D. Specificity

The cross reactivity of the ABRAXIS® Coplanar PCBAssay for various congeners and Aroclors can be expressed as the least detectable dose (LDD) which is estimated at 90% B/B_0, or as the dose required for the 50% absorbance inhibition (50% B/B_0).

Compound	LOD (ppt)	50% B/B0 (ppt)	TEF [1]
Congener 126	14	270	0.1
Congener 169	4	90	0.01
Congener 77	300	5,100	0.0001
Congener 189	700	9,000	0.0001
Congener 81	700	10,000	0.0001
Congener 123	2200	270,000	0.0001
Congener 167	3000	54,000	0.00001
Congener 105	3000	400,000	0.0001
Congener 156	3300	50,000	0.0005
Congener 114	5000	115,000	0.0005
Congener 157	10,000	140,000	0.0005
Congener 118	26,000	240,000	0.0001
Congener 170	NR	NR	
Congener 180	NR	NR	
Aroclor 1246	7,000	480,000	
Aroclor 1248	70,000	440,000	
Aroclor 1056	90,000	3,200,000	
Aroclor 1254	100,000	1,500,000	
Aroclor 1262	90,000	10,000,000	
Aroclor 1221	120,000	4,200,000	
Aroclor 1268	120,000	40,000,000	
Aroclor 1016	540,000	4,000,000	
Biphenyl	NR	NR	

NR = non-reactive up to 1,000,000 ppt

The following compounds demonstrated no reactivity in the ABRAXIS® Coplanar PCB Assay at concentrations up to 1000 ppb: aldicarb, aldicarb sulfoxide, aldicarb sulfone, alachlor, atrazine, benomyl,butachlor, butylate, captan, carbaryl, carbendazim, carbofuran, 2,4-D, 1,3-dichloropropene, dinoseb, MCPA, metolachlor, metribuzin, pentachlorophenol, picloram, propachlor, terbufos, thiabendazole, and thiophanate- methyl.

General Limited Warranty: Gold Standard Diagnostics 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. **Gold Standard Diagnostics makes no other warranty, expressed or implied. There is no warranty of merchantability or fitness for a particular purpose.**

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ABRAXIS® Coplanar PCB ELISA Microtiter Plate

Product No. 530011

1. General Description

For the detection of coplanar Polychlorinated Biphenyls (PCBs) in water (groundwater, surface water, well water). For soil, and other sample matrices contact Gold Standard Diagnostics for application bulletins and/or specific matrix validation guidelines.

2. Storage and Stability

Store all reagents at 2-8°C. Do not freeze. Reagents may be used until the last day of the month as indicated bythe expiration date on the box. Consult state, local and federal regulations forproper disposal of all reagents.

3. Test Principle

The ABRAXIS® Coplanar PCB Kit applies theprinciples of enzyme linked immunosorbent assay (ELISA) to the determination of Coplanar PCBs. The test is a direct competitive ELISA. The sample (please refer to reagent preparation section) to be tested, along with an antibody specific for Coplanar PCBs are added to microtiter wells coated with Goat Ant-Rabbit Antibody. At this point, a competitive reaction occurs between the Coplanar PCBs which may be in the sample for the antibody binding sites. The reaction is allowed to continue for thirty (30) minutes. After the above incubation, a coplanar PCB ligand labeled with an ezyme (HRP) is added and incubated for ninety (90) minutes. After a washing step and addition of a substrate (color solution), a color signal (blue color) is generated. Thecolor reaction is stopped and stabilized after twenty (20-30) minutes by the addition of diluted acid (stopping solution). The color is then evaluated using an ELISA reader. The intensity of the yellow color is inversely proportional to the concentration of the coplanar PCB present in the sample.

4. Limitations of the ABRAXIS® Coplanar PCB ELISA

The ABRAXIS® Coplanar PCB Assay will detect PCBsto different degrees. Refer to specificity table for data on various congeners and Aroclors. The ABRAXIS® Coplanar 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.

5. Working Instructions

A. Materials Provided

1. Microtiter Plate (8 X 12 strips) coated with Goat-Anti Rabbit Antibody
2. Coplanar PCB Antibody Solution Rabbit anti-coplanar PCB solution, 6 mL
3. Coplanar PCB Standards (7) (Congener 126) 0, 25, 50, 100, 250, 500, 1000 ppt, 1 mL
4. Coplanar PCB-HRP Enzyme Conjugate, 6 mL
5. Diluent/Zero Standard, 30 mL
6. Substrate (Color) Solution, 16 mL
7. Stop Solution, 6 mL
8. ABRAXIS® Wash Buffer 5X Concentrate Buffer 100 mL

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

1. Micro Pipettes* Precision pipets capable ofdelivering 50,100, and 150 uL, and tips
2. Plate reader* capable of readings at 450 nm.
3. Distilled or deionized water.
4. Methanol, reagent grade.
5. Transfer pipettes, 5 Ml
6. Disposable glass tubes or glass vials with Tefloncaps.
7. Parafilm.

C. Reagent Preparation

All reagents must be allowed to come to room temperature. PCBs tend to absorb to surfaces, therefore sample dilutions should be prepared fresh before use in disposable glass tubes or glass vials. If collecting water samples, please use glass vials with teflon caps, immediately after the collection of the sample, an equal volume of methanol should be added to the sample for preservation (prevents the absorption of PCBs to the glass surface) such as to obtain a 50% methanol/sample solution.

