Polychlorinated Biphenyls (PCB) in Soil

DOC316.53.01107

Immunoassay[1]

Method 10050

Scope and application: For soil.

[1] This test is semi-quantitative. Results are shown as more or less than the threshold value used.

Test preparation

Instrument specific information

Table 1 shows all of the instruments that can be used for this test. The table also shows adapter requirements for the instruments that use them.

To use the table, select an instrument, then read across to find the corresponding information for this test.

Table 1 Instrument-specific information

Instrument	Adapter	Light shield
DR 6000, DR 5000	—	—
DR 3900	—	LZV849
DR 3800, DR 2800, DR 2700	—	LZV646

Before starting

This method analyzes for PCBs in soil samples. In this procedure, the user adds sample extracts, calibrators and reagents to cuvettes that are layered with PCB-specific antibodies. The color that develops is measured and compared with the color measurements of the calibrators. The test requires approximately 20 minutes. This method provides semi-quantitative screening based on thresholds for PCB in the concentrations 1, 5 or 10 ppm and/or 50 ppm as Aroclor 1248.

There are two protocols for this procedure: The first is for levels 1 ppm and 5 ppm and the second is for 10 ppm and 50 ppm. Each protocol uses a different quantity of calibrator and sample extracts. Refer to PCB protocols on page 6.

Before the procedure starts, read the full procedure. Identify and prepare all the necessary reagents, cuvettes and other apparatus, then start the procedure.

Timing is very important in this procedure. Follow the instructions carefully.

It is very important to use a consistent technique to mix the solution in the cuvettes. Refer to Use of the 12-mm MicroCuvette rack on page 6. If the cuvettes are individually mixed, the results can be less consistent.

DR 3900, DR 3800, DR 2800 and DR 2700: Install the light shield in Cell Compartment #2 before this test is started.

The cuvette rack can be inverted with the cuvettes in the rack. This lets the user prepare many samples at the same time. The cuvettes stay in the rack until the results are read in the instrument.

Be careful with the cuvettes. A scratch on the inner or outer cuvette surfaces can cause incorrect results. Carefully clean the outer surfaces with a clean, absorbent cloth or tissue before use.

Each reagent set has 20 antibody cuvettes. Use one antibody cuvette for each calibrator and each sample. Cuvettes are not reusable.

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

Keep the color developing solution out of direct sunlight to prevent deterioration.

The recommended temperature for reagent storage is 4 °C (39.2 °F). Let the reagent temperature increase to room temperature before analysis.

The Soil Extractant contains methyl alcohol, which is poisonous and flammable. Review the Safety Data Sheets (MSDS/SDS) for the chemicals that are used. Use the recommended personal protective equipment.

Use protective nitrile gloves for this procedure.
Dispose of reacted solutions according to local, state and federal regulations. Refer to the Safety Data Sheets for disposal information for unused reagents. Refer to the environmental, health and safety staff for your facility and/or local regulatory agencies for further disposal information.

Items to collect

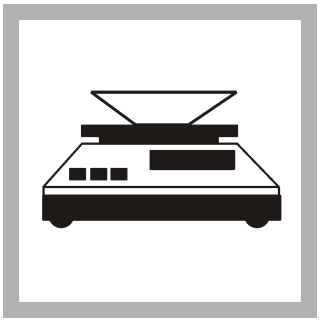
Description	Quantity
PCB Reagent Set	1
Analytical balance	1
Caps, flip spout	1
Cylinder, graduated 10-mL	1
Light shield (refer to Instrument specific information on page 1)	1
Marker, laboratory	1
Pipet, TenSette, 0.1–1.0 mL	1
Pipet tips, for TenSette Pipet, 0.1–1.0-mL	1
Rack, for 12-mm Micro Cuvettes	1
Scoop, 5 g	1
Soil extraction kit	varies
Water, deionized	varies
Wipes, disposable	1
Wiretrol pipet	1

Refer to Consumables and replacement items on page 8 for order information.

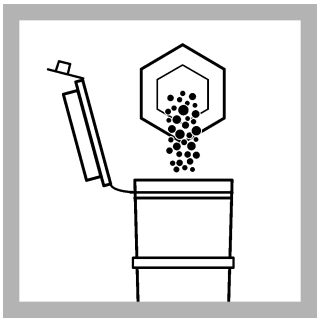
Sample collection

- Analyze the samples as soon as possible for best results.
- If sample storage is necessary, collect the samples in glass or PTFE containers. Clean the containers with soap and water, then rinse the containers with methanol. Use PTFE-lined caps for the containers. If PTFE-lined caps are not available, use aluminum foil as a substitute cap liner. Rinse the aluminum foil with methanol before use.

