Importance of Alachlor Determination

Alachlor is an acetanilide herbicide which is used to target broadleaf weeds and grasses on agricultural land. It is used for weed control with corn, soybeans, and peanuts and can be applied to fields either before or after planting.

According to the U.S. Environmental Protection Agency (EPA) Registration Eligibility Decision facts sheet (December 1998), "Alachlor presents a clear hazard to groundwater quality. Reliable monitoring studies have demonstrated that alachlor, even when used according to label directions, results in significant groundwater contamination" and that "monitoring studies show that alachlor levels in surface water result in effects on aquatic plants and indirectly on aquatic animals." The EPA has established tolerance levels in various foods ranging from 0.02 ppm to 10 ppm. The maximum contaminant level (MCL) in drinking water in the U.S., regulated under the Safe Drinking Water Act (SDWA), is 2 ppb. The use of Alachlor has been banned in the European Union (EU).

The Alachlor ELISA allows for the analysis of 41 samples in duplicate determination. Less than 1 mL of sample is required. The test can be performed in less than 90 minutes.

Performance Data

Test sensitivity: The limit of quantitation for Alachlor (90% B/B_0) is approximately 0.08 ng/mL. The middle of the test (50% B/B_0) is approximately 0.9 ng/mL. Determinations closer to the middle of the calibration curve give the most accurate results.

Test reproducibility: Coefficients of variation (CVs) for standards: <10%; CVs for samples: <15%.

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/B_0 or as the dose required for 50% absorbance inhibition (50% B/B_0).

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	110
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.

Performance Data:

Precision

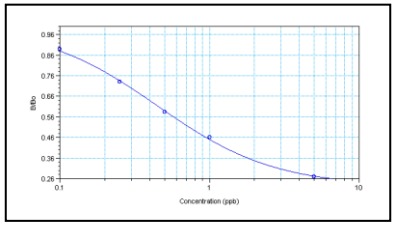
Control	1	2	3	4
Replicates	3	3	3	3
Days	5	5	5	5
N	15	15	15	15
Mean (ppb)	0.26	0.78	1.26	1.83
% CV (within assay)	12.1	6.0	8.2	10.7
% CV (between assay)	15.0	12.5	14.2	15.8

Recovery

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

Conc. (ppb)	Mean Rec. (ppb)	Std. Dev. (ppb)	% Rec.
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

Standard Curve:



For demonstration purposes only. Not for use in sample interpretation.

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Alachlor ELISA (Microtiter Plate)

Enzyme-Linked Immunosorbent Assay for the Determination of Alachlor in Water Samples

Product No. 500076

1. General Description

The Abraxis Alachlor ELISA is an immunoassay for the quantitative and sensitive screening of Alachlor and related acetanilides. This test is suitable for the quantitative and/or qualitative screening of Alachlor in water samples (groundwater, surface water, and well water). For soil, crop, and food matrices, please contact Abraxis technical services for application bulletins and/or specific matrix validation guidelines. Samples requiring regulatory action should be confirmed by HPLC, GC/MS, or other conventional methods.

2. Safety Instructions

The standard solutions in the test kit contain small amounts of Alachlor. The substrate solution contains tetramethylbenzidine (TMB) and the stop solution contains diluted sulfuric acid. Avoid contact of the TMB and stopping solution with skin and mucous membranes. If these reagents come in contact with skin, wash with water.

3. Storage and Stability

The Alachlor ELISA kit should be stored in the refrigerator (4–8°C). The solutions must be allowed to reach room temperature (20-25°C) before use. Reagents may be used until the expiration date on the box.

4. Test Principle

The test is a direct competitive ELISA based on the recognition of Alachlor by specific antibodies. Alachlor, when present in a sample, and an Alachlor-HRP analogue compete for the binding sites of the anti-Alachlor antibodies in solution. The Alachlor antibodies are then bound by a second antibody (anti-rabbit) immobilized on the wells of the microtiter plate. After a washing step and addition of the substrate solution, a color signal is generated. The intensity of the blue color is inversely proportional to the concentration of Alachlor present in the sample. The color reaction is stopped after a specified time and the color is evaluated using an ELISA reader. The concentrations of the samples are determined by interpolation using the standard curve constructed with each run.

