GOLD STANDARD DIAGNOSTICS

Glufosinate Test Kit Validation

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Glufosinate is a broad-spectrum, non-selective systemic herbicide used to control a wide range of broadleaf weeds, grasses, and some woody species. It acts by inhibiting glutamine synthetase, a key enzyme in nitrogen metabolism, leading to rapid plant death.

In recent years, the widespread emergence of glyphosate-resistant weed populations has driven increased reliance on glufosinate as an alternative mode of action. In addition, glufosinate is frequently used in tank mixtures and rotational programs to enhance weed control efficacy and delay resistance development.

The global adoption of glufosinate-resistant genetically modified crops has further expanded its use across major agricultural systems, increasing the need for reliable screening-level residue monitoring in food and environmental matrices.

ABRAXIS® Glufosinate ELISA Test Kit Validation

Comprehensive validations have been completed to confirm that the Gold Standard Diagnostics Horsham ABRAXIS® Glufosinate ELISA 96-well test kit is accurate, specific, reproducible, and rugged. Validation activities provide documented evidence that the method performs as intended and delivers reliable results under normal operating conditions.

The performance data presented below demonstrates:

- Specificity
- Limit of Quantitation
- Lot-to-lot Variability
- Precision
- Sensitivity (Limit of Detection)/Assay Range

GLUFOSINATE TEST METHOD

The ABRAXIS® Glufosinate ELISA 96-well plate kit is a semi-quantitative competitive ELISA that recognizes glufosinate using specific polyclonal antibodies. Glufosinate present in the sample competes with enzyme-labeled glufosinate for binding sites on antibodies immobilized on the microtiter plate. Following substrate addition, a colorimetric signal is produced. Color intensity is inversely proportional to the concentration of glufosinate present in the sample.

Sample Matrices

For the performance validation study, extracts from various sample matrices were evaluated for Glufosinate using the Gold Standard Diagnostics' ABRAXIS® Glufosinate ELISA Test Kit method.

- Surface Water
- Durum Wheat
- Whole Oats
- Soybeans
- Yellow Peas

Specificity

The assay exhibits **high specificity for glufosinate**, with minimal cross-reactivity toward related metabolites and structurally distinct herbicides. Glyphosate and AMPA showed negligible or no detectable response, confirming that the assay selectively detects glufosinate without interference from commonly co-occurring compounds.

Metabolites and Analogues of Glufosinate	Minimum Tested Detectable Concentration	Concentration of Glufosinate Detected As
2-[n-(phosphonomethyl)acetamido]acetic acid	1mg/mL	6.9 ppb
3-(Methylphosphinico)propionic acid	1µg/mL	0.019 ppb
Glufosinate-N-Acetyl	1 ppb	0.1 ppb
Alternative Herbicides		
AMPA	Undetectable	N/A
Glyphosate	10 mg/mL	1 ppb

Sensitivity (Limit of Detection)/Assay Range

The assay demonstrates high analytical sensitivity, with a limit of detection (LOD) of 0.015 ppb and an assay range of 0.015–10 ppb, enabling detection of glufosinate at trace levels well below regulatory thresholds.

ppb	Ave B/Bo	%CV
0.015	0.871	4.0
0.4	0.544	7.1
10	0.220	25

N=39 (across 4 lots)

Interpretation	ppb
Non-detect	<0.015
Low	0.015-0.4
Medium	0.4 -10
High	>10

Precision

Precision studies showed low intra- and inter-run variability, with %CV values consistently within acceptable limits, confirming the assay's reproducibility under normal laboratory operating conditions.

Intermediate Precision

(n=5/run, 3 runs/day, 3 days, 1-2 operators, n=45/90)

Matrix	Spike, ppb	%CV (Intra)	%CV (Inter)
	30	**5.3**	**4.8**
Durum Wheat	260	**6.4**	**5.5**
(2 operators)	2000	**7.7**	**7.4**
	30	**7.9**	**7.1**
Water	260	**9.8**	**8.7**
(2 operators)	2000	**14.2**	**13.0**
	30	**7.4**	**6.8**
Whole Oats	260	**8.7**	**7.4**
(2 operators)	2000	**12.9**	**12.7**
	30	**11.0**	**11.4**
Yellow Peas	260	**14.5**	**15.0**
	2000	**17.1**	**17.7**
	30	**11.6**	**12.0**
Soybeans	260	**12.8**	**13.3**
	2000	**17.4**	**18.1**