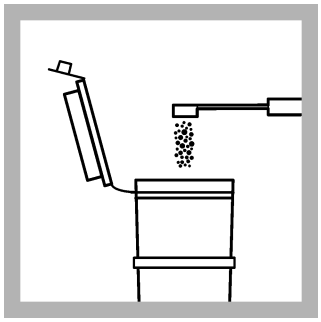
Soil extraction procedure



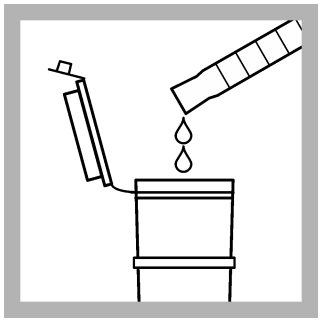
1. Weigh 5 g of soil in the plastic weighing boat.



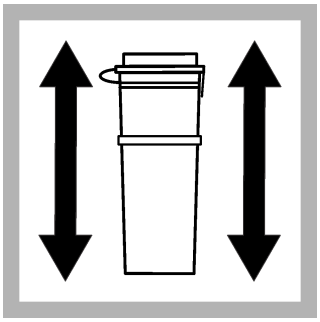
2. Carefully pour the soil into an extraction vial.



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

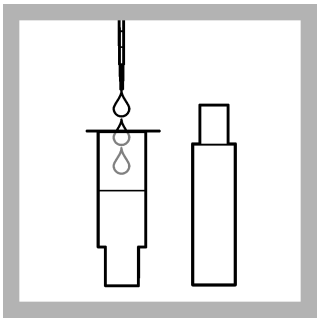


4. Use the graduated cylinder to add 10 mL of Soil Extractant into the extraction vial.

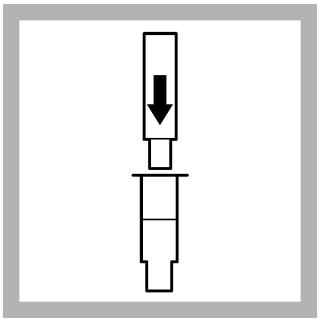


5. Put the cap on the extraction vial tightly. Shake vigorously for 1 minute.

6. Let the particles settle for a minimum of 1 minute. Carefully open the extraction vial.



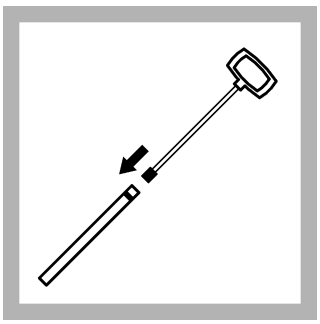
7. Use the disposable pipet to remove 1.0 to 1.5 mL from the top of the liquid layer. Add the removed liquid to the filtration barrel. Do not use more than 1.5 mL. The pipet can measure in 0.25-mL increments.



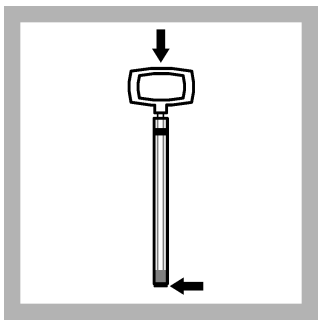
8. Put the filtration plunger into the filtration barrel. Set the filtration assembly on a table or flat surface. Push firmly on the plunger until the sample extract is forced upward into the center of the plunger.
Use the resulting filtrate for the immunoassay procedure.

Use of the Wiretrol Pipet

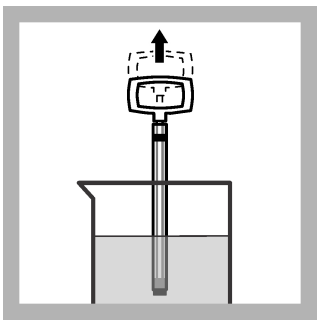
The Wiretrol Pipet accurately measures small quantities of liquids. The Wiretrol Pipet has two parts: a PTFE-tipped plunger and a calibrated capillary tube. The plunger can be used many times. Discard the capillary tubes after one use.



