Glyphosate

• 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.

• Principle

The Abraxis 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 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**.

• Reagents

The Abraxis Glyphosate Kit contains the following items:

1. Glyphosate Antibody Coupled Paramagnetic Particles
Glyphosate antibody (rabbit anti-glyphosate) covalently bound to paramagnetic particles suspended in a buffered solution with preservative and stabilizers.
120 test kit: one 65 mL vial
2. Glyphosate Enzyme Conjugate
Horseradish peroxidase (HRP) labeled glyphosate analog diluted in a buffered solution with preservative and stabilizers.
120 test kit: one 35 mL vial
3. Glyphosate Standards
Four concentrations (75, 200, 750, and 4000 parts per trillion) of glyphosate standards in distilled water with preservative and stabilizers. Each vial contains 2.0 mL.
4. Control
A concentration (approximately 500 ppt of glyphosate 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 glyphosate.
120 test kit: one 65 mL vials
6. Color Solution
A solution of hydrogen peroxide and 3,3',5,5'-tetramethylbenzidine in an organic base.
120 test kit: one 65 mL vial
7. Stopping Solution
A solution of diluted acid.
120 test kit: one 65 mL vial
8. Washing Solution
Preserved deionized water.
120 test kit: one 250 mL vial
9. Assay Buffer
Dissolved buffer salts.
120 test kit: one 125 mL vial
10. Derivatization Reagent
120 test kit: three 80 uL vials
11. Derivatization Reagent Diluent
Dimethyl Sulfoxide (DMSO): three 4 mL vials
12. Test Tubes
Glass (36) are packaged in boxes.
120 test kit: 4 X 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, 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.

• 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 100, 250, 500, 750 uL and a 1.0 mL repeating pipet. Disposable 5 mL pippete.
Vortex Mixer*	Thermolyne 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 fo use with water samples. Other samples may require modifications to the procedure and should be

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.

Derivatization of Standards, Control, and Samples

1. Dilute Derivatization Reagent with 3.5 mL of Derivatization Reagent Diluent (Diluted Reagent needs to be used within the same day). Mixed thoroughly.

2. Label single test tubes for standards, control, and samples.

3. Pipette 250 uL of standard, control, sample(s) 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. Perform the ELISA as in Assay Procedure, start with step 1 of Assay procedure.

Alternative Derivatization Procedure

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 the same day). Mixed thoroughly.

2. Label test tubes in duplicate for standards, control, and samples.

3. Pipette in duplicate, 50 uL of standard, control, sample(s) into disposable assay tubes.

4. Add 200 uL of Assay buffer, vortex to mix.

5. Add 20 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. Perform the ELISA as in Assay Procedure, starting with step 3 of Assay Procedure.

• 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, 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.

Do not use the diluted derivatization reagent after 24 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.

• Limitations

The Abraxis Glyphosate Assay will detect glyphosate. Refer to specificity table for data on several of related compounds. The Abraxis 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.

• Quality Control

A control solution at approximately 500 ppt of Glyphosate is provided with the Abraxis Glyphosate 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 ppt
3,4	Standard 1, 75 ppt
5,6	Standard 2, 200 ppt
7,8	Standard 3, 750 ppt
9,10	Standard 4, 4000 ppt
11,12	Control
13,14	Sample 1
15,16	Sample 2
17,18	Sample 3

2. Add 300 uL of the appropriate derivatized standard, control, or sample.
3. Mix the Glyphosate Antibody Coupled Paramagnetic Particles thoroughly and add 500 uL to each tube.
4. Vortex for 1 to 2 seconds minimizing foaming.
5. Incubate for 30 minutes at room temperature.
6. Add 250 uL 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 uL 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 uL 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 ppbof glyphosate 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 Glyphosate HS Assay on instruments allowing data tranformation the following parameter settings are recommended:

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

Calibrators:

# of Cals	:	5
# of Reps	:	2

Concentrations:

#1:	0.00	PPT
#2:	75	PPT
#3:	200	PPT
#4	750	PPT
#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 .

• 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 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 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 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).

B/Bo Compound	LDD (ppb)	50% (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 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.

