## H. Evaluation

The evaluation of the ELISA can be performed using commercial ELISA evaluation programs. For a manual evaluation, calculate the mean absorbance value for each of the standards and samples. Samples with lower absorbances than a standard will have concentrations of Glufosinate greater than that standard. Samples with higher absorbances than a standard will have concentrations of Glufosinate less than that standard. Samples showing a higher absorbance than standard 1 (0.015 ppb) should be reported as containing < 0.015 ppb of Glufosinate. Samples showing a lower absorbance than standard 3 (10.0 ppb) should be reported as containing > 10.0 ppb of Glufosinate or must be diluted using Diluent/Zero Standard (Sample Diluent) and re-analyzed to obtain more accurate results. Results can also be determined using a spreadsheet macro available from Gold Standard Diagnostics, Horsham upon request.

| Mean Absorbance | Concentration |
|---|---|
| Greater than Standard 0 | <0.015 ppb |
| Between Standard 0 and Standard 1 | <0.015 ppb |
| Between Standard 1 and Standard 2 | 0.015 – 0.4 ppb |
| Between Standard 2 and Standard 3 | 0.4 – 10 ppb |
| Less than Standard 3 | >10 ppb |

As with any analytical technique (GC, HPLC, etc.), positive results requiring regulatory action should be confirmed by an alternative method.

### Importance of Glufosinate Determination

Glufosinate ((±)-2-amino-4-(hydroxymethylphosphinyl)butanoic acid) is a broad-spectrum herbicide that is commonly used to control weeds in crops, orchards, vineyards, and other non-crop settings, as well as pre-harvest desiccation to increase harvest yield Glufosinate or phosphinothricin and is also known by various trade names by many different manufacturers. It is often used as an alternative to the pesticide glyphosate, which has come under scrutiny due to concerns about its potential health and environmental effects. Release of GMO Glufosinate-resistant crops (canola in 1995, corn in 1997, cotton in 2004, soybeans in 2011) has led to increased use of Glufosinate, allowing farmers to control weeds without harming their crops. The long-term impact on the environment and human health are growing concerns. Glufosinate is persistent ; it has been found to be prevalent in spinach, radishes, wheat and carrots that were planted 120 days after the treatment of the herbicide. Residues can remain in frozen food for up to two years and the chemical is not easily destroyed by cooking the food item in boiling water. The EPA classifies the chemical as 'persistent' and 'mobile' based on its lack of degradation and ease of transport through soil. The toxicity of Glufosinate depends on the dose and the route of exposure (e.g., inhalation, ingestion, or skin contact). Acute exposure to high doses of Glufosinate can cause symptoms such as headache, dizziness, nausea, vomiting, abdominal pain, and difficulty breathing. In severe cases, it can lead to seizures, coma, and even death. Chronic exposure to lower doses of Glufosinate over a long period of time may cause adverse health effects, such as developmental and reproductive effects, and may increase the risk of cancer. The WHO/FAO recommended acceptable daily intake (ADI) for Glufosinate is 0.02 mg/kg. The European Food Safety Authority has set an ADI of 0.021 mg/kg. The Acute reference dose (ARfD) for child-bearing women is 0.021 mg/kg

### Performance Data

Test sensitivity: The Glufosinate ELISA has an estimated detection limit (90% $B/B_0$) of 0.015 ppb (µg/L). The middle of the test (50% $B/B_0$) is approximately 0.4 ppb.

Test reproducibility: The absorbance Coefficient of variation (CVs) for standards should be ≤10% and for the samples should be ≤15%. The Standard 0 absorbance value should be between 0.8 - 3.000.

Specificity: The cross-reactivity of the Glufosinate ELISA for various related analogues expressed as the least detectable dose (LDD) or 90% $B/B_0$ and as the dose required for 50% inhibition (50% $B/B_0$) are as follows:

| Compound | LDD (ppb) | 50% (ppb) |
|---|---|---|
| 2-[n-(phosphonomethyl)acetamido]acetic acid | 1,000,000 | N/A |
| 3-(Methylphosphinico)propionic acid | 1,000 | 10,500 |
| Glufosinate-N-Acetyl | 1 | 11 |
| AMPA | Undetectable at 10,000,000 | N/A |
| Glyphosate | 10,000,000 | N/A |

**General Limited Warranty**: Gold Standard Diagnostics 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. **Gold Standard Diagnostics makes no other warranty, expressed or implied. There is no warranty of merchantability or fitness for a particular purpose.**

**For ordering or technical assistance contact:**
Gold Standard Diagnostics
795 Horsham Road
Horsham, PA 19044
WEB: www.abraxiskits.com
Tel.: (215) 357-3911
Ordering: info.abraxis@us.goldstandarddiagnostics.com
Technical Support: support.abraxis@us.goldstandarddiagnostics.com
Date these instructions are effective : 01/08/2026
Version: 03

