Importance of PCB Determination

Polychlorinated biphenyls (PCBs) are a group of synthetic industrial compounds which contain a varying number of chlorine atoms substituted on a biphenyl molecule. Although now legally prohibited, several industrialized countries produced PCBs, which were marketed under various trade names (Aroclors®, Kaneclors®, etc.), for such uses as dielectric fluids for capacitors and transformers, heat transfer fluids, hydraulic fluids, and lubricating and cutting oils. PCBs are chemically inert and stable when heated, allowing them to persist in the environment for long periods of time.

The PCB ELISA allows for the analysis of 42 samples in duplicate determination. Less than 1 mL of water or 10 g of soil sample is required. The test can be performed in less than 2 hours.

Performance Data

Test sensitivity: The limit of detection for PCB (90% B/B_0 calculated from the average of 10 calibration curves) is approximately 0.2 ng/mL. The middle of the test (50% B/B_0 calculated from the average of 10 calibration curves) is approximately 23.5 ng/mL. Determinations closer to the middle of the calibration curve give the most accurate results.

Test reproducibility: Precision:

Control	1	2	3
Replicates	5	5	5
Days	3	3	3
n	15	15	15
Mean (ppb)	2.59	10.91	49.97
%CV (within assay)	12.9	11.4	8.3
%CV (between assay)	6.5	6.4	5.7

Recoveries:

Spiked Water Recoveries:

Level	Mean	%CV	% Recovery
5 ppb	5.17	17.8	103.5
25 ppb	25.36	16.8	101.5
100 ppb	109.54	14.6	109.5
250 ppb	279.29	7.1	111.7

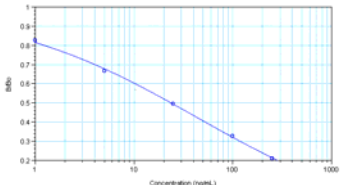
20 ppm Spiked Soil Recoveries:

Dilution Factor	Mean Result (ppb)	%CV	% Recovery
200	108.25	9.4	108.3
400	57.38	11.6	114.8
1000	23.47	3.2	117.3
2000	12.24	5.8	122.4
4000	4.91	0.5	98.2

Specificity: Cross-reactivity of the Abraxis PCB Kit for various Aroclor mixtures:

Aroclor 1254	100.0%
Aroclor 1268	0.2%
Aroclor 1262	35.7%
Aroclor 1260	64.0%
Aroclor 1248	183.7%
Aroclor 1242	112.1%
Aroclor 1016	69.3%
Aroclor 1232	50.4%
Aroclor 1221	16.7%

Standard Curve:



For demonstration purposes only. Not for use in sample interpretation.

General Limited Warranty: Abraxis LLC warrants the products manufactured by the Company, against defects and workmanship when used in accordance with the applicable instructions for a period not to extend beyond the product's printed expiration date. **Abraxis makes no other warranty, expressed or implied. There is no warranty of merchantability or fitness for a particular purpose**

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ABRAXIS

PCB ELISA (Microtiter Plate)

Enzyme-Linked Immunosorbent Assay for the Determination of PCBs in Water and Soil Samples

Product No. 530041

1. General Description

The Abraxis PCB ELISA is an immunoassay for the quantitative and sensitive screening of Polychlorinated Biphenyls (PCBs). This test is suitable for the quantitative and/or qualitative screening of PCBs in water and soil samples (please refer to the appropriate technical bulletins for additional matrices procedures). Samples requiring regulatory action should be confirmed by HPLC, GC/MS, or other conventional methods.

2. Safety Instructions

The standard solutions in the test kit contain small amounts of Aroclor 1254. In addition, the substrate solution contains tetramethylbenzidine and the stop solution contains diluted sulfuric acid. Avoid contact of stopping solution with skin and mucous membranes. If these reagents come in contact with skin, wash with water.

3. Storage and Stability

The PCB ELISA should be stored in the refrigerator (4–8°C). The solutions must be allowed to reach room temperature (20-25°C) before use. Reagents may be used until the expiration date on the box. The conjugate is supplied in lyophilized form (3 vials). Before each assay, the required volume of lyophilized conjugate must be reconstituted with conjugate diluent (see Test Preparation section). Reconstitute only the amount needed for the samples to be run, as the reconstituted solution will only remain viable for one week.

4. Test Principle

The test is a direct competitive ELISA based on the recognition of Polychlorinated Biphenyls by specific antibodies. The PCBs, when present in a sample, and a PCB-HRP analogue compete for the binding sites of anti-PCB antibodies in solution. The PCB antibodies are then bound by a second antibody immobilized on the wells of the microtiter plate. After a washing step and addition of the substrate solution, a color signal is generated. The intensity of the blue color is inversely proportional to the concentration of PCBs present in the sample. The color reaction is stopped after a specified time and the color is evaluated using an ELISA reader. The concentrations of the samples are determined by interpolation using the standard curve constructed with each run.

