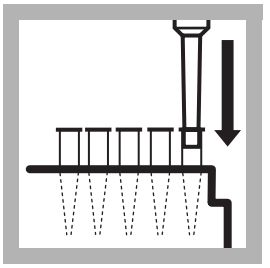


Loading the Rack — The cuvette rack is designed so that it may be inverted with the cuvettes in place. Identify each cuvette with a sample or calibrator number and place all the cuvettes in the rack before beginning the procedure. Fit the cuvettes snugly into the rack, but do not force them or they may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

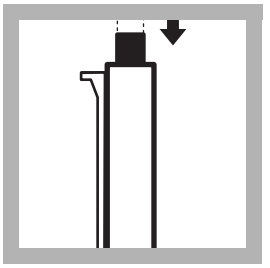
Mixing — Set the rack on a hard, flat surface that is at least twice the length of the rack. Hold the rack by one end and vigorously slide it back and forth along its long axis for 30 seconds.

The rack should move through a distance equal to its own length in each direction.

Using the Precision Pipettor

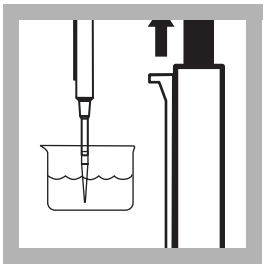


1. Press a pipette tip firmly onto the fixed-volume Precision Pipettor.



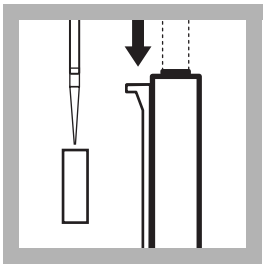
2. Depress the plunger gently to the first stop.

Note: *This is approximately half way. Do not depress the plunger past this first stop or measured quantities may be inaccurate and the Pipettor body may be damaged.*



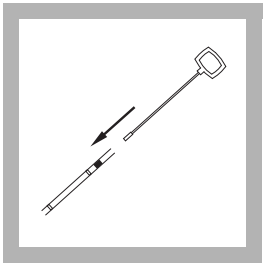
3. With the tip immersed in the solution to be pipeted, allow the plunger to return slowly to its original position

Note: *Do not let the plunger snap into place. This will decrease accuracy.*



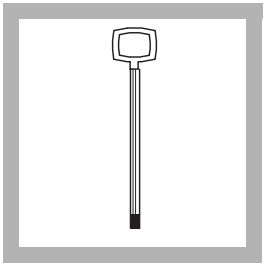
4. Position the Pipettor tip over the receiving vessel. Depress the plunger fully (past the stop) to dispense the sample. Allow the plunger to return slowly to the starting position.

Using the WireTrol™ Pipet

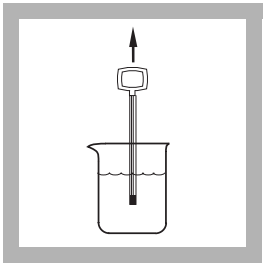


1. Gently insert the orange-tipped end of the WireTrol™ plunger into the end of the capillary tube with the colored band on it.

Note: *The pipet will operate more freely after the Teflon® tip is wetted.*

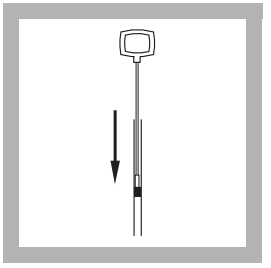


2. Push the tip to the other end of the capillary tube until it barely extends beyond the end of the capillary tube.



3. Submerge the capillary tube below the sample or standard surface. 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 standard and sample.

Instrument Operation

Key Functions

Key	Description	Function
	**POWER** Key	On/Off/Backlight To turn on backlight, turn on the instrument, then press and hold the power key until the backlight turns on. Press and hold again to turn off the backlight. This key functions the same in all instrument modes and ranges.
	**ZERO/SCROLL** Key	In measurement mode, sets the instrument to zero. In menu mode, scrolls through menu options. Also scrolls numbers when entering or editing a value.
	**READ/ENTER** key	In measurement mode, initiates sample measurement. In menu mode, selects a menu option. When entering numbers, moves one space to the right and executes the function when the entry is complete.
	**MENU** Key	Enter/Exit for the menu mode Press and hold for about 5 seconds to enter user-entered method mode.

