

## Performance Data

Test sensitivity: The GlyphoCheck Strip Test will detect Glyphosate in water samples at concentrations between 2.5 ppb and 100 ppb, at which the test line exhibits moderate intensity. At levels greater than 100 ppb, the test line is extremely faint or not visible. For food samples, please refer to Section H. Interpretation of Results for detection ranges.

Samples: A sample correlation between the GlyphoCheck 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-enolpyruvylshikimic 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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**In using the GlyphoCheck test you acknowledge that you have read and understand these Terms and Conditions and by accepting the Kit you take subject to these Terms and Conditions.**

For ordering or technical assistance contact:

The Detox Project®

Email: [info@detoxproject.org](mailto:info@detoxproject.org)

WEB: <https://detoxproject.org>

*GlyphoCheck Strip Test Kits are available exclusively on [www.detoxproject.org](http://www.detoxproject.org).*

## GlyphoCheck™ Strip Test

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

Product No. 500096 (5 Test), 500097 (2 Test)



### 1. General Description

The GlyphoCheck Strip Test is a rapid immunochemical test designed solely for use in the qualitative screening of Glyphosate in water and food, including honey, beer, baby food, and wheat/oat cereal, which require sample extraction and preparation before testing. Please contact [detoxproject.org](http://detoxproject.org) for sample preparation procedures for other food types. The GlyphoCheck Strip Test provides only preliminary qualitative test results. If necessary, positive samples can be confirmed by ELISA, HPLC or other conventional methods. Please contact [detoxproject.org](http://detoxproject.org) for information on testing laboratories.

### 2. Safety Instructions

Consult state, local, and federal regulations for the proper disposal of all reagents. All prepared samples and reagents used for this test are not for consumption (do not eat prepared samples or reagents).

### 3. Storage and Stability

The GlyphoCheck Strip Kit should be stored between 5-30°C (40-85°F). The test strips, test vials, 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. A control line, produced by a different antibody/antigen reaction, is also present on the membrane area of the strip. The glyphosate analogue conjugate on the membrane strip competes for antibody binding sites with glyphosate that may be present in the sample. 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 the compound at or below the cut-off point established for the compound. If the compound is present in the sample, it competes with the immobilized Glyphosate analogue 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 level of detection (>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). Please contact [detoxproject.org](http://detoxproject.org) for purchasing information.

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

Numerous 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 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 10°C (40°F) or higher than 30°C (85°C)).

This test is designed for use with water and food samples. The GlyphoCheck 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 GlyphoCheck Strip Test is for the screening of water and food samples. Food samples must undergo an appropriate sample preparation procedure prior to analysis to obtain accurate results (See Section F: Sample Preparation).

-The test strips, conical test 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 droppers for each sample.

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

-Use distilled or deionized water for sample preparations. Do not use tap water, as this may produce inaccurate results. Distilled water are available at drug stores and Amazon.com.

-Avoid contact of reagents with skin and mucous membranes.

-Use reasonable judgement when interpreting the test results.

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

-Use of the GlyphoCheck Strips without the other provided reagents (in vial #2, vial #3, and vial #4) will adversely affect the performance of the test, producing inaccurate results.

### A. Reagents and Materials Provided

1. Glyphosate test strips in a desiccated pouch or container
2. 50 mL sample tubes with green caps (Vial #1)
3. Self-standing 2.0 mL mixing vial containing 1 mL buffer solution (Vial #2)
4. Derivatization vials containing small amount of reagent (Vial #3) - Please note that the reagent in vial #3 is a chemical similar to vinegar and will have a vinegar-like odor.
5. Conical 1.5 mL test vials (Vial #4)
6. Disposable transfer droppers
7. Disposable graduated droppers
8. Pop-up tube holder stand
9. User's guide and flow chart
10. Disposable scoop/spoon

### B. Additional Materials

1. Distilled or deionized water (Do not use tap water for food sample preparation)
2. Timer
3. Permanent marker
4. Scale with at least 2-decimal capability for weighing samples
5. Container/storage vials or bottles for sample preparation

### C. Sample Collection and Handling

Water samples should be collected in the 50 mL sample tube (Vial #1) provided in the kit. Chlorinated drinking water samples should be tested immediately upon collection to prevent degradation of Glyphosate by water treatment chemicals, which may cause biased low results.

Non-chlorinated water samples (water from streams, lakes, ponds, etc.) can be stored refrigerated for up to one week.

### 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) should be analyzed with each lot of test strips to provide a reference for line intensity to be expected. Please contact Detox Project for purchasing information for positive controls.

### E. Test Preparation

1. Allow test strips, kit materials, and samples to reach room temperature before use.
2. Samples **must** be derivatized (Vial #3) prior to each analysis (**refer to Section F, Procedures**).

### F. Sample Preparation

#### 1. Honey

- a. Add distilled water (DO NOT USE TAP WATER) to the 50 mL mark of Vial #1.
- b. Pour honey sample into the provided disposable spoon, filling until level at the top, scraping off excess, if necessary.
- c. Place the spoon with the honey sample into Vial #1 containing the water.

### I. Additional Analysis

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

### 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 enabled the increased use of Glyphosate, by 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.

