Sensitivity
The ABRAXIS® Metolachlor Assay has anestimated minimum detectable concentration, based on a 90% B/Bo of 74 ppt.

Recovery
Five (5) groundwater samples were spiked withvarious levels of Metolachlor and then assayed using the ABRAXIS® Metolachlor Assay. The following results were obtained:

Amount of Metolachlor Added (ppb)	Recovery Mean (ppb)	Recovery S.D. (ppb)	Recovery %
0.50	0.54	0.11	108
1.0	1.06	0.14	106
2.0	1.96	0.27	98
3.0	2.76	0.35	92
4.0	3.60	0.54	90
Average			101

Specificity
The cross-reactivity of the ABRAXIS® Metolachlor 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)
Metolachlor	0.074	1.90
Acetochlor	0.35	26
Butachlor	2.2	56
Alachlor	3.0	110
Metalaxyl	9	260
Propachlor	140	4,200

The following compounds demonstrated no reactivity in the ABRAXIS® Metolachlor 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.

General Limited Warranty: Gold Standard Diagnostics warrants the products manufactured by the Company, against defects and workmanship when used in accordance with the applicable instructions for a period not to extend beyond the product's printed expiration date. **Gold Standard Diagnostics makes no other warranty, expressed or implied. There is no warranty of merchantability or fitness for a particular purpose.**

For ordering or technical assistance contact:
Gold Standard Diagnostics
795 Horsham Road
Horsham, PA 19044
WEB: www.abraxiskits.com
Phone: (215) 357-3911
Fax: (215) 357-5232
Ordering: info.abraxis@us.goldstandarddiagnostics.com
Technical Support: support.abraxis@us.goldstandarddiagnostics.com

Date this User Guide is effective: 05/15/2024
Version: 01

ABRAXIS® Metolachlor ELISA Tube Particle

Product No. 500061

Intended Use
For the detection and quantitation of Metolachlorand 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.

Storage and Stability
Store all reagents at 2-8°C. Do not freeze. Reagents may be used until the last day of the month as indicated by the expiration date on thebox. *The test tubes and Washing Solution require no special storage condition and may bestored separately from the reagents to conserverefrigerator space.*
Consult state, local and federal regulations forproper disposal of all reagents.

Assay Principle
The ABRAXIS® Metolachlor Kit applies the principles of enzyme linked immunosorbent assay (ELISA) to the determination of Metolachlor 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 metolachlor At this point a competitive reaction occurs between the Metolachlor or other acetanilides which may be in the sample and the enzyme labeled Metolachlor analog for the antibody binding sites on the magnetic particles. The reaction is allowed to continue for twenty (30) minutes. At the end of the incubation period, a magnetic field is applied to hold in the test tube the para- magnetic particles (with Metolachlor and labeled Metolachlor 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 Metolachlor is detected by adding the "Color Solution", which contains the enzyme substrate (hydrogen peroxide) and the chromogen (3,3',5,5'-tetramethylbenzidine). Theenzyme-labeled Metolachlor bound to the Metolachlor antibody catalyzes the conversion ofthe substrate/ chromogen mixture to a colored product. After an incubation period, the reactionis stopped and stabilized by the addition of a diluted acid (Stopping Solution). Since the labeled Metolachlor (conjugate) was in competition with the unlabeled Metolachlor (sample) for the antibody sites, the color developed is inversely proportional to the concentration of Metolachlor in the sample.

Limitations of the ABRAXIS® Metolachlor ELISA Tube Particle
The ABRAXIS® Metolachlor Assay will detect Metolachlor and related acetanilides to different degrees. Refer to specificity table fordata on several of the acetanilides. The ABRAXIS® Metolachlor 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 magneticparticles should be kept to two (2) minutes or less, therefore the total number of tubes that canbe assayed in a run should be adjusted accordingly.

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 reagentsand 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 sampleaddition and by avoiding contact between reagent 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, coupledparamagnetic 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 rackand 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 particlesjust prior to pipetting. • Do not use any reagents beyond their statedshelf 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.

