Importance of Glyphosate Determination

Glyphosate, a broad-spectrum systemic herbicide, was introduced in 1974 by Monsanto under the trade name Roundup®. Glyphosate (N-(phosphonomethyl)glycine or 2-[(hydroxy-oxidophosphoryl)methylamino]acetic acid) is the largest selling agrochemical in the world and is marketed under dozens of trade names by many different manufacturers. Glyphosate is used for vegetation control of perennial and annual plants, broad-leaf weeds, grasses, woody plants, and aquatic weeds, as well as grain desiccation to increase harvest yield. The introduction of genetically modified crops resistant to Glyphosate (i.e. Roundup Ready®) has caused an increased use of Glyphosate, allowing farmers to control weeds without harming their crops. The emergence of Glyphosate-resistant weeds has also caused increases in frequency and quantity of applications of Glyphosate in combination with other herbicides. Due to its widespread use, Glyphosate has become ubiquitous in the environment and food supply.

Glyphosate can adsorb to soil and is highly water soluble, which can cause surface and ground water contamination from run-off, soil erosion, and leaching especially after heavy rainfall. The long-term impact on the environment and human health are growing concerns worldwide. In March 2015, the World Heath Organization's International Agency for Research on Cancer classified Glyphosate as "probably carcinogenic in humans" (category 2A). Some studies show a correlation between exposure to Glyphosate-based herbicides and non-Hodgkin's Lymphoma in humans while others show evidence of Glyphosate causing cancers in laboratory animals.

Performance Data

Test sensitivity: The Abraxis Glyphosate Strip Test will detect Glyphosate in water samples in the range of 2.5 ppb to 100 ppb. At this level, the test line exhibits moderate intensity. At levels greater than 100 ppb, the test line is very faint or not visible.

Samples: A sample correlation between the Abraxis Strip Test and ELISA methods showed a good correlation.

References

1) US patent 3799758, Franz JE, N-phosphonomethyl-glycine phytotoxicant compositions, issued 1974-03-26, assigned to Monsanto Company.
2) Steinrucken HC, Amrhein N(Jun 1980). The herbicide glyphosate is a potent inhibitor of 5-enolpyruvyl-shikimic acid-3-phosphate synthase. Biochemical and Biophysical Research Communications. **94** (4):1207-12.
3) Press release: IARC Monographs Volume 112: Evaluation of five organophosphate insecticides and herbicides. International Agency for Research on Cancer, World Health Organization. March 20, 2015.
4) Glyphosate: EPSA updates toxicological profile, European Food Safety Authority. www.efsa.europa.eu. Retrieved 2016-05-23.

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R071018

Glyphosate Strip Test

Immunochromatographic Strip Test for the Detection of Glyphosate in Water and Food Samples

Product No. 500098 (5 Test), 500095 (20 Test)

1. General Description

The Abraxis Glyphosate Strip Test is a rapid immunochromatographic test designed solely for use in the qualitative screening of Glyphosate in water and food samples. For food samples such as honey, lentils, baby food, wheat/oat cereal, a sample extraction is necessary. For these and other matrices of interest, please contact Abraxis for the appropriate technical bulletin and/or matrix validation guidelines. The Abraxis Glyphosate Strip Test provides only preliminary qualitative test results. If necessary, positive samples can be confirmed by ELISA, HPLC, or other conventional methods.

2. Safety Instructions

Consult state, local, and federal regulations for the proper disposal of all reagents. All samples and reagents used in this test are not for consumption. Please do not eat or drink samples during preparation, testing, or after contact with any reagents.

3. Storage and Stability

The Glyphosate Strip Kit should be stored between 5-30°C. The test strips, vials, assay buffer, and samples to be analyzed should be at room temperature before use.

4. Test Principle

The test is based on the recognition of Glyphosate by specific antibodies. The sample to be tested is derivatized and then added to the conical test vial containing specific antibodies for Glyphosate labeled with a gold colloid. The Glyphosate conjugate on the membrane strip competes for antibody binding sites with the Glyphosate that may be present in the sample. A control line, produced by a different antibody/antigen reaction, is also present on the membrane strip. The control line is not influenced by the presence or absence of Glyphosate in the sample and, therefore, should be present in all reactions.