Menu Selections

Press the **MENU** key to access the menu selections.

Switching Ranges

1. Press the **MENU** key. The display will show "SEL". A flashing arrow indicates the current range.
2. Press the **READ/ENTER** key to toggle between ranges.
3. Press menu again to accept and exit back to the measurement screen.

Setting the Time

1. Press the **MENU** key, then press the **ZERO/SCROLL** key until the display shows a time in the "00:00" format.
2. Press **READ/ENTER**. The digit to be edited will flash.
3. Use the **ZERO/SCROLL** key to change the entry, then press **READ/ENTER** to accept and advance to the next digit. The time is entered in 24-hour format.

Recalling Stored Measurements

1. Press the **MENU** key, then press the **ZERO/SCROLL** key until the display shows "RCL". The instrument automatically stores the last 10 measurements.

2. In RCL mode, press **READ/ENTER** to recall the stored measurements, beginning with the most recent measurement taken. The meter stores the measurement number as 01 (most recent) through 10 (oldest), the time the measurement was taken, and the measurement value. The **ZERO/SCROLL** key allows for selection of a specific measurement by number. The **READ/ENTER** key scrolls through all stored data points.

Battery Installation

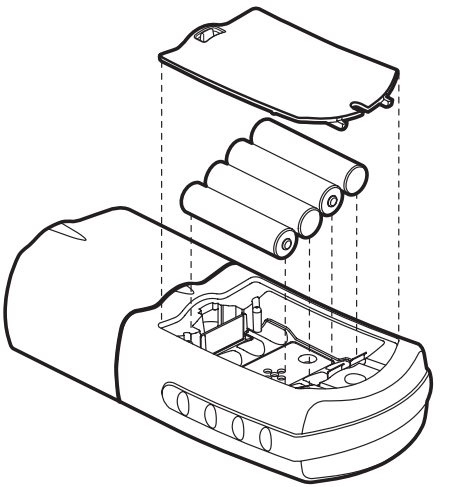
Figure 1 on page 2–6 provides an exploded view of battery installation.

1. Unhook the latch and remove the battery compartment cover. The polarities are shown on the battery holder.
2. Place the four batteries provided with the instrument in the holder as indicated and replace the battery compartment cover. The display will show the software version number (e.g., “P 1.6”) after correct battery installation.

When replacing discharged batteries, always replace the complete set of four alkaline batteries. **Rechargeable batteries are not recommended** and cannot be recharged in the instrument.

Note: *The Low Battery icon will appear on the display when the batteries have 10% battery life remaining. The battery icon will flash when the batteries are too low to complete measurements. See Instrument Keys and Display on page 1—7.*

Figure 1 Battery Installation



Error Codes

When the instrument cannot perform the function initiated by the operator, an error message will appear in the display. Refer to the appropriate message information below to determine what the problem is and how it can be corrected. Resolve error messages in the order that they appear on the display. Service Centers are listed on page 2–20.

Error Messages

1. **E-0 No Zero (User mode)**

   Error occurs when trying to read a standard in the user calibration mode before setting the meter to zero.

   - Zero the instrument on an appropriate blank.

2. **E-1 Ambient Light Error**

   There is too much light present to take a valid measurement.

   - Verify instrument cap is correctly seated.
   - Contact a Service Center.

3. **E-2 LED Error**

   The LED (light source) is out of regulation.

   - Replace the batteries.
   - Verify LED lights up (inside the cell holder) when the **READ/ENTER** or **ZERO/SCROLL** key is pressed.
   - Contact a Service Center.

   Note: *When an E-1 or E-2 error occurs on a measurement, the display will show "_.__". (The decimal place is determined by the chemistry.) If the E-1 or E-2 error occurs while zeroing the meter, the meter will require the user to re-zero.*

4. **E-3 Standard Adjust Error**

   The value obtained on the prepared standard exceeds the adjustment limits allowed for the standard concentration, or the concentration of the standard is outside the concentration range allowed for standard calibration adjust.

