

Intended Use

The Beacon Atrazine Plate Kit is an immunoassay for the detection of Atrazine residues in water samples. This product is intended for research use only.

Principles

Calibrators, Controls, and the Sample Extract(s) are pipetted into the test wells followed by Atrazine HRP Enzyme Conjugate. During an incubation, Atrazine in the calibrator/control/sample and Atrazine HRP Enzyme Conjugate compete for binding to the polyclonal Atrazine antibody immobilized on the test wells surface. Following the incubation, the wells are washed to remove any unbound Atrazine and Atrazine HRP Enzyme Conjugate. After washing, a colorless substrate is added to the wells and any bound enzyme conjugate will convert the substrate to a blue color. Following an incubation, the reaction is stopped with the addition of Stop Solution and the amount of color in each well is measured. The color of the unknown sample is compared to the color of the calibrators and the Atrazine concentration of the sample is derived.

Reagents and Materials Provided

- 1 Unit Plate containing 12 test strips of 8 wells each that are vacuum sealed in an aluminized pouch with a desiccant.
- 4 X 2 mL Vials of Atrazine Calibrators (0, 0.05, 0.5, 5 ppb).
- 1 X 2 mL Vial of Atrazine Control (3 ppb).
- 1 X 12 mL Bottle of Atrazine HRP Enzyme Conjugate.
- 1 X 14 mL Bottle of Substrate.
- 1 X 14 mL Bottle of Stop Solution.

Reagents and Materials Required but Not Provided

- Pipette(s) with disposable tips capable of dispensing the required volume(s).
- Multichannel pipette(s) (8 channels) with disposable tips capable of dispensing the required volume(s) (recommended if running more than two strips at once).
- Laboratory quality distilled or deionized water.
- Reagents and materials for sample preparation.
- Personal protective equipment.
- Paper towels or equivalent absorbent material.
- Wash bottle (optional).
- Timer.
- Microtiter plate or strip reader capable of reading at 450 nm.

Kit Handling Notes and Precautions

- Read the product brochure in its entirety prior to use.
- The kit, in its original packaging, can be used until the end of the month indicated on the box label.
- Do not use reagents after expiration date.
- Store all kit components at 2°C to 8°C (36°F to 46°F) when not in use.
- Reagents should be brought to room temperature, 20°C to 28°C (68°F to 82°F), prior to use. Avoid prolonged (> 24 hours) storage at room temperature.
- Do not freeze kit components or expose them to temperatures greater than 37°C (99°F).
- Running Calibrators, Controls and Samples in duplicate will improve assay precision and accuracy.
- Precise transfer of samples and reagents by using a calibrated pipette that is capable of dispensing the required volume is critical to obtain proper assay results.
- If running more than two strips at once, the use of a multi-channel pipette is recommended when adding the Substrate and Stop Solution.
- All procedural steps should be completed without interruption. Ensure all reagents, materials and equipment are ready at the appropriate time.
- Each reagent is optimized for use in the Beacon Atrazine Plate Kit. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other Beacon Atrazine Plate Kits with different lot numbers.
- Do not reuse test wells.
- Dilution or adulteration of reagents or samples not called for in the procedure may result in inaccurate results.
- Damage to or obstruction of the optical surface may cause unsatisfactory results.

Specificity

The Beacon Atrazine Plate Kit cannot differentiate between the various triazines and metabolites but detects their presence to differing degrees. The following table shows the relative values for 50% B₀ and the percent cross-reactivity versus Atrazine. All concentrations are in ppb.

Compound	50% B ₀	% Cross-Reactivity
Atrazine	0.5	100
Prometryne*	0.12	420
Ametryne*	0.16	310
Propazine	0.38	130
Simetryne**	0.9	56
Prometon	1.3	38
Terbutryne**	1.4	36
Simazine	6.2	8.1
Terbuthylazine	15	3.3
De-Ethylated Atrazine	19	2.6
Cyanazine	77	0.65
De-Isopropyl Atrazine	120	0.42
Cyromazine	100	0.25

* Registered for specialty crops only. Rarely found in ground water.

