Importance of Triazine Metabolite Determination

Pesticides are frequently applied in agriculture to protect crops from pests and to protect the yield of the harvest. However, a part of the active substance does not reach the target plant but evaporates during application or remains in the soil and degrades into metabolites. Due to their wide application and relatively high persistence, they can be detected in rain, surface water, and ground water. Atrazine and its metabolites are among the more frequently detected compounds in surface and ground water in the United States. It is desirable to monitor water samples and food for residues of triazines. According to the USEPA SWDA drinking water guidelines, the MCL for atrazine in drinking water is 3 ppb. Triazine metabolites are listed on the Drinking Water Contaminant Candidate List 2.

The DACT ELISA allows the determination of 41 samples in duplicate determination. Only a few uL of sample are required. The test can be performed in about 1 hour.

Performance Data

Test sensitivity: The detection limit for DACT is 0.1 ng/mL (90% B/Bo). The concentration of residue necessary to cause a 50% inhibition (50% B/B_0) is approximately1.3 ng/mL. Determinations closer to the middle of the calibration range of the test yields the most accurate results.

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

Selectivity: The ELISA for DACT recognizes DACT and other triazine metabolites.

Cross-reactivities:

DACT	100% (per definition)
Atrazine Despropyl	8.5%
Atrazine Desethyl	2.3%
Cryomazine	2.8%

Atrazine, Cyanazine, Prometon, Propachlor, Propazine, Terbutryne, Atrazine 2-OH, Atrazine Desethyl 2-OH exhibited no reactivity until greater than 1,000 ppb.

Cross-reactivities with pesticide classes other than triazines have not been observed.

Samples: Drinking water, ground water, and surface water were tested for matrix effects in the ELISA. No matrix effects were determined.

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R013108

Triazine Metabolite ELISA (Microtiter Plate)

Enzyme-Linked Immunosorbent Assay for the Determination of Diaminochlorotriazine (DACT) in Water Samples

Product No. 520006

1. General Description

The Triazine metabolite ELISA is an immunoassay for the quantitative and sensitive detection of diaminochlorotriazine and other triazine herbicide metabolites. This test is suitable for the quantitative and/or qualitative detection of these metabolites in water samples. A previous sample preparation is not required. For other sample matrices, contact Abraxis for application bulletins and/or specific matrix validation guidelines.

2. Safety Instructions

The standard solutions in the test kit contain diaminochlorotriazine. In addition, the substrate solution contains tetramethylbenzidine and the stop solution contains diluted sulfuric acid. Avoid contact of stopping solution with skin and mucous membranes. If these reagents come in contact with the skin, wash with water. Consult state, local and federal regulations for proper disposal of all reagents.

3. Storage and Stability

The DACT 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 based on the recognition of DACT by specific antibodies. DACT present in the sample and a triazine-enzyme-conjugate compete for the binding sites of the antibodies immobilized on the plate. After a washing step and addition of the substrate solution, a color signal is produced. The intensity of the blue color is inversely proportional to the concentration of the DACT 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 DACT ELISA, Possible Test Interference

Numerous organic and inorganic compounds commonly found in water samples have been tested and found not to interfere with this test. However, due to the high variability of compounds that might be found in water samples, test interferences caused by matrix effects can't be completely excluded. Mistakes in handling the test also can cause errors. Possible sources for such errors can be:

Inadequate storage conditions of the test kit, wrong pipetting sequence or inaccurate volumes of the reagents, too long or too short incubation times during the immune and/or substrate reaction, extreme temperatures during the test performance (lower than 10°C or higher than 30°C).

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

Working Instructions

A. Test Preparation

Micro-pipetting equipment and pipette tips for pipetting the standards and the samples are necessary. We recommend using a multi-channel pipette or a stepping pipette for adding the enzyme conjugate, the substrate solution and the stop solution in order to equalize the incubations periods of the standard solutions and the samples on the entire microtiter plate. Please only use the reagents and standards from one package lot in one test, as they have been adjusted in combination.

1. Adjust the microtiter plate and the reagents to room temperature before use.
2. Remove the number of microtiter plate strips required from the foil pouch. The remaining strips are stored in the foil pouch and ziplocked closed. Store the remaining kit in the refrigerator (4-8°C).
3. The standard solutions, positive and negative controls, enzyme conjugate, substrate and stop solution are ready to use and do not require any further dilutions.
4. Dilute the wash buffer at a ratio of 1:5. If using 100 mL of concentrate, add to 400 mL of deionized or distilled water.
5. The stop solution should be handled with care as it contains diluted H_2SO_4.

B. Assay Procedure

1. Add 100 µL of the standard solutions, the control or the samples into the wells of the test strips according to the working scheme given. We recommend using duplicates or triplicates.
2. Add 50 µL of enzyme conjugate solution to the individual wells successively using a multi-channel pipette or a stepping pipette. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a rapid circular motion on the benchtop for about 30 seconds. Be careful not to spill contents.
3. Incubate the strips for 30 min at room temperature.
4. After incubation, remove the covering and vigorously shake the contents of the wells into a sink.
5. Wash the strips four times using the 1X washing buffer solution. Please use at least a volume of 250 µL of washing buffer for each well and each washing step. Remaining buffer in the wells should be removed by patting the plate dry on a stack of paper towels.
6. Add 100 µL of substrate solution to the wells. The strips are incubated for 30 min at room temperature. Protect the strips from sunlight.
7. Add 100 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 the stopping solution.

C. Evaluation

The evaluation of the ELISA can be performed using commercial ELISA evaluation programs (4-parameters (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 of each of the standards by the Zero Standard (Standard 0) mean absorbance. Construct a standard curve by plotting the $\%B/B_0$ for each standard on a vertical linear (y) axis versus the corresponding DACT concentration on horizontal logarithmic (x) axis on graph paper. $\%B/B_0$ for controls and samples will then yield levels in ppb of DACT by interpolation using the standard curve.
The concentrations of the samples are determined using this standard curve. Samples showing a lower concentration of DACT compared to standard 1 (0.125 ng/mL) are considered as negative. Samples showing a higher concentration than standard 5 (5 ng/mL) must be diluted further to obtain accurate results. The concentration of the control should be in the range given on the test vial (±20%).

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

1. Micro-pipettes with disposable plastic tips (50-200 µL, 100-1000 µL)
2. Multi-channel pipette (50-250 µL) or stepper pipette with plastic tips (50-250 µL)
3. Microtiter plate washer (optional)
4. Microtiter plate reader (wavelength 450 nm)
5. Shaker for microtiter plates (optional)

E. 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.

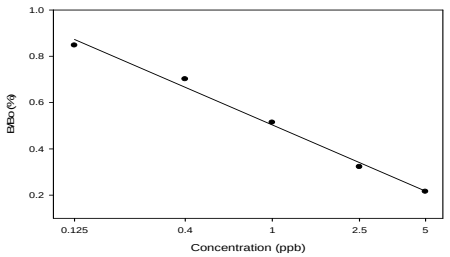
St0-St5: Standards
(0, 0.125, 0.40, 1.0, 2.5, 5.0 ng/mL)

PC (Positive Control): 3 ng/mL +/- 20%

Sam1, Sam2, Sam3, etc.: Samples

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

F. Standard Curve (These values are used for demonstration purposes; do not use these values for your determinations)



G. References

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