   - Prepare the standard and rerun according to the procedure.
   - Prepare a standard at or near the recommended concentrations given in the procedure.
   - Verify that the concentration of the standard has been entered correctly.
   - Contact a Service Center.

5. **E-6 Abs Error (User mode)**

   Indicates that the absorbance value is invalid, or indicates an attempt to make a curve with less than two points.

   - Enter or measure the absorbance value again.
   - Contact a Service Center.

6. **E-7 Standard Value Error (User mode)**

   Standard concentration is equal to another standard concentration that is already entered.

   - Enter the correct standard concentration.
   - Contact a Service Center.

7. **E-9 Flash Error**

   The meter is unable to save data.

   - Contact a Service Center.

8. **Underrange—flashing number below stated test range**

   - Verify instrument cap is correctly seated.
   - Check zero by measuring a blank. If error recurs, re-zero the instrument.
   - Contact a Service Center.

   Note: *See Maximum/Minimum Displayed Value on page 2—16 for more information.*

9. **Overrange—flashing number above stated test range**

   Note: *Flashing value will be 10% over the upper test limit.*

   - Check for light blockage.
   - Dilute and retest sample.

   Note: *See Maximum/Minimum Displayed Value on page 2—16 for more information.*

Standard Calibration Adjust

The Pocket Colorimeter™ II instrument is factory-calibrated and ready for use without calibration by the user. Use of the factory calibration is recommended when permitted. The Standard Calibration Adjust can be used to meet regulatory requirements.

This feature allows the factory default calibration curve to be adjusted with a known standard. Use the standard described in the procedure.

1. Place a blank in the meter. Press **ZERO/SCROLL**.
2. Place the reacted standard in the meter. Press **READ/ENTER**.
3. Press **MENU**, then press the **ZERO/SCROLL** key until the display shows "SCA".
4. Press **READ/ENTER** to display the standard calibration adjust value.
5. Press **READ/ENTER** to adjust the curve to the displayed value. The meter will return to the measurement mode and the "calibration adjusted" icon will appear in the display window.

If an alternate concentration is used or if a standard concentration is not given:

6. Repeat steps 1–4.
7. Press **ZERO/SCROLL** to access the Edit function, then press **READ/ENTER** to begin editing. The digit to be edited will flash. Use the **ZERO/SCROLL** key to change the entry, then press **READ/ENTER** to accept and advance to the next digit.

   When the last digit is entered, press **READ/ENTER** and the meter will adjust the curve to the value entered. The meter will return to the measurement mode and the "calibration adjusted" icon will appear in the display window.

To turn off Standard Calibration Adjust:

1. Press **MENU**.
2. Press **ZERO/SCROLL** until "SCA" appears in the display.
3. Press **READ/ENTER**, then press **ZERO/SCROLL** until "Off" appears in the display.
4. Press **READ/ENTER** to turn off SCA.

Note: *Another standard calibration adjust must be performed to turn SCA "on" again.*

Note: *For meters with factory-calibrated ranges or methods, Standard Calibration Adjust (SCA) will be disabled when a user-entered method is programmed into the meter. To turn SCA back on, restore the meter to factory default calibration. See* *Retrieving the Factory Calibration on page 2—23.*

User-Entered Calibration

Overview

The Pocket Colorimeter™ II will accept a user-prepared calibration curve. The curve can extend from 0 to 2.5 absorbance. A user-prepared calibration curve may be entered into any channel that does not contain a factory-programmed curve. These channels are labeled “abs” on instruments having a single factory calibration or are labeled “1” and “2” on the uncalibrated single wavelength instruments. Any chemistry that can be run at the instrument wavelength may be user-entered in these channels.

Using prepared standard solutions that cover the analyst’s range of interest, the meter generates a calibration curve by calculating the straight-line segments between each standard entered. A calibration curve may be entered using the keypad. Factory-entered calibration curves may also be recalculated or adjusted using the same procedure.

To enter the user-entered calibration mode, press the **MENU** key and hold it down until the display shows “USER” (about 5 seconds), followed by “CAL”. Press **ZERO/SCROLL** to scroll through the options.