** Not registered for use in the United States.

The following compounds are not detectable at 10,000 ppb with the Beacon Atrazine Plate Kit.

Alachlor	Metolachlor	Carbofuran	Aldicarb
Diamino Atrazine	Carbendazim	Melamine	2,4-D

Precision

Intra-Assay:

Two controls were run in four sets of duplicates in four different assays over four days. The average intra-assay precision is shown below:

Control Identification	Average Concentration (ppb)	Standard Deviation	Relative Standard Deviation (%)
1	0.09	0.010	11
2	3.2	0.13	4.1

Inter-Assay:

Two controls were run in duplicate in eight different assays over four days. The average inter-assay precision is shown below.

Control Identification	Average Concentration (ppb)	Standard Deviation	Relative Standard Deviation (%)
1	0.087	0.009	10
2	3.2	0.12	3.8

Assay Procedure

1. Allow kit components and the sample extract(s) to reach room temperature prior to running the test.
2. Place the appropriate number of test wells into a holder. Be sure to re-seal unused test wells in the zip-lock bag with the desiccant to limit exposure to moisture.
3. Dispense **100 µL of Calibrators, Controls, and Sample Extract(s)** into the appropriate well. Be sure to use a clean pipette tip for each solution to avoid cross contamination.
4. Dispense **100 µL of Enzyme Conjugate** into each well.
5. Gently shake the wells for 30 seconds using a back-and-forth motion and incubate for **60 minutes** at room temperature.
6. Decant the contents of the wells into an appropriate waste container. Fill the wells to overflowing with laboratory quality distilled or deionized water and then decant. Repeat this wash step four times for a total of five washes. Following the last wash, tap the inverted wells onto absorbent paper to remove excess wash solution.
7. Dispense **100 µL of Substrate** into each well.
8. Incubate for **30 minutes** at room temperature.
9. Dispense **100 µL of Stop Solution** into each well in the same order of addition as the Substrate.
10. Gently shake the wells for 30 seconds using a back-and-forth motion.
11. Carefully wipe the optical surface with a soft, lint-free wipe. Measure and record the absorbance (Optical Density; OD) of each well at 450 nm using a plate or strip reader within 10 minutes of stopping the assay. If the reader has dual wavelength capability, read at 450 nm minus 605 or 650 nm.
12. Dispose of used test wells in an appropriate waste container.

Quality Control (QC) Criteria

- The correlation coefficient (R^2) of the calibration curve, analyzed using a 4-parameter logistic regression, must be ≥ 0.99 .
- The average absorbance of the zero calibrator replicates must be ≥ 1.0 .
- The average absorbance of calibrator and control replicates must have a coefficient of variation (%CV) $< 15\%$.
- The average absorbance of sample replicates must have a coefficient of variation (%CV) $< 20\%$.
- The concentration of the control must fall between 2.4 and 3.6 ppb.

Result Interpretation

It is preferred for quantitative results to be determined using commercially available software for ELISA evaluation using a 4-parameter curve fit. Alternatively, a semi-log curve fit can be used if 4-parameter software is not available. A spreadsheet that will perform the curve fit and sample concentration calculations is available upon request. Please contact Beacon for further details.

To ensure the validity of the results, please adhere to the following:

- Ensure QC criteria are met.
- The concentration of Atrazine in a sample is determined by comparing the average sample absorbance to the standard curve. This value must then be multiplied by the dilution factor used.
- In the event that the average absorbance of the sample is lower than the highest calibrator, further dilute the sample extract in laboratory quality distilled or deionized water to fit into the standard curve and retest alongside the calibrators and controls. Sample results must be multiplied by the total dilution factor used.

Technical Assistance

For questions regarding this kit or for additional information about Beacon products, contact us.

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Safety

Stop Solution is 1N hydrochloric acid. Handle with care. To receive complete safety information on this product, contact Beacon Analytical Systems, Inc., and request Safety Data Sheets.

General Limited Warranty

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