

RESULTS INTERPRETATION

1. Semi-quantitative results can be derived by simple comparison of the sample absorbances to the absorbance of the calibrator wells: Sample containing less color than a calibrator well have a concentration of Alachlor greater than the concentration of the calibrator. Samples containing more color than a calibrator well have a concentration less than the concentration of the calibrator.
2. Quantitative interpretation requires graphing the absorbances of the calibrators (y-axis) versus the log of the calibrator concentration (x-axis) on semi-log graph paper. A straight line is drawn through the calibrator points and the sample absorbances are located on the line. The corresponding point on the x-axis is the concentration of the sample. Samples with absorbances greater than the lowest calibrator or less than the highest calibrator must be reported as < 0.1ppb or >0.75 ppb, respectively.

SAMPLE CALCULATIONS

Well Contents	OD	Average OD ± SD**	%RSD	%Bo*
Negative Control	1.538 1.508	1.523 ± 0.021	1.4	100.0
0.1 ppb Calibrator	1.280 1.259	1.269 ± 0.015	1.2	83.3
0.3 ppb Calibrator	0.789 0.758	0.774 ± 0.022	2.8	50.8
0.75 ppb Calibrator	0.334 0.331	0.332 ± 0.002	0.6	21.8

This data is for example purposes only.

* %Bo = (OD₄₅₀ / 0 ppb OD₄₅₀)*100

** standard deviation

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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Alachlor Plate Kit

Cat.# 20-0166

Instructional Booklet

READ COMPLETELY BEFORE USE.

INTENDED USE

The Alachlor Plate Kit is an immunological laboratory test for the quantitation of alachlor herbicide residues in water.

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ASSAY PRINCIPLES

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

•Add a sample containing Alachlor to a test well, followed by Alachlor-enzyme conjugate. The conjugate competes with any Alachlor 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 well. In the presence of bound Alachlor-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 Alachlor-enzyme conjugate molecules, a sample containing a low concentration of Alachlor allows the antibody to bind many Alachlor-enzyme conjugate molecules. The result is a dark blue solution.

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

NOTE: Color is inversely proportional to Alachlor concentration.

Darker color = Lower concentration
Lighter color = Higher concentration

SPECIFICITY

The Alachlor Plate Kit is specific for acetanilide herbicides but can not distinguish between various members of the acetanilide group of compounds. The following table shows the relative values for 50% B₀ and the % cross reactivity versus alachlor (used as calibrators in the kit). All concentrations are in parts per billion (ppb).

Compound	50% B ₀	%CR
Alachlor	0.30	100
Acetochlor	0.75	40
Metolachlor	0.82	36
Metolachlor-ESA	23	1.3
Alachlor-ESA	52	0.6
Propachlor	150	0.2

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

Atrazine	Carbofuran	Chlorpyrifos
Aldicarb	2,4-D	Carbendazim
Diazinon		

PRECAUTIONS

1. Each reagent is optimized for use in the Beacon Alachlor Plate Kit. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other Beacon Alachlor Plate Kits with different Lot numbers.
2. Dilution or adulteration of reagents or samples not called for in the procedure may result in inaccurate results.
3. Do not use reagents after expiration date.
4. Store all plate 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)
5. Reagents should be brought to room temperature, 20 – 28°C (62 – 82°F) prior to use. Avoid prolonged (> 24 hours) storage at room temperature.
6. Use approved methodologies to confirm any positive results.
7. The Stop Solution is 1N hydrochloric acid. Avoid contact with skin and mucous membranes. Immediately clean up any spills and wash area with copious amounts of water. If contact should occur, immediately flush with copious amounts of water.

REAGENTS AND MATERIALS PROVIDED

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

- 1 Frame containing 12 antibody coated test strips of 8 wells each vacuum-packed in aluminized pouch with indicating desiccant.
- 1 vial each of 0, 0.1, 0.3 and 0.75 ppb alachlor calibrators
- 1 vial of Alachlor-HRP Enzyme Conjugate.
- 1 vial containing 14 mL of Substrate.
- 1 vial containing 14 mL of Stop Solution. (Caution! 1N HCl. Handle with care.)

MATERIALS REQUIRED BUT NOT PROVIDED

- Wash bottle containing deionized water.
- Glass tubes or vials for calibrator preparation.
- Tape or Parafilm®
- Orbital shaker (optional)
- Pipet with disposable tips capable of dispensing 100 µL.
- Multi-channel pipet; 8 channel capable of dispensing 100 µL.
- Paper towels or equivalent absorbent material.
- Microwell plate or strip reader with 450 nm filter.
- Timer

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	0.24	0.014	5.8
Control II	0.58	0.043	7.4

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	0.26	0.020	7.7
Control II	0.59	0.049	8.3

TEST PROCEDURE (Note: Running calibrators and samples in duplicate will improve assay precision and accuracy.)

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 dessicant 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. Swirl the plate rapidly to mix the contents and cover the wells with tape or Parafilm. Alternately, the plate may be incubated on a rotater 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 deionized or distilled water, then shake to empty. Repeat this wash step four times for a total of five washes. Invert the plate on absorbant 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.
11. Read the plate on a microtiter plate reader at 450 nm. If the plate reader has dual wavelength capability, read at 450 nm minus 605 or 650 nm.
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.