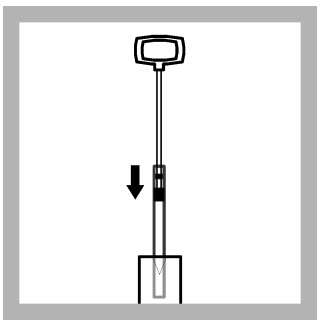
1. Make sure that the plunger tip is wet with the liquid. Carefully insert the plunger tip into the end of the capillary tube with the colored band.



2. Push the plunger tip to the other end of the capillary tube. Stop when the plunger tip barely extends beyond the end of the capillary tube.

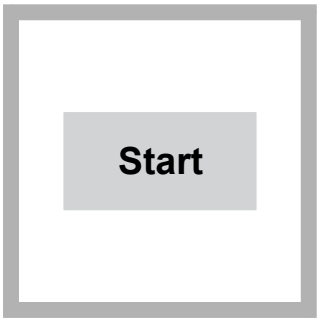


3. Insert the capillary tube below the surface of the liquid. Slowly and smoothly, pull the plunger up until the bottom of the plunger tip reaches the applicable volume line. Touch the end of the tube to the side of the vessel to release drops that remain on the capillary tube tip.

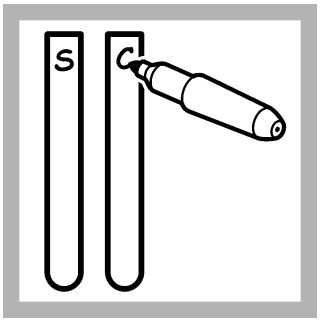


4. To release the liquid, insert the tip of the capillary tube **below the surface of the receiving solution**, and push the plunger downward in one smooth motion. Change capillary tubes for each calibrator and sample.

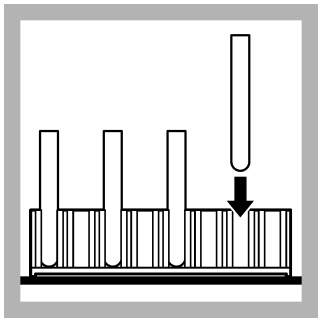
Immunoassay procedure



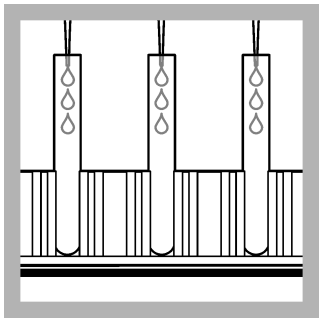
1. Push **SINGLE WAVELENGTH**>**OPTIONS**, then the **λ** key. Enter **450 nm** and push OK. For information about adapters, refer to Instrument specific information on page 1.



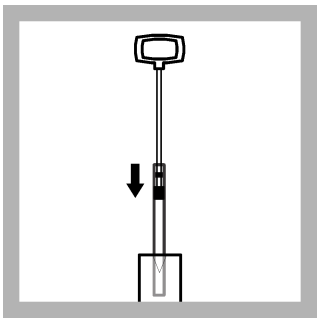
2. Put marks on the cuvettes to identify the samples and calibrators.



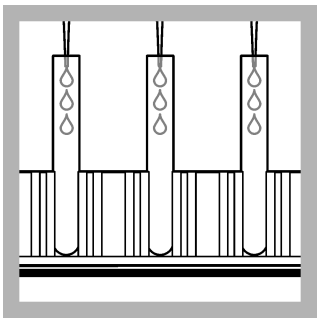
3. Insert the cuvettes into the rack. Make sure that the cuvettes are secure. Do not use force to put them into position because the cuvettes can spill or can be difficult to remove.



4. Use a pipet to add 0.5 mL of Diluent Solution into each cuvette. The same pipette tip can be used for this step.

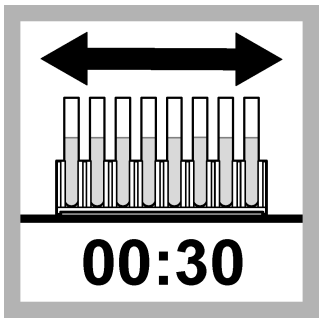


5. Use a Wiretrol pipet to add the correct volume of calibrator or sample extract into each cuvette. Use a separate capillary tube for each solution. Refer to PCB protocols on page 6.
Have the necessary apparatus ready for this step and the next four steps. Do not wait—do these steps quickly.

