11. After the final wash, shake the rack upside down firmly three times. Remove most of the remaining wash by holding the rack upside down and tapping the rims of all tubes gently on a clean paper towel. Small droplets of wash water may remain in the tubes but will not affect the assay results.
12. Pipette 500 µL of substrate solution into each tube in the same fashion as the previous solutions were dispensed. The pipette tip need not be changed between additions.
13. Set the timer for 30 minutes and allow the tubes to incubate at room temperature. Shake the rack gently every 2-½ minutes during this incubation.
14. Substrate within the tubes will gradually turn varying shades of blue.
15. Pipette 500 µL of Stop Solution into each tube in the same order as the addition of the Substrate above. The blue color in each tube will turn yellow. Measure the color at 450 nm within 15 minutes of stopping the reaction.
16. Measure and record the absorbance of each tube at 450nm using a Source Scientific MicroChem tube photometer, or equivalent. Carefully wipe the outside of each tube with soft, lint-free wipe prior to reading.

CALCULATE RESULTS

1. After you read all of the tubes, average the OD of each set of calibrators, controls and samples.
2. Graph the average absorbance of each calibrator on the Y (linear) axis against its Diamino Atrazine concentration on the X (log) axis using semi-log graph paper. Draw the best-fit line through the calibrator points. Alternatively, the linear regression of the formula $y = m \ln(x) + b$ can be calculated using a calculator or computer.
3. Determine the Diamino Atrazine concentration of each sample by finding its absorbance and the corresponding concentration level on the graph.
4. Samples which fall outside the quantitation range of the assay must be reported as <0.1 ppb or greater than 5.0 ppb. Samples greater than 5.0 ppb can be diluted and re-assayed. See the table below for recommended dilution schemes.

SAMPLE CALCULATIONS

Tube Contents	OD	Average OD ± SD*	%RSD	%Bo**	Diamino atrazine conc. (ppb)
Negative Control	1.57 1.6	1.59 ± 0.021	1.34	100	N/A
0.1 ppb Calibrator	1.39 1.37	1.38± 0.014	1.02	87	0.1
0.2 ppb Calibrator	1.23 1.26	1.25 ± 0.021	1.7	79	0.2
1.0 ppb Calibrator	0.78 0.76	0.77 ± 0.017	2.2	49	1
5.0 ppb Calibrator	0.32 0.34	0.34 ± 0.001	0.21	21	5

*Actual values may vary; this data is for example purposes only.

* standard deviation

** %Bo equals average sample absorbance divided by average negative control absorbance times 100%.

TECHNICAL ASSISTANCE

For questions regarding this kit or for additional information about Beacon products, call (207) 571-4302.

SAFETY

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

General Limited Warranty

Beacon Analytical Systems, Inc. ("Beacon") warrants the products manufactured by it against defects in materials and workmanship when used in accordance with the applicable instructions for a period not to extend beyond a product's printed expiration date. BEACON MAKES NO OTHER WARRANTY, EXPRESSED OR IMPLIED. THERE IS NO WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE. The warranty provided herein and the data, specifications and descriptions of Beacon products appearing in published catalogues and product literature may not be altered except by express written agreement signed by an officer of Beacon. Representations, oral or written, which are inconsistent with this warranty or such publications are not authorized and, if given, should not be relied upon.

In the event of a breach of the foregoing warranty, Beacon's sole obligation shall be to repair or replace, at its option, any product or part thereof that proves defective in materials or workmanship within the warranty period, provided the customer notifies Beacon promptly of any such defect. The exclusive remedy provided herein shall not be deemed to have failed of its essential purpose so long as Beacon is willing and able to repair or replace any nonconforming Beacon product or part. Beacon shall not be liable for consequential, incidental, special or any other indirect damages resulting from economic loss or property damage sustained by a customer from the use of its products. However, in some states the purchaser may have rights under state law in addition to those provided by this warranty.

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Beacon Analytical Systems Inc.

Diamino Atrazine Tube Kit

Cat.# 20- 0164

Instructional Booklet

READ COMPLETELY BEFORE USE.

INTENDED USE

The Beacon Diamino atrazine Tube Kit is an immunological laboratory test for the quantitation of diamino atrazine residues in water.

ASSAY PRINCIPLES

The Beacon Diamino Atrazine Tube Kit uses polyclonal antibodies that bind both diamino Atrazine and a Diamino Atrazine-enzyme conjugate. Diamino atrazine in the sample compete with diamino atrazine-enzyme conjugate for a limited number of antibody binding sites. Antibodies, which bind Diamino Atrazine, are immobilized to the inside of the test tubes.
In the assay procedure you will:

- Add samples and calibrators containing known amounts of Diamino Atrazine and a Diamino Atrazine enzyme conjugate to test tubes coated with anti-Diamino Atrazine antibodies. The conjugate competes with any Diamino Atrazine in the sample for the same antibody binding sites.
- Wash away any unbound molecules, after you incubate this mixture for 60 minutes.
- Add clear substrate solution to each tube. In the presence of bound Diamino Atrazine-enzyme conjugate, the substrate is converted to a blue compound. One enzyme molecule can convert many substrate molecules.

