PCB

• Intended Use

For detection of Polychlorinated Biphenyls (PCBs). Please refer to the attached specific procedures for water (groundwater, surface water, well water, effluent), and soil. Application procedures for other sample matrices can be obtained from Abraxis.

• Principle

The Abraxis PCB Assay applies the principles of enzyme linked immunosorbent assay (ELISA) to the determination of PCB. The sample to be tested is added, along with an enzyme conjugate, to a disposable test tube, followed by paramagnetic particles with antibodies specific to PCB attached. Both the PCB (which may be in the sample) and the enzyme labeled PCB (the enzyme conjugate) compete for antibody binding sites on the magnetic particles. At the end of an incubation period, a magnetic field is applied to hold the paramagnetic particles (with PCB and labeled PCB analog bound to the antibodies on the particles, in proportion to their original concentration) in the tube and allow the unbound reagents to be decanted. After decanting, the particles are washed with Washing Solution.

The presence of PCB is detected by adding the enzyme substrate (hydrogen peroxide) and the chromogen (3,3',5,5'-tetramethylbenzidine). The enzyme-labeled PCB analog bound to the PCB antibody catalyzes the conversion of the substrate/chromogen mixture to a colored product. After an incubation period, the reaction is stopped and stabilized by the addition of acid. Since the labeled PCB (conjugate) was in competition with the unlabeled PCB (sample) for the antibody sites, **the color developed is inversely proportional to the concentration of PCB in the sample**.

• Reagents

1. PCB Antibody Coupled Paramagnetic Particles
The PCB antibody (rabbit anti-PCB) is covalently bound to paramagnetic particles, which are suspended in buffered saline containing preservative and stabilizers.
100 test kit: one 60 mL vial

2. PCB Enzyme Conjugate
The horseradish peroxidase (HRP) labeled PCB analog is diluted in buffered saline containing preservative and stabilizers.
100 test kit: one 30 mL vial

3. PCB Standards
Three concentrations (0.25, 2.5, 25.0 ppb) of PCB (as Aroclor 1254) standards in a methanolic solution with preservative and stabilizers are supplied. Other aroclor standards can be supplied as per customer request. Each vial contains 2.0 mL.

4. Control
A concentration (approximately 5 ppb) of PCB (as Aroclor 1254) in a methanolic solution containing preservative and stabilizers. Other aroclor controls can be supplied as requested by the customer. A 2.0 mL volume is supplied in one vial.

5. Diluent/Zero Standard
Buffered saline containing preservative and stabilizers without any detectable PCB.
100 test kit: one 35 mL vial

6. Color Solution
A solution of hydrogen peroxide and 3,3',5,5'-tetramethylbenzidine in an organic base.
100 test kit: one 65 mL vial

7. Stopping Solution
A solution of sulfuric acid (0.5%).
100 test kit: one 60 mL vial

8. Washing Solution
Preserved deionized water.
100 test kit: one 250 mL vial

9. Test Tubes
Polystyrene tubes (36) are packed in a box.
100 test kit: three 36 tube boxes

• Reagent Storage and Stability

Store all reagents at 2-8°C. Do not freeze. Reagents may be used until the expiration date on the box. *The test tubes require no special storage condition and may be stored separately from the reagents to conserve refrigerator space.*

Consult state, local and federal regulations for proper disposal of all reagents.

• Materials Required but Not Provided

In addition to the reagents provided, the following items are essential for the performance of the test:

Pipets* Precision pipets capable of delivering 200, 250 and 500 uL and a 1.0 mL repeating pipet.

Vortex Mixer* Thermolyne Maxi Mix, Scientific Industries Vortex Genie, or equivalent

Magnetic Separation Rack*

Photometric Analyzer* capable of readings at 450 nm

* These items are available from Abraxis.

• Sample Information

Refer to sample preparation information contained under individual procedure (i.e. water, soil) or application notes.

