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PBDE

ABRAXIS

• Intended Use

For detection of Polybrominated Diphenyl Ether (PBDEs). 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 PBDE Microtiter Plate Kit applies the principles of enzyme linked immunosorbent assay (ELISA) to the determination of PBDE. In the assay system, standards or samples are added, along with an antibody specific to PBDE, to microtiter wells coated with Goat Anti-Rabbit Antibody and incubated for thirty (30) minutes. The PBDE enzyme conjugate is then added. At this point, a competitive reaction occurs between the PBDE, which may be in the sample, and the enzyme-labeled PBDE analog for the antibody binding sites on the microtiter well. The reaction is allowed to continue for thirty (30) minutes. After a washing step, the presence of PBDE is detected by adding the "Color Solution," which contains the enzyme substrate (hydrogen peroxide) and the chromogen (3,3',5,5'-tetramethylbenzidine). The enzyme-labeled PBDE bound to the PBDE antibody catalyzes the conversion of the substrate/chromogen mixture to a colored product. The color reaction is stopped and stabilized after a twenty (20) minute incubation period by the addition of diluted acid (stopping solution). The color is then evaluated using an ELISA reader.

A dose response curve of absorbance vs. concentration is generated using results obtained from the standards. The concentration of PBDE present in the samples is determined directly from this curve. Since the labeled PBDE (conjugate) was in competition with the unlabeled PBDE (sample) for the antibody sites, t**he intensity of the color developed is inversely proportional to the concentration of Triclosan present in the sample.**

• Reagents

The Abraxis PBDE Plate Kit contains the following items:

1. Microtiter Plate coated with Goat-Anti Rabbit Antibody

96 test kit: 12 strips of 8 antibody coated wells and strip holder (1).

2. PBDE Antibody Solution
PBDE antibody (rabbit anti-PBDE) solution in a buffered saline solution with preservative and stabilizers.

96 test kit: One vial containing 6 mL

3. PDBE Enzyme Conjugate
Horseradish peroxidase (HRP) labeled PBDE analog in a buffered solution with preservative and stabilizers.

96 test kit: One vial containing 6 mL

4. PBDE Standards
Eight concentrations (0, 0.04, 0.1, 0.25, 0.5, 1.0, 2.0, and 4.0 ppb) of PBDE Congener 47 in DMSO with preservative and stabilizers.

96 test kit: Each vial contains 1.0 mL

5. Diluent/Zero Standard (Sample Diluent)
DMSO with preservative and stabilizers without any detectable PBDE.

96 test kit: One bottle containing 30 mL

7. Color Solution
A solution of hydrogen peroxide and 3,3',5,5'-tetramethyl benzidine in an organic base.

96 test kit: One bottle containing 16 mL

8. Stopping Solution
A solution of diluted acid.

96 test kit: One bottle containing 6 mL each

9. Washing Buffer (5x) Concentrate
Buffered salts with detergent and preservatives.

96 test kit: One bottle containing 100 mL

• 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.

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:

Precision pipets capable of delivering 50, 100 and 250 uL, and tips*

Tape or Parafilm®*

Timer*

Distilled or deionized water for diluting Wash Buffer

Storage bottle with 1000 mL capacity for storage of 1x Wash Buffer*

Microplate or strip reader capable of reading absorbance at 450 nm*

* Please contact Abraxis for supplier information.

• Sample Information

This procedure is recommended for use with water samples. Other samples may require modifications to the procedure and should be thoroughly validated.

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 PBDE concentration of a sample exceeds 4.0 ppb, 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 1000 ppm were found to have no significant effect on the PBDE Assay results: zinc, manganese, calcium, magnesium, sodium, phosphate, sulfate, thiosulfate, and nitrate. Humic acid, copper, and $FeSO_4$ up to 100 ppm was found to have no significant effect.

• Reagent Preparation

All reagents must be allowed to come to room temperature.

Wash Buffer

In a 1000 mL container, dilute the wash buffer concentrate 1:5 by the addition of distilled or deionized water (i.e., 100 mL of wash buffer concentrate plus 400 mL of H_2O). This solution is used to wash the antibody coated wells.

• 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 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.

The microtiter plate consists of 12 strips of 8 wells. If fewer than twelve strips are used, remove the unneeded strips and store refrigerated in the resealable foil bag (with desiccant) provided.

If more than 3 strips are being used per run, the use of a multi-channel pipette is recommended for the addition of conjugate, antibody, color, and stopping solutions.

Do not use any reagents beyond their stated shelf life. Each component used in any one assay should be of the same lot number and stored under identical conditions.

