Sensitivity

The ABRAXIS® Atrazine ELISA Tube Particle has an estimated minimum detectable concentration, based on a 90% B/Bo of50 ppt.

Recovery

Five (5) groundwater samples were spiked with variouslevels of atrazine and then assayed using the ABRAXIS® Atrazine ELISA Tube Particle. The following results were obtained:

Amount of Atrazine Added (ppb)	Recovery Mean (ppb)	Recovery S.D. (ppb)	Recovery %
0.50	0.55	0.09	110
1.0	1.09	0.15	109
2.0	2.16	0.14	108
4.0	3.92	0.27	98
Average			106

Specificity

The cross-reactivity of the ABRAXIS® Atrazine ELISA Tube Particle for various triazine analogues can be expressed as the leastdetectable dose (LDD) which is estimated at 90% B/Bo, or asthe dose required for 50% absorbance inhibition (50% B/Bo).

Compound	LDD (ppb)	50% B/Bo (ppb)	Compound	LDD (ppb)	50% B/Bo (ppb)
Atrazine	0.050	0.70	Terbutryn	0.340	210
Propazine	0.084	1.18	Simazine	0.760	3.40
Ametryn	0.022	0.44	Desisopropyl Atrazine	29	970
Prometryn	0.052	0.80	Cyanazine	0.800	47
Prometon	0.140	2.20	2-Hydroxy Atrazine	0.960	20
Desethyl Atrazine	0.250	4.75			

The following compounds demonstrated no reactivity in the ABRAXIS® Atrazine ELISA Tube Particle at concentrations up to 1000ppb: aldicarb, aldicarb sulfoxide, aldicarb sulfone, alachlor, benomyl, butachlor, butylate, captan, carbaryl, carbendazim,carbofuran, 2,4-D, 1,3-dichloropropene, dinoseb, MCPA, metolachlor, metribuzin, pentachlorophenol, picloram, propachlor, terbufos, thiabendazole, and thiophanate-methyl.

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Atrazine ELISA Tube Particle

Product No. 500001

Intended Use

For the detection and quantitation of atrazine and related triazines 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 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.

Assay Principle

The ABRAXIS® Atrazine ELISA Tube Particle Kit applies the principles of enzyme linked immunosorbent assay (ELISA) to the determination of atrazine and related triazines. 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 triazines. At this point, a competitive reaction occurs between the triazine, which may be in the sample, and the enzyme labeled atrazine for theantibody binding sites on the magnetic particles. The reaction is allowed to continue for fifteen (15) minutes. At theend of the incubation period, a magnetic field is applied to hold in the test tube the para-magnetic particles (with atrazineand labeled atrazine 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 atrazine is detected by adding the "Color Solution", which contains the enzyme substrate (hydrogen peroxide) and the chromogen (3,3',5,5'-tetramethyl- benzidine). The enzyme-labeled atrazine bound to the atrazine antibody catalyzes the conversion of the substrate/ chromogen mixture to a colored product. After an incubationperiod, the reaction is stopped and stabilized by the additionof a diluted acid (Stopping Solution). Since the labeled atrazine (conjugate) was in competition with the unlabeled atrazine (sample) for the antibody sites, the color developedis inversely proportional to the concentration of atrazine in the sample.

Limitations of the ABRAXIS® Atrazine ELISA Tube Particle

The ABRAXIS® Atrazine ELISA Tube Particle will detect atrazine andrelated triazines to different degrees. Refer to specificity table for data on several of the triazines. The ABRAXIS® Atrazine ELISA Tube Particle 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.

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 reagent droplets on the tubes and pipet tips. • Avoid foam formation during vortexing. • The Magnetic Separation System consists of two parts: anupper 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 theincubation. • For separation steps (washing and decanting), the rack and the separator are combined to pull the paramagneticparticles to the sides of the tubes. • To obtain optimum assay precision, it is important to performthe separation steps carefully and consistently. Decant the

Magnetic Separation System (combined rack and separator)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 MagneticSeparation System on an absorbent pad and allow to drain. Lifting the Magnetic Separation System and replacing gentlyonto the pad several times will ensure complete removal of the liquid from the rim of the tube. Do not bang or shake theMagnetic Separation System. • Mix the antibody coupled paramagnetic particles just prior topipetting. • Do not use any reagents beyond their stated shelf life. • Avoid contact of Stopping Solution (diluted sulfuric acid) withskin and mucous membranes. If this reagent comes in contact with skin, wash with water.

Working Instructions

Materials Provided

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

Materials Required (Not Provided)

In addition to the reagents provided, the following items are essential for the performance of the test:
Precision pipets capable of delivering 250 -500 µL 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 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 monochloroaceticacid or other acids, should be neutralized with strong base e.g. 6N NaOH, prior to assay. • If the atrazine concentration of a sample exceeds 5 ppb, thesample is subject to repeat testing using a diluted sample. Aten-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 µLof 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. The antibody coupled paramagnetic particles should be mixed thoroughly before use.

Quality Control

A control solution at approximately 3 ppb of atrazine is provided with the ABRAXIS® Atrazine ELISA Tube Particle 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 andPrecautions before proceeding.

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

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 or 250 uL of the appropriate standard, control,or sample**.
3. **Add 250 uL of Atrazine Enzyme Conjugate** to each tube.
4. Mix the Atrazine Antibody Coupled ParamagneticParticles thoroughly and **add 500 uL** to each tube.
5. **Vortex each tube for 1 to 2 seconds** minimizing foaming.
6. **Incubate for 15 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 System 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 ofColor 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. Useas blank in Step 17.
17. Read results at 450 nm within 15 minutes after addingthe Stopping Solution.

Results

Manual Calculations

- Calculate the mean absorbance value for each of the standards.
- 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.
- Construct a standard curve by plotting the %B/Bo for each standard on vertical logit (Y) axis versus the corresponding atrazine concentration on horizontal logarithmic (X) axis on the graph paper provided.
- %B/Bo for controls and samples will then yield levels in ppb of atrazine by interpolation using the standard curve.

Photometric Analyzer

Some instrument manufacturers make available photometersallowing calibration curves to be automatically calculatedand stored. Refer to instrument operating manual for detailedinstructions. To obtain results 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: 0.10 ppb
Wavelength:	450 nm		#3: 1.00 ppb
Units:	ppb		#4: 5.00 ppb
# Rgt Blk:	0		
Range:	0.05 - 5.00		
Correlation:	0.990		
Rep. %CV:	10%		

Expected Results

In a study with water samples from locations across the U.S.,the ABRAXIS® Atrazine ELISA Tube Particle was shown to correlate well with another commercial Atrazine immunoassay (r = 0.971).
The following results were obtained:

Performance Data

Precision

Control	1	2	3
Replicates	5	5	5
Days	5	5	5
n	25	25	25
Mean (ppb)	1.34	2.65	3.99
% CV (within assay)	6.6	7.0	7.9
% CV (between assay)	2.9	3.0	5.1