# ABRAXIS® Glufosinate ELISA Microtiter Plate

Enzyme-Linked Immunosorbent Assay for the Determination of Glufosinate in Water, Durum Wheat, and Whole Oats Samples

**Product No. 500060**

## 1. General Description

The ABRAXIS® Glufosinate ELISA Plate Kit is an immunoassay test suitable for the semiquantitative and/or qualitative screening of Glufosinate in surface water, durum wheat and whole oats samples (refer to section C, Sample Collection and Handling). For additional crop and food sample applications, contact Gold Standard Diagnostics for the appropriate technical bulletin and/or matrix validation guidelines. Samples requiring regulatory action should be confirmed by HPLC, GC/MS, or other conventional methods.

## 2. Safety Instructions

The standard and solutions in the test kit contain small amounts of Glufosinate. The ABRAXIS® Derivatization Reagent Diluent is Dimethyl Sulfoxide (DMSO). The ABRAXIS® Derivatization Reagent is succinic anhydride. In addition, the substrate solution contains tetramethylbenzidine and the stop solution contains diluted sulfuric acid. Avoid contact of these solutions with skin and mucous membranes. If these reagents come in contact with skin, wash thoroughly with water.

## 3. Storage and Stability

The ABRAXIS® Glufosinate ELISA Kit should to be stored in the refrigerator (2-8°C). The solutions must be allowed to reach room temperature (20-25°C) before use. Unopened reagents may be used until the expiration date on the box. Consult state, local, and federal regulations for the proper disposal of all reagents.

## 4. Test Principle

The test is a competitive ELISA based on the recognition of Glufosinate by polyclonal antibodies. The sample to be tested is derivatized (please refer to Section E, Test Preparation, Step 8), diluted after derivatization, and then added to microtiter wells coated with rabbit anti-Glufosinate antibody. The derivatized, diluted samples are allowed to incubate for 30 minutes. The Glufosinate enzyme conjugate is then added and a competitive reaction occurs between the Glufosinate, which may be present in the sample, and the enzyme labeled Glufosinate for the binding sites of the rabbit anti-Glufosinate antibodies which are immobilized on the microtiter plate. The reaction is allowed to continue for 30 minutes. After a washing step and addition of the substrate solution, a color signal is generated. The intensity of the blue color is inversely proportional to the concentration of Glufosinate present in the sample. The color reaction is stopped after a specified time and the color is evaluated using an ELISA reader. The concentrations of the samples are determined by comparing sample results to the standard curve constructed with each run.

## 5. Limitations of the ABRAXIS® Glufosinate ELISA, 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, cannot be completely excluded. The presence of the following substances at the specified concentrations were found to have no significant effect on the ABRAXIS® Glufosinate ELISA results: **up to 10,000 ppm** – calcium, chloride, magnesium, potassium, sodium chloride, sodium thiosulfate, sulfate; **up to 2,500 ppm** – sodium fluoride; **up to 1,000 ppm** – L-ascorbic acid, manganese II, sodium phosphate (mono and dibasic); **up to 100 ppm** – iron II and III, potassium phosphate (monobasic), sodium chlorite, zinc; **up to 85.5 ppm** – aluminum, calcium oxide; **up to 10 ppm** – copper II, Humic acid.

Standards and samples must be derivatized prior to each analysis with the ABRAXIS® Glufosinate ELISA kit (See Section E, Test Preparation, Step 8).

Mistakes in handling the test can also cause errors. Possible sources for such errors include: inadequate storage conditions of the test kit, incorrect pipetting sequence or inaccurate volumes of the reagents, too long or too short incubation times during the immune, enzyme conjugate, and/or substrate reaction, exposure to direct or indirect sunlight during the substrate reaction, allowing Wash solution to remain in wells of plates longer than necessary or performing less than 3 washes (See Section A, Reagents and Materials provided); or extreme temperatures (lower than 10°C or higher than 30°C) during the test performance. As with any analytical technique (GC, HPLC, etc.), positive results requiring regulatory action should be confirmed by an alternative method.

## 6. Working Instructions

### A. Reagents and Materials Provided (*Additional quantities purchasable from Gold Standard Diagnostics)

1. ABRAXIS® Glufosinate Microtiter Plate (96T) – goat anti-rabbit antibody microtiter plate coated with rabbit anti-Glufosinate antibodies in a re-sealable aluminum pouch with desiccant
2. ABRAXIS® Glufosinate Standards (4): 0, 0.015, 0.4, 10 ppb; 4 mL each
3. ABRAXIS® Glufosinate Enzyme Conjugate (Lyophilized), 3 vials, must be reconstituted before use (See Section E,Test Preparation, Step 4)
4. ABRAXIS® Glufosinate Conjugate Diluent, 12.5 mL