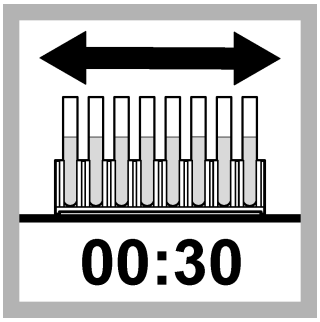


6. Immediately use a pipet to add 0.5 mL of PCB Enzyme Conjugate into each calibrator and sample cuvette. The same pipette tip can be used for this step.

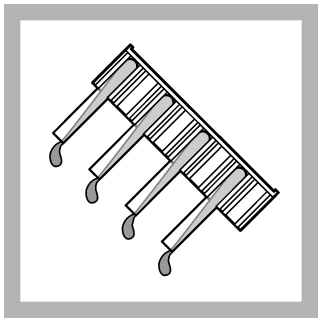
7. Start the instrument timer. The reaction time starts.



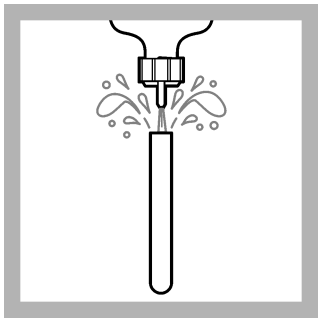
8. Immediately mix the cuvettes for 30 seconds. Refer to Use of the 12-mm MicroCuvette rack on page 6 for the correct mixing procedure.



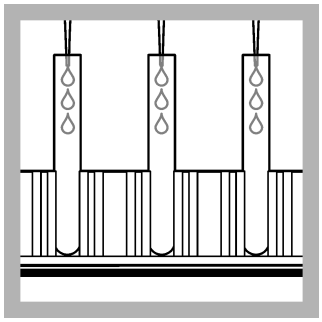
9. After 5 minutes, mix the contents of the rack a second time for 30 seconds.



10. At the end of the 10-minute reaction period, discard the contents of all the cuvettes into a waste container for disposal.

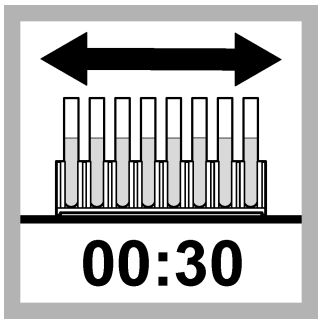


11. Fully rinse each cuvette with deionized water four times. Discard the contents into the waste container for disposal. Turn the cuvettes and rack upside down on a paper towel to dry. Carefully tap the cuvettes on the towel to remove the liquid.

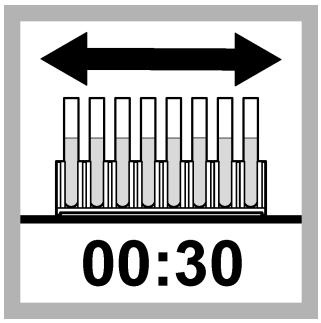


12. Start color development: Timing is very important. Make sure that the cuvettes are still in position in the rack. Use the pipet to add 0.5 mL of Color Developing Solution into each Antibody Cuvette. Use a new pipette tip for each cuvette.

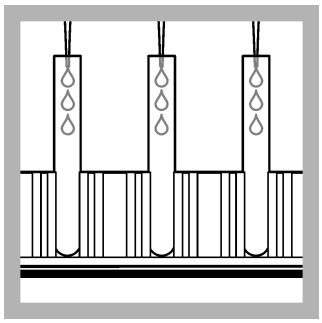
13. Start the instrument timer. The reaction time starts.



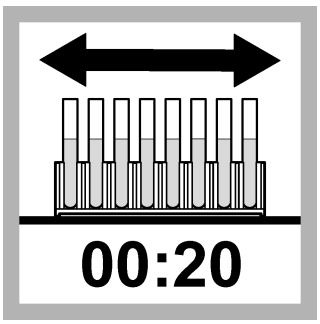
14. Immediately mix the cuvettes for 30 seconds.



