

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 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 solution), 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

Horseshoe 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 obtained by the dilution factor e.g. 10.

• Reagent Preparation

All reagents must be allowed to come to room temperature.

Cyclodienes tend to adsorb 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 uL of 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 desiccant) 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

	1	2	3	4	5	6	7	8	9	10	11	12
A	Std 0	Std 0	Std 0	Std 0	etc.	etc.						
B	Std1	Std1	PC	PC								
C	Std2	Std2	NC	NC								
D	Std3	Std3	Samp1	Samp1								
E	Std4	Std4	Samp2	Samp2								
F	Std5	Std5										
G	Std6	Std6										
H	Std7	Std7										

● **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)	1.05	2.49	7.23
% CV (within assay)	10.2	11.2	7.0
% CV (between assay)	14.5	18.6	9.3

● **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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Sensitivity

The Abraxis Cyclodienes Assay has an estimated minimum detectable concentration, based on a 90% B/Bo of 100 ppt.

Recovery

Five (5) groundwater samples, were spiked with various levels of cyclodienes and then assayed using the Abraxis Cyclodienes Assay. The following results were obtained:

Amount of Cyclodienes Added (ppb)	Mean (ppb)	S.D. (ppb)	Recovery %
2.5	2.25	0.39	90
5.0	4.75	0.89	95
10.0	8.61	0.96	86
Average			90

1. Add 25 uL of the appropriate standard, control, or sample. We recommend using duplicates or triplicates.
2. Add 100 uL of Cyclodienes antibody solution successively to each well. 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. Incubate at room temperature for 60 minutes.
3. After the incubation, remove the covering and vigorously shake the contents of the wells into a container. Wash the strips 3 times using the 1X wash solution with a volume of at least 250 uL per each wash step. Any remaining buffer in the wells should be removed by patting the plate on a dry stack of paper towels.
4. Add 100 uL 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. Incubate at room temperature for 30 minutes.
5. After the incubation, remove the covering and vigorously shake the contents of the wells into a container. Wash the strips 3 times using the 1X wash solution with a volume of at least 250 uL per each wash step. Any remaining buffer in the wells should be removed by patting the plate on a dry stack of paper towels.
6. Add 100 uL of color solution successively to each well. Incubate for 20 minutes.
7. Add 50 uL of Stopping Solution to each well in the same sequence as for the other reagents.
8. 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 (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 value for the zero standard (Standard 1). Construct a standard curve by plotting the %B/Bo for each standard on a the vertical (y) axis versus the corresponding dieldrin 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 1.25 to account for the initial sample dilution (methanol addition).**

Samples exhibiting a lower concentration than 0.25 ppb are considered to be negative. Samples exhibiting a higher concentration than 25 ppb must be diluted to obtain accurate results.

Specificity

The cross-reactivity of the Abraxis Cyclodienes Assay for various cyclodiene analogues can be expressed as the 50% inhibition of each cyclodiene analogue divided by the 50% inhibition of dieldrin.

Compound	Cross-reactivity (%)
Dieldrin	100
Endosulfan	150
Heptachlor	58
Aldrin	26
Chlordane	26
Toxaphene	8.2

The following compounds demonstrated no reactivity in the Abraxis Cyclodienes 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.

● **Ordering information**

Abraxis Cyclodienes Assay Kit 100T	PN 540021
Sample Diluent	PN 500022
Standard Stock (additional)	PN 500023

India Contact:

Life Technologies (India) Pvt. Ltd.

306, Aggarwal City Mall, Road No. 44, Pitampura, Delhi – 110034, India

Mobile: +91-98105-21400, Tel: +91-11-42208000, 8111, 8222, Fax: +91-11-42208444

Email: customerservice@lifetechindia.com, www.atzlabs.com ; www.lifetechindia.com