PBDE

• 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 Eurofins Abraxis.

• Principle

The Eurofins Abraxis PBDE Assay applies the principles of enzyme linked immunosorbent assay (ELISA) to the determination of PBDE. The sample to be tested is added, along with paramagnetic particles attached with antibodies specific to PBDE, to a disposable glass test tube. This is followed by the addition of an PBDE enzyme conjugate. Both the PBDE (which may be in the sample) and the enzyme labeled PBDE (the enzyme conjugate) compete for antibody binding sites on the magnetic particles. At the end of a twenty minute (20) incubation period, a magnetic field is applied to hold the paramagnetic particles (with PBDE and labeled PBDE 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 PBDE is detected by adding the enzyme substrate (hydrogen peroxide) and the chromogen (3,3',5,5'-tetramethylbenzidine). The enzyme-labeled PBDE analog bound to the PBDE antibody catalyzes the conversion of the substrate/ chromogen mixture to a colored product. After an incubation period of twenty (20) minutes, the reaction is stopped and stabilized by the addition of acid. Since the labeled PBDE (conjugate) was in competition with the unlabeled PBDE (sample) for the antibody sites, **the color developed is inversely proportional to the concentration of PBDE in the sample**.

• Reagents

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

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

3. PBDE Standards
Five concentrations (0.025, 0.05, 0.1, 0.5, 1.0 ppb) of PBDE Congener 47 in a methanolic solution with preservative and stabilizers. Each vial contains 2.0 mL.

4. Control
A concentration (approximately 0.25 ppb) of PBDE in a methanolic solution containing preservative and stabilizers.
A 2.0 mL volume is supplied in one vial.

5. Diluent/Zero Standard
A methanolic solution containing preservative and stabilizers without any detectable PBDE.
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 diluted sulfuric acid (0.5%).
100 test kit: one 60 mL vial

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

9. Test Tubes
Glass 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 last day of the month as indicated by 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 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 Eurofins Abraxis.

• Sample Information

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

** Water samples should be collected in glass vessels (teflon cap liners). Immediately upon collection, samples and controls should be diluted with an equal volume (1:1) of methanol (HPLC grade) to prevent adsorptive losses to the glass containers.

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

After samples are diluted, those samples containing gross particulate matter should be filtered (e.g. 0.2 um Anotop™ 25 Plus, Whatman, Inc.) to remove particles.

If the PBDE concentration of a sample exceeds 1.0 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: copper, zinc, manganese, calcium, magnesium, sodium, phosphate, sulfate, thiosulfate, and nitrate Humic acid 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 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 Eurofins Abraxis PBDE Assay will detect PBDEs to different degrees. Refer to specificity table for data on various Aroclors and congeners. The PBDE 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 0.25 ppb of PBDE is provided with the Eurofins Abraxis PBDE 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 Eurofins Abraxis.
2. Label **glass** test tubes for standards, control, and samples.

Tube Number	Contents of Tube
1,2	Diluent/Zero Standard, 0 ppb
3,4	Standard 1, 0.025 ppb
5,6	Standard 2, 0.05 ppb
7,8	Standard 3, 0.1 ppb
9,10	Standard 4, 0.5 ppb
11,12	Standard 5, 1.0 ppb
13	Control
14	Sample 1
15	Sample 2
16	Sample 3

3. Add 250 uL of the appropriate standard, control, or sample.
4. Mix the PBDE Antibody Coupled Paramagnetic Particles thoroughly and add 500 uL to each tube.
5. Vortex for 1 to 2 seconds minimizing foaming.
6. Incubate for 20 minutes at room temperature.
7. Add 250 uL of PBDE Enzyme Conjugate to each tube.
8. Vortex for 1 to 2 seconds minimizing foaming.
9. Incubate for 20 minutes at room temperature.
10. Separate in the Magnetic Separation Rack for **two (2) minutes**.
11. Decant and **gently** blot all tubes briefly in a consistent manner.
12. Add 1 mL of Washing Solution T 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**.
13. Decant and **gently** blot all tubes briefly in a consistent manner.
14. Repeat Steps 10 and 11 an additional time.
15. Remove the rack from the separator and add 500 uL of Color Solution to each tube.
16. Vortex for 1 to 2 seconds minimizing foaming.
17. Incubate for 20 minutes at room temperature.
18. Add 500 uL of Stopping Solution to each tube.
19. Add 1 mL Washing Solution to a clean test tube. Use as blank in Step 17.
20. 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 Ln (Y) axis versus the corresponding PBDE concentration on horizontal Ln (X) axis on the graph paper provided.
4. %B/Bo for controls and samples will then yield levels in ppt of PBDE by interpolation using the standard curve.

Photometric Analyzer

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

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 Eurofins Abraxis PBDE Assay on instruments allowing data transformation, the following parameter settings are recommended:

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

Calibrators:

# of Cals	:	6
# of Reps	:	2

Concentrations:

#1:	0.00	PPT
#2:	25	PPT
#3:	50	PPT
#4:	100	PPT
#5:	500	PPT
#6:	1000	PPT

Range	:	20 - 1000
Correlation	:	0.990
Rep. %CV	:	10%

**Multiply the sample and control results by a factor of 2 to account for the initial 1:1 dilution of sample with methanol, or alternatively program the Photometric Analyzer to automatically correct for the dilution factor.

• Expected Results

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

• Performance Data

Sensitivity

The Eurofins Abraxis PBDE Assay has an estimated minimum detectable concentration, based on a 90% B/Bo of 17 ppt (0.017 ppb). Refer to appropriate application notes or procedures for sensitivity in specific sample matrices.

Specificity

The cross-reactivity of the Eurofins Abraxis PBDE 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).

B/Bo Compound	LDD (ppb)	50% (ppb)
PBDE Congener 47	0.017	0.135
PBDE Congener 99	0.02	0.15
PBDE Congener 28	0.045	0.9
PBDE Congener 100	0.055	5.5
PBDE Congener 153	0.075	10
PBDE Congener 154	3.5	580
PBDE Congener 183	13.5	2000
PBDE Congener 209	370	3000
5'methoxy-PBDE-47	0.017	0.084
5'methoxy-PBDE-99	2	13
4'OH-2,4,4'-PBDE	0.012	0.65
3'OH-2,4,4'-PBDE	0.68	1.65
2'OH-2,4,4'-PBDE	0.14	4.9
3'OH-2,4-PBDE	0.135	8.0
2'OH-2,4-PBDE	2.3	>100
5'OH-PBDE-47	0.030	1.6
6'OH-PBDE-47	0.040	13
Methyl Triclosan	0.1	4.6
Triclosan	0.25	11
PCB Aroclor 1254	3	180
PCB Congener 37	160	2000
PCB Congener 77	>1000	>1000
T3	>1000	>1000
Thyroxine (T4)	>1000	>1000
PCP	3300	>10,000
2-4-D	>10,000	>10,000

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.

• Assistance

For ordering or technical assistance contact:

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• Availability

Eurofins Abraxis PBDE Assay Kit, 100T	PN	500090
PBDE Sample Diluent	PN	500091

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