Cyclodienes ELISA KIT

**Cat. No.: DEIA6861**  
**Pkg. Size: 96T**

**Intended use**

The Cyclodienes ELISA KIT is suitable for the detection and quantitation of cyclodienes in water (groundwater, surface water, well water).

**General Description**

Hexachlorocyclopentadiene, also known as C-56, is an organochlorine compound that is a precursor to several pesticides. This colourless liquid is an inexpensive reactive diene. Many of its derivatives proved to be highly controversial, as studies showed them to be persistent organic pollutants. Collectively, the pesticides derived from hexachlorocyclopentadiene are called the cyclodienes. An estimated 270,000 tons were produced until 1976, and some are still manufactured by Velsicol in the US and by Jiangsu Anpon Electrochemicals Co. in China.

**Principle Of The Test**

The 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 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 minutes. After a washing step, a second antibody-HRP label is added and incubated for thirty 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 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 And Materials Provided**

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.  
   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

### Materials Required But Not Supplied

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 us for supplier information.

### Storage

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.

### Specimen Collection And Handling

Samples containing gross particulate matter should be filtered 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.

### Plate Preparation

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

Figure 1:
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:
Table 1.
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.

Assay Steps

1. Add 25 uL of the appropriate standard, control, or sample. We recommend using duplicates or triplicates.
2. Add 100 uL of Cycloides 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.

### Quality Control

Control solutions (negative and positive solution) of cyclodiene 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.

### Evaluation

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 intial 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.

### Sensitivity

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

### Specificity

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

Table 2.
The following compounds demonstrated no reactivity in the 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, thiafendazole, and thiophanate-methyl.

### Recovery

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

Table 3:

<table>
<thead>
<tr>
<th>Amount of Cyclodienes Added (ppb)</th>
<th>Mean (ppb)</th>
<th>S.D. (ppb)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5</td>
<td>2.25</td>
<td>0.39</td>
<td>90</td>
</tr>
<tr>
<td>5.0</td>
<td>4.75</td>
<td>0.89</td>
<td>95</td>
</tr>
<tr>
<td>10.0</td>
<td>8.61</td>
<td>0.96</td>
<td>86</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td></td>
<td>90</td>
</tr>
</tbody>
</table>

### Reproducibility

- Intra-Assay: 7.0%-11.2%
- Inter-Assay: 9.3%-18.6%

### Precautions
1. 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.

2. 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.

3. 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.

4. Do not use any reagents beyond their stated shelf life.

5. Avoid contact of Stopping Solution (diluted sulfuric acid) with skin and mucous membranes. If this reagent comes in contact with skin, wash with water.

6. 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.

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