Alachlor

• Intended Use

For the detection and quantitation of Alachlor and related acetanilides in water (groundwater, surface water, well water). For soil, crop, and food use contact the company for application bulletins and/or specific matrix validation guidelines.

• Principle

The Abraxis Alachlor Microtiter Plate Kit applies the principles of enzyme linked immunosorbent assay (ELISA) to the determination of Alachlor. In the assay system, standards, controls, or samples are added, along with an enzyme conjugate, to microtiter wells coated with Goat Anti-Rabbit Antibody. An antibody specific for Alachlor is then added. At this point, a competitive reaction occurs between the Alachlor or other acetanilides, which may be in the sample and the enzyme-labeled Alachlor analog for the antibody binding sites on the microtiter well. The reaction is allowed to continue for sixty (60) minutes. After a washing step, the presence of Alachlor is detected by adding the "Color Solution," which contains the enzyme substrate (hydrogen peroxide) and the chromogen (3,3',5,5'-tetramethylbenzidine). The enzyme-labeled Alachlor bound to the Alachlor antibody catalyzes the conversion of the substrate/chromogen mixture to a colored product. The color reaction is stopped and stabilized after a twenty (20) minute incubation period by the addition of diluted acid (stopping solution). The color is then evaluated using an ELISA reader.

A dose response curve of absorbance of the colored product formed vs. concentration is generated using results obtained from the standards. The concentration of Alachlor present in the control and samples are determined directly from this curve. Since the labeled Alachlor (conjugate) was in competition with the unlabeled Alachlor (sample) for the antibody sites, t**he intensity of the color developed is inversely proportional to the concentration of Alachlor present in the sample.**

• Reagents

The Abraxis Alachlor Plate Kit contains the following items:

1. Microtiter Plate coated with Goat-Anti Rabbit Antibody
96 test kit: 12 strips of 8 antibody coated wells and strip holder (1).

2. Alachlor Antibody Solution
Alachlor antibody (rabbit anti-alachlor) solution in a buffered saline solution with preservative and stabilizers.
96 test kit: One vial containing 6 mL

3. Alachlor Enzyme Conjugate
Horseradish peroxidase (HRP) labeled Alachlor analog in a buffered solution with preservative and stabilizers.
96 test kit: One vial containing 6 mL

4. Alachlor Standards
Six concentrations (0, 0.1, 0.25, 0.5, 1.0, 5.0 ppb) of Alachlor standards in distilled water with preservative and stabilizers.
96 test kit: Each vial contains 1.0 mL

5. Control
A concentration (approximately 0.75 ppb) of Alachlor in distilled water with preservative and stabilizers.
96 test kit: One vial containing 1.0 mL

6. Diluent/Zero Standard (Sample Diluent)
Distilled water with preservative and stabilizers without any detectable Alachlor.
96 test kit: One bottle containing 30 mL

7. Color Solution
A solution of hydrogen peroxide and 3,3',5,5'-tetramethyl benzidine in an organic base.
96 test kit: One bottle containing 16 mL

8. Stopping Solution
A solution of diluted acid.
96 test kit: One bottle containing 12 mL

9. Washing Buffer (5x) Concentrate
Buffered salts with detergent and preservatives.
96 test kit: One bottle containing 100 mL

• Reagent Storage and Stability

Store all reagents at 2-8°C. Do not freeze. Reagents may be used until the expiration date on the box.

Consult state, local and federal regulations for proper disposal of all reagents.

• Materials Required but Not Provided

In addition to the reagents provided, the following items are essential for the performance of the test:

Precision pipets capable of delivering 25, 50, 100, and 250 µL, and tips*

Tape or Parafilm®*

Timer*

Distilled or deionized water for diluting Wash Buffer

Storage bottle with 1000 mL capacity for storage of 1x Wash Buffer*

Microplate or strip reader capable of reading absorbance at 450 nm*

* Please contact Abraxis for supplier information.

• Sample Information

This procedure is recommended for use with water samples. Other samples may require modifications to the procedure and should be thoroughly validated.

