

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 Abraxis.

• Principle

The 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 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 μ L and a 1.0 mL repeating pipet.

Vortex Mixer* ThermoMylene 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 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 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 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 FeSO₄ 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 Abraxis PBDE Assay will detect PBDEs to different degrees. Refer to specificity table for data on various Aroclors and congeners. The Abraxis 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 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 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 μ L of the appropriate standard, control, or sample.

4. Mix the PBDE Antibody Coupled Paramagnetic Particles thoroughly and add 500 μ L to each tube.

5. Vortex for 1 to 2 seconds minimizing foaming.

6. Incubate for 20 minutes at room temperature.

7. Add 250 μ L of PBDE Enzyme Conjugate to each tube.