5. Limitations of the PCB ELISA, Possible Test Interference

Although many organic and inorganic compounds commonly found in samples have been tested and found not to interfere with this test, due to the high variability of compounds that might be found in samples, test interferences caused by matrix effects cannot be completely excluded.

Samples must be extracted and diluted as instructed in the sample preparation section (Section D) or appropriate technical bulletin before testing in the ELISA.

Mistakes in handling the test also can cause errors. Possible sources for such errors include: inadequate storage conditions of the test kit, incorrect pipetting sequence or inaccurate volumes of the reagents, too long or too short incubation times during the immune and/or substrate reaction, extreme temperatures (lower than 10°C or higher than 30°C) during the test performance.

The Abraxis PCB ELISA kit provides screening results. As with any analytical technique (GC, HPLC, etc.), samples requiring some regulatory action should be confirmed by an alternative method.

A. Reagents and Materials Provided

1. Microtiter plate (12 X 8 strips) coated with a secondary antibody, in a resealable aluminum pouch
2. PCB Calibrators/Standards as Aroclor 1254* (6): 0, 1, 5, 25, 100, 250 ng/mL (ppb), 1 mL each
3. Anti-PCB Antibody Solution, 6 mL
4. PCB-HRP Conjugate, 3 vials (lyophilized), 2.5 mL/vial after reconstitution
5. Conjugate Diluent, 12 mL
6. Wash Solution (5X) Concentrate, 100 mL, must be diluted before use, see Test Preparation (Section C)
7. Sample Diluent, 1 bottle, 25 mL (additional Sample Diluent is available for purchase)
8. Substrate (Color) Solution (TMB), 16 mL
9. Stop Solution, 12 mL (handle with care)

NOTE: Other Aroclor Standards are available.

B. Additional Materials (not delivered with the test kit)

1. Micro-pipettes with disposable plastic tips (20-1000 µL)
2. Multi-channel pipette (50-250 µL) or stepper pipette with disposable plastic tips (50-250 µL)
3. Microtiter plate reader (wave length 450 nm)
4. 50-100 mL glass sample container with Teflon-lined cap
5. Vortex mixer
6. Deionized or distilled water
7. Sodium Sulfate, anhydrous
8. Methanol, HPLC grade
9. Paper towels or equivalent absorbent material
10. Timer
11. Centrifuge capable of spinning at 3,000 x g
12. 4 mL, 20 mL, and 60 mL glass vials with Teflon-lined caps
13. Glass fiber syringe filter (1.2 µm, Environmental Express or equivalent) with syringe
14. Scoopula
15. Pipette bulbs
16. Pasteur pipettes
17. Serological glass pipettes
18. Analytical 3 place balance

C. Test Preparation

Micro-pipetting equipment and pipette tips for pipetting the standards and the samples are necessary. In order to equalize the incubation periods on the entire microtiter plate, a multi-channel pipette or a stepping pipette is recommended for adding the enzyme conjugate, antibody, substrate, and stop solutions. Please only use the reagents and standards from one package lot in one test, as they have been adjusted in combination.

1. Adjust the microtiter plate and the reagents to room temperature before use.
2. Remove the number of microtiter plate strips required from the aluminum pouch. The remaining strips are stored in the aluminum pouch and zip-locked closed. Store the remaining kit in the refrigerator (4-8°C).
3. The standard solutions, antibody, substrate and stop solutions are ready to use and do not require any further dilutions.
4. The conjugate provided is lyophilized (3 vials). Before each assay, calculate the volume of conjugate needed (when reconstituted, each vial will provide enough conjugate for approximately 50 wells). Reconstitute only the amount necessary for the samples to be analyzed. Once reconstituted, the conjugate solution will only remain viable for 1 week. If additional samples are to be analyzed greater than one week from reconstitution, a new vial of conjugate will need to be prepared. To reconstitute, add 2.5 mL of Conjugate Diluent to each vial of conjugate required and vortex thoroughly. If multiple vials are necessary at one time, reconstitute each vial, then combine the reconstituted solutions and vortex thoroughly.
5. Dilute the Wash Solution (5X) Concentrate at a ratio of 1:5. If using the entire bottle (100 mL), add to 400 mL of deionized or distilled water and mix thoroughly.
6. The stop solution must be handled with care as it contains diluted H_2SO_4.

D. Sample Preparation

Water Samples

1. Water samples should be collected in glass jars with Teflon-lined caps.
2. **Immediately** upon collection, samples must be diluted with an equal volume of methanol (HPLC grade) to prevent adsorptive losses to the glass. Mix well.
3. After samples are diluted, those samples containing gross particulate matter should be filtered using a syringe and glass fiber syringe filter (Environmental Express or equivalent).
4. Analyze as sample (Assay Procedure, step 1).

The PCB concentration in the sample is determined by multiplying the ELISA result by a factor of 2. Highly contaminated samples, those outside of the calibration range of the assay, must be diluted further and re-analyzed.