D. Samples to be analyzed:

Prior to analysis (if not previously done), each sample needs to be diluted in methanol to obtain a methanol concentration of 50% (v/v), as follows: add 250 uL of methanol to a disposable test tube, add 250 uL of sample and vortex gently. Cover sample with parafilm until use.

E. Wash Buffer

In a 1000 mL container, dilute the wash buffer concentrate 1:5 by the addition of deionized or distilled water. If using the entire bottle (100 mL) of ABRAXIS® Wash Buffer 5X Concentrate dilute with 400 mL of deionized or distilled water.

F. Sample Information

Refer to sample preparation information contained underindividual procedure (i.e. water) or application notes.
Samples containing gross particulate matter should be filtered (e.g. 0.2 um Anotop™ 25 Plus, Whatman, Inc.) to remove particles.
Samples which have been preserved with monochloroaceticacid or other acids, should be neutralized with strong base e.g. 6N NaOH, prior to assay.
If the PCB concentration of a sample exceeds 1000 ppt, thesample 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 glass 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.

G. 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 well in an identical manner.
Add reagents directly to the bottom of the well while **avoiding contact between the reagents and the pipet tip**. This will help assure consistent quantities of reagent in thetest 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.
Do not use any reagents beyond their stated shelf life.
Avoid contact of Stopping Solution (diluted sulfuric acid) with skin and mucous membranes. If this reagent comes in contact with skin, wash with water.
The microtiter plate consists of 12 strips of 8 wells, when you use fewer than 12 strips, remove the unneeded strips and store them refrigerated in the re- sealable bag (with desiccant) provided.
If more than three strips are being used per run, it isrecommended that a multi-channel pipette be used for the addition of antibody, conjugate, color, and stopping solution.

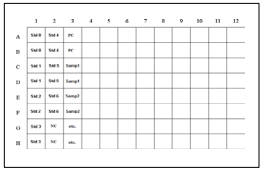
H. Quality Control

Control solutions (negative and positive solution) of PCBs should be assayed with each run. It is recommended that they be included in every run and treated in the samemanner as unknown samples. Acceptable limits should be established by each laboratory.

I. Working Scheme

Read Reagent Preparation, Procedural Notes and Precautions before proceeding.

Std0-Std6: Standards
NC: Negative Control (standard 0)
PC: Positive Control (supplied by lab)
Samp1, Samp2, etc.: Samples



J. Assay Procedure

1. Add 50 µL of anti-coplanar PCB antibody solution successively to each well.
2. Add 50 µL of the appropriate standard, control, or sample. We recommend using duplicates or triplicates. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop. Be careful not to spill the contents.
3. Incubate at room temperature for 30 minutes.
4. After the incubation, remove the covering and add 50µL of enzyme conjugate solution to the individual wells successively. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop. Be careful not to spill the contents.
5. Incubate at room temperature for 90 minutes. .After the incubation, remove the covering and vigorously shake the contents of the wells into a waste container.
6. Wash the strips 3 times using the 1X wash buffer with a volume of at least 250 µL per each wash step. Any remaining buffer in the wells should be removed by patting the plate on a dry stack of paper towels.
7. Add 150 µL of color solution successively to each well. Incubate for 20-30 minutes.
8. Add 50 µL of Stopping Solution to each well inthe same sequence as for the other reagents.
9. Read absorbance using a microplate reader at 450 nmwithin 15 minutes after adding the Stop Solution.

K. Results

The evaluation of the ELISA can be performed using commercial ELISA evaluation programs (4-Parameters, Logit/Log or alternatively point to point). For a manual evaluation, calculate the mean absorbance value for each of the standards. Calculate the % B/Bo for each standard by dividing the mean absorbance obtained with each standard by the mean absorbance value for the zero standard (Standard 0). Construct a standard curve by plotting the % B/Bo for each standard on the vertical (y) axis versus the corresponding Congener 126 concentration on the horizontal (x) axis on a graph paper. Calculate the %B/Bo for each control and sample(s) and obtain concentration by interpolation using the constructed standard curve.
The results obtained will then need to be multiplied by the appropriate factor to account for the initial sample dilution (methanol addition), i.e. by 2 if testing water samples.
Samples exhibiting a lower concentration than 25 ppt are considered to be negative. Samples exhibiting a higher concentration than 1000 ppt must be diluted to obtain accurate results.

6. Performance Data

A. Precision

The following results were obtained:

Control	1	2	3
Replicates	5	5	5
Days	3	3	3
n	15	15	15
Mean (ppt)	108	236	479
%CV within assay	12.9	11.2	10.2
%CV between assay	8.4	7.0	7.4

B. Sensitivity

The ABRAXIS® Coplanar PCB Assay has an estimated minimum detectable concentration, based on a 90% B/Bo of 14 ppt for congener-126, please refer to cross-reactivity table for other congeners or aroclors.

C. Recovery

Four (4) samples, including a municipal water source, drinking water from a local pond and a small creek were spiked with various levels of PCB (Congener #126), diluted 1:1 with methanol and then assayed using the ABRAXIS® Coplanar PCB Assay. The following results were obtained:

PCBs (ppt)	Mean (ppt)	S.D. (ppt)	% Recovery
50	50.0	9.9	100.0
100	100.9	17.1	100.9
200	187.2	21.9	93.6
400	383.6	39.1	95.9
800	786.4	40.2	98.3
Average			97.7