- CAL—Used to enter and edit standard values and measure absorbance values, or review the existing calibration.
- Edit—Used to enter and edit standard values and absorbance values with the keypad or review the existing calibration. Used to enter a predetermined calibration curve.
- dFL—Used to return the instrument back to the default factory calibration. User-entered calibrations are stored upon exit from the calibration or edit modes.

If the instrument is shut off or loses power during data entry, all edits will be lost. Automatic shut-off in user calibration entry mode is 60 minutes.

“CAL” and “Edit” Submenus

In CAL mode, standard values are entered and absorbance values are measured. In Edit mode, standard and absorbance values are entered.

To select CAL from the User menu, press **READ/ENTER.**

To select Edit from the User menu, press **ZERO/SCROLL** and **READ/ENTER.**

Once in the CAL or Edit option, press the **READ/ENTER** key to navigate through each option.

Note: *Press* **ZERO/SCROLL** *to quickly scroll through each option.*

Calibration Procedure Using Prepared Standards

Note: *Deionized water or a reagent blank can be used to zero during the calibration procedure. Calibrations generated with deionized water as the zero will give less accurate results if the reagent blank is significantly more turbid or colored than deionized water. Use the deionized water or the reagent blank as the zero concentration point (S0) in the following calibration procedure.*

Note: *Deionized water or a reagent blank can be used to zero during the calibration procedure. Calibrations generated with deionized water as the zero will give less accurate results if the reagent blank is significantly different from deionized water. Use the deionized water or the reagent blank as the zero concentration point (S0) in the following calibration procedure.*

1. Turn on the instrument and select the range to be calibrated. An arrow at the top of the display will point to the selected range. To change ranges, press the **MENU** key, then use the **READ/ENTER** key to toggle between ranges 1 and 2. Press **MENU** again to return to measurement mode.
2. Follow the procedure for the chemical method to be calibrated. Prepare a reagent blank (if needed) and a standard solution. Allow the color to develop fully.
3. Insert the reagent blank or deionized water into the meter and cover with the cap. Press the **ZERO/SCROLL** key. The meter will display "- - - -", followed by "0.000". This initializes (zeroes) the meter.
4. Press the **MENU** key and hold it down until the display shows "USER", followed by "CAL". Press **READ/ENTER** to enter the calibration mode.
5. In factory-calibrated meters, "S0" will appear in the display.

   Note: *When recalibrating a factory-calibrated meter or range, RES (resolution) cannot be changed.*
6. In uncalibrated meters or meters with ranges labeled Abs, "RES" will appear. Press **ZERO/SCROLL** to review the current resolution. Press **ZERO/SCROLL** again to accept the current resolution. To change the resolution, press **READ/ENTER**, then **ZERO/SCROLL** to change the resolution (decimal placement). Press **READ/ENTER** to accept the new resolution. "S0" will appear on the display.
7. Press the **READ/ENTER** key again, then enter the blank value.

   Note: *Press the* **READ/ENTER** *key to move from digit to digit. Use the* **ZERO/SCROLL** *key to change the number.*
8. After completing entry of the blank value, press the **READ/ENTER** key. The display will show "A0".
9. Insert the reagent blank or deionized water into the cell holder. Cover the blank with the instrument cap.
10. Press the **READ/ENTER** key. The meter will measure and display the absorbance value for S0.

11. Remove the sample blank. Press the **ZERO/SCROLL** key. "S1" will appear. Press the **READ/ENTER** key, then enter the first standard value.

    Note: *Press the* **READ/ENTER** *key to move from digit to digit. Use the* **ZERO/SCROLL** *key to change the number.*

12. After completing entry of the first standard value, press the **READ/ENTER** key. The display will show "A1".
13. Insert the first reacted standard solution into the cell holder. Cover the prepared standard with the instrument cap.
14. Press the **READ/ENTER** key. The meter will measure and display the absorbance value for S1.
15. The calibration is complete with two points. If additional standards are required, press **ZERO/SCROLL** until "Add" appears on the display. Repeat steps 11–14 to enter additional standards.
16. Press the **MENU** key twice to exit and accept the changes. The instrument will use this calibration to determine the displayed concentration of future sample measurements.

