DDE/DDT ELISA KIT

Cat. No.: DEIA6862
Pkg. Size: 96T

Intended use

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

General Description

Dichlorodiphenyldichloroethylene (DDE) is a chemical compound formed by the loss of hydrogen chloride (dehydrohalogenation) from DDT, of which it is one of the more common breakdown products. DDE is fat soluble which tends to build up in the fat of animals. Due to its stability in fat, DDE is rarely excreted from the body, and body levels tend to increase throughout life. The major exception is the excretion of DDE in breast milk, which delivers a substantial portion of the mother's DDE burden to the young animal or child.

DDE and its parent, DDT, are reproductive toxicants for certain bird species, and major reasons for the decline of the bald eagle, brown pelican, peregrine falcon, and osprey. These compounds cause egg shell thinning in susceptible species, which leads to the birds’ crushing their eggs instead of incubating them, due to the latter’s lack of resistance. Birds of prey, waterfowl, and song birds are more susceptible to eggshell thinning than chickens and related species, and DDE appears to be more potent than DDT.

The biological mechanism for the thinning is not entirely known, but it is believed that p,p’-DDE impairs the shell gland’s ability to excrete calcium carbonate onto the developing egg. Multiple mechanisms may be at work, or different mechanisms may operate in different species. Some studies have shown that although DDE levels have fallen dramatically, eggshell thickness remains 10-12 percent thinner than before DDT was first used.

Some studies have indicated that DDE is an endocrine disruptor and contributes to breast cancer, but more recent studies provide strong evidence that there is no relationship between DDE exposure and breast cancer. What is more clear is that DDE is a weak antiandrogen.

Animal studies show that organochlorine pesticides—such as DDE—are neurotoxic, cause oxidative stress, and damage the brain’s dopaminergic system.

DDT (dichlorodiphenyltrichloroethane) is an organochlorine insecticide which is a white, crystalline solid, tasteless, and almost odorless. Technical DDT has been formulated in almost every conceivable form including solutions in xylene or petroleum distillates, emulsifiable concentrates, water-wettable powders, granules, aerosols, smoke candles, and charges for vaporisers and lotions.

First synthesized in 1874, DDT’s insecticidal properties were not discovered until 1939, and it was used with great success in the second half of World War II to control malaria and typhus among civilians and troops. The Swiss chemist Paul Hermann Müller was awarded the Nobel Prize in Physiology or Medicine in 1948 "for his discovery of the high efficiency of DDT as a contact poison against several arthropods." After the war, DDT was made available for use as an agricultural insecticide, and soon its production and use skyrocketed.

In 1962, Silent Spring by American biologist Rachel Carson was published. The book catalogued the environmental impacts of the indiscriminate spraying of DDT in the US and questioned the logic of releasing large amounts of chemicals into the environment without fully understanding their effects on ecology or human health. The book suggested that DDT and other pesticides may cause cancer and that their agricultural use was a threat to wildlife, particularly birds. Its publication was one of the signature events in the birth of the environmental movement, and resulted in a large public outcry that eventually led to DDT
being banned in the US in 1972. DDT was subsequently banned for agricultural use worldwide under the Stockholm Convention, but its limited use in disease vector control continues to this day and remains controversial.

**Principle Of The Test**

The DDE/DDT Kit applies the principles of enzyme linked immunosorbent assay (ELISA) to the determination of DDE/DDT. The test is a direct competitive ELISA. The sample to be tested and an antibody specific for DDE/DDT are added to microtiter wells coated with goat anti-mouse antibody and incubated for thirty minutes. The DDE/DDT enzyme conjugate is then added. At this point, a competitive reaction occurs between the DDE/DDT which may be in the sample and the enzyme-labeled DDE/DDT analogue for the antibody binding sites. This competitive reaction is allowed to continue for thirty minutes. After a washing step, the presence of DDE/DDT is detected by adding the substrate ("color solution"), which contains the enzyme substrate (hydrogen peroxide) and the chromogen (3,3',5,5'-tetramethylbenzidine). The enzyme-labeled DDE/DDT bound to the DDE/DDT antibody catalyzes the conversion of the substrate/chromogen to a colored product. The color reaction is stopped and stabilized, after a twenty minute incubation period, 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 DDE/DDT present in the sample.