• Ordering informattion

Abraxis Glyphosate Assay Kit,120T	PN 500081
Sample Diluent	PN 500082
Standard Set	PN 500083
High Sensitivity Reagent Set	PN 500084
High Sensitivity Standard Set	PN 500085

• Assistance

For ordering or technical assistance contact:

Abraxis LLC
Sales Department
Northhampton Center
54 Steamwhistle Drive
Warminster, Pennsylvania, 18974

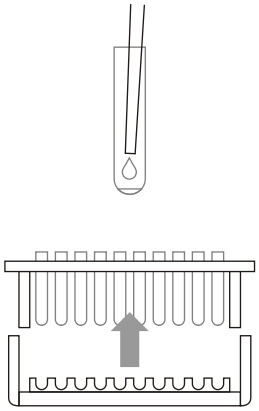
Phone: (215) 357-3911
Fax: (215) 357-5232
Email: info@abraxiskits.com
WEB: www.abraxiskits.com

• General Limited Warranty

Abraxis LLC 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. **Abraxis makes no other warranty, expressed or implied. There is no warranty of merchantability or fitness for a particular purpose.**

GLYPHOSATE DETAILED FLOWCHART

1.



Remove upper rack from magnetic base. Label test tubes for Standards, Control, and Samples.

Tube #	Content
1, 2	Diluent/Zero Standard 0 ppb
3, 4	Standard 1, 0.15 ppb
5, 6	Standard 2, 1.0 ppb
7, 8	Standard 3, 5.0 ppb
9,10	Control
11,12	Sample 1
13,14	Sample 2
15,16	Sample 3

Add 300 µL of either **Derivatized** Standards, Control or Samples to the bottom of each test tube by inserting the pipette tip all the way into the bottom of the tube without touching the sides of the tube.

2.

Add 500 µL of thoroughly mixed Glyphosate Antibody Coupled Magnetic Particles down the inside wall of each tube by using the technique described in Box 2. *Vortex* for 1 to 2 seconds (at low speed to minimize foaming).

3.

React 30 minutes at room temperature (15 °- 30°C).

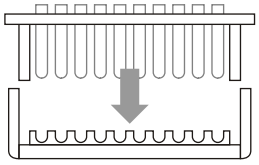
4.

Add 250 µL of Glyphosate Enzyme Conjugate down the inside wall of each tube by aiming the pipet tip 1/4" to ½" below the tube rim without touching the rim or tube wall with the pipet tip; deliver liquid gently.

5.

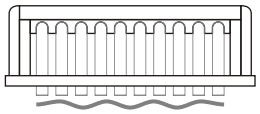
React 30 minutes at room temperature (15 °- 30°C).

6.



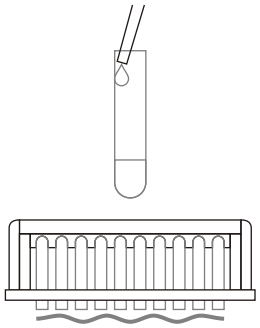
Combine the upper rack with the magnetic base; press all tubes into base; allow 2 minutes for the particles to separate.

7.



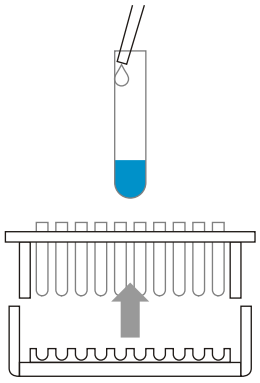
Do not separate upper rack from lower base. Using a smooth motion, *invert* the combined rack assembly over a sink and pour out the tube contents; keep inverted and **gently blot** the test tube rims on several layers of paper toweling.

8.



Add 1 mL of Washing Solution down the inside wall of each tube by using the technique described in Box 2. *Wait 2 minutes.* Using a smooth motion, invert the combined rack assembly over a sink and pour out the tube contents: keep inverted and **gently blot** the test tube rims on several layers of paper toweling. Repeat this step **two** times.

9.

