

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/Bo) is at about 0.5 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%.

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

Cross-reactivities:	atrazine	100% (per definition)
	ametryn	3.9%
	deethylatrazine	1.3%
	hydroxyatrazine	1.8%
	propazine	81%
	simazine	6.9%
	terbutylazine	1%

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. Parallel determinations using HPLC and GC/MS methods showed a good correlation in the atrazine concentrations found.

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R042808

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. Consult state, local and federal regulations for proper disposal of all reagents.

3. Storage and Stability

The atrazine ELISA should to be stored in the refrigerator (4–8°C). The solutions have to 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 atrazine by specific antibodies. Atrazine 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 atrazine present in the sample. The color reaction is stopped after a specified time and the color is evaluated using an ELISA reader.

5. 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 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 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 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 aluminum foil. The remaining strips are stored back in the aluminum foil and zip locked 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 H₂SO₄.

B. 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. We recommend using duplicates or triplicates.
3. Add 50 µL of enzyme conjugate solution to the individual wells successively using a multi-channel pipette or a stepping pipette.
4. Incubate the strips for 30 min at room temperature (if possible use an orbital shaker).
5. Wash the strips three times using the 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.
6. Add 100 µL of substrate solution to the wells. The strips are incubated for 15-20 min at room temperature away from sunlight, if possible on a shaker. Protect the strips from sunlight.
7. Add 50 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.

C. 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/B₀ for each standard by dividing the mean absorbance value for the Zero Standard (Standard 0). Construct a standard curve by plotting the %B/B₀ for each standard on a vertical linear (y) axis versus the corresponding atrazine concentration on horizontal logarithmic (x) axis on graph paper. %B/B₀ 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%).

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

1. Micro-pipettes with disposable plastic tips (10-250 µL, 100-1000 µL)
2. Multi-channel pipette (10-250 µL) or stepper pipette with plastic tips (10-250 µL)
3. Microtiter plate washer
4. Microtiter plate reader (wave length 450 nm)
5. Shaker for microtiter plates (optional)

E. 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.

St0-St6: Standards

(0, 0.05, 0.10, 0.25, 1.0, 2.5, 5.0 ng/mL)

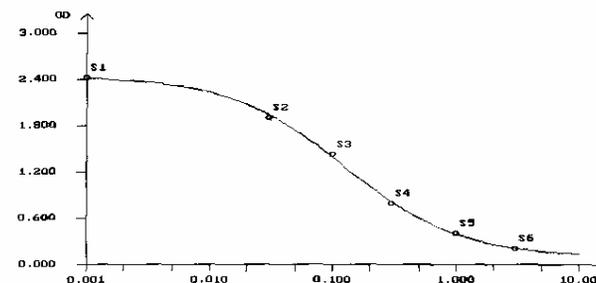
NC (Negative Control): <0.05 ng/mL

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 6	Std 4	PC		etc.	etc.						
B	Std 6	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										

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



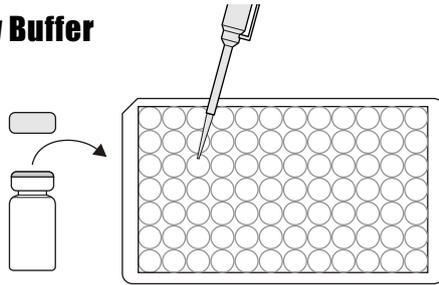
G. References

- (1) A. Dankwardt, E.M. Thurman, B. Hock, Terbutylazine and deethylterbutylazine in rain and surface water – Determination by enzyme immunoassay and gas chromatography/mass spectrometry, Acta hydrochim. hydrobiol. 25, 1997, 5-10.
- (2) 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.
- (3) S. Wüst, B. Hock, A sensitive enzyme immunoassay for the detection of atrazine based upon sheep antibodies, Anal. Lett. 25, 1992, 1025-1037.
- (4) 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.

Atrazine Plate, Detailed ELISA Procedure

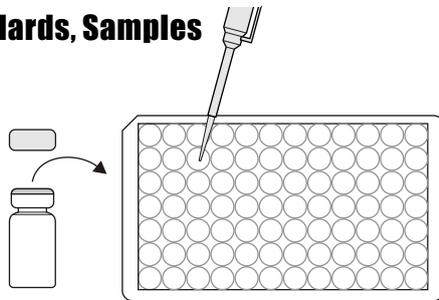
1. Addition of Assay Buffer

Add 25 μ l of the Assay Buffer into the wells of the test strips according to the working scheme given. We recommend using duplicates or triplicates.