**Reagents And Materials Provided**

   96 test kit: 12 strips of 8 antibody coated wells and strip holder.
2. DDE/DDT Antibody Solution
   Monoclonal mouse anti-DDE/DDT antibody solution in a buffered saline solution with preservative and stabilizers.
   96 test kit: one 6 mL vial
3. p,p'-DDE Standard Stock
   p,p'-DDE standard stock at a concentration of 5μg/mL (5,000 ppb) in methanol.
   96 test kit: one 0.5 mL vial
4. DDE/DDT-HRP Enzyme Conjugate Horseradish peroxidase (HRP) labeled DDE/DDT analog in a buffered solution with preservative and stabilizers.
   96 test kit: one 6 mL vial
5. Diluent/Zero Standard
   10% methanol in distilled water (v/v) without any detectable DDE/DDT.
   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 16 mL vial
7. Stopping Solution
   A solution of diluted acid.
   96 test kit: one 12 mL vial
8. Washing Buffer (5X) Concentrate
   Buffered salts with detergent and preservatives.
   96 test kit: one 100 mL vial

**Materials Required But Not Supplied**

1. Micro Pipettes* Precision pipettes capable of delivering 2-20 μL, 20-200 μL, and 200-1000 μL with disposable tips.
2. Multi-channel or stepper pipette* capable of delivering 50-250μL with disposable tips.
3. Vortex Mixer* Thermolyne Maxi Mix, Scientific Industries Vortex Genie, or Equivalent.
4. Microplate or strip reader* capable of readings at 450 nm.
5. Timer.*
6. Distilled or deionized water.
7. Methanol, reagent grade.
8. Transfer pipettes, 5 mL.
9. Disposable glass test tubes or glass vials with Teflon lined caps.
10. Tape or Parafilm.
11. 500 mL bottle for diluted (1X) wash buffer.
*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

This procedure is recommended for use with water samples. Water samples should be collected in glass vessels with Teflon lined caps. Immediately upon collection, methanol (HPLC grade) should be added to the samples (10% v/v final concentration of methanol) to prevent adsorptive losses to the glass containers.

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 analysis.

If the DDE/DDT concentration of a sample exceeds 25 ppb, the sample must be diluted and re-analyzed. A ten-fold or greater dilution of the sample is recommended with an appropriate amount of Diluent/Zero Standard. For example, in a separate glass test tube, make a ten-fold dilution by adding 100 μL of the sample to 900 μL of Diluent/Zero Standard and mix thoroughly.

Perform the assay according to the Assay Procedure and obtain final results by multiplying the value obtain by 1.1 (to account for the initial preservation with methanol) and then by the dilution factor (e.g. 10).

Plate Preparation

Std. 0 - Std. 6: Standards
S1-Sx: Samples

Figure 1:
Reagent Preparation

All reagents and samples must be allowed to come to room temperature prior to analysis.

**Standards**

Organochlorine compounds tend to adsorb to surfaces, therefore standards should be prepared fresh before use in disposable glass tubes or glass vials with Teflon lined caps.

Table 1.
Wash Buffer
In a 500 mL container, dilute the wash buffer concentrate 1:5 with deionized or distilled water (i.e. 100 mL of 5X wash buffer concentrate into 400 mL of deionized or distilled water).

