This method analyzes for PCB that has been extracted from soil samples. Sample extracts, calibrators, and reagents are added to cuvettes coated with PCB-specific antibodies. The color that develops is then measured and compared with the color measurements of the calibrators. The test requires about 20 minutes for complete analysis. As many as 10 cuvettes can be run simultaneously.

Tips and Techniques

- **Read the entire procedure before starting.** Identify and have ready all the necessary reagents, cuvettes, and other apparatus before beginning the analysis.

- **Timing is critical:** follow instructions carefully.

- **A consistent technique when mixing the cuvettes is critical to this test.** The best results come from using the cuvette rack and mixing as described in *Using the 1-cm MicroCuvette Rack*. Cuvettes can be mixed individually, but test results may not be as consistent.

- Handle the cuvettes carefully. Scratches on the inside or outside may cause erroneous results. Carefully clean the outside of the cuvettes with a clean absorbent cloth or tissue before placing them into the instrument. (Kimwipe® tissues are provided with the kit.)

- Antibody cuvettes and enzyme conjugate are made in matched lots. Do not mix reagent lots.

- Twenty Antibody Cuvettes are provided with each reagent set. One Antibody Cuvette will be used for each calibrator or sample. Antibody Cuvettes are not reusable.

- To avoid damaging the Color Developing Solution, do not expose it to direct sunlight.

- There are two protocols in this procedure, one for levels of 1 ppm and 5 ppm, and another for 10 ppm and 50 ppm. Each uses a different quantity of calibrator and sample extract as follows:

<table>
<thead>
<tr>
<th>Range</th>
<th>Volume of calibrator and sample extract used</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 ppm and 5 ppm</td>
<td>50 µL</td>
</tr>
<tr>
<td>10 ppm and 50 ppm</td>
<td>10 µL</td>
</tr>
</tbody>
</table>

- To test across ranges, such as 1 and 50 ppm, test the lower concentration first. If the result is positive then test at the higher level. If the result of the test at the lower concentration is negative, the higher range test will be negative also, and need not be performed.

- The same filtered extract can be used for both protocols if it is tightly capped between assays. The maximum time between assays cannot exceed one-half hour.

- Store the reagents at 4 °C when they are not in use. Allow the reagents to reach room temperature before using them in an analysis. Actual testing may be done at temperatures ranging from 1 °C to 38 °C.

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

**Note:** *Hach Company recommends wearing protective nitrile gloves for this procedure.*
Soil Extraction Procedure

1. Weigh out 5 g of soil in the plastic weighing boat. 
2. Carefully pour the soil into an extraction vial. 
3. Use the 5-gram scoop to add one scoop of sodium sulfate to the extraction vial. 
4. Use the graduated cylinder to transfer 10 mL of Soil Extractant into the extraction vial. 
5. Cap the extraction vial tightly and shake vigorously for one minute. 
6. Allow to settle for at least one minute. Carefully open the extraction vial. 
7. Using the disposable bulb pipet, withdraw 1.0–1.5 mL from the liquid layer at the top of the extraction vial. Transfer it into the filtration barrel (the bottom part of the filtering assembly into which the plunger inserts). 
   Note: Do not use more than 1.5 mL. The bulb is marked in 0.25–mL increments. 
8. Insert the filtration plunger into the filtration barrel. Press firmly on the plunger until the sample extract is forced upward into the center of the plunger. Use the resultant filtrate for the immunoassay in the Immunoassay Procedure for Soil Extracts. 
   Note: It may be necessary to place the filtration assembly on a table and press down on the plunger.
Immonoassay Procedure for Soil Extracts

1. Press the soft key under **SINGLE** λ.
   Press the soft key under **GO TO** λ.
   Select 450 nm by pressing the numeric keys **4 5 0**.
   Press: **ENTER**

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

2. The display will show: **ZERO REQUIRED**

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

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

4. Place the cuvettes into the rack snugly.

5. Pipet 0.5 mL of Diluent Solution into each cuvette.

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

   *Note:* Have the necessary apparatus at hand for the next four steps as they must be done without delay.