Entering a Predetermined Calibration Curve

Note: *Entering a predetermined calibration curve requires at least two data pairs. Each data pair requires a concentration value and the absorbance value for the given concentration. Up to 10 data pairs may be entered. This procedure uses the Edit mode.*

1. Turn on the instrument and select the range to be calibrated. An arrow at the top of the display will point to the selected range. To change ranges, press the **MENU** key, then use the **READ/ENTER** key to toggle between ranges 1 and 2. Press **MENU** again to return to measurement mode.
2. Press the **MENU** key and hold it down until the display shows "USER", followed by "CAL". Press **ZERO/SCROLL** to scroll to EDIT. Press **READ/ENTER**.
3. In uncalibrated meters or in Abs range, "RES" will appear. Press **ZERO/SCROLL**. To change the resolution (decimal placement), press **READ/ENTER**. Press **ZERO/SCROLL** to select the new resolution, then press **READ/ENTER** the accept. "S0" will appear on the display.
4. Enter the concentration value and absorbance value of the first data pair (S0, A0).
5. To enter the S0 value, press the **READ/ENTER**. Use the **ZERO/SCROLL** key to select the numerical value, then press

the **READ/ENTER** key to accept the entry and advance to the next decimal place. Repeat this sequence until the S0 concentration value is entered.

6. After editing the S0 value, press **READ/ENTER** to accept. "A0" will appear on the display.
7. To enter the absorbance value for A0, press the **READ/ENTER** key to go to entry mode. Use the **ZERO/SCROLL** key to select the numerical value, then press the **READ/ENTER** key to accept the entry and advance to the next decimal place. Repeat this sequence until the absorbance value for A0 is entered.
8. After entering A0, press **READ/ENTER** to accept. "S1" will appear on the display.
9. Repeat steps 5 through 8 for each standard value and absorbance value pair in the calibration curve

   Note: *After A1 is entered, "Add" will appear in the display. If additional data pairs are to be entered, press* **READ/ENTER** *and continue with step 9.*

10. When all the calibration data has been entered, press **MENU** twice to return to the measurement mode.

Editing a User-entered or Factory Calibration Curve

1. Press the **MENU** key and hold it down until the display shows "USER", followed by "CAL". Press **ZERO/SCROLL** until EDIT appears.
2. Press the **READ/ENTER** key to enter Edit mode. In factory-calibrated meters, "S0" will appear in the display.

   Note: *When editing a factory-calibrated meter or range, RES (resolution) cannot be changed.*

   Note: *When RES or S0 appears in the display, press* **ZERO/SCROLL** *to quickly scroll the data to be edited.*

3. In uncalibrated meters or in Abs range, "RES" will appear. Press **ZERO/SCROLL** to review the current resolution. Press **ZERO/SCROLL** again to accept the displayed resolution. To change the resolution, press **READ/ENTER.** Press **ZERO/SCROLL** to select the new resolution, then press **READ/ENTER** the accept. S0 will appear on the display.
4. Press **READ/ENTER.** The current concentration value for S0 will appear on the display.
5. To edit the S0 value, press the **READ/ENTER.** Use the **ZERO/SCROLL** key to select the numerical value, then press the **READ/ENTER** key to accept the entry and advance to the

next decimal place. Repeat this sequence until the S0 concentration value is entered.

6. After editing the S0 value, press **READ/ENTER** to accept. "A0" will appear on the display.
7. To edit the absorbance value for A0, press the **READ/ENTER** key to go to entry mode. Use the **ZERO/SCROLL** key to select the numerical value, then press the **READ/ENTER** key to accept the entry and advance to the next decimal place. Repeat this sequence until the absorbance value for A0 is entered.
8. After editing A0, press **READ/ENTER** to accept. "S1" will appear on the display.
9. Repeat steps 4 through 8 for each standard value and absorbance value pair in the calibration curve.
10. When all calibration data has been reviewed or edited, Add will appear in the display.
11. Press **READ/ENTER** to add more calibration points, or press **MENU** twice to return to the measurement mode.