Assay Steps

1. Add 25 μL of the appropriate standard or sample to the wells of the test strips according to the working scheme shown above. Analysis in duplicates or triplicates is recommended.
2. Add 50 μL of DDE/DDT antibody solution successively to the wells using a multi-channel pipette or stepping pipette. 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.
3. After the incubation, add 50 μL of enzyme conjugate solution successively to the wells using a multi-channel pipette or stepping pipette. 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.
4. After the incubation, remove the covering and vigorously shake the contents of the wells into a waste container. Wash the strips with the diluted Wash Buffer by adding a volume of at least 250 μL of Wash Buffer to each well. Vigorously shake the contents of the wells into the waste container. Any remaining buffer in the wells should be removed by patting the plate on a stack of dry paper towels. Repeat this wash step two times, for a total of 3 rinses.
5. Add 150 μL of color solution successively to each well using a multi-channel pipette or stepping pipette. 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 20 minutes.
6. Add 100 μL of stopping solution to each well using a multi-channel pipette or stepping pipette. 
7. Read absorbance using a microplate reader at 450 nm within 15 minutes after adding the Stopping Solution.

Quality Control

Control solutions (negative and positive solutions) of DDE 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 (4-Parameter (preferred) or Logit/Log). 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 each standard by the zero standard (Standard 0) mean absorbance. Construct a standard curve by plotting the %B/Bo for each standard on a the vertical (y) axis versus the corresponding DDE concentration on the horizontal (x) axis on graph paper. The %B/Bo for each sample will then yield levels of DDE in ppb by interpolation using the constructed standard curve. The results obtained will then need to be multiplied by 1.1 to account for the initial sample preservation/dilution (methanol addition).

Samples containing lower concentrations of DDE than Standard 1 (0.625 ppb) are considered to be negative. Samples containing a higher concentration than Standard 6 (25 ppb) must be diluted to obtain accurate results.

Sensitivity

The DDE/DDT Assay has an estimated minimum detectable concentration, based on a 90% B/Bo, of 0.37 ppb.

Specificity

The cross-reactivity of the DDE/DDT Assay for various organochlorine compounds can be expressed as the 50% inhibition of p,p’-DDE divided by the 50% inhibition of each analogue.

<table>
<thead>
<tr>
<th>Compound (%)</th>
<th>Cross-reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>p,p’-DDE</td>
<td>100</td>
</tr>
<tr>
<td>p,p’-DDD</td>
<td>1189</td>
</tr>
<tr>
<td>p,p’-DDT</td>
<td>238</td>
</tr>
<tr>
<td>o,p’-DDD</td>
<td>146</td>
</tr>
<tr>
<td>o,p’-DDT</td>
<td>40</td>
</tr>
<tr>
<td>o,p’-DDE</td>
<td>13</td>
</tr>
</tbody>
</table>

Recovery
Four groundwater samples were spiked with various levels of DDE and then assayed using the DDE/DDT Assay. The following results were obtained:

Table 3:

<table>
<thead>
<tr>
<th>Amount of DDE Added (ppb)</th>
<th>Recovery Mean (ppb)</th>
<th>Recovery S.D. %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2.943</td>
<td>0.285</td>
</tr>
<tr>
<td>2.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.0</td>
<td>4.120</td>
<td>0.328</td>
</tr>
<tr>
<td>7.5</td>
<td>6.697</td>
<td>0.420</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Reproducibility**

Intra-Assay: 4.7%-10.9%
Inter-Assay: 7.2%-11.5%

**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. Add reagents directly to the bottom of the well while avoiding contact between the reagents and the pipette tip. This will help assure consistent quantities of reagent in the test mixture.
2. Avoid cross-contaminations and carryover of reagents by using clean pipette tips for each sample addition and by avoiding contact between reagent droplets on the tubes and pipette tips.
3. Do not use any reagents beyond their stated shelf life.
4. Avoid contact of Stopping Solution (diluted sulfuric acid) with skin and mucous membranes. If this reagent comes in contact with skin, wash with water.
5. The microtiter plate consists of 12 strips of 8 wells. If fewer than 12 strips are used, remove the unneeded strips and store refrigerated in the re-sealable bag (with desiccant) provided.
6. If more than three strips are used per run, the use of a multi-channel pipette or stepper pipette is recommended for the addition of antibody, conjugate, color, and stopping solutions.

**Limitations**

The DDE/DDT Assay will detect DDE and related organochlorine compounds to different degrees. Refer to the specificity table for data on several of the organochlorine compounds. The DDE/DDT 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.