Glyphosate Plate

• 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 Plate Kit applies the principles of enzyme linked immunosorbent assay (ELISA) to the determination of glyphosate. The sample (please refer to reagent preparation section) to be tested is derivatized and then added, along with an antibody specific for Glyphosate to microtiter wells coated with Goat Anti-Rabbit Antibody 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 microtiter well. The reaction is allowed to continue for sixty (60) minutes. After a washing step and addition of a substrate (color solution), a color signal (blue color) is generated.

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 Plate Kit contains the following items:

1. *Microtiter Plate coated with Goat-Anti Rabbit Antibody*
   96 test kit: 8 X 12 strips
2. *Glyphosate Antibody Solution*
   Glyphosate antibody (rabbit anti-glyphosate) solution in a buffered saline solution with a non-mercury preservative and stabilizers.
   96 test kit: one 6 mL vial
3. *Glyphosate Enzyme Conjugate*
   Horseradish peroxidase (HRP) labeled glyphosate analog diluted in a buffered solution with a non-mercury preservative and stabilizers.
   96 test kit: one 6 mL vial
4. *Glyphosate Standards*
   Six concentrations (0, 0.075, 0.2, 0.5, 1.0, 4.0 ppb) of glyphosate standards in distilled water with a non-mercury preservative and stabilizers.
   96 test kit: one 2 mL vial each
5. *Control*
   A concentration (approximately 0.75 ppb) of glyphosate in distilled water with a non-mercury preservative and stabilizers.
   96 test kit: one 2 mL vial
6. *Diluent/Zero Standard (Sample Diluent)*
   Distilled water with a non-mercury preservative and stabilizers without any detectable glyphosate.
   96 test kit: one 30 mL vial
7. *Color Solution*
   A solution of hydrogen peroxide and 3,3',5,5'-tetramethylbenzidine in an organic base.
   96 test kit: one 16 mL vial
8. *Stopping Solution*
   A solution of diluted acid.
   96 test kit: one 12 mL vial
9. *Washing Buffer 5X Concentrate*
   Buffer salts with detergent and a non-mercury preservative.
   96 test kit: one 100 mL vial
10. *Assay Buffer*
    Dissolved buffer salts.
    96 test kit: one 125 mL vial
11. *Derivatization Reagent*
    96 test kit: three 100 µL vials
12. *Derivatization Reagent Diluent*
    Dimethyl Sulfoxide (DMSO):
    96 test kit: three 4 mL vials

• 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 Washing Solution requires 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.

• 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 50, 100, 150, 250 µL
A 1.0 mL repeating pipet
Disposable 5 mL pipette

Parafilm

Disposable Test Tubes

Distilled or deionized Water

Vortex Mixer* Thermolyne Maxi Mix, Scientific Industries Vortex Genie, or equivalent

Plate Reader* capable of readings at 450 nm

* Please contact Abraxis for supplier information.

• 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 µL of the sample to 900 µL 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 10,000 ppm were found to have no significant effect on the Glyphosate Assay results: nitrate, phosphate, sulfate, sodium fluoride, calcium, magnesium, copper, zinc, iron and sodium thiosulfate. Manganese up to 100 ppm. Humic acid up to 10 ppm. Sodium chloride up to 1.0 M.

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 Plate immunoassay up to 100%.

• Reagent Preparation

All reagents must be allowed to come to room temperature.

Wash Buffer

In a 1000 mL container, dilute the wash buffer concentrate 1:5 by the addition of deionized or distilled water (i.e. 100 mL of wash buffer 5X concentrate plus 400 mL of water.

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 µL of standard, control, sample(s) into separate disposable tubes.
4. Add 1.0 mL of Assay buffer, vortex to mix.
5. Add 100 µL of the diluted derivatization reagent, vortex each tube immediately after addition of reagent until no swirling lines are present.
6. Incubate at room temperature for 10 minutes.
7. Perform the ELISA as in Assay Procedure, start with step 1 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 well in an identical manner.

Add reagents directly to the bottom of the well 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 wells and pipet tips.

Avoid foam formation during vortexing.

The microtiter plate consists of 12 strips of 8 wells, when you use fewer than 8 strips, remove the unneeded strips and store them refrigerated in the re-sealable bag (with desiccant) provided.