6. Use a Wiretrol® pipet to transfer the appropriate volume of calibrator or sample extract into each cuvette.

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

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

7. Immediately pipet 0.5 mL of PCB Enzyme Conjugate into each calibrator and sample cuvette.

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

8. Key **1000** to bring up a 10-minute timer.

   Press **START TIMER**.

   A 10-minute reaction time will begin. Proceed immediately to the next step.
9. Mix the contents of the cuvettes for 30 seconds using the technique described in Using the 1-cm MicroCuvette Rack.

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

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

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

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

Color Development

   Note: Timing is critical; follow instructions carefully

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

   Note: Use a new pipette tip for each cuvette.

14. Key 500. Press the soft key under START TIMER.

   A 5-minute reaction period will begin. Mix following the instructions in Using the 1-cm MicroCuvette Rack.

15. After 2.5 minutes, mix the contents of the rack a second time for a period of 30 seconds using the same technique.

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

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

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

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

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

18. Place the filled zeroing cuvette into the cell holder with the arrow pointing left. Orient the arrow in the same direction for all cuvettes.

19. Press the soft key under ZERO. The display will show: 0.000 ABS

20. Place the prepared sample into the cell holder. Read the results. The display will give an absorbance reading. Record the results for each calibrator and sample. 

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

21. Repeat step 20 for all remaining calibrators and samples.

See Interpreting and Reporting Results for help with interpretation of results.
Using the Wiretrol® Pipet

The Wiretrol Pipet can accurately measure small quantities of liquids. It consists of two parts: a Teflon®-tipped plunger and a calibrated capillary tube. Use Figure 1 to determine the quantity measured at each line on the capillary tube.

The plunger can be re-used; the capillary tubes must be discarded after one use.

Figure 1  Wiretrol Pipet

1. Wet the orange Teflon® tip of the Wiretrol plunger in the sample and carefully insert it into the end of the capillary tube with the colored band on it.

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

3. Submerge the capillary tube below the surface of the liquid to be pipetted. Slowly and smoothly draw the Wiretrol plunger up until the bottom of the plunger tips reaches the appropriate volume line.

   Note: Touch the end of the tube to the side of the vessel to release drops on the capillary tube tip.

4. To discharge the pipet, place the tip of the capillary tube below the surface of the solution and push the Wiretrol plunger down in one smooth motion. Change capillary tubes for each calibrator and sample.

Using the 1-cm MicroCuvette Rack

This rack (see Figure 2) has been designed specifically to aid in achieving precise and accurate results when using the immunoassay technique to analyze several samples at the same time.
Loading the Rack — The cuvette rack is designed so that it may be inverted with the cuvettes in place. Identify each cuvette with a sample or calibrator number and place all the cuvettes in the rack before beginning the procedure. Fit the cuvettes snugly into the rack, but do not force them or they may be difficult to remove and their contents may spill. The cuvettes should remain in place when the rack is inverted and tapped lightly.

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

Interpreting and Reporting Results

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

<table>
<thead>
<tr>
<th>If the sample reading is...</th>
<th>the sample TPH Concentration is...</th>
</tr>
</thead>
<tbody>
<tr>
<td>...less than calibrator reading</td>
<td>...greater than the calibrator concentration</td>
</tr>
<tr>
<td>...greater than calibrator reading</td>
<td>...less than the calibrator concentration</td>
</tr>
</tbody>
</table>

Example

Readings:
1 ppm PCB Calibrator: 0.775 Abs
5 ppm PCB Calibrator: 0.430 Abs
Sample #1: 0.200 Abs
Sample #2: 0.600 Abs
Sample #3: 0.900 Abs
Interpretation

Interpretation for a soil sample:

Sample #1 — Sample reading is less than the readings for both calibrators. Therefore the sample concentration of PCB is greater than both 1 ppm and 5 ppm as Aroclor 1248.

Sample #2 — Sample reading is between the readings for the 1 ppm and 5 ppm PCB calibrators. Therefore the sample concentration of PCB is between 1 ppm and 5 ppm as Aroclor 1248.

