Cyclodienes

• Intended Use

For the detection and quantitation of cyclodienes in water (groundwater, surface water, well water). For soil, and other sample matrices contact the company for application bulletins and/or specific matrix validation guidelines.

• Principle

The Abraxis Cyclodienes Kit applies the principles of enzyme linked immunosorbent assay (ELISA) to the determination of cyclodienes. The test is an indirect competitive ELISA. The sample (please refer to reagent preparation section) to be tested, along with an antibody specific for cyclodienes are added to microtiter wells containing an immobilized cyclodiene-protein analogue. At this point a competitive reaction occurs between the cyclodienes which may be be in the sample and the immobilized cyclodienes analogue for the antibody binding sites. The reaction is allowed to continue for sixty (60) minutes. After a washing step, a second antibody-HRP label is added and incubated for thirty (30) minutes. After a washing step and addition of the substrate (color soution), a color signal (blue color) is generated. The color reaction is stopped and stabilized after twenty (20) 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 cyclodienes present in the sample.**

• Reagents

The Abraxis Cyclodienes Kit contains the following items:

1. Microtiter Plate coated with an analogue of Cyclodiene conjugated to a protein.
Immobilized Cyclodiene analogue conjugated to a protein.
96 test kit: 12 X 8 strips

2. Cyclodiene Antibody Solution
Rabbit anti-cyclodiene solution in a colored buffered saline solution with preservative and stabilizers.
96 test kit: one 11 mL vial

3. Cyclodienes Standards
Dieldrin standard stock at a concentration of 250 ng/mL in methanol. **See reagent preparation section.**
96 test kit: one 1 mL vial

4. Anti-Rabbit-HRP Enzyme Conjugate
Horseradish peroxidase (HRP) labeled anti-rabbit diluted in a buffered solution with preservative and stabilizers.
96 test kit: one 11 mL vial

5. Diluent/Zero Standard
25% methanol in distilled water (v/v) without any detectable cyclodienes.
96 test kit: one 30 mL vial

6. Color Solution
A solution of hydrogen peroxide and 3,3',5,5'-tetramethylbenzidine in an organic base.
96 test kit: one 11 mL vial

7. Stopping Solution
A solution of diluted acid.
96 test kit: one 6 mL vial

8. Washing Buffer 5X Concentrate
Buffer salts with detergent and preservatives.
96 test kit: one 100 mL vial

• 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:

1. Micro Pipettes* Precision pipets capable of delivering 25, 50,100, and 250 uL, and tips.
2. Vortex Mixer* Thermolyne Maxi Mix, Scientific Industries Vortex Genie, or Equivalent.
3. Plate reader* capable of readings at 450 nm.
4. Distilled or deionized water.
5. Methanol, reagent grade.
6. Transfer pipettes, 5 mL
7. Disposable glass tubes or glass vials with Teflon caps.
8. Parafilm.

* Please contact Abraxis for supplier information.

• Sample Information

Refer to sample preparation information contained under individual 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 monochloroacetic acid or other acids, should be neutralized with strong base e.g. 6N NaOH, prior to assay.

If the cyclodienes concentration of a sample exceeds 25 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 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.

• Reagent Preparation

All reagents must be allowed to come to room temperature.

Cyclodienes tend to absorb to surfaces, therefore sample dilutions should be prepared fresh before use in disposable glass tubes or glass vials.

Standards:

A reasonable Standard dilution scheme:

Std. Concentration (ppb)	Standard Diluent (mL)	Dieldrin Stock (250 ppb) to Add (uL)
25	0.900	100
10	0.960	40
5	0.980	20
2.5	0.900	100 uLof **25 ppb std.**
1.0	0.900	100 uL of **10 ppb std.**
0.50	0.900	100 uL of **5 ppb Std.**
0.25	0.900	100 uL of **2.5 ppb Std.**
0	1.000	0

Samples to be analyzed:

At collection time and prior to analysis, each sample needs to be diluted in methanol to obtain a methanol concentration of 25% (v/v), as follows: add 50 uL of methanol to a disposable test tube, add 150 uL of sample and vortex gently. Cover sample with parafilm until use.

Wash Buffer

In a 1000 mL container, dilute the wash buffer concentrate 1:5 by the addition of deionized or distilled water (i.e. 100 mL of wash buffer 5X concentrate plus 400 mL of water.

• 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.

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 8 strips of 12 wells, when you use fewer than 8 strips, remove the unneeded strips and store them refrigerated in the re-sealable bag (with dessicant) provided.

If more than three strips are being used per run, it is recommended that a multi-channel pipette be used for the addition of antibody, conjugate, color, and stopping solution.

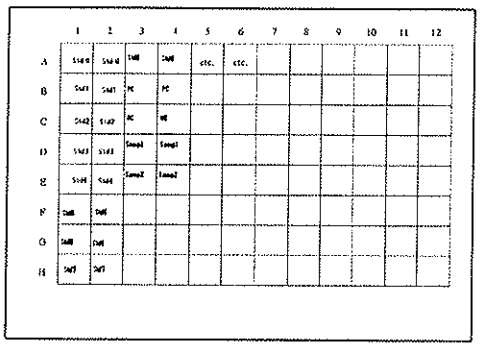
• Limitations

The Abraxis Cyclodienes Assay will detect dieldrin and related cyclodienes to different degrees. Refer to specificity table for data on several of the cyclodienes. The Abraxis Cyclodienes 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.

• Quality Control

Control solutions (negative and positive solution) of cyclodienes should be assayed with each run. It is recommended that they 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.

St1-St 8: Standards
NC: Negative Control (standard 1)
PC: Positive Control (supplied by lab)
Samp1-Sx: Samples