5. Limitations of the Alachlor ELISA, Possible Test Interference

Although many organic and inorganic compounds commonly found in samples have been tested and found not to interfere with this test, due to the high variability of compounds that might be found in samples, test interferences caused by matrix effects cannot be completely excluded.

The presence of the following substances were found to have no significant effect on the Alachlor assay results: sodium chloride up to 100,000 ppm; calcium chloride, calcium sulfate, magnesium chloride, magnesium sulfate, manganese sulfate, potassium phosphate, sodium nitrate, and sodium thiosulfate up to 10,000 ppm; copper chloride, ferric sulfate, sodium fluoride, and zinc sulfate up to 1,000 ppm; humic acid up to 100 ppm.

Mistakes in handling the test can cause errors. Possible sources for such errors include: inadequate storage conditions of the test kit, incorrect pipetting sequence or inaccurate volumes of the reagents, too long or too short incubation times during the immune and/or substrate reaction, extreme temperatures (lower than 10°C or higher than 30°C) during the test performance.

Each reagent is optimized for use in the Abraxis Alachlor ELISA kit. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other Abraxis Alachlor ELISA Kits with different lot numbers.

The Abraxis Alachlor ELISA kit provides screening results. As with any analytical technique (GC/MS, HPLC, etc.), samples requiring regulatory action should be confirmed by an alternative method.

A. Reagents and Materials Provided

1. Microtiter plate (12 X 8 strips) coated with a secondary antibody, in a resealable aluminum pouch
2. Alachlor Standards (6): 0, 0.1, 0.25, 0.5, 1.0, 5.0 ng/mL (ppb), 1 mL each
3. Control: 0.75 ± 0.15 ppb, 1 mL
4. Antibody Solution (anti-Alachlor), 6 mL
5. Alachlor-HRP Conjugate Solution, 6 mL
6. Wash Buffer (5X) Concentrate, 100 mL, must be diluted before use, see Test Preparation (Section D)
7. Sample Diluent, 30 mL
8. Substrate (Color) Solution (TMB), 16 mL
9. Stop Solution, 12 mL (handle with care)

B. Additional Materials (not delivered with the test kit)

1. Micro-pipettes with disposable plastic tips (50-200 µL)
2. Multi-channel pipette (50-250 µL) or stepper pipette (50-250 µL), or electronic repeating pipette with disposable plastic tips
3. Container with 500 mL capacity (for 1X diluted wash buffer, see Test Preparation, Section D)
4. Graduated cylinder
5. Deionized or distilled water
6. Paper towels or equivalent absorbent material
7. Timer
8. Tape or parafilm
9. Microtiter plate reader (wave length 450 nm)
10. Microtiter plate washer (optional)

C. Notes and Precautions

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

Micro-pipetting equipment and pipette tips for pipetting the standards and the samples are necessary. The use of a multi-channel pipette, stepping pipette, or electronic repeating pipette is recommended for adding the enzyme conjugate, antibody, substrate, and stop solutions in order to equalize the incubation periods on the entire microtiter plate.

To avoid drift and obtain accurate results, the addition of the antibody, conjugate, color, and stop solutions should be performed in less than 2 minutes for each reagent. If additions to the entire microtiter plate cannot be completed in less than 2 minutes, run size should be decreased to the number of rows which can be pipetted in less than 2 minutes.

Samples containing gross particulate matter should be filtered (e.g. 0.2 µm 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 analysis.

Highly contaminated samples (those outside of the calibration range of the assay) must be diluted further in sample diluent and re-analyzed in order to obtain accurate quantitative results.

Please only use the reagents and standards from one package lot in one test, as they have been adjusted in combination.