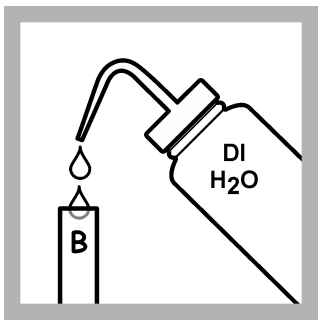
15. After 2.5 minutes, mix the cuvettes for 30 seconds. The solutions in some or all of the cuvettes change to blue.



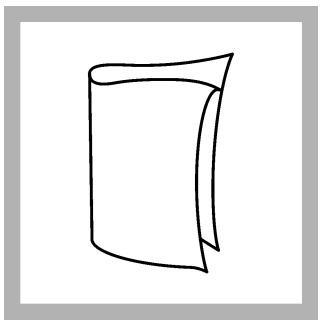
16. When the timer expires, use a pipette to add 0.5 mL of Stop Solution into each cuvette with the same pipette tip. Consistent technique is very important. Add the solution in the same sequence that was used for the Color Developing Solution addition.



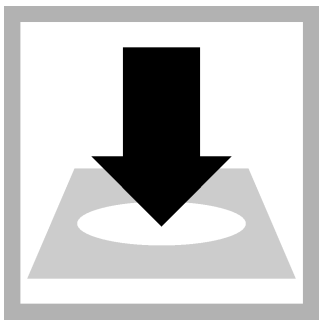
17. Slide the rack back and forth for 20 seconds. The blue solution color changes to yellow.



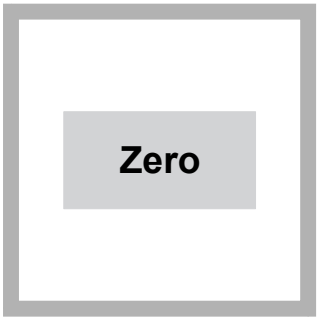
18. Put a mark on a zeroing cuvette to identify it as the blank. Fill the cuvette with deionized water.



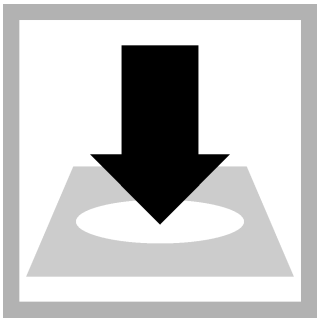
19. Clean all of the cuvettes.



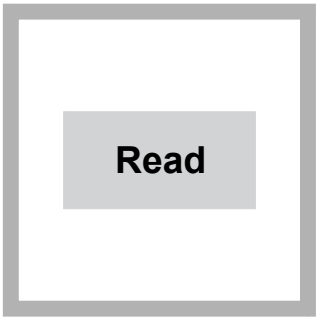
20. Insert the blank into the circular cell holder.



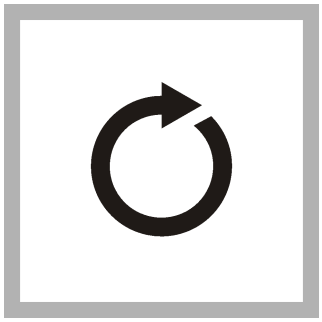
21. Push **ZERO**. The display shows 0.000 Abs.



22. Insert the first calibrator into the circular cell holder.



23. Push **READ**. Results show in Abs. Record the result.



24. Read the absorbance values of the remaining calibrators and samples. Record the results. Refer to Interpret and report the results on page 7.

PCB protocols

There are two PCB protocols in this procedure, one is for levels of 1 to 5 ppm and the other is for 10 to 50 ppm. Each test procedure uses a different volume of calibrator and sample extract. Refer to Table 2.

Table 2 PCB protocols

Range (as Arochlor 1248)	Volume of calibrator and sample extract
1 to 5 ppm	50 µL
10 to 50 ppm	10 µL

To do a test procedure across ranges, such as 1 and 50 ppm, do a test of the lower concentration first. If the result is positive, then do a test procedure at the higher level. If the result of the lower concentration is negative, the higher range test will be negative. It is not necessary to do a higher range test.

Use the same filtered extract if the cap is tightly closed between assays. Do not wait more than 30 minutes between the assays.