Samples which have been preserved with monochloroacetic acid or other acids, should be neutralized with strong base e.g. 6N NaOH, prior to assay.

If the PCB concentration of a sample exceeds 25 ppb (Aroclor 1254), the sample is subject to repeat testing using a diluted sample. A ten-fold or greater dilution of the sample is recommended with an appropriate amount of Diluent/Zero Standard or Sample Diluent. For example, in a separate test tube make a ten-fold dilution by adding 100 uL of the sample to 900 uL of Diluent/Zero Standard. Mix thoroughly before assaying. Perform the assay according to the Assay Procedure and obtain final results by multiplying the value obtain by the dilution factor e.g. 10.

The presence of the following substances up to 250 ppm were found to have no significant effect on the PCB Assay results: copper, nickel, zinc, mercury, manganese, phosphate, sulfate, sulfite, magnesium, calcium, nitrate and thiosulfate. Humic acid up to 25 ppm and iron to 100 ppm were found to have no significant effect. In addition, sodium chloride concentrations up to 1.0 M showed no effect on results.

• Reagent Preparation

All reagents must be allowed to come to room temperature and the antibody coupled paramagnetic particles should be mixed thoroughly before use.

• Procedural Notes and Precautions

As with all immunoassays, a consistent technique is the key to optimal performance. To obtain the greatest precision, be sure to treat each tube in an identical manner.

Add reagents directly to the bottom of the tube while **avoiding contact between the reagents and the pipet tip**. This will help assure consistent quantities of reagent in the test mixture.

Avoid cross-contaminations and carryover of reagents by using clean pipets for each sample addition and by avoiding contact between reagent droplets on the tubes and pipet tips.

Avoid foam formation during vortexing.

The magnetic separation rack consists of two parts: an upper rack which will securely hold the test tubes and a lower separator which contains the magnets used to attract the antibody coupled paramagnetic particles. During incubations the upper rack is removed from the lower separator so that the paramagnetic particles remain suspended during the incubation. **For separation steps, the rack and the separator are combined to pull the paramagnetic particles to the sides of the tubes.**

To obtain optimum assay precision, it is important to perform the separation steps carefully and consistently. Decant the rack by slowly inverting away from the operator using a smooth turning action so the liquid flows consistently along only one side of the test tube. While still inverted, place the rack on an absorbent pad and allow to drain. Lifting the rack and replacing gently onto the pad several times will ensure complete removal of the liquid from the rim of the tube.

Mix the antibody coupled paramagnetic particles just prior to pipetting.

> Standard and Control vials should remain capped when not in use, to prevent evaporation.

Do not use any reagents beyond their stated shelf life.

Avoid contact of Stopping Solution (sulfuric acid) with skin and mucous membranes. If this reagent comes in contact with skin, wash with water.

• Limitations

The Abraxis PCB Assay will detect PCBs to different degrees. Refer to specificity table for data on various Aroclors and congeners. The Abraxis PCB Assay kit provides screening results. As with any analytical technique (GC, HPLC, etc...) positive results requiring some action should be confirmed by an alternative method.

The total time required for pipetting the magnetic particles should be kept to two (2) minutes or less, therefore the total number of tubes that can be assayed in a run should be adjusted accordingly.

• Quality Control

A control solution at approximately 5 ppb of PCB (as Aroclor 1254) is provided with the Abraxis PCB Assay kit. It is recommended that it be included in every run and treated in the same manner as unknown samples. Acceptable limits should be established by each laboratory.

• Assay Procedure

Read Reagent Preparation, Procedural Notes and Precautions before proceeding.

1. Perform the appropriate sample preparation according to the attached water or soil procedure. For any other sample matrices refer to specific procedures available from Abraxis.
2. Label test tubes for standards, control, and samples.