Samples containing gross particulate matter should be filtered (e.g. 0.2 um Anotop™ 25 Plus, Whatman, Inc.) to remove particles.

Samples which have been preserved with monochloroacetic acid or other acids, should be neutralized with strong base e.g. 6N NaOH, prior to assay.

If the Alachlor concentration of a sample exceeds 5.0 ppb, the sample is subject to repeat testing using a diluted sample. A ten-fold or greater dilution of the sample is recommended with an appropriate amount of Diluent/Zero Standard or Sample Diluent. For example, in a separate test tube make a ten-fold dilution by adding 100 µL of the sample to 900 µL of Diluent/Zero Standard. Mix thoroughly before assaying. Perform the assay according to the Assay Procedure and obtain final results by multiplying the value obtain by the dilution factor, e.g. 10.

• Reagent Preparation

All reagents must be allowed to come to room temperature.

Wash Buffer
In a 1000 mL container, dilute the wash buffer concentrate 1:5 by the addition of distilled or deionized water (i.e., 100 mL of wash buffer concentrate plus 400 mL of H_2O). This solution is used to wash the antibody coated wells.

• Procedural Notes and Precautions

As with all immunoassays, a consistent technique is the key to optimal performance. To obtain the greatest precision, be sure to treat each well in an identical manner.

Add reagents directly to the bottom of the well while **avoiding contact between the reagents and the pipet tip**. This will help assure consistent quantities of reagent in the test mixture.

Avoid cross-contaminations and carryover of reagents by using clean pipets for each sample addition and by avoiding contact between reagent droplets on the tubes and pipet tips.

The microtiter plate consists of 12 strips of 8 wells. If fewer than twelve strips are used, remove the unneeded strips and store refrigerated in the resealable foil bag (with desiccant) provided.

If more than 3 strips are being used per run, the use of a multi-channel pipette is recommended for the addition of conjugate, antibody, color, and stopping solutions.

Do not use any reagents beyond their stated shelf life. Each component used in any one assay should be of the same lot number and stored under identical conditions.

Avoid contact of Stopping Solution (diluted sulfuric acid) with skin and mucous membranes. If this reagent comes in contact with skin, wash with water.

• Limitations

The Abraxis Alachlor Plate Assay will detect Alachlor and related acetanilides to different degrees. Refer to the specificity table for data on several of the acetanilides. The Abraxis Alachlor Plate Assay kit provides screening results. As with any analytical technique (GC, HPLC, etc...) positive results requiring some action should be confirmed by an alternative method.

• Quality Control

A control solution at approximately 0.75 ppb of Alachlor is provided with the Abraxis Alachlor Plate Assay kit. It is recommended that it be included in every run and treated in the same manner as unknown samples. Acceptable limits should be established by each laboratory.

• Assay Procedure

Read Reagent Preparation, Procedural Notes and Precautions before proceeding.

St0-St5: Standards
C: Control
S1-Sx: Samples

	1	2	3	4	5	6	7	8	9	10	11	12
A	St 0	St 4	S2									
B	St 0	St 4	S2									
C	St 1	St 5	etc.									
D	St 1	St 5	etc.									
E	St 2	C										
F	St 2	C										
G	St 3	S1										
H	St 3	S1										

1. Add 25 µL of the appropriate standard, control, or sample. Analysis in duplicates or triplicates is recommended.
2. Add 50 µL of enzyme conjugate solution successively to each well.
3. Add 50 µL of Alachlor antibody solution successively to each well. Cover wells with parafilm or tape to prevent contamination and evaporation.
4. Thoroughly mix the contents of the wells by moving the strip holder in a rapid circular motion on the benchtop for a full 20-30 seconds. Be careful not to spill the contents.
5. Incubate at ambient temperature for 60 minutes.
6. After incubation, carefully remove the covering and vigorously shake the contents of the wells into a waste container. Wash the strips with the diluted Wash Buffer (see Reagent Preparation) by adding a volume of at least 250 µL of Wash Buffer to each well. Vigorously shake the contents of the wells into a waste container. Any remaining buffer in the wells should be removed by patting the plate on a dry stack of paper towels. Repeat this wash step two times, for a total of 3 rinses.
7. Add 150 µL of Color Solution successively to each well. Cover wells with parafilm or tape. Thoroughly mix the contents of the wells by moving the strip holder in a rapid circular motion on the benchtop for a full 20-30 seconds Incubate at ambient temperature for 20 minutes.

