

CALCULATE RESULTS

1. After you read all of the wells, average the OD of each set of calibrators, controls and samples, and calculate the %Bo as follows:
$$\%B^{\circ} = (\text{average OD of calibrator, control or sample} \times 100) \div \text{average OD of negative control}$$
2. Graph the %Bo of each calibrator on the Y (linear) axis against its atrazine concentration on the X (log) axis using semi-log graph paper. Draw the best-fit line through the calibrator points.
3. Determine the atrazine concentration of each sample by finding its %Bo value and the corresponding concentration level on the graph.
4. Calculation of sample concentration is only valid if the %Bo of the sample falls within the range of the %Bo's set by the calibrators. If the sample falls outside of that range, the results must be reported as less than the lowest calibrator value or greater than the highest calibrator value.

QUALITY CONTROL

1. The %Bo ranges for the calibrators should fall within the following ranges:

Atrazine Calibrator (ppb)	%Bo Range
0.05	78 - 88
0.5	46 - 56
5.0	16 - 27

2. The assay control value should fall within the range printed on the vial.

SAMPLE CALCULATIONS

Plate Contents	OD	Average OD \pm SD*	%RSD	%Bo**	Atrazine conc. (ppb)
Negative Control	1.538	1.523 \pm 0.021	1.4	100.0	N/A
	1.508				
0.05 ppb Calibrator	1.280	1.269 \pm 0.015	1.2	83.3	N/A
	1.259				
0.5 ppb Calibrator	0.789	0.774 \pm 0.022	2.8	50.8	N/A
	0.758				
5.0 ppb Calibrator	0.334	0.332 \pm 0.002	0.6	21.8	N/A
	0.331				
Sample	0.428	0.418 \pm 0.014	3.4	27.4	3.14
	0.408				

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.

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Atrazine Plate Kit

Cat.# 20-0002

Instructional Booklet

READ COMPLETELY BEFORE USE.

INTENDED USE

The Beacon Atrazine Plate Kit is an immunological laboratory test for the quantitation of atrazine pesticide residues in water.

USE PRINCIPLES

The Beacon Atrazine Plate Kit uses polyclonal antibodies that bind both atrazine and an atrazine-enzyme conjugate. Any atrazine present in the sample competes with an atrazine-enzyme conjugate for a limited number of antibody binding sites. Antibodies, which bind atrazine, are immobilized to the inside of the test wells. In the assay procedure you will:

- Add samples or calibrators containing known amounts of atrazine and an atrazine enzyme conjugate to test wells coated with anti-atrazine antibodies.
- Wash away any unbound molecules, after you incubate this mixture for 60 minutes.
- Add clear substrate solution to each Plate. In the presence of bound 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 well, and each well receives the same number of atrazine-enzyme conjugate molecules, a sample containing a low concentration of atrazine allows the antibody to bind many atrazine-enzyme conjugate molecules. The result is a dark blue solution.

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

MATERIALS PROVIDED IN THE BEACON ATRAZINE PLATE KIT

This plate kit contains the following items:

- 1 plate containing 12 strips of 8 wells coated with rabbit anti-atrazine antibodies
- 1 vial of Negative Control (0.0 ppb Atrazine)
- 1 vial each of 0.05 ppb, 0.5 ppb, and 5.0 ppb Atrazine Calibrator
- 1 vial of Atrazine Control (exact value range printed on vial)
- 1 vial of Atrazine-HRP Enzyme Conjugate
- 1 vial of Substrate
- 1 vial of Stop Solution
- 1 Instructional Booklet

You also need these items:

- Microtiter plate reader
- Tape or Parafilm®
- Watch or timer
- Clean running water or a wash bottle containing tap or deionized water.
- Orbital shaker (optional)

PERFORMANCE CHARACTERISTICS

SPECIFICITY

The Beacon Atrazine Plate Kit can not 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 % cross reactivity versus atrazine. All concentrations are in parts per billion (ppb).

Compound	50% B ₀	%CR
Atrazine	0.50	100
Prometryne*	0.12	420
Ametryne*	0.16	310
Propazine	0.38	130
Simetryne**	0.90	56
Prometon	1.3	38
Terbutryne**	1.4	36
Simazine	6.2	8.1
Terbutylazine	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 speciality crops only. Rarely found in ground water.

** Not registered for use in U.S.

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

Alachlor, Metolachlor, Carbofuran, Aldicarb, 2,4-D, Diaminoatrazine, Carbendazim, Melamine

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:

	Average (ppb)	Std. Dev. (ppb)	%RSD
Control I	0.090	0.010	11
Control II	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:

	Average (ppb)	Std. Dev. (ppb)	%RSD
Control I	0.087	0.009	10
Control II	3.2	0.12	3.8

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 Plates from Plate kits with different lot numbers.
- Transfer of samples and reagents by pipette requires constant monitoring of technique. Pipetting errors are the major source of error in immunoassay methodology.
- Samples found to have or expected to have concentrations of atrazine greater than 5.0 ppb should be diluted prior to analysis.
- Use approved methodologies to confirm any positive results.

ASSAY PROCEDURE

1. Bring all kit reagents and samples to be run to room temperature.
2. Remove the required number of antibody coated strips from the zip lock bag. Be sure to re-seal the bag with the desiccant to limit exposure of the strips to moisture.
3. Pipet **100 µL of calibrators, control and samples** into the appropriate wells. Be sure to use a clean pipet tip for each solution to avoid cross contamination.
4. Add **100 µL of Enzyme Conjugate** to each well.
5. Cover the wells with tape or Parafilm Swirl the plate rapidly on bench surface to mix the contents. Alternately, the plate may be incubated on a rotator for continuous mixing during incubation.
6. Incubate for **60 minutes**.
7. After incubation, remove the covering and vigorously shake the contents of the wells into a sink. Flood the wells completely with cool running tap water, then shake to empty. Repeat this wash step four times for a total of five washes. Invert the plate on absorbent paper and tap out as much water as possible.
8. Add **100 µL of Substrate** to each well.
9. Cover the wells and incubate for **30 minutes**.
10. Add **100 µL of Stop Solution** to each well in the same order of addition as the Substrate.
WARNING: Stop Solution is 1N hydrochloric acid. Handle carefully.
11. Read the plate on a microtiter plate reader at 450nm. If the plate reader has dual wavelength capability, read at 450nm minus 605 or 650nm.
12. If the microtiter plate reader has data reduction capabilities, use either a semi-log linear or 4 parameter curve fit. If manual data reduction is required, proceed with next section.