Recovery

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

Amount of Fluridone Added (ppb)	Recovery Mean (ppb)	Recovery S.D. (ppb)	Recovery %
1.0	1.09	0.16	109
2.0	2.27	0.31	113
4.0	4.51	0.23	113
8.0	8.68	0.78	109
12.0	12.0	1.12	100
Average			109

Specificity

The cross-reactivity of the ABRAXIS® Fluridone Assay for various 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).

Compound	LDD (ppb)	50% B/Bo (ppb)
Fluridone	0.15	3.0
TSN 125670	0.03	0.56
TSN 92891	21	920
Toluic acid	60	>10,000
Tolualdehyde	110	>10,000
Benzoic acid	1,000	>10,000
Benzaldehyde	8,100	>10,000
Endothal	>10,000	>10,000
2,4-D	>10,000	>10,000
Penoxsulam	>10,000	>10,000

The following compounds demonstrated no reactivity in the ABRAXIS® Fluridone 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, thiophanate-methyl, triclopyr, and trifularin.

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Date this User Guide is effective 18JAN2023
Version : 02

ABRAXIS® Fluridone ELISA Tube Particle

Product No. 500511

Intended Use

For the detection and quantitation of Fluridone 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® Fluridone ELISA Tube Particle Kit applies the principles of enzyme linked immunosorbent assay (ELISA) to the determination of Fluridone. 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 Fluridone. At this point, a competitive reaction occurs between the Fluridone, which may be in the sample, and the enzyme labeled Fluridone 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 Fluridone and labeled Fluridone 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 Fluridone 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 Fluridone bound to the Fluridone 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 Fluridone (conjugate) was in competition with the unlabeled Fluridone (sample) for the antibody sites, the color developed is inversely proportional to the concentration of Fluridone in the sample.

Limitations of the ABRAXIS® Fluridone ELISA Tube Particle

The ABRAXIS® Fluridone ELISA Tube Particle Kit will detect Fluridone and related pyridazinone compounds to different degrees. Refer to specificity table for data on several of the compounds. The ABRAXIS® Fluridone 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.

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 excessive 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 (washing and decanting), 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 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 Magnetic Separation System on an absorbent pad and allow to drain. Lifting the Magnetic Separation System and replacing gently onto the pad several times will ensure complete removal of the liquid from the rim of the tube. Do not bang or shake the Magnetic Separation System.

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.

Working Instructions

Materials Provided

1. Fluridone Antibody Coupled Paramagnetic Particles, 65 mL
2. Fluridone Enzyme Conjugate, 35 mL
3. Fluridone Standards (0.5, 2.0, 7.5, 15.0 ppb), 2.0 mL
4. Control, 2.0 mL
5. Diluent/Zero Standard, 35 mL
6. Color Solution, 60 mL
7. Stopping Solution, 60 mL
8. Washing Solution T, 250 mL X 2
9. Glass Test Tubes, 36 tube boxes 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 150, 250 and 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. Uniprep 0.45 µm, 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 Fluridone concentration of a sample exceeds 15.0 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 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 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.

Quality Control

A control solution at approximately 6.0 ppb of Fluridone is provided with the ABRAXIS® Fluridone 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

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, 0.5 ppb
5,6	Standard 2, 2.0 ppb
7,8	Standard 3, 7.5 ppb
9,10	Standard 4, 15.0 ppb
11,12	Control
13, 14	Sample 1
15,16	Sample 2
17, 18	Sample 3

2. **Add 150 µL of the appropriate standard, control, or sample**.
3. **Add 250 µL of Fluridone Enzyme Conjugate** to each tube.
4. **Mix the Fluridone Antibody Coupled Paramagnetic Particles thoroughly and add 500 µL** 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. A**dd 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 two (2) additional times**, for a total of 3 washes.
12. Remove the rack from the separator and **add 500 µL 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 µL 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 logit (Y) axis versus the corresponding Fluridone 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 Fluridone by interpolation using the standard curve.

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® Fluridone Assay on instruments allowing data transformation the following parameter settings are recommended:

		Calibrators:	Concentrations:
Data Reduct:	Lin. Regression		
Xformation:	Ln/LgtB	# of Cals: 5	#1: 0.00 PPB
Read Mode:	Absorbance	# of Reps: 2	#2: 0.5 PPB
Wavelength:	450 nm		#3: 2.0 PPB
Units:	PPB		#4: 7.5 PPB
# Rgt Blk:	0		#5: 15.0 PPB
Range:	0.50 – 15.0		
Correlation:	0.990		
Rep. %CV:	10%		

Expected Results

In a study with water samples from locations across the U.S., the ABRAXIS® Fluridone Assay was shown to correlate well with HPLC ($r^2 = 0.985$)

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)	2.54	6.18	10.85
% CV (within assay)	8.8	8.4	9.6
% CV (between assay)	5.9	6.0	7.2

Sensitivity

The ABRAXIS® Fluridone Assay has an estimated minimum detectable concentration, based on 4 SD from zero = 0.24 ppb.