Importance of Cyclodienes Determination

Cyclodienes are a group of organochlorine pesticides. Examples of Cyclodienes include Dieldrin, Endosulfan, and Chlordane. After widespread use against insects in agricultural fields, lawns and gardens, and structures, the use of Cyclodienes has been severely restricted or banned in most countries due to their persistence in the environment and the adverse health effects associated with them. Cyclodienes accumulate in body fat and are excreted in breast milk; they can also cross the placenta. In addition to the negative effects on human health which have been documented, they also possess varying degrees of toxicity to birds, fish, and bees.

The Cyclodienes ELISA allows for the analysis of 40 samples in duplicate determination. Less than 1 mL of sample is required. The test can be performed in less than 2 hours.

Performance Data

Test sensitivity: The limit of quantitation for Cyclodienes (90% B/B_0) is approximately 0.138 ng/mL. The middle of the test (50% B/B_0) is approximately 3.311 ng/mL. Determinations closer to the middle of the calibration curve give the most accurate results.

Test reproducibility: Coefficients of variation (CVs) for standards: <10%; CVs for samples: <15%.

Specificity: The cross-reactivity of the Abraxis Cyclodienes Plate Assay for various Cyclodiene analogues can be expressed as the 50% inhibition of Dieldrin divided by the 50% inhibition of each Cyclodiene analogue.

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, pentachlorphenol, picloram, propachlor, terbufos, thiabendazole, and thiophanate-methyl.

Performance Data:

Precision

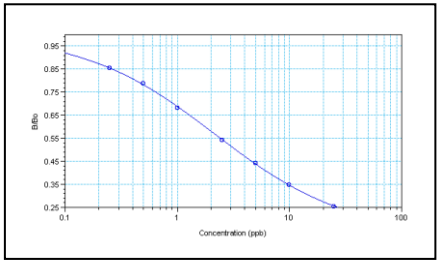
Control	1	2	3
Replicates	5	5	5
Days	3	3	3
N	15	15	15
Mean (ppb)	1.05	2.49	7.23
% CV (within assay)	10.2	11.2	7.0
% CV (between assay)	14.5	18.6	9.3

Recovery

Five (5) groundwater samples were spiked with various levels of Cyclodienes and then assayed using the Abraxis Cyclodienes Assay:

Conc. (ppb)	Mean Rec. (ppb)	Std. Dev. (ppb)	% Rec.
2.5	2.25	0.39	90
5.0	4.75	0.89	95
10.0	8.61	0.96	86
Average			90

Standard Curve:



For demonstration purposes only. Not for use in sample interpretation.

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R051117

Cyclodienes ELISA (Microtiter Plate)

Enzyme-Linked Immunosorbent Assay for the Determination of Cyclodienes in Water Samples

Product No. 540021

1. General Description

The Abraxis Cyclodienes ELISA is an immunoassay for the quantitative and sensitive screening of Cyclodienes. This test is suitable for the quantitative and/or qualitative screening of Cyclodienes in water samples (groundwater, surface water, and well water). For soil and other sample matrices, please contact Abraxis technical services for application bulletins and/or specific matrix validation guidelines. Samples requiring regulatory action should be confirmed by HPLC, GC/MS, or other conventional methods.

2. Safety Instructions

The stock standard solution in the test kit contains a small amount of Dieldrin. The substrate solution contains tetramethylbenzidine (TMB) and the stop solution contains diluted sulfuric acid. Avoid contact of the TMB and stopping solution with skin and mucous membranes. If these reagents come in contact with skin, wash with water. Consult federal, state, and local regulations for proper disposal of all reagents.

3. Storage and Stability

The Cyclodienes ELISA should be stored in the refrigerator (4–8°C). The solutions must be allowed to reach room temperature (20-25°C) before use. Reagents may be used until the expiration date on the box.

4. Test Principle

The test is an indirect competitive ELISA based on the recognition of Cyclodienes by specific antibodies. The sample to be tested, along with an antibody specific for Cyclodienes, are added to microtiter plate wells containing an immobilized Cyclodiene-protein analogue and incubated. A competitive reaction occurs between the Cyclodienes which may be present in the sample and the immobilized Cyclodienes analogue for the antibody binding sites. After a washing step, a second antibody-HRP label is added and incubated. After a second washing step and the addition of the substrate solution, a color signal is generated. The intensity of the blue color is inversely proportional to the concentration of Cyclodienes present in the sample. The color reaction is stopped after a specified time and the color is evaluated using an ELISA reader. The concentrations of the samples are determined by interpolation using the standard curve constructed with each run.

5. Limitations of the Cyclodienes ELISA, Possible Test Interference

Although many organic and inorganic compounds commonly found in samples have been tested and found not to interfere with this test, due to the high variability of compounds that might be found in samples, test interferences caused by matrix effects cannot be completely excluded.

The presence of the following substances were found to have no significant effect on the Cyclodienes assay results: calcium sulfate, potassium phosphate, sodium fluoride, sodium nitrate up to 10,000 ppm; magnesium chloride, magnesium sulfate, sodium chloride up to 1,000 ppm; calcium chloride, copper chloride, humic acid up to 100 ppm.