In the absence of Glyphosate in the sample, the colloidal gold labeled antibody complex moves with the sample by capillary action to react with the immobilized Glyphosate conjugate. An antibody-antigen reaction occurs forming a visible line in the 'test' area. The formation of two visible lines of similar intensity indicates a negative test result, meaning the test did not detect Glyphosate at or below the established cut-off point for the test. If the Glyphosate is present in the sample, it competes with the immobilized Glyphosate conjugate in the test area for the antibody binding sites on the colloidal gold labeled complex. If a sufficient amount of Glyphosate is present, it will fill all of the available binding sites, thus preventing attachment of the labeled antibody to the Glyphosate conjugate, therefore preventing the development of a colored line. If a colored line is not visible in the test line region, or if the test line is lighter than the control line, Glyphosate is present at a detectable level (>2.5 ppb). Semi-quantitative results can be obtained by comparing the sample test strip appearance to the appearance of test strips from solutions of known Glyphosate concentrations (control solutions). Glyphosate controls are available through Abraxis.

5. Limitations of the Glyphosate Strip Test, Possible Test Interference

Numerous organic and inorganic compounds commonly found in samples have been tested and found not to interfere with this test. However, due to the high variability of compounds that might be found in samples, test interferences caused by matrix effects can't be completely excluded.

Mistakes in handling the test can also cause errors. Possible sources for such errors include: Inadequate storage conditions of the test strip, too long or too short incubation times, and extreme temperatures during the test performance (lower than 5°C or higher than 30°C).

This test is designed for use with water and food samples. The Glyphosate Strip Test provides only a preliminary qualitative test result. Use another more quantitative analytical method such as ELISA or instrumental analysis to obtain a confirmed quantitative analytical result. Apply good judgement to any test result, particularly when preliminary positive results are observed.

6. Warnings and Precautions

-The Glyphosate Strip Test is for the screening of water and food samples (see Section C, Sample Collection and Handling).

-The test strips, vials, and samples should be allowed to reach room temperature before testing.

-Prior to use, ensure that the product has not expired by verifying that the date of use is prior to the expiration date on the label.

-For test strips packaged in a desiccated vial, the vial should be kept completely closed except for opening to remove tests strips. When re-closing, snap lip firmly.

-Avoid cross-contamination of samples by using a new sample vial and disposable pipette for each sample.

-Use only the test strips, mixing vials, derivatization vials, and conical vials from one kit lot (do not mix with other lots), as they have been adjusted in combination.

-Use reasonable judgement when interpreting the test results.

-Results should be interpreted within 5-10 minutes after completion of the test.

A. Reagents and Materials Provided

1. Glyphosate test strips in a desiccated container
2. Derivatization vials - contain a small amount of liquid reagent
3. Conical test vials
4. Disposable transfer pipettes
5. Disposable graduated pipettes
6. Self-standing, 2.0 mL mixing vials - contain 1.0 mL Glyphosate Assay Buffer
7. User's guide and flow chart

B. Additional Materials (not provided with the test kit)

1. Timer
2. Marking pen
3. Container/storage vials or bottles for sample collection/preparation

C. Sample Collection and Handling

Water Samples

Water samples should be collected in clean glass or plastic sample containers. Chlorinated drinking water samples should be tested immediately upon collection, as contact with chlorine will degrade Glyphosate, producing biased low results.

Food Samples

Food samples must undergo appropriate sample preparation procedures prior to analysis to obtain accurate results. Please contact Abraxis for additional information regarding sample preparation (extraction) for various food matrices.

Store samples refrigerated for up to 1 week. For storage periods greater than 1 week, samples should be stored frozen.

D. Controls

It is a good laboratory practice to use positive and negative controls to ensure proper test performance. Samples containing known quantities of Glyphosate (positive controls) and samples known to be free of Glyphosate (negative controls) should be analyzed with each lot of test strips to provide a reference for line intensities to be expected. Controls can be purchased from Abraxis, Inc.

E. Test Preparation

1. Allow test strips, vials, and samples to reach room temperature before use.
2. Remove the number of test strips required from the package. The remaining strips are stored in the tightly closed desiccated container.
3. Samples **must** be derivatized prior to each analysis (refer to Section F, Testing of Samples). Failure to derivatize samples will cause inaccurate results.