Tube Number	Contents of Tube
1,2	Diluent/Zero Standard, 0 ppb
3,4	Standard 1, 0.25 ppb
5,6	Standard 2, 2.5 ppb
7,8	Standard 3, 25.0 ppb
9	Control
10	Sample 1
11	Sample 2
12	Sample 3

3. Add 200 uL of the appropriate standard, control, or sample.
4. Add 250 uL of PCB Enzyme Conjugate to each tube.
5. Mix the PCB Antibody Coupled Paramagnetic Particles thoroughly and add 500 uL to each tube.
6. Vortex for 1 to 2 seconds minimizing foaming.
7. Incubate for 15 minutes at room temperature.

8. Separate in the Magnetic Separation Rack for **two (2) minutes**.
9. Decant and **gently** blot all tubes briefly in a consistent manner.
10. Add 1 mL of Washing Solution to each tube and vortex tubes for 1-2 seconds. Return tubes and allow to remain in the magnetic separation unit for **two (2) minutes**.
11. Decant and **gently** blot all tubes briefly in a consistent manner.
12. Repeat Steps 10 and 11 an additional time.
13. Remove the rack from the separator and add 500 uL of Color Solution to each tube.
14. Vortex for 1 to 2 seconds minimizing foaming.
15. Incubate for 20 minutes at room temperature.
16. Add 500 uL of Stopping Solution to each tube.
17. Add 1 mL Washing Solution to a clean test tube. Use as blank in Step 17.
18. Read results at 450 nm within 15 minutes after adding the Stopping Solution.

• Results

Manual Calculations

1. Calculate the mean absorbance value for each of the standards.
2. Calculate the %B/Bo for each standard by dividing the mean absorbance value for the standard by the mean absorbance value for the Diluent/Zero Standard.
3. Construct a standard curve by plotting the %B/Bo for each standard on vertical logit (Y) axis versus the corresponding PCB concentration on horizontal logarithmic (X) axis on the graph paper provided.
4. %B/Bo for controls and samples will then yield levels in ppb of PCB by interpolation using the standard curve.

(Contact Abraxis for detailed application information on specific photometers.)

Photometric Analyzer

Some instrument manufacturers make available photometers allowing for calibration curves to be automatically calculated and stored. Refer to the instrument operating manual for detailed instructions. To obtain results from the Abraxis PCB Assay on instruments allowing data transformation, the following parameter settings are recommended when using Arochlor 1254 as calibrators (Refer to application bulletin when using other aroclors as calibrators):

Data Reduct	:	Lin. Regression
Xformation	:	Ln/LogitB
Read Mode	:	Absorbance
Wavelength	:	450 nm
Units	:	PPB
# Rgt Blk	:	0

Calibrators:

# of Cals	:	4
# of Reps	:	2

Concentrations:

#1:	0.00	PPB
#2:	0.25	PPB
#3:	2.50	PPB
#4:	25.0	PPB

Range	:	0.10 - 25.0
Correlation	:	0.990
Rep. %CV	:	10%

• Expected Results

Refer to the expected result section in the appropriate application note or procedure.

• Performance Data

Sensitivity

The Abraxis PCBR Assay has an estimated minimum detectable concentration, based on a 90% B/Bo of 100 ppt. Refer to appropriate application notes or procedures for sensitivity in specific sample matrices.

Specificity

The cross-reactivity of the Abraxis PCB Assay for various Aroclors can be expressed as the least detectable dose (LDD) which is estimated at 90% B/Bo, or as the dose required to displace 50% (50% B/Bo).

Compound	LDD (ppb)	50% B/Bo (ppb)
Aroclor 1254	0.11	9.0
Aroclor 1260	0.35	4.4
Aroclor 1248	0.40	18
Aroclor 1242	1.3	38
Aroclor 1262	0.25	4.0
Aroclor 1232	0.60	46
Aroclor 1268	0.36	20
Aroclor 1016	0.46	38
Aroclor 1221	8.3	550

The following compounds demonstrated no reactivity in the PCB RaPID Assay at concentrations up to 10,000 ppb: Biphenyl, 2,5-Dichlorophenol, 2,3,5-Trichlorophenol, Di-n-octyl-phthalate.