8. Add 100 µL of Stopping Solution successively to each well.
9. Read absorbance using a microplate reader at 450 nm within 15 minutes after adding the Stopping Solution.

• Results

The evaluation of the ELISA can be performed using commercial ELISA evaluation programs (Logit/Log or alternatively point to point). For manual evaluation, calculate the mean absorbance value for each of the standards. Calculate the %B/Bo for each standard by dividing the mean absorbance value for each standard by the mean absorbance value for the Diluent/Zero Standard (Standard 0). Construct a standard curve by plotting the %B/Bo for each standard on the vertical linear (Y) axis versus the corresponding Alachlor concentration on the horizontal log (X) axis on the graph paper provided. Calculate the %B/Bo for the control and sample(s) and obtain the concentration of Alachlor (in ppb) by interpolation using the constructed standard curve.

Samples exhibiting a concentration lower than 0.08 ppb should be assumed to be below the detection limit of the assay. Samples exhibiting a concentration higher than 5.0 ppb must be diluted to obtain accurate results.

• Performance Data

Precision

The following results were obtained:

Control	1	2	3	4
Replicates	3	3	3	3
Days	5	5	5	5
n	15	15	15	15
Mean (ppb)	0.26	0.78	1.26	1.83
% CV (within assay)	12.1	6.0	8.2	10.7
% CV (between assay)	15.0	12.5	14.2	15.8

Limit of Detection

The Abraxis Alachlor Plate Assay has an estimated minimum detection concentration based on a 90% B/Bo of 0.08 parts per billion (ppb).

Recovery

Four (4) groundwater samples were spiked with various levels of Alachlor and then assayed using the Abraxis Alachlor Plate Assay. The following results were obtained:

Amount of Alachlor Added (ppb)	Recovery Mean (ppb)	Recovery S.D. (ppb)	Recovery %
0.25	0.226	0.031	90
0.75	0.816	0.047	109
1.5	1.498	0.145	100
2.5	2.253	0.290	90
Average			97

Specificity

The cross-reactivity of the Abraxis Alachlor Plate Assay for various acetanilide analogues can be expressed as the least detectable dose (LDD) which is estimated at 90% B/Bo, or as the dose required for 50% absorbance inhibition (50% B/Bo).

B/Bo Compound	LDD (ppb)	50% (ppb)
Alachlor	0.08	0.9
Acetochlor	1.75	40
Metolachlor	2.20	110
Butachlor	0.90	190
Alachlor Sulfonic Acid	0.80	650
Alachlor Oxalinic Acid	5	450
Metalaxyl	25	1100
Propachlor	730	1780

The following compounds demonstrated no reactivity in the Abraxis Alachlor Plate Assay at concentrations up to 1000 ppb: Atrazine ametryn, cyanazine, 2,4-D, propazine, and simazine.

• Ordering information

Microtiter Plate Kit

Abraxis Alachlor Plate Assay Kit, 96T	PN 500076
Sample Diluent	PN 500072
Plate Standard Set	PN 500074

Test Tube Kit

Abraxis Alachlor Assay Kit, 100T	PN 500071
Sample Diluent	PN 500072
Standard Set	PN 500073

• Assistance

For ordering or technical assistance contact:

Abraxis, Inc.
124 Railroad Drive
Warminster, Pennsylvania 18974

(215) 357-3911 * Fax(215) 357-5232
WEB: www.abraxiskits.com
Email: info@abraxiskits.com

• General Limited Warranty

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