If more than three strips are being used per run, it is recommended that a multi-channel pipette be used for the addition of antibody, conjugate, color, and stopping solution.

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.

• Limitations

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

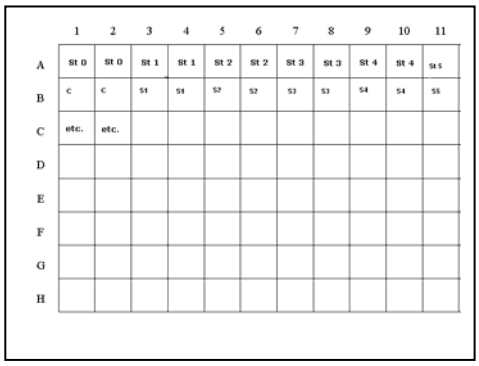
• Quality Control

A control solution at approximately 0.75 ppb of Glyphosate is provided with the Abraxis Glyphosate Plate 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.

St 0-St 5: Standards
C: Control sample
S1-Sx: Samples



1. Add 50 µL of the appropriate **derivatized** standard, control, or sample (see Reagent Preparation). We recommend using duplicates or triplicates (See example above.)
2. Add 50 µL of the anti-Glyphosate antibody solution successively to each well. .) Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop. Be careful not to spill the contents. Incubate at room temperature for 30 minutes.
3. After the incubation, remove the covering and add 50 µl of enzyme conjugate solution to the individual wells successively. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop. Be careful not to spill the contents. Incubate at room temperature for 60 minutes.
4. After the incubation, remove the covering and vigorously shake the contents of the wells into a waste container. Wash the strips 3 times using the 1X wash solution (see Reagent Preparation) with a volume of at least 250 µL per each wash step. Any remaining buffer in the wells should be removed by patting the plate on a dry stack of paper towels.
5. Add 150 µL of color solution successively to each well. Incubate for 20-30 minutes.
6. Add 100 µL of Stopping Solution to each well in the same sequence as for the other reagents.
7. Read absorbance using a microplate reader 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 $\%B/B_o$ 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 $\%B/B_o$ for each standard on vertical linear (Y) axis versus the corresponding glyphosate concentration on horizontal log (X) axis on the graph paper provided. The analysis can also be performed using a a Log/Logit or 4-Parameter data calculation programs.
4. $\%B/B_o$ for controls and samples will then yield levels in ppb of glyphosate by interpolation using the standard curve.

(Contact Abraxis for detailed application information on specific photometers.)

NOTE: Any results obtained with a calculated glyphosate concentration of less than 0.05 ppb 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 Plate Assay was shown to correlate well with another analytical technique.

• Performance Data

Precision

The following results were obtained:

Control	1	2	3	4
Replicates	5	5	5	5
Days	3	3	3	3
n	15	15	15	15
Mean (ppb)	0.41	0.77	1.54	2.81
% CV (within assay)	12.2	8.2	4.1	5.5
% CV (between assay)	11.7	16.9	11.8	8.0

Sensitivity

The Abraxis Glyphosate Plate Assay has an estimated minimum detectable concentration based on a 90% B/B_o of 0.05 parts per billion (ppb).

Recovery

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

Amount of Glyphosate Added (ppb)	Recovery Mean (ppb)	Recovery S.D. (ppb)	Recovery %
0.25	0.25	0.05	102
0.5	0.53	0.05	105
1.0	1.03	0.14	103
2.0	2.12	0.16	106
Average			104

Specificity

The cross-reactivity of the Abraxis Glyphosate Plate Assay for various related analogues can be expressed as the least detectable dose (LDD) which is estimated at 90% B/B_o, or as the dose required for 50% absorbance inhibition (50% B/B_o).

B/B_o Compound	LDD (ppb)	50% (ppb)
Glyphosate	0.05	0.5
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 information

Microtiter Plate Kit

Abraxis Glyphosate Plate Assay Kit, 96T	PN 500086
Sample Diluent	PN 500082
Derivatization Reagent Set	PN 500087
Plate Standard Set	PN 500088

Magnetic Particle Tube Kit

Abraxis Glyphosate HS Assay Kit, 120T	PN 500081
Sample Diluent	PN 500082
HS Derivatization Reagent Set	PN 500084
HS 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.**