Expected Results

In a study with water samples from various locations, the ABRAXIS® Glyphosate HS Assay was shown to correlate well with another analytical technique

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)	0.98	2.82	5.80
% CV (within assay)	6.0	3.5	6.9
% CV (between assay)	15.5	11.6	9.5

Sensitivity

The Eurofins Abraxis Glyphosate HS Assay has an estimated minimum detectable concentration based on a 90% B/Bo of 50 parts per trillion (ppt).

Recovery

Five (5) groundwater samples were spiked with various levels of glyphosate and then assayed using the Eurofins Abraxis Glyphosate HS Assay. The following results were obtained:

Amount of Glyphosate Added (ppb)	Recovery Mean (ppb)	Recovery S.D. (ppb)	Recovery %
0.50	0.47	0.09	95
1.0	1.04	0.13	104
2.5	2.70	0.41	108
Average			102

Specificity

The cross-reactivity of the Eurofins Abraxis Glyphosate HS Assay for various related 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)
Glyphosate	0.05	2.40
Glyphosine	50	3,000
Glufosinate	2000	70,000
AMPA	35,000	>1,000,000
Glycine	>10,000	>1,000,000

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

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ABRAXIS® HS Glyphosate ELISA Tube Particle

Product No. 500081 / 500084

Intended Use

For the detection and quantitation of glyphosate 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, except for derivatization reagent (use the same day as diluted). The test tubes and Washing Solution require no special storage condition and may be stored separately from the reagents to conserve refrigerator space. The Derivatization Reagent Diluent may freeze if stored cool, thaw reagent by placing on a 37 C incubator. Consult state, local and federal regulations for proper disposal of all reagents.

Assay Principle

The Gold Standard Diagnostics Glyphosate Kit applies the principles of enzyme linked immunosorbent assay (ELISA) to the determination of glyphosate. The sample to be tested is derivatized and then added, along with paramagnetic particles attached with antibodies specific to glyphosate and incubated for 30 minutes. The glyphosate enzyme conjugate is then added; at this point, a competitive reaction occurs between the glyphosate, which may be in the sample, and the enzyme labeled glyphosate analog for the antibody binding sites on the magnetic particles. The reaction is allowed to continue for thirty (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 glyphosate and labeled glyphosate 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 glyphosate 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 glyphosate bound to the glyphosate 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 glyphosate (conjugate) was in competition with the unlabeled glyphosate (sample) for the antibody sites, the color developed is inversely proportional to the concentration of glyphosate in the sample.

Limitations of the ABRAXIS® Glyphosate ELISA Tube Particle

The Gold Standard Diagnostics Glyphosate Assay will detect glyphosate. Refer to specificity table for data on several of related compounds. The Gold Standard Diagnostics Glyphosate 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 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. • Mix the antibody coupled paramagnetic particles just prior to pipetting. • Do not use any reagents beyond their stated shelf life. • Do not use the diluted Derivatization Reagent after 8 hours from dilution. • 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. Glyphosate Antibody Coupled Paramagnetic Particles, 65 mL
2. Glyphosate Enzyme Conjugate, 35 mL
3. (4) Glyphosate Standards (75, 200, 750, and 4000 (ppt), 2.0 mL
9. Glass Test Tubes, 4 X 36 tube boxes
4. Control (500 ppt), 2.0 mL
10. Assay Buffer, 125 mL
5. Diluent/Zero Standard, 65 mL
11. Derivatization Reagent, 100 µL X3
6. Color Solution, 65 mL
12. Derivatization Reagent Diluent, 4 mL X3
7. Stopping Solution, 65 mL
8. Washing Solution, 250 mL (1 in each kit) X2

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 100, 250, 500, 750 uL
- Disposable 5 mL serological pipette
- 1.0 mL repeating pipet
- Vortex Mixer
- Disposable glass test tubes
- 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 monochloroacetic acid or other acids, should be neutralized with strong base e.g. 6N NaOH, prior to assay.

If the glyphosate concentration of a sample exceeds 4 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 obtain by the dilution factor (e.g. 10).

The presence of the following substances up to 20,000 ppm were found to have no significant effect on the Glyphosate Assay results: calcium, magnesium, nitrate, sodium fluoride, copper, carbonate. Sulfate and potassium up to 2,000 ppm. Phosphate up to 100 ppm. Humic acid up to 20 ppm. Sodium chloride up to 1.0 M; and HCl up to 0.25 N.

Solvents usually used to extract pesticides from soil or plant matrices such as methanol and acetone were found to be acceptable for use in the Glyphosate immunoassay up to 50%.

