Importance of the Atrazine 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. According to their wide application and the relatively high persistence they can be detected in rain, surface water, and in ground water. The application of the herbicide atrazine is prohibited in several countries, e.g. Germany. In the U.S., according to the USEPA SWDA drinking water guidelines, the MCL for atrazine in drinking water is not allowed to exceed 3 ppb. It is desirable, to check water samples or food for possible residues of triazines as these herbicides frequently occur in water and soil.

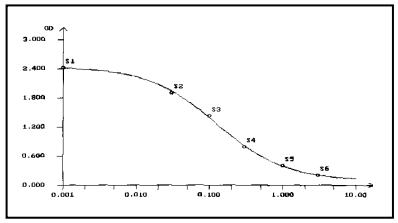
The atrazine ELISA allows the determination of 40 samples in duplicate determination. Only few mL of sample are required. The test can be performed in less than 1 hour.

Performance Data

Test sensitivity: The detection limit for atrazine is 0.04 ng/mL (90% B/Bo). The middle of the test (50% B/B_0) is at about 0.7 ng/mL. Determinations close to the middle of the tests give the most accurate results.

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

Standard curve:



Selectivity: The ELISA for atrazine recognizes beside atrazine also propazine.

Cross-reactivities:

atrazine	100% (per definition)
ametryn	1.5%
deethylatrazine	3.08%
hydroxyatrazine	0.01%
propazine	96%
simazine	14.3%
terbutylazine	0.33%

*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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R030617

Atrazine ELISA (Microtiter Plate)

Enzyme-Linked Immunosorbent Assay for the Determination of Atrazine in Water Samples

Product No. 520005

1. General Description

The Atrazine ELISA is an immunoassay for the quantitative and sensitive detection of atrazine, a triazine herbicide. This test is suitable for the quantitative and/or qualitative detection of atrazine in water samples. A previous sample preparation is not required.

2. Safety Instructions

The standard solutions of the test kit contain the herbicide atrazine. 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.

1. Storage and Stability

The atrazine ELISA should to 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. Consult state, local, and federal regulations for proper disposal of all reagents.

2. Test Principle

The test is a direct competitive ELISA is based on the recognition of atrazine by specific antibodies. Atrazine present in a water 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 atrazine 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.

3. Limitations of the Atrazine 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 cannot 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, incorrect pipetting sequence or inaccurate volumes of the reagents, too long or too short incubation times during the immune and/or substrate reaction, extreme outside temperatures during the test performance (lower than 10°C or higher than 30°C).

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

A. Materials Provided

1. Microtiter plate (8 wells X 12 strips) coated with a capture antibody, in a resealable pouch
2. Standards (7): 0, 0.05, 0.10, 0.25, 1.0, 2.5, 5.0 ppb, 1 mL each
3. Negative control, 1 mL
4. Positive control: 3.0 ppb, 1 mL
5. Assay buffer, 6 mL
6. Triazine enzyme conjugate solution, 6 mL
7. Wash Solution (5X) Concentrate, 100 mL, must be diluted before use, see Test Preparation (Section C)
8. Substrate (Color) Solution (TMB), 12 mL
9. Stop Solution, 6 mL (handle with care)

B. Additional Materials

1. Micro-pipettes with disposable plastic tips (20-200 µL)
2. Multi-channel pipette (50-300 µL), stepper pipette (50-300 µL), or electronic repeating pipette with disposable plastic tips
3. Deionized or distilled water
4. Paper towels or equivalent absorbent material
5. Timer
6. Tape or parafilm
7. Microtiter plate reader (wavelength 450 nm)
8. Microtiter plate washer (optional)

C. 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 aluminum foil. The remaining strips are stored back in the pouch with desiccant, tightly 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 then add to 400 mL of deionized or distilled water.
5. The stop solution has to be handled with care as it contains diluted H_2SO_4.

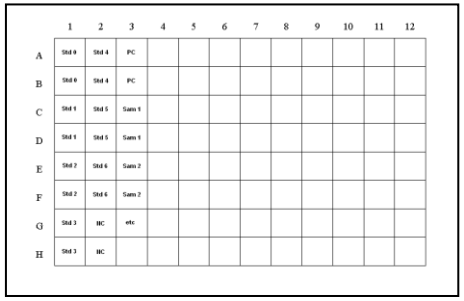
F. Working Scheme

The microtiter plate consists of 12 X 8 strips, which can be used individually for the test. The standards have to 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, 0.05, 0.10, 0.25, 1.0, 2.5, 5.0 ng/mL)

NC (Negative Control): <0.05 ng/mL
PC (Positive Control): 3 ng/mL +/- 20%

Sa1, Sa2, Sa3, etc.: Samples



D. Assay Procedure

1. Add **25 µL of the assay buffer** into each individual well using a multi-channel or stepping pipet
2. Add **25 µL of the standard solutions, the controls or the samples** into the wells of the test strips according to the working scheme given. Analysis in duplicate or triplicate is recommended.
3. Add **50 µL of enzyme conjugate solution** to the individual wells successively using a multi-channel pipette or a stepping pipette. Cover wells with parafilm or tape and mix the contents by moving the plate in a circular motion on the bench top for 30 seconds. Be careful not to spill the contents. Incubate the strips for **30 minutes** at room temperature.
4. Remove the covering and decant contents of wells into a sink. Wash the strips **three 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 stock of paper.
5. Add **100 µL of substrate (color) solution** to the individual wells successively using a multi-channel or stepping pipet. Cover wells with parafilm or tape and mix the contents by moving the plate in a circular motion on the bench top for 30 seconds. Be careful not to spill the contents. Incubate the strips for **20 minutes** at room temperature. Protect the strips from sunlight.
6. Add **50 µL of stop solution** to the wells in the same sequence as for the substrate solution.
7. Read the absorbance at 450 nm using a microplate ELISA photometer within 10 minutes of adding the stopping solution.

E. Evaluation

The evaluation of the ELISA can be performed using commercial ELISA evaluation programs (Logit/Log or 4-Parameter). 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 the Zero Standard (Standard 0). Construct a standard curve by plotting the $\%B/B_0$ for each standard on a vertical linear (y) axis versus the corresponding atrazine concentration on horizontal logarithmic (x) axis on graph paper. $\%B/B_0$ for controls and samples will then yield levels in ppb of atrazine by interpolation using the standard curve.

The concentrations of the samples are determined using this standard curve. Samples showing a lower concentrations of atrazine compared to standard 1 (0.05 ng/mL) are considered as negative. Samples showing a higher concentration than standard 6 (5 ng/mL) must be diluted further to obtain more accurate results. The concentration of the negative and positive controls should be in the range given in the test instructions (±20%).

H. References

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