

• Intended Use

For the detection and quantitation of acetochlor 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 Acetochlor Kit applies the principles of enzyme linked immunosorbent assay (ELISA) to the determination of acetochlor and related acetanilides. The sample to be tested is added, along with an enzyme conjugate, to a disposable test tube, followed by paramagnetic particles attached with antibodies specific to acetanilides. At this point a competitive reaction occurs between the acetochlor or other acetanilides which may be in the sample and the enzyme labeled acetochlor analog for the antibody binding sites on the magnetic particles. The reaction is allowed to continue for twenty (20) minutes. At the end of the incubation period, a magnetic field is applied to hold in the test tube the para-magnetic particles (with acetochlor and labeled acetochlor bound to the antibodies on the particles, in proportion to their original concentration), and allow the unbound reagents to be decanted. After decanting, the particles are washed with Washing Solution.

The presence of acetochlor 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 acetochlor bound to the acetochlor antibody catalyzes the conversion of the substrate/ chromogen mixture to a colored product. After an incubation period, the reaction is stopped and stabilized by the addition of a diluted acid (Stopping Solution). Since the labeled acetochlor (conjugate) was in competition with the unlabeled acetochlor (sample) for the antibody sites, **the color developed is inversely proportional to the concentration of acetochlor in the sample.**

• Reagents

The Abraxis Acetochlor Kit contains the following items:

1. Acetochlor Antibody Coupled Paramagnetic Particles

Acetochlor antibody (rabbit anti-acetochlor) covalently bound to paramagnetic particles suspended in a buffered solution with preservative and stabilizers.

100 test kit: one 65 mL vial

2. Acetochlor Enzyme Conjugate

Horseradish peroxidase (HRP) labeled acetochlor analog diluted in a buffered solution with preservative and stabilizers.

100 test kit: one 35 mL vial

3. Acetochlor Standards

Three concentrations (0.1, 0.5, 2.5 ppb) of acetochlor standards in distilled water with preservative and stabilizers. Each vial contains 2.0 mL.

4. Control

A concentration (approximately 1 ppb) of acetochlor in distilled water with preservative and stabilizers. A 2.0 mL volume is supplied in one vial.

5. Diluent/Zero Standard

Distilled water with preservative and stabilizers without any detectable acetochlor.

100 test kit: one 35 mL vial

6. Color Solution

A solution of hydrogen peroxide and 3,3',5,5'-tetramethylbenzidine in an organic base.

100 test kit: one 65 mL vial

7. Stopping Solution

A solution of diluted acid.

100 test kit: one 60 mL vial

8. Washing Solution

Preserved deionized water.

100 test kit: one 250 mL vial

9. Test Tubes

Polystyrene tubes (36) are packaged in a box.

100 test kit: three 36 tube boxes

• 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. *The test tubes and Washing Solution require no special storage condition and may be stored separately from the reagents to conserve refrigerator space.*

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:

Pipets* Precision pipets capable of delivering 200, and 500 μ L and a 1.0 mL repeating pipet.

Vortex Mixer* ThermoMylne Maxi Mix, Scientific Industries Vortex Genie, or equivalent

Magnetic Separation System*

Photometer* capable of readings 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 μ m 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 acetochlor concentration of a sample exceeds 2.5 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 μ L of the sample to 900 μ L of Diluent/Zero Standard. Mix thoroughly before assaying. Perform the assay according to the Assay Procedure and obtain final results by multiplying the value obtained by the dilution factor e.g. 10.

• Reagent Preparation

All reagents must be allowed to come to room temperature. The antibody coupled paramagnetic particles should be mixed thoroughly before use.

• 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 tube in an identical manner.

Add reagents directly to the bottom of the tube 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 enzyme droplets on the tubes and pipet tips.

Avoid foam formation during vortexing.

The magnetic separation system consists of two parts: an upper rack which will securely hold the test tubes and a lower separator which contains the magnets used to attract the antibody coupled paramagnetic particles. During incubations the upper rack is removed from the lower separator so that the paramagnetic particles remain suspended during the incubation. **For separation steps, the rack and the separator are combined to pull the paramagnetic particles to the sides of the tubes.**

To obtain optimum assay precision, it is important to perform the separation steps carefully and consistently. Decant the rack by slowly inverting away from the operator using a smooth turning action so the liquid flows consistently along only one side of the test tube. While still inverted, place the rack on an absorbent pad and allow to drain. Lifting the rack and replacing gently onto the pad several times will ensure complete removal of the liquid from the rim of the tube. Do not bang the rack.

Mix the antibody coupled paramagnetic particles just prior to pipetting.

Do not use any reagents beyond their stated shelf life.

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 Acetochlor Assay will detect acetochlor and related acetanilides to different degrees. Refer to specificity table for data on several of the acetanilides. The Abraxis Acetochlor 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.

The total time required for pipetting the magnetic particles should be kept to two (2) minutes or less, therefore the total number of tubes that can be assayed in a run should be adjusted accordingly.

• Quality Control

A control solution at approximately 1 ppb of acetochlor is provided with the Abraxis Acetochlor 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.

