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 Diamino 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 to fit into the standard curve and retest alongside the calibrators. 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.

Beacon Analytical Systems, Inc.
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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

Intended Use

The Beacon Diamino Atrazine 100 Tube Kit is an immunoassay for the detection of Diamino Atrazine residues in water samples. This product is intended for research use only.

Principles

Calibrators and the Sample Extract(s) are pipetted into the test tubes followed by Diamino Atrazine HRP Enzyme Conjugate. During an incubation, Diamino Atrazine in the calibrator/ sample and Diamino Atrazine HRP Enzyme Conjugate compete for binding to the polyclonal Diamino Atrazine antibody immobilized on the test tubes surface. Following the incubation, the tubes are washed to remove any unbound Diamino Atrazine and Diamino Atrazine HRP Enzyme Conjugate. After washing, a colorless substrate is added to the tubes 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 tube is measured. The color of the unknown sample is compared to the color of the calibrators and the Diamino Atrazine concentration of the sample is derived.

Reagents and Materials Provided

5 Units	Bags each containing 20 test tubes that are vacuum sealed in an aluminized pouch with a desiccant.
5 X 10 mL	Vials of Diamino Atrazine Calibrators (0, 0.1, 0.2, 1, and 5 ppb).
1 X 60 mL	Bottle of Diamino Atrazine HRP Enzyme Conjugate.
1 X 50 mL	Bottle of 50X Wash Concentrate (dilute prior to use).
1 X 60 mL	Bottle of Substrate.
1 X 60 mL	Bottle of Stop Solution.

Reagents and Materials Required but Not Provided

- Pipette(s) with disposable tips capable of dispensing the required volume(s).
- Repeater pipette(s) with disposable tips capable of dispensing the required volume(s) (recommended if running more than five tubes at once).
- Laboratory quality distilled or deionized water.
- Materials for 1X wash solution preparation.
- Reagents and materials for sample preparation.
- Personal protective equipment.
- Paper towels or equivalent absorbent material.
- Permanent Marker.
- Tube rack.
- Timer.
- Photometer capable of reading absorbance at 450 nm in 12 mm x 75 mm tubes.

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 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 five tubes at once, the use of a repeater 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 Diamino Atrazine 100 Tube Kit. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other Beacon Diamino Atrazine 100 Tube Kits with different lot numbers.
- Do not reuse test tubes.
- 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 Diamino Atrazine 100 Tube 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% B_0 and the percent cross reactivity versus Diamino Atrazine. All concentrations are in ppb.

Compound	50% B_0	% Cross-Reactivity
G-11355*	3.4	14
G-28279*	4.7	11
G-11354*	16	2.9
Cyromazine	24	2.3
G-30033*	25.5	2
Melamine	44	1.1
GS-14626*	81	< 1

* Reference: Hydroxyatrazine and Atrazine Determination in Soil and Water by Enzyme-Linked Immunosorbent Assay Using Specific Monoclonal Antibodies, Schlaeppi J.M., J. Agric. Food Chem., 1989, 37, 1532-1538.

The following compounds are not detectable at 10,000 ppb with the Beacon Diamino Atrazine 100 Tube Kit.

Atrazine	OH-Propazine	Prometron
Cyanazine	OH-Atrazine	Propazine
Ametryn	Simazine	

1X Wash Solution Preparation

1. Measure 490 mL of laboratory quality distilled or deionized water and transfer to a clean container with a tight-fitting lid.
2. Measure 10 mL of the 50X Wash Concentrate and add to the container.
3. Gently swirl to mix.
4. Transfer the 1X Wash Solution to a wash bottle to use in the assay.

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 tubes into a tube rack. Label the tubes one inch from the top with the calibrator concentration or sample identification. Be sure to re-seal unused tubes in the zip-lock bag with the desiccant to limit exposure to moisture.
3. Dispense **500 µL of Calibrators and Sample Extract(s)** into the appropriate tube. Be sure to use a clean pipette tip for each solution to avoid cross contamination.
4. Dispense **500 µL of Enzyme Conjugate** into each tube.
5. Pick up the tube rack and gently shake for 30 seconds using a back-and-forth motion. Incubate for **60 minutes** at room temperature.
6. Decant the contents of the tubes into an appropriate waste container. Fill the tubes to overflowing with 1X Wash Solution and then decant. Repeat this wash step three times for a total of four washes. Following the last wash, tap the inverted tubes onto absorbent paper to remove excess wash solution.
7. Dispense **500 µL of Substrate** into each tube.
8. Incubate for **30 minutes** at room temperature. Pick up the tube rack and gently shake for 30 seconds using a back-and-forth motion every 2.5 minutes during the incubation.
9. Dispense **500 µL of Stop Solution** into each tube in the same order of addition as the Substrate.
10. Gently shake the tubes 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 tube at 450 nm using a tube reader within 10 minutes of stopping the assay. Be sure to blank the reader with laboratory quality distilled or deionized water prior to measuring.
12. Dispose of used test tubes 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 replicates must have a coefficient of variation (%CV) < 15%.
- The average absorbance of sample replicates must have a coefficient of variation (%CV) < 20%.