• Assistance

For ordering or technical assistance contact:

Abraxis LLC
Sales Department
54 Steamwhistle Drive
Warminster, PA 18974
(215) 357-3911 * Fax (215) 357-5232

• Availability

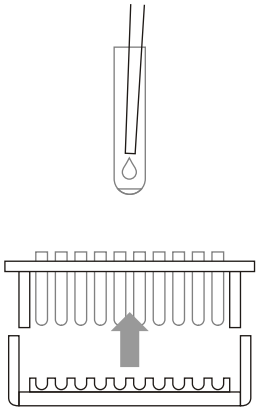
Abraxis PCB Assay Kit, 100T	PN 530001
PCB Sample Diluent	PN 530002
PCB Extraction Solution	PN 530005

PCB Calibrator sets (1254, 1260, 1248, 1242, 1262, 1232, 1268, 1016, 1221)

R112803

PCB DETAILED FLOWCHART

1.



Remove upper rack from magnetic base. Label test tubes for Standards, Control, and Samples.

Tube #	Content
1, 2	Diluent/Zero Standard 0 ppb
3, 4	Standard 1, 0.25 ppb
5, 6	Standard 2, 2.5 ppb
7, 8	Standard 3, 25.0 ppb
9	Control
10	Sample 1
11	Sample 2
12	Sample 3

Add 200 µL of either Standards, Control or Samples to the bottom of each test tube by inserting the pipette tip all the way into the bottom of the tube without touching the sides of the tube.

2.

Add 250 µL of PCB Enzyme Conjugate down the inside wall of each tube by aiming the pipet tip 1/4" to 1/2" below the tube rim without touching the rim or tube wall with the pipet tip; deliver liquid gently.

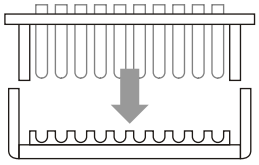
3.

Add 500 µL of thoroughly mixed PCB Antibody Coupled Magnetic Particles down the inside wall of each tube by using the technique described in Box 2. *Vortex* for 1 to 2 seconds (at low speed to minimize foaming).

4.

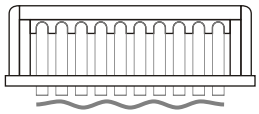
React 15 minutes at room temperature (15 °- 30°C).

5.



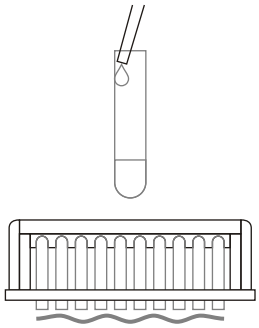
Combine the upper rack with the magnetic base; press all tubes into base; allow 2 minutes for the particles to separate.

6.



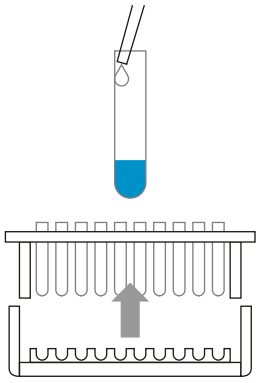
Do not separate upper rack from lower base. Using a smooth motion, *invert* the combined rack assembly over a sink and pour out the tube contents; keep inverted and **gently blot** the test tube rims on several layers of paper toweling.

7.



Add 1 mL of Washing Solution down the inside wall of each tube by using the technique described in Box 2. *Wait 2 minutes.* Using a smooth motion, invert the combined rack assembly over a sink and pour out the tube contents: keep inverted and **gently blot** the test tube rims on several layers of paper toweling. Repeat this step.

8.

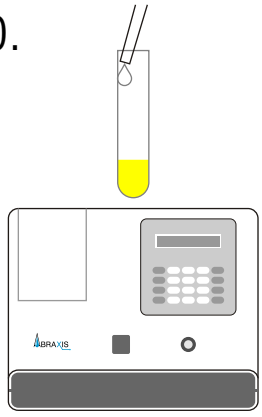


Lift the upper rack (with its tubes) off the magnetic base; *add* 500 µL of Color Reagent down the inside wall of each tube by using the technique described in Box 2. *Vortex* for 1 to 2 seconds (at low speed to minimize foaming).