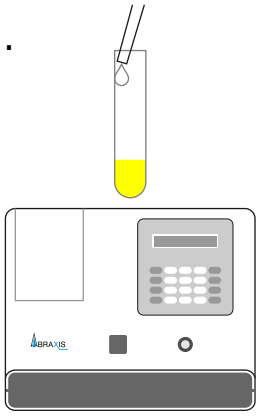


Lift the upper rack (with its tubes) off the magnetic base; *add* 500 µL of Color Reagent down the inside wall of each tube by using the technique described in Box 2. *Vortex* for 1 to 2 seconds (at low speed to minimize foaming).

10.

React for 20 minutes at room temperature (15°- 30° C). During this period, add 1 mL of Washing Solution into a clean tube for use as an instrument blank in Step 10.

11.



Add 500 µL of Stopping Solution down the inside wall of each tube by using the technique previously described. *Read* results at 450 nm within 15 minutes after adding the Stopping Solution. *Multiply* results of samples by the appropriate dilution factor (if any).

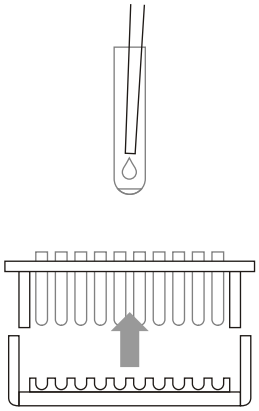
[**Safety Caution:** Stopping Solution contains diluted sulfuric acid.]

For Ordering or Technical Assistance Contact:
ABRAXIS, LLC 54 Steamwhistle Drive, Warminster, PA 18974
Phone: 215-357-3911 Fax: 215-357-5232
Web: www.abraxiskits.com

Glyphosate Magnetic Particle Kit Part # 500080, 120 Test

GLYPHOSATE CONCISE FLOWCHART

1.



Separate the rack.

Add 300 µL of either **Derivatized** Standards, Control or Samples to the bottom of each test tube.

2.

Add 500 µL of mixed Magnetic Particles to each test tube.

Vortex.

3.

Incubate for 30 minutes.

4.

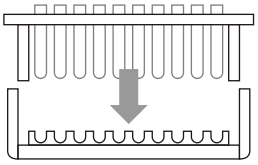
Add 250 µL of Enzyme Conjugate to each test tube.

Vortex.

5.

Incubate for 30 minutes.

6.

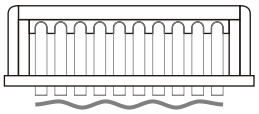


Combine the rack and magnetic base.

Seat all tubes.

Wait 2 minutes.

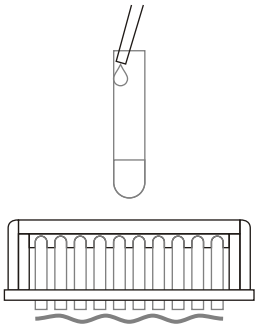
7.



Invert the combined rack.

Blot **gently**.

8.



Add 1 mL of Washing Solution.

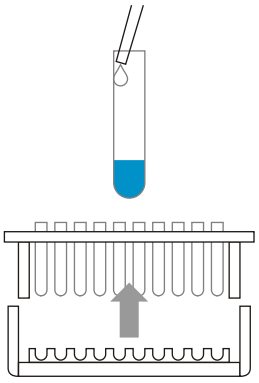
Wait 2 minutes.

Invert the combined rack.

Blot **gently**.

Repeat this step **two** times

9.



Separate the rack.

Add 500 µL of Color Reagent to each test tube.

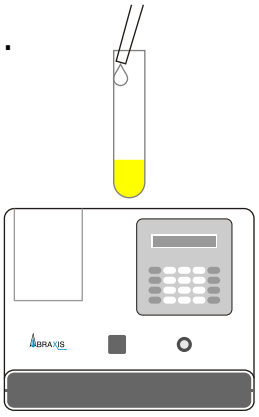
Vortex.

10.

Incubate for 20 minutes.

Prepare blank.

11.



Add 500 µL of Stopping Solution to each test tube.

Read OD 450

For Ordering or Technical Assistance Contact:
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Web: www.abraxiskits.com

Glyphosate Magnetic Particle Kit Part # 500080, 120 Test

ABRAXIS