Limit of Quantification (LOQ)

Table: Replicate absorbance and B/B0 responses at the LOQ level

	DI Water		Durum Wheat		Whole Oats	
	Absorbance	**B/B0**	**Absorbance**	**B/B0**	**Absorbance**	**B/B0**
	0.905	0.73	1.058	0.82	0.945	0.72
	0.926	0.74	1.051	0.81	0.994	0.76
	0.915	0.73	1.014	0.78	0.976	0.75
	0.945	0.76	1.085	0.84	0.955	0.73
Run 1	0.901	0.72	1.079	0.83	0.937	0.72
	0.985	0.79	1.022	0.74	0.987	0.72
	0.98	0.78	1.043	0.76	1.014	0.74
	0.978	0.78	1.022	0.74	0.998	0.72
	0.962	0.77	1.122	0.82	1.004	0.73
Run 2	0.969	0.78	1.139	0.83	1.051	0.76
	0.928	0.77	0.891	0.74	0.928	0.71
	0.886	0.73	0.907	0.76	0.889	0.68
	0.855	0.71	0.883	0.74	0.879	0.67
	0.896	0.74	0.973	0.81	0.919	0.70
Run 3	0.857	0.71	0.905	0.76	0.949	0.73
	0.97	0.84	1.004	0.75	0.936	0.72
	0.925	0.80	0.99	0.74	0.926	0.71
	0.852	0.74	0.995	0.74	0.886	0.68
	0.95	0.82	1.034	0.77	0.93	0.72
Run 4	0.958	0.83	1.034	0.77	0.943	0.73
Average	0.927	0.76	1.013	0.78	0.952	0.72
%CV	4.6	5.1	7.2	4.7	4.8	3.2

Replicate analyses across multiple runs demonstrated consistent absorbance and B/B responses in water, durum wheat, and whole oats, with %CV values ≤ 7.2%, confirming excellent repeatability at low-level concentrations near the detection limit.

LOQ Summary:

- Water / Durum Wheat / Whole Oats: 20 ppb (~1:1,350-fold dilution)
- Soybeans: 20 ppb (Lipid Removal Adsorber)
- Yellow Peas: 10 ppb (PSA SPE, ~1:650-fold dilution)

The method provides reliable quantification at 20 ppb in water, durum wheat, and whole oats following routine dilution, with comparable LOQs achieved in soybeans and yellow peas using matrix-appropriate cleanup procedures (as described in the relevant Technical Bulletins). This supports robust semi-quantitative screening across diverse matrices.

Lot-to-Lot Variability and Plate Drift

Lot-to-lot and plate drift evaluations across three transfer lots demonstrated good manufacturing consistency and assay stability. Observed plate drift values (-0.2% to 2.2%) were well within the predefined acceptance criterion (<10%), indicating minimal positional effects across the plate. Low %CVs were observed at zero, low, and mid-range concentrations. Higher %CVs at the upper end of the range are expected for a semi-quantitative assay and reflect curve flattening near saturation rather than manufacturing variability.

	ppb	Lot 1	Lot 2	Lot 3	Average	%CV
Absorbance	0	2.00	2.21	2.33	2.18	7.6
	0.015	1.83	1.93	2.24	2.00	10.7
	0.4	1.05	1.13	1.44	1.21	17.0
	10	0.38	0.54	0.73	0.55	31.5
B/B0	0.015	0.91	0.87	0.96	0.92	5.0
	0.4	0.53	0.51	0.62	0.55	10.7
	10	0.19	0.24	0.31	0.25	24.2

Plate Drift

Zero	
Samp Rep	42
%Drift	**-0.2**
AvgA450	1.717
Stdev	0.027
%CV	1.6

1.0 ppb	
Samp Rep	42
%Drift	**2.2**
AvgA450	0.930
Stdev	0.019
%CV	2.1

Ordering Information

The ABRAXIS® Glufosinate ELISA 96-well test kit includes all reagents and materials required for sample analysis.

Part Number	Product Description
500060	ABRAXIS® Glufosinate, ELISA, 96-well

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