9.

React for 20 minutes at room temperature (15°- 30° C). During this period, add 1 mL of Washing Solution into a clean tube for use as an instrument blank in Step 10.

10.



Add 500 µL of Stopping Solution down the inside wall of each tube by using the technique previously described. *Read* results at 450 nm within 15 minutes after adding the Stopping Solution. *Multiply* results of samples by the appropriate dilution factor (if any).

[**Safety Caution:** Stopping Solution contains diluted sulfuric acid.]

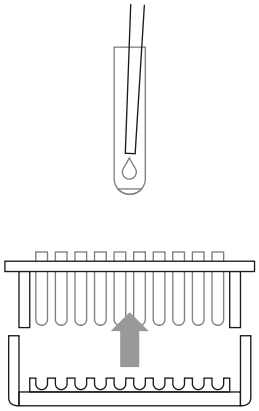
For Ordering or Technical Assistance Contact:
ABRAXIS, LLC 54 Steamwhistle Drive, Warminster, PA 18974
Phone: 215-357-3911 Fax: 215-357-5232
Web: www.abraxiskits.com

PCB Magnetic Particle Kit Part # 530001, 100 Test

ABRAXIS

PCB CONCISE FLOWCHART

1.



Separate the rack.

Add 200 µL of either Standards, Control or Samples to the bottom of each test tube.

2.

Add 250 µL of PCB Enzyme Conjugate to each test tube.

3.

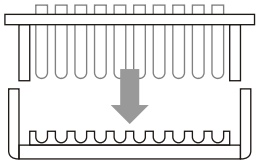
Add 500 µL of mixed Magnetic Particles to each test tube.

Vortex.

4.

Incubate for 15 minutes.

5.

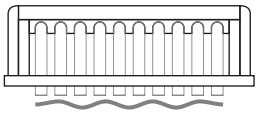


Combine the rack and magnetic base.

Seat all tubes.

Wait 2 minutes.

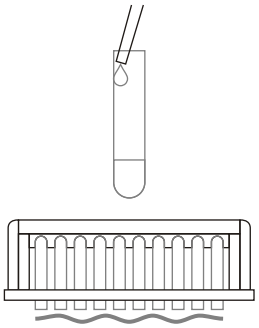
6.



Invert the combined rack.

Blot **gently**.

7.



Add 1 mL of Washing Solution.

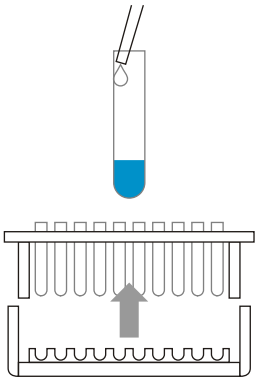
Wait 2 minutes.

Invert the combined rack.

Blot **gently**.

Repeat this step.

8.



Separate the rack.

Add 500 µL of Color Reagent to each test tube.

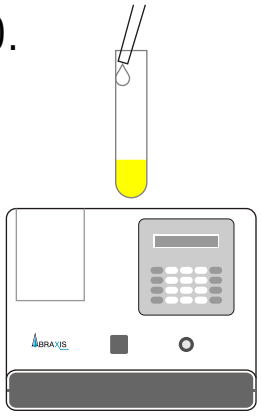
Vortex.

9.

Incubate for 20 minutes.

Prepare blank.

10.



Add 500 µL of Stopping Solution to each test tube.

Read OD 450

For Ordering or Technical Assistance Contact:
ABRAXIS, LLC 54 Steamwhistle Drive, Warminster, PA 18974
Phone: 215-357-3911 Fax: 215-357-5232
Web: www.abraxiskits.com

ABRAXIS