Mistakes in handling the test can cause errors. Possible sources for such errors include: inadequate storage conditions of the test kit, incorrect pipetting sequence or inaccurate volumes of the reagents, too long or too short incubation times during the immune and/or substrate reaction, extreme temperatures (lower than 10°C or higher than 30°C) during the test performance.

Each reagent is optimized for use in the Abraxis Cyclodienes ELISA kit. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other Abraxis Cyclodienes ELISA kits with different lot numbers.

The Abraxis Cyclodienes ELISA kit provides screening results. As with any analytical technique (GC/MS, HPLC, etc.), samples requiring regulatory action should be confirmed by an alternative method.

Working Instructions

A. Reagents and Materials Provided

1. Microtiter plate (12 X 8 strips) coated with a Cyclodiene analogue conjugated to a protein, in a resealable aluminum pouch
2. Cyclodiene Stock Standard (1): 250 ng/mL (ppb) in methanol, 1 mL, for the preparation of the calibration curve, see Test Preparation (Section E)
3. Diluent/Zero Standard, 30 mL
4. Antibody Solution (anti-Cyclodienes), 11 mL
5. Anti-Rabbit-HRP Conjugate Solution, 11 mL
6. Wash Buffer (5X) Concentrate, 100 mL, must be diluted before use, see Test Preparation (Section E)
7. Substrate (Color) Solution (TMB), 11 mL
8. Stop Solution, 6 mL (handle with care)

B. Additional Materials (not delivered with the test kit)

1. Micro-pipettes with disposable plastic tips (50-200 µL)
2. Multi-channel pipette, stepper pipette (50-250 µL), or electronic repeating pipette with disposable plastic tips
3. Container with 500 mL capacity (for 1X diluted Wash Buffer, see Test Preparation, Section E)
4. Graduated cylinder
5. Deionized or distilled water
6. Methanol (reagent grade)
7. Disposable glass test tubes or glass vials with Teflon lined caps
8. Vortex mixer (Thermolyne Maxi Mix, Scientific Industries Vortex Genie, or equivalent)
9. Paper towels or equivalent absorbent material
10. Timer
11. Tape or parafilm
12. Microtiter plate reader (wave length 450 nm)

C. Notes and Precautions

This procedure is recommended for use with freshwater samples. Other sample types may require modifications to the procedure and should be thoroughly validated. Water samples should be collected in glass sample containers. In order to avoid adsorptive loss of analyte, water samples should be preserved with methanol at a ratio of 3:1 (75% water sample/25% methanol). See Sample Preparation, section D for sample preservation procedure.

Cyclodienes tend to adsorb to surfaces, therefore calibration standards and sample dilutions should be prepared fresh before use in disposable glass test tubes or glass vials.

Micro-pipetting equipment and pipette tips for pipetting the standards and the samples are necessary. The use of a multi-channel pipette, stepping pipette, or electronic repeating pipette is recommended for adding the enzyme conjugate, antibody, substrate, and stop solutions in order to equalize the incubation periods on the entire microtiter plate.

To avoid drift and obtain accurate results, the addition of the antibody, conjugate, color, and stop solutions should be performed in less than 2 minutes for each reagent. If additions to the entire microtiter plate cannot be completed in less than 2 minutes, run size should be decreased to the number of rows which can be pipetted in less than 2 minutes.

Samples containing gross particulate matter should be filtered (e.g. 0.2 µm 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 analysis.

Highly contaminated samples (those outside of the calibration range of the assay) must be diluted further in sample diluent and re-analyzed in order to obtain accurate quantitative results.

Please only use the reagents and standards from one package lot in one test, as they have been adjusted in combination.

D. Sample Preparation

Water samples should be collected in glass sample containers. Immediately upon collection, in order to avoid adsorptive loss of analyte, water samples must be preserved with methanol at a ratio of 3:1 (75% water sample/25% methanol). For example, a 75 mL water sample would be added to 25 mL of methanol (cap tightly and mix thoroughly).

The Cyclodienes concentration contained in water samples is determined by multiplying the ELISA result by the dilution factor of 1.25. Highly contaminated samples (those outside of the calibration range of the assay) must be diluted further in Diluent/Zero Standard and re-analyzed.

