DDE/DDT

• Intended Use

For the detection and quantitation of DDE/DDT 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 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 (please refer to Sample Information section) 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 (30) 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 (30) 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 (20) 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

The Abraxis DDE/DDT Kit contains the following items:

1. Microtiter Plate coated with Goat Anti-Mouse Antibody.
96 test kit: 12 strips of 8 antibody coated wells and strip holder (1).

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 5 µg/mL (5,000 ppb) in methanol. **See Reagent Preparation section.**
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

• 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:

Micro Pipettes* Precision pipettes capable of delivering 2-20 µL, 20-200 µL, and 200-1000 µL with disposable tips.

Multi-channel or stepper pipette* capable of delivering 50-250µL with disposable tips.

Vortex Mixer* Thermolyne Maxi Mix, Scientific Industries Vortex Genie, or Equivalent.

Microplate or strip reader* capable of readings at 450 nm.

Timer.*

Distilled or deionized water.

Methanol, reagent grade.

Transfer pipettes, 5 mL.

Disposable glass test tubes or glass vials with Teflon lined caps.

Tape or Parafilm.

500 mL bottle for diluted (1X) wash buffer.

*Please contact Abraxis for supplier information.

• Sample Information

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. To account for this initial preservation, final results will be obtained by multiplying the ELISA results by 1.1 (see Results section).

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 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).

• 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.

A reasonable Standard Dilution Scheme:

Standard Concentration (ppb)	Diluent (mL)	p, p'- DDE
25	3.98	20 µL of **5,000 ppb Stock Std.**
10	3.992	8 µL of **5,000 ppb Stock Std.**
5	1.6	0.4 mL of **25 ppb Std.**
2.5	1.5	0.5 mL of **10 ppb Std.**
1.25	1	1.0 mL of **2.5 ppb Std.**
0.625	1	1.0 mL of **1.25 ppb Std.**
0	1	0

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).

• 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 pipette tip**. This will help assure consistent quantities of reagent in the test mixture.

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.

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 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.

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 Abraxis 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.

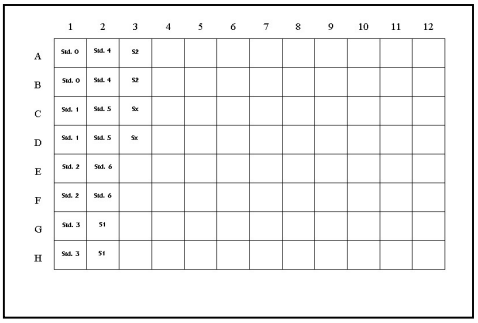
• Quality Control

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.

• Assay Procedure

Read Sample Information, Reagent Preparation, and Procedural Notes and Precautions before proceeding.

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



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 (see Reagent Preparation) 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.

• Results

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.

• Performance Data

Precision

The following results were obtained:

Control	1	2	3
Replicates	3	3	3
Days	2	2	2
n	6	6	6
Mean (ppb)	1.15	2.68	7.01
% CV (within assay)	10.9	6.8	4.7
% CV (between assay)	11.5	7.2	7.2

Sensitivity

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

Recovery

Four (4) groundwater samples were spiked with various levels of DDE and then assayed using the Abraxis DDE/DDT Assay. The following results were obtained:

Amount of DDE Added (ppb)	Recovery Mean (ppb)	Recovery S.D. (ppb)	Recovery %
2.5	2.943	0.285	118
4.0	4.120	0.328	103
7.5	6.697	0.420	89
Average			103

Specificity

The cross-reactivity of the Abraxis 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.

Compound	Cross-reactivity (%)
p,p'-DDE	100
p,p'-DDD	1189
p,p'-DDT	238
o,p'-DDD	146
o,p'-DDT	40
o,p'-DDE	13

• Ordering information

Abraxis DDE/DDT Assay Kit 96T	PN 540041
Sample Diluent	PN 540042
Standard Stock (additional)	PN 540043

• Assistance

For ordering or technical assistance contact:

Abraxis, Inc.
124 Railroad Drive
Warminster, Pennsylvania, 18974

Phone: (215) 357-3911 * Fax: (215) 357-5232
Email: info@abraxiskits.com
WEB: www.abraxiskits.com

• General Limited Warranty

Abraxis, Inc. 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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