Note: *When a factory calibration curve has been edited, the "calibration adjust" icon will appear in the display.*

Exiting the Calibration Routine

Exit the calibration routine by pressing the **MENU** key to step back through to measurement mode. The instrument uses the last completed user-entered calibration or the factory calibration if no user-entered calibration has been completed.

Deleting Calibration Points

1. Select the range containing user-entered calibration points. See Switching Ranges on page 2–4.
2. Press and hold the **MENU** key until "USER", then "CAL" appears. Press **READ/ENTER.**

   Note: *Calibration points can also be deleted in Edit mode.*
3. Press **ZERO/SCROLL** to select the point to delete (e.g., S0 or S1 or S2). Press **READ/ENTER.**
4. The left digit will flash. Press **ZERO/SCROLL** until "dEL" appears. ("dEL" will appear after the numeral 9.)
5. Press **READ/ENTER** to delete. Repeat for all points to be deleted.

   Note: *The minimum number of valid points is two. For example, if five points have been entered, three can be deleted using this feature.*

6. Press **MENU** to return to the measurement mode.

Retrieving the Factory Calibration

1. Select the range to restore factory default calibration. See Switching Ranges on page 2–4.
2. Press and hold the **MENU** key until “User”, then “CAL” appears.
3. Press the **ZERO/SCROLL** key to find dFL.
4. Press the **READ/ENTER** key to select dFL and restore the instrument to the factory default calibration.

Note: *For meters with factory-calibrated ranges or methods, Standard Calibration Adjust (SCA) will be disabled when a user-entered method is programmed into the meter. To turn SCA back on, restore the meter to factory default calibration.*

Maximum/Minimum Displayed Value

In meters with absorbance (Abs) ranges, the maximum displayed value and minimum displayed value is related to the value of the standards entered in a user calibration.

Measurements that exceed the minimum or maximum standards entered in the user calibration will return a flashing number indicating “underrange” or “overrange”. See Error Messages on page 2–7 for more information.

Example 1

For a calibration with the following standards:

S0=0.000
S1=1.000

Maximum Displayed Value	1.000
Minimum Displayed Value	0.000

Example 2

For a calibration with the following standards:
S0=1.00
S1=2.00
S2=4.00

Maximum Displayed Value	4.00
Minimum Displayed Value	1.00

For factory-calibrated programs, the maximum and minimum displayed values always equal the factory-calibrated values, and cannot be changed.

Certification

Hach Company certifies this instrument was tested thoroughly, inspected, and found to meet its published specifications when it was shipped from the factory.

The Pocket Colorimeter™ II instrument has been tested and is certified as indicated to the following instrumentation standards:

EMC Immunity:
Per **89/ 336/ EEC** EMC: **EN 61326: 1998** (Electrical Equipment for measurement, control and laboratory use—EMC requirements). Supporting test records by Hach Company, certified compliance by Hach Company.

Standard(s) include:

IEC 1000-4-2: 1995 (EN 61000-4-2: 1995) Electrostatic Discharge Immunit (Criteria B)

IEC 1000- 4- 3: 1995 (EN 61000- 4- 3: 1996) Radiated RF Electromagnetic Field Immunity (Criteria A)

Additional Immunity Standard(s) include:

ENV 50204: 1996 Radiated Electromagnetic Field from Digital Telephones (Criteria A) Radio Frequency Emissions:

Per **89/ 336/ EEC** EMC: **EN 61326: 1998** (Electrical Equipment for measurement, control and laboratory use—EMC requirements) "Class B" emission limits. Supporting test records from Hach EMC Test Facility, certified compliance by Hach Company.

Additional Radio Frequency Emissions Standard(s) include:
EN 55022 (CISPR 22), Class B emissions limits.

Canadian Interference-causing Equipment Regulation, IECS-003, Class A: Supporting test records from Hach EMC Test Facility, certified compliance by Hach Company.

This Class A digital apparatus meets all requirements of the Canadian Interference-Causing Equipment Regulations.