1. Label test tubes for standards, control, and samples.

Tube Number	Contents of Tube
1,2	Diluent/Zero Standard, 0 ppb
3,4	Standard 1, 0.1 ppb
5,6	Standard 2, 0.5 ppb
7,8	Standard 3, 2.5 ppb
9	Control
10	Sample 1
11	Sample 2
12	Sample 3

2. Add 200 μ L of the appropriate standard, control, or sample.
3. Add 250 μ L of Acetochlor Enzyme Conjugate to each tube.
4. Mix the Acetochlor Antibody Coupled Paramagnetic Particles thoroughly and add 500 μ L to each tube.
5. Vortex for 1 to 2 seconds minimizing foaming.
6. Incubate for 20 minutes at room temperature.
7. Separate in the Magnetic Separation System for **two (2) minutes**.
8. Decant and **gently** blot all tubes briefly in a consistent manner.

9. Add 1 mL of Washing Solution to each tube and allow them to remain in the magnetic separation unit for **two (2) minutes**.
10. Decant and **gently** blot all tubes briefly in a consistent manner.
11. Repeat Steps 9 and 10 an additional time.
12. Remove the rack from the separator and add 500 uL of Color Solution to each tube.
13. Vortex for 1 to 2 seconds minimizing foaming.
14. Incubate for 20 minutes at room temperature.
15. Add 500 uL of Stopping Solution to each tube.
16. Add 1 mL Washing Solution to a clean test tube. Use as blank in Step 17.
17. Read results at 450 nm within 15 minutes after adding the Stopping Solution.

• Results

Manual Calculations

1. Calculate the mean absorbance value for each of the standards.
2. Calculate the %B/Bo for each standard by dividing the mean absorbance value for the standard by the mean absorbance value for the Diluent/Zero Standard.
3. Construct a standard curve by plotting the %B/Bo for each standard on vertical linear (Y) axis versus the corresponding acetochlor concentration on horizontal logarithmic (X) axis on the graph paper provided.
4. %B/Bo for controls and samples will then yield levels in ppb of acetochlor by interpolation using the standard curve.
(Contact Abraxis for detailed application information on specific photometers.)

Photometric Analyzer

Some instrument manufacturers make available photometers allowing for calibration curves to be automatically calculated and stored. Refer to instrument operating manual for detailed instructions. To obtain results for the Abraxis Acetochlor Assay on instruments allowing data transformation the following parameter settings are recommended:

Data Reduct : Lin. Regression
 Xformation : Ln/Linear
 Read Mode : Absorbance
 Wavelength : 450 nm
 Units : PPB
 # Rgt Blk : 0

Calibrators:
 # of Cals : 4
 # of Repts : 2

Concentrations:
 #1: 0.00 PPB
 #2: 0.10 PPB
 #3: 0.50 PPB
 #4: 2.50 PPB

Range : 0.07 – 2.50
 Correlation : 0.990
 Rep. %CV : 10%

• Expected Results

In a study with water samples from locations across the U.S., the Abraxis Acetochlor Assay was shown to correlate well with another analytical technique ($r = 0.898$).

• Performance Data

Precision

The following results were obtained:

Control	1	2	3
Replicates	5	5	5
Days	5	5	5
n	25	25	25
Mean (ppb)	0.42	1.07	2.53
% CV (within assay)	11.9	8.4	5.9
% CV (between assay)	7.5	3.7	0.3

Sensitivity

The Abraxis Acetochlor Assay has an estimated minimum detectable concentration, based on a 90% B/Bo of 42 ppt.

Recovery

Five (5) groundwater samples, were spiked with various levels of acetochlor and then assayed using the Abraxis Acetochlor Assay. The following results were obtained:

Amount of Acetochlor Added (ppb)	Recovery -----		
	Mean (ppb)	S.D. (ppb)	%
0.25	0.25	0.04	101
0.50	0.56	0.06	112
1.0	1.13	0.11	113
2.0	1.96	0.14	98
Average			106

Specificity

The cross-reactivity of the Abraxis Acetochlor Assay for various acetanilides 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)
Acetochlor	0.042	0.60
Alachlor	0.045	0.70
Metolachlor	0.110	1.60
Butachlor	0.920	20
Alachlor Sulfonic Acid	9.2	224
Acetochlor Sulfonic Acid	15	78
Alachlor Oxalonic Acid	16.8	496
Metalaxyl	68	1600
Acetochlor Oxalonic Acid	130	680
Propachlor	8,000	> 10,000

The following compounds demonstrated no reactivity in the Abraxis Acetochlor Assay at concentrations up to 1000 ppb: aldicarb, aldicarb sulfoxide, aldicarb sulfone, atrazine, ametryn, benomyl, butylate, captan, carbaryl, carbendazim, carbofuran, cyanazine, 2,4-D, 1,3-dichloropropene, dinoseb, MCPA, metribuzin, pentachlorophenol, picloram, propazine, simazine, terbufos, thiabendazole, and thiophanate-methyl.

• Ordering information

Abraxis Acetochlor Assay Kit 100T PN 500021
 Sample Diluent PN 500022
 Standard Set PN 500023

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