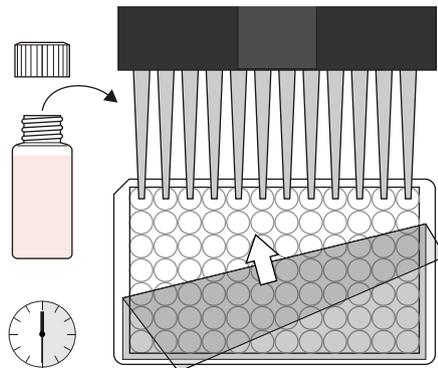
2. Addition of Standards, Samples

Add 25 μ l of the standard solutions, control or samples into the wells of the test strips according to the working scheme given. We recommend using duplicates or triplicates.



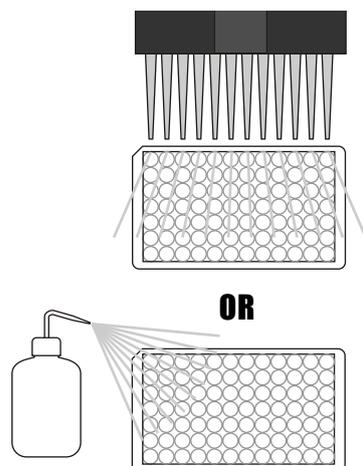
3. Addition of Enzyme Conjugate

Add 50 μ l of the enzyme conjugate 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 30 seconds. Be careful not to spill contents. Incubate for 30 minutes at room temperature.



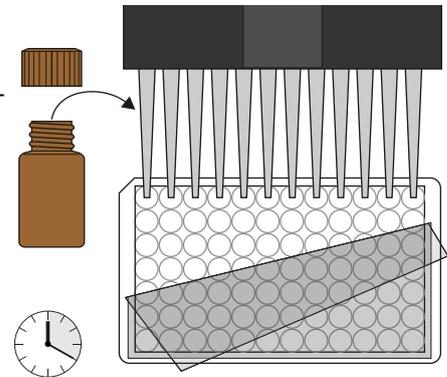
4. Washing of Plates

After incubation, remove the covering and vigorously shake the contents of the wells into a sink. Wash the strips three times with a multi-channel pipette or wash bottle using the 1X washing buffer solution. Please use at least a volume of 250 μ l 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.



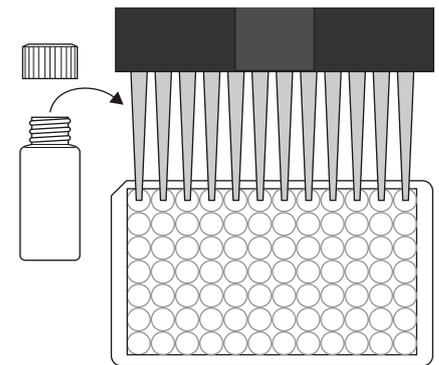
5. Addition of Substrate/Color Solution

Add 100 μ l of substrate/color 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. Be careful not to spill contents. Incubate the strips for 15-20 min at room temperature.



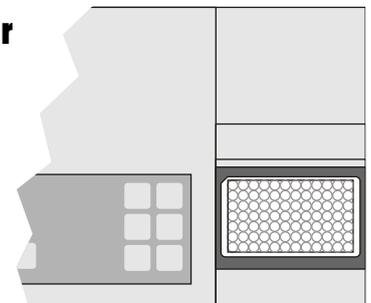
6. Addition of Stopping Solution

Add 50 μ l of stop solution to the wells in the same sequence as for the substrate solution using a multi-channel pipette or a stepping pipette.



7. Measurement of Color

Read the absorbance at 450 nm using a microplate ELISA reader. Calculate results.