5. Empty amber HDPE bottle for combining reconstituted Enzyme Conjugate (See Section E, Test Preparation, Step 4)
6. ABRAXIS® Glufosinate Sample Diluent/Zero Standard (Standard and Sample Diluent), 50 mL
7. ABRAXIS® Wash Buffer (5X) Concentrate,100 mL, must be diluted before use (see Section E, Test Preparation,Step 2)
8. Substrate (Color) Solution (TMB), 18 mL
9. Stop Solution, 13.5 mL (handle with care)
10. ABRAXIS® Glufosinate Derivatization Reagent (Lyophilized), 3 vials, must be reconstituted before use (see Section E, Test Preparation, Step 8 (handle with care))
11. ABRAXIS® Glufosinate Derivatization Reagent Diluent, 1.7 mL
12. ABRAXIS® Glufosinate Post Derivatization Diluent, 50 mL

## B. Additional Materials (not delivered with the test kit)

1. Syringe filters (see Section C, Sample Collection and Handling)
2. Disposable glass test tubes or vials
3. Deionized or distilled water
4. 15 mL plastic centrifuge tube
5. Rotator
6. 2 mL plastic microcentrifuge tube
7. Microcentrifuge
8. Micro-pipettes with disposable plastic tips (10-200 and 200-1000 µL)
9. Multi-channel pipette (10-300 µL), stepper pipette (10-300 µL), or electronic repeating pipette with disposable plastic tips (capable of delivering 50-1000 µL)
10. Container with 500 mL capacity (for diluted 1X Wash Buffer, see Section E, Test Preparation, Step 2)
11. Vortexer
12. Timer
13. Parafilm or microtiter plate cover slip
14. Paper towels or equivalent absorbent material
15. Microtiter plate washer (optional)
16. Microtiter plate reader (wavelength 450 nm)

## C. Sample Collection and Handling

Collect water samples in glass or plastic sample containers.

Samples containing gross particulate matter should be filtered prior to analysis using any of the following syringe filters: Environmental Express 0.2 µm PES (PN SF020E), Pall Acrodisc® 0.2 µm PVDF (PN 4450), Whatman™ 0.2 µm Anotop™ 25 Plus (Cat. No. 6809-4022), or Environmental Express 1.2 µm Glass Fiber (PN SF012G).

## D. Preparation of Samples

Samples should be analyzed immediately after preparation, if possible, or can be stored 2-8°C for 5 days or -20°C indefinitely. Please direct inquiry about preparation of samples for other matrices to Technical Support at Gold Standard Diagnostics.

Water Samples ***(After completing, proceed to Final Preparation, Step 1, below)***

1. Dilute 50 µL of sample in 950µL of deionized or distilled water in 4 mL glass vial, vortex to mix.

Durum wheat and Whole Oats Samples ***(After completing, proceed to Final Preparation, Step 1, below)***

1. Weigh 0.5 ± 0.05 gram of sample into a 15 mL plastic centrifuge tube.
2. Add 10.0 mL of deionized water, vortex to suspend. Extract using a rotator for 10 minutes.
3. Let extract settle for at least 2 minutes.
4. Add 1.5 mL of extract solution to 2 mL microcentrifuge tube. Centrifuge for 5 min at 8,100 X g or 10,000 rpm in microcentrifuge. Remove and transfer supernatant into a clean glass vial. Optional: Use any syringe filter listed in Section C, Sample Collection and Handling.

**Final Preparation *(Necessary for all Samples)***

1. Dilute supernatant, filtrate, or diluted sample 6.7-fold by adding 150 µL of solution to 850 µL Glufosinate Sample Diluent in 4 mL glass vial or 12 x 75 mm borosilicate glass tube. Vortex to mix.
2. Proceed to Section E, Test Preparation, Step 1. *Note: Following step 8g of Section E, the sample will be diluted 10-fold further in post-derivatization diluent. The cumulative Glufosinate concentration contained in sample is then determined by* ***multiplying the ELISA result by the dilution factor of 1333 for Water, Durum Wheat, or Whole Oats***.

## E. Test Preparation

Micro-pipetting equipment and pipette tips for pipetting the standards and the samples are necessary. A multi-channel, stepping, or electronic repeating pipette is recommended for adding the enzyme conjugate, substrate (color), and stop solutions in order to equalize the incubation periods across the entire microtiter plate. Please only use the reagents and standards from one package lot in one test, as they have been adjusted in combination.