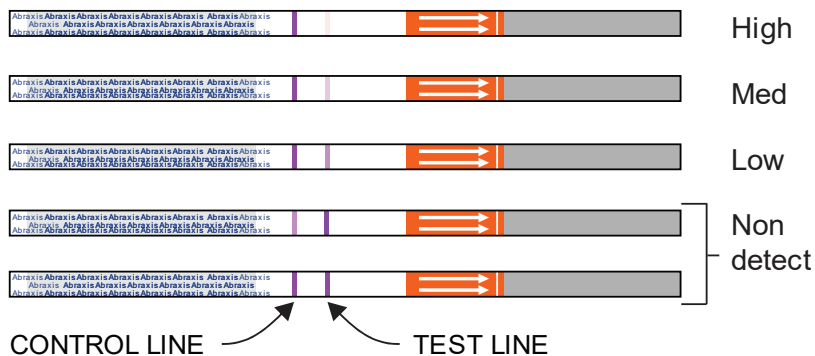
Glyphosate can adsorb to soil and is highly water soluble, which can cause surface and ground water contamination from run-off, soil erosion, and leaching especially after heavy rainfall. The long-term impact on the environment and human health are growing concerns worldwide. In March 2015, the World Health 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.

## H. 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. For example with water samples, test strips with a test line which is lighter than the control line indicates a result which is  $\geq 2.5$  and  $\leq 100$  ppb. Test strips with no test line visible or very faint test line indicates a result which is  $\geq 100$  ppb. Results should be determined within 5-10 minutes after completion of the strip test procedure. Determinations 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.

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.

### Appearance:



### Corresponding Glyphosate Concentration:

Visual	Water Glyphosate levels	Honey Glyphosate levels	Beer Glyphosate levels	Cereal/Oats/Wheat Glyphosate levels	Corn/Soybeans Glyphosate levels
High	~100 ppb	~2000 ppb	~400 ppb	~2000 ppb	~2000 ppb
Med	~10ppb	~200 ppb	~40 ppb	~200 ppb	~200 ppb
Low	~2.5ppb	~50 ppb	~10 ppb	~50 ppb	~50 ppb
Non detect	~0 to <2.5ppb	~0 to <50ppb	~0 to <10ppb	~0 to <50ppb	~0 to <50ppb

- d. Cap and shake (with the spoon inside) until the honey sample has completely dissolved.
- e. With spoon inside, proceed to Sample Testing Instructions in Section G.

### 2. Beer

- a. Add distilled water (DO NOT USE TAP WATER) to the 30 mL mark of vial #1.
- b. Add beer to the distilled water up to the 40 mL mark of vial #1.
- c. Cap the vial and shake for 10 seconds.
- d. Unscrew the cap to vent carbonation and then repeat **step c**. Repeat **step d** two (2) more times.
- e. Proceed to Sample Testing Instructions in Section G.

### 3. Cereal and Infant Cereal, Whole Oats, Wheat, Corn, and Soybeans

- a. Samples must be ground to ensure accurate sample results. Grind samples using a blender or food processor, or put sample in a zip-top bag and crush with a mallet or hammer. If samples are provided as ground samples, for example wheat flour samples, then no additional grinding is necessary.
- b. Add distilled water (DO NOT USE TAP WATER) to the 30 mL mark of vial #1.
- c. Using the provided spoon, add 4 scoops of infant cereal or 2 scoops of cereal, oats, wheat, corn, or soybeans into vial #1. Discard spoon after use.
- d. Cap and shake for 30 seconds.
- e. Let the sample tube sit for 2 minutes and then shake again for 30 seconds.
- f. Repeat Step e. 3 more times to ensure all glyphosate is released from the sample into the liquid.
- g. Let the sample sit for at least 5 minutes to settle.
- h. Proceed to test the upper portion of the prepared sample as described in the Sample Testing Instructions in Section G.

### 4. Water (Tap water, bottled water, or water from streams, ponds, or lakes)

- a. Collect sample up to the 40 mL mark in vial #1.
- b. Proceed to Sample Testing Instructions in Section G.

## G. Sample Testing

1. Label vials 1, 2, and 3 (provided in the kit) for each sample to be tested. Remove and discard the blue cap from vial #3.
2. Using a **new disposable graduated dropper for each sample**, draw up the sample to the 1 mL mark on the dropper and dispense the entire 1.0 mL into vial #2, which contains 1 mL of a reagent solution. Cap and mix well by shaking for 15-30 seconds.
3. Using the **same graduated dropper from step 2**, transfer the entire sample mixture (2.0 mL) from vial #2 to vial #3. Replace the rubber stopper/cap on vial #3 and mix for 30 seconds. Allow to sit and incubate at room temperature for 10 minutes.
5. With the **new small disposable transfer dropper**, transfer 6 drops (approximately 0.2 mL) of the sample from vial #3 to vial #4, which contains a dried purple reagent. Discard the dropper after use.
6. Snap shut vial #4 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. Allow to sit and incubate at room temperature for 10 minutes.
8. Insert test strip (arrows down) into the solution in vial #4.
9. Allow the test strip to develop for 10 minutes.
10. After 10 minutes, remove the test strip. Lay the strip flat on a piece of white paper or paper towel and allow to continue developing for 5-10 minutes.
11. Read the results visually, as explained below in section H. Interpretation of Results.