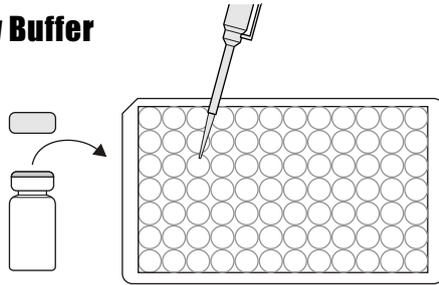
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Atrazine Plate Kit Part # 520005

Atrazine Plate, Concise ELISA Procedure

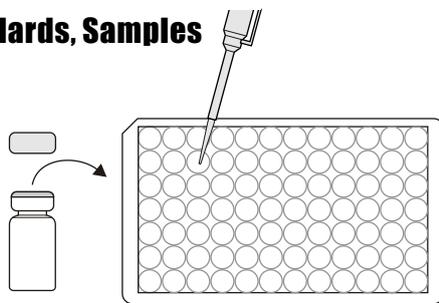
1. Addition of Assay Buffer

Add 25 μ L of the Assay Buffer.



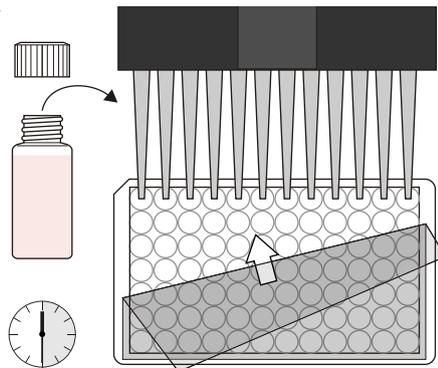
2. Addition of Standards, Samples

Add 25 μ L of standard solutions, control or samples.



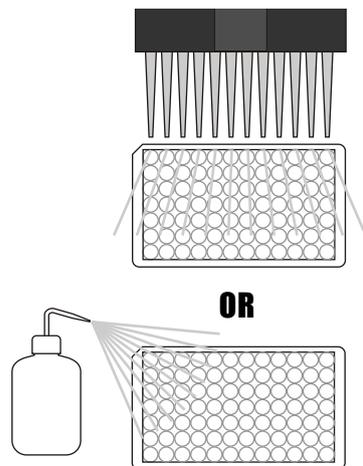
3. Addition of Enzyme Conjugate

Add 50 μ L of enzyme conjugate. Cover and mix for 30 seconds by rotating on benchtop. Incubate for 30 minutes at room temperature.



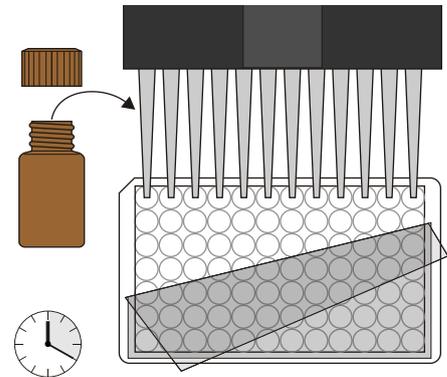
4. Washing of Plates

Wash the plates three times with 250 μ L of 1X washing buffer.



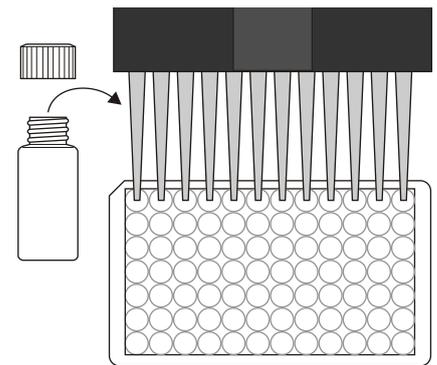
5. Addition of Substrate/Color Solution

Add 100 μ L of substrate/color solution. Incubate 15-20 minutes at room temperature and away from direct sunlight.



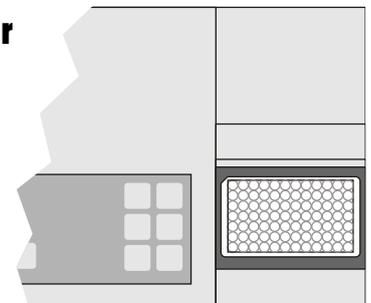
6. Addition of Stopping Solution

Add 50 μ L of stop solution.



7. Measurement of Color

Measure color at 450 nm.
Calculate results.



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