1. Allow the microtiter plate, reagents, and samples to reach room temperature before use.
2. Dilute the ABRAXIS® Wash Buffer (5X) Concentrate at a ratio of 1:5. If using the entire bottle (100 mL) add to 400 mL of deionized or distilled water.
3. The standard solutions, sample diluent, derivatization reagent diluent, post-derivatization diluent, conjugate diluent, substrate (color) and stop solutions are ready to use and do not require any further dilutions.
4. The enzyme conjugate must be reconstituted prior to use. Add 3 mL of the Glufosinate Conjugate Diluent to the Glufosinate Enzyme Conjugate (Lyophilized) Vial, vortex well. ***If more than one vial is required for testing, combine the reconstituted enzyme conjugate vials in the empty amber HDPE bottle. Reconstituted Enzyme Conjugate should only be used up to one month after reconstitution.***
5. The stop solution must be handled with care as it contains diluted $H_2SO_4$. The Derivatization Reagent must be handled with care before and after reconstitution as it contains succinic anhydride.
6. Remove the number of microtiter plate strips required from the foil bag. The remaining strips are stored in the foil bag with desiccant and zip-locked closed.
7. After analysis, store the remaining kit components in the refrigerator (2-8°C).
8. **Derivatization of Standards and Samples (must be performed prior to each analysis)**:
   a. Reconstitute the Glufosinate Derivatization Reagent (Lyophilized) with 0.5 mL of Glufosinate Derivatization Reagent Diluent. Vortex to mix until Derivatization Reagent has fully dissolved and no solid reagent is visible in vial. ***Note: Reconstituted Derivatization Reagent must be used within 8 hours of preparation. If additional samples are to be analyzed more than 8 hours after reconstitution, discard the vial, and a new vial of Derivatization Reagent should be diluted for use.***
   b. Label single disposable glass test tubes for standards and samples.
   c. Pipette 1 mL of standard or sample into appropriately labeled glass test tube.
   d. To each tube of standard or sample, add 10 µL of reconstituted Derivatization Reagent. After addition of reconstituted derivatization reagent to all standards or samples, **vortex each tube immediately until no swirling lines are present.**
   e. Incubate at room temperature for 15 minutes.
   f. For each standard or sample, prepare a separate, appropriately labelled glass tube. Pipette 0.9 mL of Glufosinate Post-derivatization Diluent into each of these tubes.
   g. After derivatization, add 100 µL of each derivatized standard or sample into a separate, labeled glass tube containing 900 µL of Post-derivatization diluent per tube (prepared in step f) . Vortex to mix.
   h. Standards and samples are ready to be analyzed. Proceed to Section G, Assay Procedure,Step 1.

   **Note: Discard derivatized standards and samples after use. Do not use for re-analysis.**

## F. Working Scheme

The microtiter plate consists of 12 strips of 8 wells, which can be used individually for the test. The standards must be run with each test. Never use the values of standards which have been determined in a test performed previously.

Std 0-Std 3: Derivatized Standards
Sam 1, Sam 2, etc: Derivatized Samples

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | Std 0 | Sam 1 | | | | | | | | | | |
| B | Std 0 | Sam 1 | | | | | | | | | | |
| C | Std 1 | Sam 2 | | | | | | | | | | |
| D | Std 1 | Sam 2 | | | | | | | | | | |
| E | Std 2 | etc. | | | | | | | | | | |
| F | Std 2 | etc. | | | | | | | | | | |
| G | Std 3 | | | | | | | | | | | |
| H | Std 3 | | | | | | | | | | | |

## G. Assay Procedure

1. **Add 100 µL of derivatized, diluted standards or samples** (see Section E, Test Preparation, Step 8) into the wells of the test strips according to the working scheme given. Analysis in duplicate or triplicate is recommended. Cover wells with parafilm or tape and mix contents by moving strip holder in a circular motion on the benchtop for 30 seconds. Be careful not to spill contents. **Incubate the strips for 30 minutes at room temperature.**
2. Remove the covering and **add 50 µL of the reconstituted Enzyme Conjugate** (see Section E, Step 4) to the individual wells successively using a multi-channel pipette, stepping, or electronic repeating pipette. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop for 30 seconds. Be careful not to spill the contents. **Incubate the strips for 30 minutes at room temperature.**
3. Remove the covering, decant the contents of the wells into a sink, and blot the inverted plate on a stack of paper towels. **Wash the strips three times using the diluted wash buffer.** Please use at least 250 µL of 1X wash buffer for each well and each washing step. Blot the inverted plate after each wash step on paper towels. After the last wash/blot, check for any remaining buffer in wells, and if necessary, remove by additional blotting.
4. **Add 150 µL of substrate (color) solution** to the individual wells successively using a multi-channel pipette, stepping, or electronic repeating pipette. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop for 30 seconds. Be careful not to spill the contents. **Incubate the strips for 20 minutes at room temperature.** Protect the strips from sunlight.
5. **Add 100 µL of stop solution** to the wells in the same sequence as for the substrate (color) solution using a multi-channel pipette, stepping, or electronic repeating pipette.
6. Read the absorbance at 450 nm using a microtiter plate ELISA photometer within 15 minutes after the addition of the stopping solution.