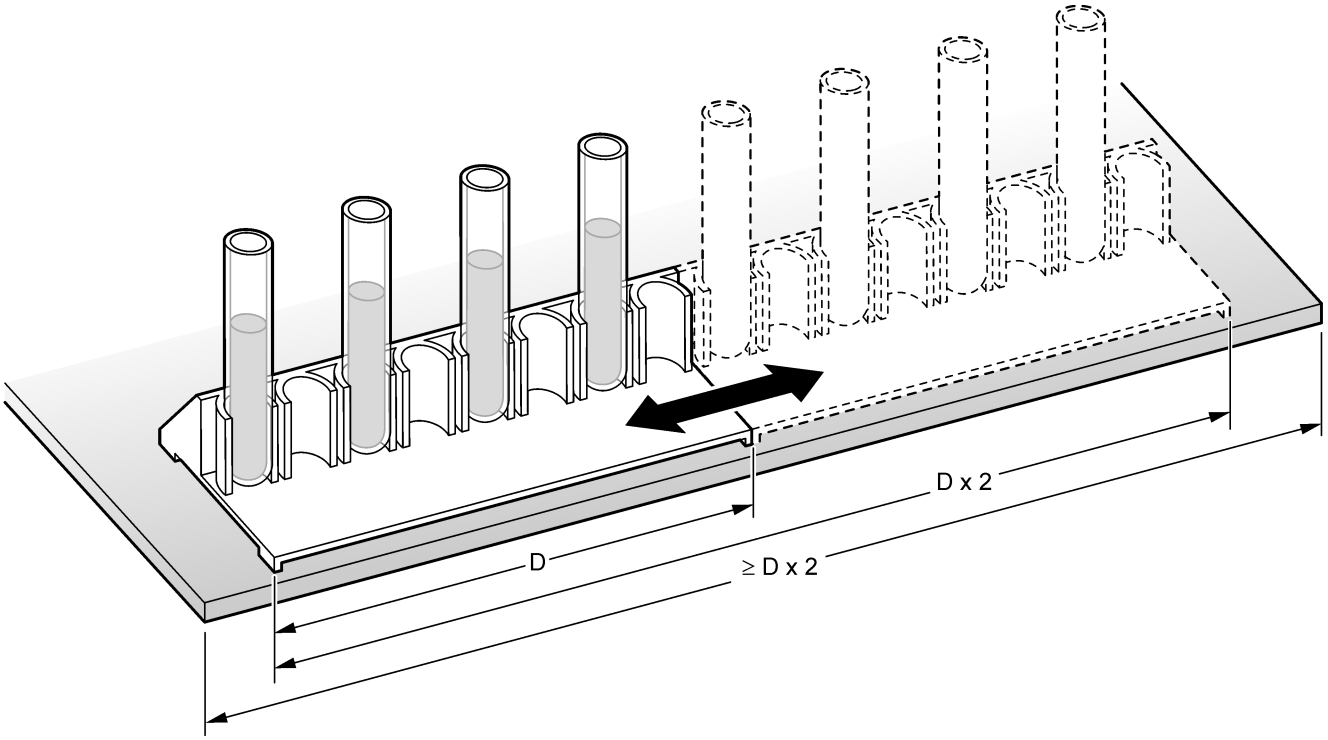
Use of the 12-mm MicroCuvette rack

Use the MicroCuvette rack to get accurate and precise results for the immunoassay procedure during the analysis of several samples at a time. Refer to Figure 1.

Insert the cuvettes in the rack—Use the MicroCuvette rack to securely hold cuvettes that are set in the rack. Before the procedure starts, identify each cuvette with a sample or a calibrator number. Correctly insert the cuvettes in the rack. Do not force the cuvettes into the rack because the sample can spill or the cuvettes can be difficult to remove. The cuvettes must stay in position if the rack is inverted and carefully tapped.

Mix the sample—Put the rack on a hard, flat surface that is at least twice the length of the rack. Refer to Figure 1. Hold one end of the rack, then vigorously slide the rack back and forth along its axis for 30 seconds. The rack moves through a distance equal to its own length in each direction.

Figure 1 MicroCuvette rack



Interpret and report the results

There is an inverse relationship between the concentration of PCB and the absorbance reading. In other words, the higher the reading, the lower the concentration of PCB. Refer to Table 3.