Cet appareil numérique de la classe A respecte toutes les exigences du Règlement sur le matériel brouilleur du Canada.

FCC Part 15, Class "A" Limits: Supporting test records from Hach EMC Test Facility, certified compliance by Hach Company.

This device complies with Part 15 of the FCC Rules. Operation is subject to the following two conditions:

(1) This device may not cause harmful interference, and (2) This device must accept any interference received, including interference that may cause undesired operation Changes or modifications to this unit not expressly approved by the party responsible for compliance could void the user's authority to operate the equipment.

This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to Part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference, in which case the user will be required to correct the interference at his own expense. The following techniques of reducing the interference problems are applied easily

1. Remove power from the Pocket Colorimeter II instrument by removing one of its batteries to verify that it is or is not the source of the interference.

2. Move the Pocket Colorimeter II instrument away from the device receiving the interference.

3. Reposition the receiving antenna for the device receiving the interference.

4. Try combinations of the above.

General Information

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.

How To Order

By Telephone:

6:30 a.m. to 5:00 p.m. MST
Monday through Friday
(800) 227-HACH
(800-227-4224)
By FAX: (970) 669-2932

By Mail:

Hach Company
P.O. Box 389
Loveland, CO 80539-0389
U.S.A.

Ordering information by E-mail: orders@hach.com

Information Required

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

Technical and Customer Service (U.S.A. only)

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.
Call **1-800-227-4224** or E-mail **techhelp@hach.com**.

International Customers

Hach maintains a worldwide network of dealers and distributors. To locate the representative nearest you, send E-mail to **intl@hach.com** or contact:

In Canada, Latin America, Africa, Asia, Pacific Rim:
Telephone: (970) 669-3050; FAX: (970) 669-2932

In Europe, the Middle East, or Mediterranean Africa:
HACH Company, c/o
Dr. Bruno Lange GmbH
Willstätterstr. 11
D-40549 Düsseldorf
Germany
Telephone: +49/[0]211.52.88.0
Fax: +49/[0]211.52.88.231

Repair Service

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

In the United States:
Hach Company
100 Dayton Avenue
Ames, Iowa 50010
(800) 227-4224 (U.S.A. only)
Telephone: (515) 232-2533
FAX: (515) 232-1276

In Canada:
Hach Sales & Service Canada Ltd.
1313 Border Street, Unit 34
Winnipeg, Manitoba
R3H 0X4
(800) 665-7635 (Canada only)
Telephone: (204) 632-5598
FAX: (204) 694-5134
E-mail: canada@hach.com

In Latin America, the Caribbean, the Far East, the Indian Subcontinent, Africa, Europe, or the Middle East:
Hach Company World Headquarters
P.O. Box 389
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.

HACH Technical Training Center

HACH analytical training programs

Workshops in analytical theory and procedure, and in the application of Hach products, are available in cities across the United States and at the HACH Technical Training Center in Loveland, Colorado. Find the latest schedule of classes and locations by visiting our website (www.hach.com).

HACH Technical Training Center (HTTC)

HTTC offers technical training workshops for a wide variety of applications. Our purpose is to help analysts from all backgrounds understand analytical theory, gain practical, hands-on experience with instrumentation and chemistries, and return to their jobs with increased understanding and confidence.

To register for an HTTC workshop:

Review class descriptions to determine the workshop you wish to attend, then check the class schedule and class fees before registering. There are no prerequisites for any of the workshops.

You may use the online HTTC registration form, e-mail httc@hach.com, call 800-227-4224, extension 2391, or fax 970-461-3915. From outside the United States, call 970-669-3050, extension 2391.

When you register for a workshop, please provide your HACH customer number (if you have one) and a purchase order number or credit card number to reserve your space in the class. After your registration has been processed, a confirmation packet will be sent to you. Please wait for registration confirmation before making travel arrangements. An invoice will be mailed under separate cover.

If you are outside the United States, training is frequently available directly from your local HACH Distributor. Please contact your distributor or the HACH International Department for more information.

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P.O. Box 389
Loveland, Colorado 80539-0389
Telephone: (970) 669-3050
Fax: (970) 669-2932

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**

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