Reagent Preparation

All reagents must be allowed to come to room temperature. The antibody coupled paramagnetic particles should be mixed thoroughly before use. Diluted Derivatization Reagent must be used within 8 hours of preparation. If additional samples are to be analyzed more than 8 hours after dilution, discard the vial and dilute a new vial of Derivatization Reagent for use.

Derivatization of Standards, Control, and Samples (must be performed prior to each analysis):

1. Dilute Derivatization Reagent with 3.5 mL of Derivatization Reagent Diluent (Diluted Reagent needs to be used within 8 hours of preparation). Vortex to mix thoroughly.
2. Label single glass test tubes for standards, control, and samples.
3. Pipette 250 µL of standard, control, or sample into separate disposable tubes.
4. Add 1.0 mL of Assay buffer, vortex to mix.
5. Add 100 uL of the diluted Derivatization Reagent, vortex each tube immediately after addition of reagent. We recommend vortexing until no swirl lines are seen in the tube.
6. Incubate at room temperature for 10 minutes.
7. Proceed to Assay Procedure, Step 1. Note: Discard derivative standards, control, and samples after use. Do not use for re-analysis.

Alternative Derivatization Procedure:

Note: Performing the alternative derivatization procedure allows the user to use the same derivatization tubes in the performance of the assay, therefore eliminating the use of additional assay tubes.

1. Dilute Derivatization Reagent with 3.5 mL of Derivatization Reagent Diluent (Diluted Reagent needs to be used within 8 hours of preparation). Vortex to mix thoroughly.
2. Label test tubes in duplicate for standards, control, and samples.
3. Pipette 50 µL of standard, control, or sample into duplicate disposable glass assay tubes.
4. Add 200 µL of Assay buffer, vortex to mix.
5. Add 20 µL of the diluted Derivatization Reagent, vortex each tube immediately after addition of reagent. We recommend vortexing until no swirl lines are seen in the tube.
6. Incubate at room temperature for 10 minutes.
7. Proceed to Assay Procedure, Step 3.

Quality Control

A control solution at approximately 500 ppt of Glyphosate is provided with the ABRAXIS® Glyphosate HS 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	Tube Number	Contents of Tube
1,2	Diluent/Zero Standard, 0 ppt	11,12	Control
3,4	Standard 1, 75 ppt	13,14	Sample 1
5,6	Standard 2, 200 ppt	15,16	Sample 2
7,8	Standard 3, 750 ppt	17,18	Sample 3
9,10	Standard 4, 4000 ppt		

2. **Add 300 µL of the appropriate derivatized standard, control, or sample**.
3. **Mix the Glyphosate Antibody Coupled Paramagnetic Particles thoroughly and add 500 µL** to each tube.
4. **Vortex for 1 to 2 seconds** minimizing foaming.
5. **Incubate for 30 minutes at room temperature**.
6. **Add 250 µL of Glyphosate Enzyme Conjugate** to each tube.
7. **Vortex for 1 to 2 seconds** minimizing foaming.
8. **Incubate for 30 minutes at room temperature**.
9. Separate in the Magnetic Separation System for two (2) minutes.
10. Decant and gently blot all tubes briefly in a consistent manner.
11. **Add 1 mL of Washing Solution** to each tube and allow them to remain in the magnetic separation unit for two (2) minutes.
12. Decant and gently blot all tubes briefly in a consistent manner.
13. **Repeat Steps 11 and 12 two (2) additional times**.
14. **Remove the rack from the separator and add 500 µL of Color Solution** to each tube.
15. **Vortex for 1 to 2 seconds** minimizing foaming.
16. **Incubate for 20 minutes at room temperature**.
17. **Add 500 µL of Stopping Solution** to each tube.
18. **Add 1 mL Washing Solution** to a clean test tube. Use as blank in Step 19.
19. 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 glyphosate concentration on horizontal log (X) axis on the graph paper provided.
4. %B/Bo for controls and samples will then yield levels in ppb of glyphosate 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® Glyphosate HS Assay on instruments allowing data transformation the following parameter settings are recommended:

Data Reduct:	Lin. Regression	Calibrators:	Concentrations:		
Xformation :	Ln/Ln	# of Cals :5	#1:	0.00	PPT
Read Mode :	Absorbance	# of Reps :2	#2:	75	PPT
Wavelength :	450 nm		#3:	200	PPT
Units :	PPT		#4	750	PPT
# Rgt Blk :	0		#5:	4000	PPT
Range :	75 – 4000				
Correlation :	0.990				
Rep. %CV :	15%				

NOTE: Any results obtained with a calculated glyphosate concentration of less than 50 ppt on the print out should be assumed to be below the detection limit of the assay.

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