Sample #3 — Sample reading is greater than the readings for both calibrators. Therefore the sample concentration of PCB is less than both 5 ppm and 1 ppm as Aroclor 1248.

Storing and Handling Reagents

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

Sensitivity

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

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration (ppm) to give a positive result at</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 ppm</td>
</tr>
<tr>
<td>1248</td>
<td>1</td>
</tr>
<tr>
<td>1016</td>
<td>2</td>
</tr>
<tr>
<td>1242</td>
<td>1.2</td>
</tr>
<tr>
<td>1254</td>
<td>1.4</td>
</tr>
<tr>
<td>1260</td>
<td>1.1</td>
</tr>
</tbody>
</table>

The following compounds are not detectable at 1000 ppm.

- Biphenyl
- 2,4,6-trichlorophenyl
- 1,3-dichlorobenzene
- 2,4-dichlorophenyl
- pentachlorophenol
- 1,4-dichlorobenzene
- 2,4,5-trichlorophenyl
- 1,2-dichlorobenzene
- 1,2,4-trichlorobenzene
Sample Collection and Storage
Analyze the samples as soon as possible after collection. If the samples must be stored, collect them in glass or Teflon® containers that have been washed with soap and water and rinsed with methanol. The container should be capped with a Teflon-lined cap. If a Teflon cap is not available, aluminum foil rinsed in methanol may be used as a substitute cap liner.

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

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

Required Reagents

<table>
<thead>
<tr>
<th>Description</th>
<th>Unit</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent Set, PCB *</td>
<td>20 cuvettes</td>
<td>27735-00</td>
</tr>
<tr>
<td>Deionized water</td>
<td>500 mL</td>
<td>272-48</td>
</tr>
</tbody>
</table>

Required Apparatus

<table>
<thead>
<tr>
<th>Description</th>
<th>Unit</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adapter, 1-cm MicroCell</td>
<td>each</td>
<td>48588-00</td>
</tr>
<tr>
<td>Caps, flip spout</td>
<td>2/pkg</td>
<td>25818-02</td>
</tr>
<tr>
<td>Marker, laboratory</td>
<td>each</td>
<td>20920-00</td>
</tr>
<tr>
<td>TenSette®, Pipet, 0.1–1.0 mL</td>
<td>each</td>
<td>19000-01</td>
</tr>
<tr>
<td>Tips, for TenSette®, Pipet 19000-01</td>
<td>1000/pkg</td>
<td>21856-28</td>
</tr>
<tr>
<td>Rack, for 1-cm Micro Cuvettes</td>
<td>each</td>
<td>48799-00</td>
</tr>
<tr>
<td>Wipes, disposable</td>
<td>box</td>
<td>20970-00</td>
</tr>
</tbody>
</table>

For Soil Extraction only:

<table>
<thead>
<tr>
<th>Description</th>
<th>Unit</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil Scoop, 5-g, 4.25-cc</td>
<td>each</td>
<td>26572-05</td>
</tr>
<tr>
<td>Soil Extraction Refill Kit</td>
<td>each</td>
<td>27752-00</td>
</tr>
<tr>
<td>Includes:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dropper, LDPE, 0.5 and 1.0-mL</td>
<td>20/pkg</td>
<td>21247-20</td>
</tr>
<tr>
<td>Filter and Barrel Assembly</td>
<td>20/pkg</td>
<td>25676-20</td>
</tr>
<tr>
<td>Sodium Sulfate, anhydrous</td>
<td>250 g</td>
<td>7099-29</td>
</tr>
<tr>
<td>Soil Extractant Solution</td>
<td>200 mL</td>
<td>25677-29</td>
</tr>
<tr>
<td>Soil Sample Container</td>
<td>20/pkg</td>
<td>25929-20</td>
</tr>
<tr>
<td>Weighing Boat, 8.9-cm, square</td>
<td>20/pkg</td>
<td>21790-20</td>
</tr>
</tbody>
</table>

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