E. Test Preparation

1. Allow the microtiter plate, reagents, and samples to reach room temperature before use.
2. Dilute the Wash Buffer (5X) Concentrate at a ratio of 1:5. If using the entire bottle (100 mL), add to 400 mL of deionized or distilled water and mix thoroughly.
3. Remove the number of microtiter plate strips required from the aluminum pouch. The remaining strips are stored in the aluminum pouch with the desiccant (tightly sealed) in the refrigerator (4-8°C).
4. The conjugate, antibody, substrate and stop solutions are ready to use and do not require any further dilutions.
5. Cyclodienes calibration standards must be prepared fresh before use in disposable glass test tubes or glass vials. Calibration standards are prepared using the Cyclodiene Stock Standard (250 ng/mL in methanol) provided in the test kit using the following dilution scheme:

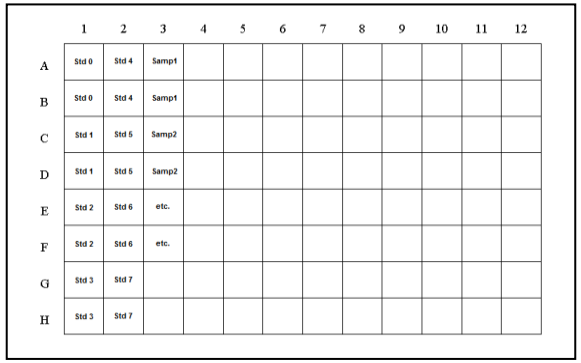
Standard Number	Concentration (ppb)	Diluent/Zero Standard (mL)	Cyclodienes Solution to add (uL)
7	25	0.900	100 µL of **250 ppb** stock
6	10	0.960	40 µL of **250 ppb** stock
5	5	0.980	20 µL of **250 ppb** stock
4	2.5	0.900	100 µL of **25 ppb** standard
3	1.0	0.900	100 µL of **10 ppb** standard
2	0.5	0.900	100 µL of **5 ppb** standard
1	0.25	0.900	100 µL of **2.5 ppb** standard
0	0	1.000	0

F. Working Scheme

The microtiter plate consists of 12 strips of 8 wells, which can be used individually for the test. The standards must be run with each test. Never use the values of standards which have been determined in a test performed previously.

Std 0-Std 7: Standards
(0; 0.25; 0.5; 1.0; 2.5; 5.0, 10.0, 25.0 ppb)

Samp1, Samp2, etc.: Samples



G. Assay Procedure

1. Add **25 µL of the standards or samples** into the wells of the test strips according to the working scheme given. Analysis in duplicate or triplicate is recommended.
2. Add **100 µL of antibody solution** to the individual wells successively using a multi-channel pipette, stepping pipette, or electronic repeating pipette. Cover the wells with parafilm or tape then mix the contents by moving the strip holder in a circular motion on the benchtop for 30 seconds. Be careful not to spill the contents. Incubate the strips at room temperature for 60 minutes.
3. Remove the covering and decant the contents of the wells into a waste container. Wash the strips **three times** using the diluted wash buffer. Please use at least a volume of **250 µL of 1X wash buffer** for each well in each washing step. Remaining buffer in the wells should be removed by patting the inverted plate dry on a stack of paper towels.
4. Add **100 µL of conjugate solution** to the individual wells successively using a multi-channel pipette, stepping pipette, or electronic repeating pipette. Cover the wells with parafilm or tape then mix the contents by moving the strip holder in a circular motion on the benchtop for 30 seconds. Be careful not to spill the contents. Incubate the strips at room temperature for 30 minutes.
5. Remove the covering and decant the contents of the wells into a waste container. Wash the strips **four times** using the diluted wash buffer. Please use at least a volume of **250 µL of 1X wash buffer** for each well in each washing step. Remaining buffer in the wells should be removed by patting the inverted plate dry on a stack of paper towels.
6. Add **100 µL of substrate (color) solution** to the wells successively using a multi-channel pipette, stepping pipette, or electronic repeating pipette. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop for 30 seconds. Be careful not to spill the contents. Incubate the strips for 20 minutes at room temperature. Protect the strips from direct sunlight.
7. Add **50 µL of stop solution** to the wells in the same sequence as for the substrate solution.
8. Read the absorbance at 450 nm using a microplate ELISA photometer within 15 minutes after the addition of stopping solution.

H. Evaluation

The concentrations of the samples are determined using the standard curve run with each test. The evaluation of the ELISA can be performed using commercial ELISA evaluation programs such as 4-Parameter (preferred) or Logit/Log. For a manual evaluation, calculate the mean absorbance value for each of the standards. Calculate the $\%B/B_0$ for each standard by dividing the mean absorbance value for each standard by the Zero Standard (Standard 0) mean absorbance. Construct a standard curve by plotting the $\%B/B_0$ for each standard on the vertical linear (y) axis versus the corresponding Cyclodienes concentration on the horizontal logarithmic (x) axis on graph paper. $\%B/B_0$ for samples will then yield levels in ppb (or ng/mL) of Cyclodienes by interpolation using the standard curve. Results can also be determined using a spreadsheet macro available from Abraxis upon request.

Results for water samples are determined by multiplying the concentration determined from the curve by the dilution factor of 1.25. Samples showing a lower concentration of Cyclodienes than standard 1 (0.25 ppb) should be reported as containing < 0.313 ppb of Cyclodienes. Samples showing a higher concentration than standard 7 (25.0 ppb) should be reported as containing > 31.25 ppb of Cyclodienes. If a quantitative result is necessary, samples must be diluted in Diluent/Zero Standard and re-analyzed.

As with any analytical technique (GC/MS, HPLC, etc.), positive results requiring regulatory action should be confirmed by an alternative method.