

• Intended Use

For detection of Polychlorinated Biphenyls – Lower Chlorinated (PCB-LC). 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-LC Assay applies the principles of enzyme linked immunosorbent assay (ELISA) to the determination of PCB-LC. 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-LC attached. Both the PCB-LC (which may be in the sample) and the enzyme labeled PCB-LC (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-LC and labeled PCB-LC 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-LC is detected by adding the enzyme substrate (hydrogen peroxide) and the chromogen (3,3',5,5'-tetramethylbenzidine). The enzyme-labeled PCB-LC analog bound to the PCB-LC 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-LC (conjugate) was in competition with the unlabeled PCB-LC (sample) for the antibody sites, **the color developed is inversely proportional to the concentration of PCB-LC in the sample.**

• Reagents

1. PCB-LC Antibody Coupled Paramagnetic Particles

The PCB-LC antibody (sheep anti-PCB-LC) 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-LC Enzyme Conjugate

The horseradish peroxidase (HRP) labeled PCB-LC analog is diluted in buffered saline containing preservative and stabilizers.

100 test kit: one 30 mL vial

3. PCB-LC Standards

Three concentrations (10, 100, 500 ppb) of PCB-LC (as Aroclor 1221) 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 250 ppb) of PCB-LC (as Aroclor 1221) 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-LC.

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 μ L 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-LC concentration of a sample exceeds 500 ppb (Aroclor 1221), 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 μ L of the sample to 900 μ L of Diluent/Zero Standard. Mix thoroughly before assaying. Perform the assay according to the Assay Procedure and obtain final results by multiplying the value obtained by the dilution factor e.g. 10.

The presence of the following substances up to 10,000 ppm were found to have no significant effect on the PCB-LC Assay results: nickel, zinc, mercury, manganese, phosphate, sulfate, sulfite, magnesium, calcium, nitrate and thiosulfate. Humic acid up to 10 ppm and copper and iron to 1,000 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-LC Assay will detect PCBs to different degrees. Refer to specificity table for data on various Aroclors and congeners. The Abraxis PCB-LC 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 250 ppb of PCB-LC (as Aroclor 1221) is provided with the Abraxis PCB-LC 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, 10 ppb
5,6	Standard 2, 100 ppb
7,8	Standard 3, 500 ppb
9	Control
10	Sample 1
11	Sample 2
12	Sample 3

3. Add 250 μ L of the appropriate standard, control, or sample.
4. Add 250 μ L of PCB-LC Enzyme Conjugate to each tube.
5. Mix the PCB-LC Antibody Coupled Paramagnetic Particles thoroughly and add 500 μ L 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-LC Assay on instruments allowing data transformation, the following parameter settings are recommended when using Arochlor 1221 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: 10.0 PPB
 #3: 100.0 PPB
 #4: 500.0 PPB

Range : 10.0 – 500.0
 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 PCB-LC Assay has an estimated minimum detectable concentration, based on a 90% B/Bo of 5 ppb. Refer to appropriate application notes or procedures for sensitivity in specific sample matrices.

Specificity

The cross-reactivity of the Abraxis PCB-LC 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 1221	5	76
Aroclor 1016	1.5	24
Aroclor 1242	1.7	26.5
Aroclor 1248	1.5	26.5
Aroclor 1254	2.3	43
Aroclor 1262	6.6	144
Aroclor 1268	43	2,500
Biphenyl	275	6,600

• Availability

Abraxis PCB-LC Assay Kit, 100T	PN 530021
PCB-LC Sample Diluent	PN 530022
PCB-LC Extraction Solution	PN 530005
PCB Calibrator sets (1221)	PN 530023

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