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

• Limitations

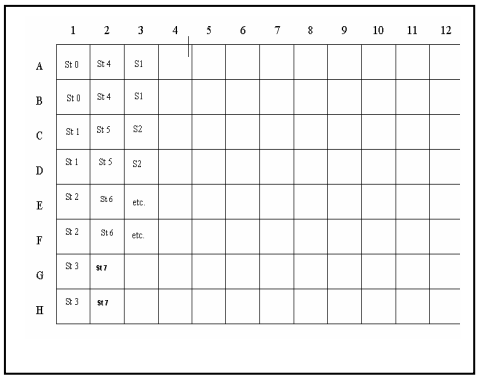
The Abraxis PBDE Plate Assay will detect PBDE and related compounds. Refer to the specificity table for data on several related compounds. The Abraxis PBDE Plate Assay kit provides screening results. As with any analytical technique (GC, HPLC, etc...) positive results requiring some action should be confirmed by an alternative method.

• Assay Procedure

Read Reagent Preparation, Procedural Notes and Precautions before proceeding.

St0-St7: Standards

S1-Sx: Samples



1. Add 50 uL of the appropriate standard or sample. Analysis in duplicates or triplicates is recommended.
2. Add 50 uL of PBDE antibody solution successively to each well. Cover wells with parafilm or tape to prevent contamination and evaporation. Thoroughly mix the contents of the wells by moving the strip holder in a rapid circular motion on the benchtop for a full 2 minutes. Be careful not to spill the contents. Incubate at ambient temperature for 30 minutes.
3. After the incubation, add 50 uL of PBDE enzyme conjugate solution successively to each well. Cover wells with parafilm or tape and thoroughly mix the contents of the wells by moving the strip holder in a rapid circular motion on the benchtop for a full 2 minutes. Incubate at ambient temperature for 30 minutes.
4. After the incubation, carefully remove the covering and vigorously shake the contents of the wells into a waste container. Wash the strips with the diluted Wash Buffer (see Reagent Preparation) by adding a volume of at least 250 uL of Wash Buffer to each well. Vigorously shake the contents of the wells into a waste container. Any remaining buffer in the wells should be removed by patting the plate on a dry stack of paper towels. Repeat this wash step three times, for a total of 4 rinses.
5. Add 100 uL of Color Solution successively to each well. Cover wells with parafilm or tape. Thoroughly mix the contents of the wells by moving the strip holder in a rapid circular motion on the benchtop for a full 20-30 seconds Incubate at ambient temperature for 30 minutes.
6. Add 100 uL of Stopping Solution successively to each well.
7. Read absorbance using a microplate reader at 450 nm within 15 minutes after adding the Stopping Solution.

• Results

The evaluation of the ELISA can be performed using commercial ELISA evaluation programs (4-parameter or alternatively point to point). For manual evaluation, calculate the mean absorbance value for each of the standards. Calculate the %B/Bo for each standard by dividing the mean absorbance value for each standard by the mean absorbance value for the Diluent/Zero Standard (Standard 0). Construct a standard curve by plotting the %B/Bo for each standard on the vertical linear (Y) axis versus the corresponding PBDE concentration on the horizontal log (X) axis on the graph paper provided. Calculate the %B/Bo for the control and sample(s) and obtain the concentration of PBDE (in ppb) by interpolation using the constructed standard curve.

Samples exhibiting a concentration lower than 0.040 ppb should be assumed to be below the detection limit of the assay. Samples exhibiting a concentration higher than 4.0 ppb must be diluted to obtain accurate results.

• Performance Data

Precision

The following results were obtained:

Control	1	2	3
Replicates	5	5	5
Days	3	3	3
n	15	15	15
Mean (ppb)	0.175	0.782	3.312
% CV (within assay)	15.4	11.5	12.1
% CV (between assay)	10.5	9.1	10.7

Limit of Detection

The Abraxis PBDE Plate Assay has an estimated minimum detection concentration based on a 90% B/Bo of 0.030 parts per billion (ppb).

Recovery

Four (4) groundwater samples were spiked with various levels of PBDE and then assayed using the Abraxis PBDE Plate Assay. The following results were obtained:

Amount of PBDE Added (ppb)	Recovery Mean (ppb)	Recovery S.D. (ppb)	Recovery %
0.10	0.093	0.028	92.9
0.25	0.285	0.071	114.1
0.50	0.586	0.117	117.2
2.00	2.086	0.301	104.3
Average			107.1

Sensitivity

The Abraxis PBDE Plate Assay has an estimated minimum detectable concentration, based on a 90% B/Bo of 30 ppt (0.030 ppb). Refer to appropriate application notes or procedures for sensitivity in specific sample matrices.

Cross-reactivities

Compound	% React
PBDE Congener 47	100.0
PBDE Congener 99	31.9
PBDE Congener 28	8.1
PBDE Congener 100	2.0
PBDE Congener 153	0.0
5'methoxy-PBDE-47	159.7
5'methoxy-PBDE-99`	1.1
3'OH-2,4,4'-PBDE	7.8
2'OH-2,4,4'-PBDE	3.7
5'OH-PBDE-47	14.2
Triclosan	1.1
PCB Aroclor 1254	0.1

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

• Ordering Information

Abraxis PBDE Assay Kit, 96T	PN	530045
PBDE Sample Diluent	PN	530047

• 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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