Importance of DDE/DDT Determination

DDT (Dichlorodiphenyltrichloroethane) is an organochlorine pesticide; DDE (Dichlorodiphenyldichloroethylene) and DDD (Dichlorodiphenyldichloroethane) are breakdown products of DDT. Initially used by the United States military during World War II to control insects responsible for transmitting diseases including malaria and typhus, DDT was also used extensively within the U.S. against insects in agricultural fields, lawns and gardens, and structures. Due to decreased effectiveness due to resistance among insects and increased concerns regarding the safety of animals and the environment, the U.S. Department of Agriculture began instituting regulations on the use of DDT in the late 1950s and 1960s. The use of DDT in agriculture was banned by the U.S. Environmental Protection Agency (EPA) in 1972 and DDT can now only be used in the United States for the control of disease-causing insects during public health emergencies. Although the use of DDT has declined worldwide, routine use for the control of mosquitoes that transmit malaria continues in Asia, Africa, and South America.

DDT and its breakdown products are highly persistent in the environment. Considered a probable human carcinogen, DDT accumulates in body fat and is excreted in breast milk. The negative effects of DDT gained worldwide attention in 1962 with the publication of the book *Silent Spring* by Rachel Carson.

The DDE/DDT ELISA allows for the analysis of 41 samples in duplicate determination. Less than 1 mL of sample is required. The test can be performed in less than 90 minutes.

Performance Data

Test sensitivity: The DDE/DDT assay has an estimated minimum detectable concentration, based on 90% B/B_0, of 0.4 ng/mL. The middle of the test (50%B/B_0) is approximately 3.2 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 DDE/DDT assay for various organochlorine compounds can be expressed as the 50% inhibition 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

Performance Data:

Precision

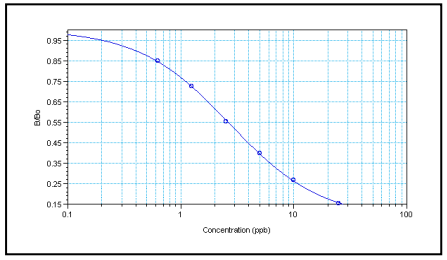
Control	1	2	3
Replicates	3	3	3
Days	2	2	2
N	6	6	6
Mean (ppb)	1.15	2.68	7.01
% CV (within assay)	10.9	6.8	4.7
% CV (between assay)	11.5	7.2	7.2

Recovery

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

Conc. (ppb)	Mean Rec. (ppb)	Std. Dev. (ppb)	% Rec.
2.5	2.943	0.285	118
4.0	4.120	0.328	103
7.5	6.697	0.420	89
Average			103

Standard Curve:



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

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DDE/DDT ELISA (Microtiter Plate)

Enzyme-Linked Immunosorbent Assay for the Determination of DDE/DDT in Water Samples

Product No. 540041

1. General Description

The Abraxis DDE/DDT ELISA is an immunoassay for the quantitative and sensitive screening of DDE/DDT. This test is suitable for the quantitative and/or qualitative screening of DDE/DDT 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 DDE. 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 DDE/DDT 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 a direct competitive ELISA based on the recognition of DDE/DDT by specific antibodies. DDE/DDT, when present in a sample, and a DDE/DDT-HRP analogue compete for the binding sites of the anti-DDE/DDT antibodies in solution. The DDE/DDT antibodies are then bound by a second antibody (anti-mouse) immobilized on the wells of the microtiter plate. After a 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 DDE/DDT 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 DDE/DDT 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.

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 DDE/DDT ELISA kit. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other Abraxis DDE/DDT ELISA kits with different lot numbers.

The Abraxis DDE/DDT 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 secondary antibody, in a resealable aluminum pouch
2. p,p'-DDE Stock Standard (1): 5 µg/mL (5,000 ppb) in methanol, 0.5 mL, for the preparation of the calibration standards, see Test Preparation (Section E)
3. Diluent/Zero Standard, 30 mL
4. Antibody Solution (anti-DDE/DDT), 6 mL
5. DDE/DDT-HRP Conjugate Solution, 6 mL
6. Wash Buffer (5X) Concentrate, 100 mL, must be diluted before use, see Test Preparation (Section E)
7. Substrate (Color) Solution (TMB), 16 mL
8. Stop Solution, 12 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 or 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 with Teflon lined caps. In order to avoid adsorptive loss of analyte, water samples should be preserved immediately upon collection using methanol (10% v/v final concentration of methanol). See Sample Preparation, section D for sample preservation procedure.

DDE/DDT tends 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 with Teflon lined caps. Immediately upon collection, in order to avoid adsorptive loss of analyte, water samples should be preserved with methanol (10% v/v final concentration of methanol). For example, a 90 mL water sample would be added to 10 mL of methanol (cap tightly and mix thoroughly).

The DDE/DDT concentration contained in water samples is determined by multiplying the ELISA result by the dilution factor of 1.1. 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. DDE/DDT calibration standards must be prepared fresh before use in disposable glass test tubes or glass vials. Calibration standards are prepared using the p,p'-DDE Stock Standard (5 µg/mL in methanol) provided in the test kit using the following dilution scheme:

Standard Number	Concentration (ppb)	Diluent/Zero Standard (mL)	DDE/DDT Solution to add
6	25	3.98	20 µL of **5,000 ppb** p,p'-DDE stock
5	10	3.992	8 µL of **5,000 ppb** p,p'-DDE stock
4	5	1.6	0.4 mL of **25 ppb** standard
3	2.5	1.5	0.5 mL of **10 ppb** standard
2	1.25	1.0	1 mL of **2.5 ppb** standard
1	0.625	1.0	1 mL of **1.25 ppb** standard
0	0	1.0	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 6: Standards
(0; 0.625; 1.25; 2.5; 5.0, 10.0, 25.0, ppb)

Samp1, Samp2, etc.: Samples

	1	2	3	4	5	6	7	8	9	10	11	12
A	Std 0	Std 4	Samp2									
B	Std 0	Std 4	Samp2									
C	Std 1	Std 5	etc.									
D	Std 1	Std 5	etc.									
E	Std 2	Std 6										
F	Std 2	Std 6										
G	Std 3	Samp1										
H	Std 3	Samp1										

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 **50 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 **30 minutes**.
3. After the incubation, remove the covering and add **50 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**.
4. 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.
5. Add **150 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.
6. Add **100 µL of stop solution** to the wells using a multi-channel pipette, stepping pipette, or electronic repeating pipette in the same sequence as for the substrate solution.
7. 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 DDE/DDT 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 DDE/DDT 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 1.1. Samples showing a lower concentration of DDE/DDT than standard 1 (0.625 ppb) should be reported as containing < 0.688 ppb of DDE/DDT. Samples showing a higher concentration than standard 6 (25.0 ppb) should be reported as containing > 27.5 ppb of DDE/DDT. If a quantitative result is necessary, samples must be diluted in sample diluent and re-analyzed.

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