D. Test Preparation

1. Allow the microtiter plate, reagents, and samples to reach room temperature before use.
2. Remove the number of microtiter plate strips required from the aluminum pouch. The remaining strips are stored in the aluminum pouch with the desiccant (tightly sealed) in the refrigerator (4-8°C).
3. The standard solutions, conjugate, antibody, substrate and stop solutions are ready to use and do not require any further dilutions.
4. Dilute the Wash Buffer (5X) Concentrate at a ratio of 1:5. If using the entire bottle (100 mL), add to 400 mL of deionized or distilled water and mix thoroughly.
5. The stop solution must be handled with care as it contains diluted H_2SO_4.

E. Working Scheme

The microtiter plate consists of 12 strips of 8 wells, which can be used individually for the test. The standards must be run with each test. Never use the values of standards which have been determined in a test performed previously.

Std 0-Std 5: Standards
(0; 0.1; 0.25; 0.5; 1.0; 5.0 ppb)

Contr.: Control

Samp1, Samp2, etc.: Samples

	1	2	3	4	5	6	7	8	9	10	11	12
A	Std 0	Std 4	Samp2									
B	Std 0	Std 4	Samp2									
C	Std 1	Std 5	etc.									
D	Std 1	Std 5	etc.									
E	Std 2	Contr.										
F	Std 2	Contr.										
G	Std 3	Samp1										
H	Std 3	Samp1										

F. Assay Procedure

1. Add **25 µL of the standards, control, or samples** into the wells of the test strips according to the working scheme given. Analysis in duplicate or triplicate is recommended.
2. Add **50 µL of conjugate solution** to the individual wells successively using a multi-channel pipette, stepping pipette, or electronic repeating pipette.
3. Add **50 µL of antibody solution** to the individual wells successively using a multi-channel pipette, stepping pipette, or electronic repeating pipette. Cover the wells with parafilm or tape.
4. Mix the contents by moving the strip holder in a circular motion on the benchtop for 30 seconds. Be careful not to spill the contents.
5. Incubate the strips for 60 minutes at room temperature.
6. Remove the covering and decant the contents of the wells into a waste container. Wash the strips **three times** using the diluted wash buffer. Please use at least a volume of **250 µL of 1X wash buffer** for each well in each washing step. Remaining buffer in the wells should be removed by patting the inverted plate dry on a stack of paper towels.
7. Add **150 µL of substrate (color) solution** to the wells successively using a multi-channel pipette, stepping pipette, or electronic repeating pipette. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop for 30 seconds. Be careful not to spill the contents. Incubate the strips for 20 minutes at room temperature. Protect the strips from direct sunlight.
8. Add **100 µL of stop solution** to the wells in the same sequence as for the substrate solution.
9. Read the absorbance at 450 nm using a microplate ELISA photometer within 15 minutes after the addition of stopping solution.

G. Evaluation

The evaluation of the ELISA can be performed using commercial ELISA evaluation programs such as 4-Parameter (preferred) or Logit/Log. For a manual evaluation, calculate the mean absorbance value for each of the standards. Calculate the $\%B/B_0$ for each standard by dividing the mean absorbance value for each standard by the Zero Standard (Standard 0) mean absorbance. Construct a standard curve by plotting the $\%B/B_0$ for each standard on the vertical linear (y) axis versus the corresponding Alachlor concentration on the horizontal logarithmic (x) axis on graph paper. $\%B/B_0$ for samples will then yield levels in ppb (or ng/mL) of Alachlor by interpolation using the standard curve. Results can also be determined using a spreadsheet macro available from Abraxis upon request.

The concentrations of the samples are determined using the standard curve run with each test. Samples showing a lower concentration of Alachlor than standard 1 (0.1 ppb) should be reported as containing < 0.1 ppb of Alachlor. Samples showing a higher concentration than standard 5 (5.0 ppb) should be reported as containing > 5.0 ppb of Alachlor. If a quantitative result is necessary, samples must be diluted in sample diluent and re-analyzed.

As with any analytical technique (GC/MS, HPLC, etc.), positive results requiring regulatory action should be confirmed by an alternative method.