Working Instructions

Materials Provided

1. Metolachlor Antibody Coupled ParamagneticParticles, 65 mL
2. Metolachlor Enzyme Conjugate, 35 mL
3. Metolachlor Standards (3) (0.1, 1.0, 5.0 ppb), 2.0 mL
4. Control (2 ppb), 2.0 mL
5. Diluent/Zero Standard, 35 mL
6. Color Solution, 65 mL
7. Stopping Solution, 60 mL
8. Washing Solution T, 250 mL
9. Test Tubes (36/box) X3

Materials Required (not provided)

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

• Precision pipets capable of delivering 200, and 500 uL and a 1.0 mL repeating pipet.

• Vortex Mixer • Magnetic Separation System • Photometer capable of readings at 450 nm

Sample Information

This procedure is recommended for use with water samples. Other samples may require modifications to the procedure and should bethoroughly 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, priorto assay. • If the Metolachlor concentration of a sample exceeds 5 ppb, the sample is subject to repeattesting 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 uL of the sample to 900 uL 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 roomtemperature. The antibody coupled paramagnetic particles should be mixed thoroughly before use.

Quality Control

A control solution at approximately 2 ppb of Metolachlor is provided with the ABRAXIS® Metolachlor Assay kit. It is recommended that it be included in every run and treated in the samemanner as unknown samples. Acceptable limitsshould be established by each laboratory.

Assay Procedure

Read Reagent Preparation, Procedural Notesand Precautions before proceeding.

1. Label test tubes for standards, control, andsamples.

Tube Number	Contents of Tube	Tube Number	Contents of Tube
1,2	Diluent/Zero Standard, 0 ppb	9, 10	Control
3,4	Standard 1, 0.1 ppb	11, 12	Sample 1
5,6	Standard 2, 1.0 ppb	13, 14	Sample 2
7,8	Standard 3, 5.0 ppb	15, 16	Sample 3

2. **Add 200 uL of the appropriate standard,control, or sample**.
3. **Add 250 uL of Metolachlor Enzyme Conjugate** to each tube.
4. Mix the Metolachlor Antibody Coupled Paramagnetic Particles thoroughly and add500 uL to each tube.
5. **Vortex for 1 to 2 seconds** minimizing foaming
6. **Incubate for 30 minutes at room temperature**.
7. Separate in the Magnetic Separation System for **two (2) minutes**.
8. **Decant and gently blot all tubes** briefly in aconsistent 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 aconsistent manner.
11. **Repeat Steps 8 and 9 an additional time**.
12. Remove the rack from the separator and **add500 uL of Color Solution to each tube**.
13. **Vortex for 1 to 2 seconds** minimizingfoaming.
14. **Incubate for 20 minutes at room temperature**.
15. **Add 500 uL of Stopping Solution** to eachtube.
16. **Add 1 mL Washing Solution** to a clean testtube. Use as blank in Step 17.
17. Read results at 450 nm within 15 minutesafteradding the Stopping Solution.

Results

Expected Results

In a study with water samples from locations across the U.S., the ABRAXIS® MetolachlorAssay was shown to correlate well with another commercial immunoassay ($r^2 = 0.947$).

Manual Calculations

1. Calculate the mean absorbance value for eachof 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 theDiluent/Zero Standard.
3. Construct a standard curve by plotting the
4. %B/Bo for each standard on vertical linear (Y)axis versus the corresponding Metolachlor concentration on horizontal logarithmic (X) axison the graph paper provided.
5. %B/Bo for controls and samples will then yield levels in ppb of Metolachlor by interpolation using the standard curve.

(Contact Gold Standard Diagnostics for detailed application information on specific photometers.)

Photometric Analyzer

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

Data Reduct:	Lin. Regression	Calibrators:		Concentrations:	
Xformation:	Ln/LogitB	# of Cals	: 4	#1: 0.00	PPB
Read Mode:	Absorbance	# of Reps	: 2	#2: 0.1	PPB
Wavelength:	450 nm			#3: 1.0	PPB
Units:	PPB			#4: 5.0	PPB
# Rgt Blk:	0				
Range:	0.07 –5.0				
Correlation:	0.990Rep.				
%CV:	10%				

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)	1.24	2.33	3.90
% CV (within assay)	7.9	7.9	8.1
% CV (between assay)	4.8	4.8	6.0