Soil Samples

Note: Approximately 50 g of sample should be homogenized prior to extraction to ensure a truly representative sample.

1. Weigh 10.0 g of sample into a 60 mL glass vial with Teflon-lined cap.
2. Add 5 g of anhydrous Sodium Sulfate to each sample. Mix thoroughly. *Note: Samples may harden when mixed with Sodium Sulfate. Samples must be free flowing prior to adding the Methanol to ensure proper extraction. If necessary, hardened samples can be broken up using a scoopula.*
3. Add 20 mL of Methanol to the vial.
4. Shake vigorously by hand or vortex for 2 minutes.
5. Allow the samples to settle. Transfer methanol extract to an appropriately labeled 20 mL glass vial with Teflon-lined cap.
6. Centrifuge for 10 minutes at 3000 x g at room temperature.
7. Transfer methanol extract (avoid transferring sediment) to an appropriately labeled 20 mL glass vial with Teflon-lined cap.
8. Transfer 1 mL of the extract to an appropriately labeled 4 mL glass vial. Dilute with 1 mL of deionized or distilled water. Vortex well.
9. To a second appropriately labeled 4 mL glass vial, add 980 µL of Sample Diluent. Add 20 µL of the diluted extract (from step 8) to the diluent in the vial. Vortex. This will then be analyzed as a sample (Assay Procedure, step 1).

The PCB concentration in the sample is determined by multiplying the ELISA result by a factor of 200. Highly contaminated samples, those outside of the calibration range of the assay, must be diluted further and re-analyzed.

E. Working Scheme

The microtiter plate consists of 12 strips of 8 wells, which can be used individually for the test. The standards must be run with each test. Never use the values of standards that have been determined in a test performed previously.

Std 0-Std 5: Standards
(0; 1; 5; 25; 100; 250 ppb)

Samp1, Samp2, etc.: Samples

	1	2	3	4	5	6	7	8	9	10	11	12
A	Std 0	Std 4	etc.									
B	Std 0	Std 4	etc.									
C	Std 1	Std 5										
D	Std 1	Std 5										
E	Std 2	Samp1										
F	Std 2	Samp1										
G	Std 3	Samp2										
H	Std 3	Samp2										

F. Assay Procedure

1. Add 50 µL of the **calibrator/standard solutions**, **samples**, or **sample extracts** (Section D) into the wells of the test strips according to the working scheme given. Analysis in duplicate or triplicate is recommended.
2. Add 50 µL of **reconstituted enzyme conjugate solution** to the individual wells successively using a multi-channel pipette or a stepping pipette.
3. Add 50 µL of **antibody solution** to the individual wells successively using a multi-channel pipette or a stepping pipette. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop for 60 seconds. Be careful not to spill the contents.
4. Incubate the strips for 60 minutes at room temperature.
5. Remove the covering and decant the contents of the wells into a sink. Wash the strips **four times** using the **diluted washing buffer solution**. Please use at least a volume of 250 µL of washing buffer for each well in each washing step. Remaining buffer in the wells should be removed by patting the inverted plate dry on a stack of paper towels.
6. Add 150 µL of **substrate (color) solution** to the wells. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop for 60 seconds. Be careful not to spill the contents. Incubate the strips for 30 minutes at room temperature. Protect the strips from direct sunlight.
7. Add 100 µL of **stop solution** to the wells in the same sequence as for the substrate solution.
8. Read the absorbance at 450 nm using a microplate ELISA photometer within 15 minutes after the addition of stopping solution.

G. Evaluation

The evaluation of the ELISA can be performed using commercial ELISA evaluation programs such as 4-Parameter (preferred) or Logit/Log. For a manual evaluation, calculate the mean absorbance value for each of the standards. Calculate the $\%B/B_0$ for each standard by dividing the mean absorbance value for each standard by the Zero Standard (Standard 0) mean absorbance. Construct a standard curve by plotting the $\%B/B_0$ for each standard on the vertical linear (y) axis versus the corresponding PCB concentration on the horizontal logarithmic (x) axis on graph paper. $\%B/B_0$ for samples will then yield levels in ppb (ng/mL or ng/g) of PCB by interpolation using the standard curve. Results can also be obtained by using a spreadsheet macro available from Abraxis upon request.

The concentrations of the samples are determined using the standard curve run with each test. Sample extracts showing a lower concentration of PCB than standard 1 (1 ppb) should be reported as containing < 2 ppb of PCB for Water Samples and < 200 ppb for Soil Samples. Samples showing a higher concentration than standard 5 (250 ppb) must be diluted further with the provided sample diluent and re-analyzed.

Semi-quantitative results can be derived by simple comparison of the sample absorbances to the absorbances of the calibrators. Samples with lower absorbances than a calibrator will have concentrations of PCB greater than the concentration of that calibrator. Samples which have higher absorbances than a calibrator will have concentrations of PCB less than that calibrator.