F. Testing of Samples

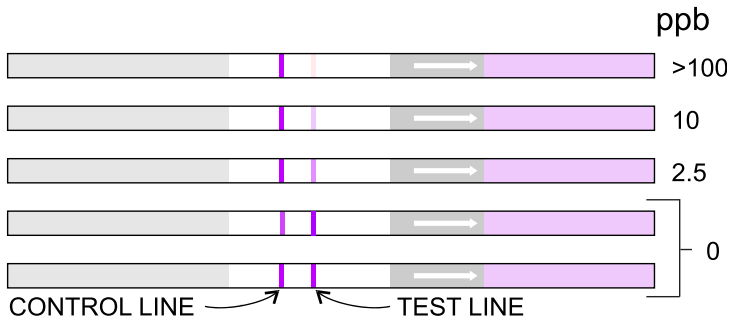
1. Label Mixing Vials, Derivatization Vials, Conical Test Vials, and disposable graduated pipettes (to be used for steps 2 and 4) provided in the kit for each sample to be tested.
2. Using the appropriate **disposable graduated pipette** for each sample, draw up the sample to the 1.0 mL mark of the pipette and dispense the entire 1.0 mL in the appropriately labeled 2.0 mL mixing vial, containing 1.0 mL Glyphosate Assay Buffer.
3. Cap the vial and mix well by shaking for 30 seconds.
4. Using the **same graduated pipette** previously used for the sample, remove the entire contents (2.0 mL) of sample mixture and transfer to the appropriately labeled derivatization vial. Mix by shaking for 30 seconds. Incubate for 10 minutes at room temperature to complete the sample derivatization.
5. Using a **new disposable transfer pipette** for each sample, transfer 6 drops (approximately 0.2 mL) of the derivatized sample to the appropriately labeled conical test vial.
6. Close the conical test vial and shake for 30 seconds. Examine the vial to ***ensure all dried reagents are completely dissolved*** (dried reagents will dissolve, turning the sample purple).
7. Incubate the conical test vial for 10 minutes at room temperature.
8. Insert test strip (arrows down) into the conical vial.
9. Allow the test to develop for 10 minutes at room temperature.
10. At the 10 minute mark, remove the test strip. Lay the strip flat and allow to continue developing for 5-10 minutes at room temperature.
11. **Immediately** read the results visually, as explained below in Section G, Interpretation of Results.

G. Interpretation of Results

Sample concentrations are determined by comparison of the intensity of the test line to the intensity of the control line on the same test strip. Although control line intensity may vary, a visible control line must be present for results to be considered valid. Test strips with a test line which is darker than or of equal intensity to the control line indicates a result which is below the limit of detection of the test. Test strips with a test line which is lighter than the control line indicates a result which is between 2.5 ppb and 100 ppb. Test strips with a very faint test line or no test line visible indicates a result which is > 100 ppb. Results should be determined within 5-10 minutes after completion of the strip test procedure. Determination made using strips which have dried for more or less than the required time may be inaccurate, as line intensities may vary with drying time.

Control Line	***Test Line***	***Interpretation***
No control line present	No test line present	Invalid result
Control line present	Very faint or no test line present	>100 ng/mL (ppb)
Control line present	Moderate intensity test line present	Between 2.5 and 100 ng/mL (ppb)

The appearance of test strips may also be compared to the illustration below to determine approximate sample concentration ranges. Please note that the illustration is intended for the demonstration of test line to control line intensity only. Results should not be determined by comparing the intensity of test lines from test strips to the test line intensity of the illustration, as the overall intensity of test strips may vary slightly with different lots of reagents. To obtain semi-quantitative results in the range of 0-100 ppb, solutions of known Glyphosate concentration (control solutions) must be tested concurrently with samples. Sample test line intensities can then be compared with control solution test line intensities, yielding approximate sample concentrations. Do not use strips run previously to determine semi-quantitative sample concentrations, as test line intensities may vary once strips are completely dry.



H. Additional Analysis

If necessary, positive samples can be confirmed by ELISA, HPLC, or other conventional methods.