Table 3 Relative PCB concentration

If the sample absorbance reading is...	then the sample concentration is...
Smaller than the calibrator reading	Larger than the calibrator reading
Larger than the calibrator reading	Smaller than the calibrator reading

For example, if the readings are:

- 1 ppb PCB Calibrator: 0.775 Abs
- 5 ppb PCB Calibrator: 0.430 Abs
    - Sample 1: 0.200 Abs
    - Sample 2: 0.600 Abs
    - Sample 3: 0.900 Abs

The interpretation for a sample:

Sample 1: The sample concentration of PCB is larger than 1 and 5 ppm as Aroclor 1248.

Sample 2: The sample concentration of PCB is between 1 and 5 ppm as Aroclor 1248.

Sample 3: The sample concentration of PCB is smaller than 5 and 1 ppm as Aroclor 1248.

Reagent storage and handling

1. Always wear gloves and eyewear for protection.
2. For long-term storage, make sure that the reagents are not in direct sunlight. Keep the reagent set at 4 °C (39.2 °F) when not in use. Warm the reagents to room temperature before use.

3. When not in use, seal the foil pouch that contains the antibody cuvettes.
4. If the Stop Solution is in contact with the eyes, rinse fully for 15 minutes with cold water and get immediate medical help.

Sensitivity

The PCB immunoassay cannot identify specific Arochlors. The PCB immunoassay reacts with different compounds at different sensitivity levels. Refer to Table 4 and Table 5.

Table 4 Various PCBs in soil

Arcochlor Compound	Concentration (ppm) to give positive results 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

Table 5 Compounds not detectable at 1000 ppm

Biphenyl	2,4,6-trichlorophenyl	1,3-dichlorobenzene
2,4-dichlorophenyl	pentachlorophenol	1,4-dichlorobenzene
2,4,5-trichlorphenyl	1,2-dichlorobenzene	1,2,4-trichlorobenzene

Summary of method

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

After incubation, the sample and unbound enzyme conjugate are rinsed from the cuvette and a color-development reagent is added. The enzyme in the conjugate catalyzes the development of color. Thus, 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 if the analyte concentration in the sample is larger or smaller than the threshold levels. The PCB concentration is inversely proportional to the color development—the lighter the color, the higher the PCB concentration. The test results are measured at 450 nm.

The method reacts with all PCBs and cannot identify samples with specific PCBs in a mixture.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Item no.
Soil Extraction Kit	1	each	2775100
PCB Reagent Set	1	20 cuvettes	2773500
Water, deionized	varies	500 mL	27248

Required apparatus

Description	Quantity/Test	Unit	Item no.
Balance, portable, 300 g capacity	1	each	2796900
Caps, flip spout (for 500-mL deionized water bottle)	1	2/pkg	2581802
Graduated cylinder, 10-mL	1	each	108138
Marker, laboratory	1	each	2092000
Gloves, nitrile, medium	1	100/pkg	2550502
Pipet, TenSette®, 0.1–1.0 mL	1	each	1970001
Pipet tips, for TenSette® Pipet, 0.1–1.0 mL	2	50/pkg	2185696
Pipet, Wiretrol®, 10–50 µL	1	each	2852200
Pipet, Wiretrol®, 50–1000 µL	1	each	2568905
Rack, for 12-mm Micro Cuvettes	1	each	4879910
Safety goggles, vented	1	each	2550700
Wipes, disposable	1	280/pkg	2097000
Soil scoop, 5-g, 4.25-cc	1	20/pkg	2657205
Timer, talking	1	each	2764400
Soil extraction refill kit, for 2775100, includes:	1	each	2775200
Dropper, LDPE, 0.5 and 1.0-mL	1	20/pkg	2124720
Filter and barrel assembly	1	20/pkg	2567620
Sodium sulfate, anhydrous	1	250 g	709929
Soil extraction solution	1	200 mL	2567729
Soil sample container	1	20/pkg	2592920
Weighing boat, 8.9-cm square	1	20/pkg	2179020
Spatula, disposable	1	2/pkg	2569320

FOR TECHNICAL ASSISTANCE, PRICE INFORMATION AND ORDERING:
In the U.S.A. – **Call toll-free 800-227-4224**
Outside the U.S.A. – **Contact the HACH office or distributor serving you.**
On the Worldwide Web – **www.hach.com**; E-mail – **techhelp@hach.com**

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

 01/2017, Edition 9