Since the same number of antibody binding sites are available in every tube, and each tube receives the same number of Diamino Atrazine-enzyme conjugate molecules, a sample containing a low concentration of Diamino Atrazine allows the antibody to bind many Diamino Atrazine-enzyme conjugate molecules. The result is a dark blue solution.

Conversely, a high concentration of Diamino Atrazine allows fewer Diamino Atrazine-enzyme conjugate molecules to be bound by the antibodies, resulting in a lighter blue solution.

NOTE: Color is inversely proportional to Diamino Atrazine concentration. Therefore:

Darker color = Lower concentration

Lighter color = Higher concentration

MATERIALS PROVIDED IN THE BEACON DIAMINO ATRAZINE TUBE KIT

The kit in its original packaging can be used until the end of the month indicated on the box label when stored at 2 – 8ºC.

40 antibody coated test tubes vacuum-packed in aluminized pouch with indicating desiccant

1 vial each of 0, 0.1 ppb, 0.2 ppb, 1.0 ppb, and 5.0 ppb Diamino Atrazine Calibrator

1 vial of Diamino Atrazine-HRP Enzyme Conjugate

1 vial of Substrate

1 vial of Stop Solution

1 Instructional Booklet

MATERIALS REQUIRED BUT NOT PROVIDED

- Photometer capable of reading 12x75 mm tubes at 450 nm
- Watch or timer
- Wash bottle with flip top cap containing PBST (0.05% tween 20)
- Pipette with disposable tips capable of delivering 500 µL
- Test tube rack that will retain tubes when inverted
- Diamino Atrazine-free water for dilution of samples

PERFORMANCE CHARACTERISTICS SPECIFICITY

The Beacon Diamino Atrazine 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_o and the % cross reactivity versus Diamino Atrazine. All concentrations are in parts per billion (ppb).

Compound	50% B_o	%CR
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

* ref: 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 Tube Kit:

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

PRECAUTIONS

- Store all kit components at 4°C to 8°C (39°F to 46°F) when not in use.
- Do not freeze kit components or expose them to temperatures greater than 37°C (99°F).
- Allow all reagents and samples to reach ambient temperature before you begin the test.
- Do not use kit components after the expiration date.
- Do not mix reagents or test tubes from tube kits with different lot numbers.
- Care should be taken to minimize scratching the outside of the antibody-coated test tubes as these are the optical surface through which the assay results will be read.
- Transfer of samples and reagents by pipette requires constant monitoring of technique. Pipetting errors are the major source of error in immunoassay methodology.
- The assay is not specific for Diamino Atrazine and will react with related triazine structures. See table in Performance Characteristics for specific information.

SAMPLE DILUTIONS

Samples found to have or expected to have concentrations of Diamino Atrazine greater than 5.0 ppb should be diluted prior to analysis. The following table illustrates some possible dilution schemes. It is recommendable to run a sample at multiple dilutions in a single assay to minimize requirements for re-assay.

These dilutions can be easily accomplished using the 500 µL pipette used in the assay and dilution vials available from Beacon. Serial dilutions, when a diluted sample is diluted further, can be useful. The following table shows the effective range of quantitation in the assay based on serial dilutions of 1:4.

Initial Sample	Dilution Factor	Final Dilution	Quantitation Range (ppb)
Neat	None	1:1	0.05 – 5.0
Neat	1:4	1:4	0.2 – 20
1:4	1:4	1:16	0.8 - 80

To determine the amount of Diamino Atrazine in a diluted sample multiply the concentration found in the assay by the dilution factor. For example, if a sample diluted 1:4 was found to contain 2.5 ppb the original sample contains 10 ppb (2.5 X 4).

ASSAY PROCEDURE

(NOTE: Running calibrators and samples in duplicate will improve assay precisions and accuracy)

1. Bring all kit reagents and samples to be run to room temperature.
2. Remove the required number of antibody coated tubes from the zip lock bag. Be sure to re-seal the bag with the desiccant to limit exposure of the tubes to moisture.
3. Label the antibody coated tubes with appropriate sample or standard identification. Arrange the labeled tubes in the tube rack such that sample and standard tubes are interspersed. The first and last tubes should always be standards with samples and other standards mixed in between.
4. NOTE: Be sure to label the tubes a maximum of one inch from the top to ensure proper photometric reading.
5. Pipette 500 µL of sample or standard solutions to the appropriately labeled antibody coated tubes. Be sure to use clean tip for each sample or standard.
6. Pipette 500 µL of Enzyme Conjugate into each tube.
7. After the addition of the enzyme conjugate, pick up the rack and shake it to mix the contents of the tubes. Set the timer for 60 minutes and allow reaction mixture to incubate at room temperature.
8. Decant the contents of all tubes by holding the rack upside down over the sink and shaking the rack vertically twice.
9. Fill each tube to overflowing with PBST using the wash bottle. Direct the stream towards the bottom of each tube. Maintain moderate pressure on the stream of water by squeezing the wash bottle. When all tubes have been filled, decant the wash as described above.
10. Wash all tubes three more times.