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. Due to the wide application and the relatively high persistence, pesticides can be detected in rain, surface water, and ground water. The application of the herbicide atrazine is prohibited in several countries, e.g. Germany. In the U.S., according to the USEPA Safe Drinking Water Act (SDWA) guidelines, the MCL for atrazine in drinking water is not allowed to exceed 3 ppb. For this reason, it is desirable to test water samples or food for possible residues of triazines as these herbicides frequently occur in water and soil.

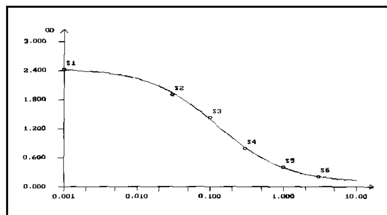
The ABRAXIS® Atrazine ELISA allows the determination of 40 samples in duplicate determination. Only a few milliliters of sample are required. The test can be performed in less than 1 hour.

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: The standard curve R^2 must be ≥ 0.98. The absorbance Coefficient of variation (CVs) for standards should be ≤10% and for the samples and Control should be ≤15%. The Control should be within its acceptable range and Standard 0 absorbance value should be between 0.8 - 3.000.

Standard curve:



Selectivity: The ABRAXIS® Atrazine ELISA detects atrazine and 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.

General Limited Warranty: Gold Standard Diagnostics 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. **Gold Standard Diagnostics makes no other warranty, expressed or implied. There is no warranty of merchantability or fitness for a particular purpose.**

For ordering or technical assistance contact:
Gold Standard Diagnostics
795 Horsham Road
Horsham, PA 19044
WEB: www.abraxiskits.com
Tel: (215) 357-3911
Ordering: info.abraxis@us.goldstandarddiagnostics.com
Technical Support: support.abraxis@us.goldstandarddiagnostics.com

Date these instructions are effective : 03/18/2025
Version: 03

ABRAXIS® Atrazine ELISA Microtiter Plate

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

Product No. 520005

1. General Description

The ABRAXIS® Atrazine ELISA is an immunoassay for the quantitative and sensitive detection of atrazine, atriazine 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.

3. Storage and Stability

The ABRAXIS® Atrazine ELISA should to be stored in the refrigerator (2-8°C). The solutions must be allowed to reach room temperature (20-25°C) before use. Reagents may be used until the last day of the month as indicated by the expiration date on the box. Consult state, local, and federal regulations for proper disposal of all reagents.

4. 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.

5. Limitations of the ABRAXIS® 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.

6. Working Instructions

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, 1.5 mL each
3. Negative control, 1.5 mL
4. Positive control: 3.0 ± 0.6 ppb, 1.5 mL
5. Sample Diluent, 25 mL, used to dilute samples above the range of the assay
6. Assay buffer, 6 mL
7. Atrazine enzyme conjugate solution, 6 mL
8. ABRAXIS® Wash Buffer (5X) Concentrate, 100 mL, must be diluted before use, see Test Preparation (Section C)
9. Substrate (Color) Solution (TMB), 12 mL
10. 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. Container with 500 mL capacity (for diluted 1X Wash Buffer, see Test Preparation, Section C)
4. Deionized or distilled water
5. Paper towels or equivalent absorbent material
6. Timer
7. Tape or parafilm
8. Microtiter plate reader (wavelength 450 nm)
9. 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, stepping, or electronic repeating 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 (2-8°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 ABRAXIS® Wash Buffer (5X) Concentrate at a ratio of 1:5. If using the entire bottle (100 mL) of concentrate then add to 400 mL of deionized or distilled water.
5. The stop solution needs to be handled with care as it contains diluted H_2SO_4.

D. 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

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

E. Assay Procedure

1. **Add 25 µL of the assay buffer** into each individual well using a multi-channel, stepping, or electronic repeating pipette.
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, stepping, or electronic repeating 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, decant the contents of the wells into a sink, and blot the inverted plate on a stack of paper towels. **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 and each washing step. Blot the inverted plate after each wash step on a stack of paper towels. After the last wash/blot, check the wells for any remaining buffer in the wells, and if necessary, remove by additional blotting.
5. **Add 100 µL of substrate (color) solution** to the individual wells successively using a multi-channel, stepping, or electronic repeating 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 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 using a multi-channel, stepping, or electronic repeating pipette.
7. Read the absorbance at 450 nm using a microplate ELISA photometer within 10 minutes of adding the stop solution.

F. 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 ($\pm$20%).

G. References

A. Dankwardt, E.M. Thurman, B. Hock, Terbuthylazine and deethylterbuthylazine in rain and surface water – Determination by enzyme immunoassay and gas chromatography/mass spectrometry, Acta hydrochim. hydrobiol. 25, 1997, 5-10.

A. Dankwardt, S. Pullen, S. Rauchalles, K. Kramer, F. Just, B. Hock, Atrazine residues in soil two years after the atrazine ban – A comparison of enzyme immunoassay with HPLC, Anal. Lett. 28, 1995, 621-634.

S. Wüst, B. Hock, A sensitive enzyme immunoassay for the detection of atrazine based upon sheep antibodies, Anal. Lett. 25, 1992, 1025-1037.

B. Hock, T. Giersch, A. Dankwardt, K. Kramer, S. Pullen, Toxicity Assessment and On-line monitoring: Immunoassays, Environ. Toxicol. Water Qual. 9, 1994, 243-262.