CERTIFICATION

AOAC Research Institute

Performance Tested Methods[SM]

Certificate No.

072104

The AOAC Research Institute hereby certifies the method known as:

ABRAXIS® Glyphosate ELISA Plate Kit

manufactured by

Gold Standard Diagnostics Horsham, LLC.
795 Horsham Road
Horsham, PA 19044

This method has been evaluated and certified according to the policies and procedures of the AOAC *Performance Tested Methods*[SM] Program. This certificate indicates an AOAC Research Institute Certification Mark License Agreement has been executed which authorizes the manufacturer to display the AOAC Research Institute *Performance Tested Methods*[SM] certification mark on the above-mentioned method for the period below. Renewal may be granted by the Expiration Date under the rules stated in the licensing agreement.

Bradley A. Stawick, Senior Director
Signature for AOAC Research Institute

Issue Date October 25, 2024
Expiration Date December 31, 2025

AUTHORS
Graham Yearwood

SUBMITTING COMPANY
Gold Standard Diagnostics Horsham, LLC. (formerly Eurofins Abraxis)
795 Horsham Road
Horsham, PA 19044

METHOD NAME
ABRAXIS® Glyphosate ELISA Plate Kit

CATALOG NUMBER
500089

INDEPENDENT LABORATORY
OMIC USA
3344 NW Industrial St.
Portland, OR 97210 USA

APPLICABILITY OF METHOD
Analyte – Glyphosate, including glyphosate isopropylamine salt and N-acetyl glyphosate.

Matrixes – Whole grain durum wheat, whole grain oats, oat groats, dried yellow peas, and dried red lentils

Performance claims – Performance in accordance with draft *Standard Method Performance Requirements for Glyphosate, its Metabolites, and Trimesium, in Fruits and Vegetables, Cereals, Food of Animal Origin, Pet Food, and Baby Food* (4) in the range 14 - 400 µg/kg for durum wheat, 18 - 400 µg/kg for whole oats, 23 - 400 µg/kg for oat groats, 12 – 400 µg/kg for dried yellow peas, and 15 - 400 µg/kg for dried red lentils.

REFERENCE METHOD

***Standard Method Performance Requirements (SMPRs) for Glyphosate, its Metabolites, and Trimesium in Fruits and Vegetables, Cereals, Food of Animal Origin, Pet Food and Baby Food* (Draft, 2021) (4)**

ORIGINAL CERTIFICATION DATE
July 16, 2021

CERTIFICATION RENEWAL RECORD
Renewed annually through December 2025.

METHOD MODIFICATION RECORD
1. **February 2023 Level 1**

SUMMARY OF MODIFICATION
1. **Corporate name change and rebranding to Gold Standard Diagnostics Horsham.**

Under this AOAC *Performance Tested Methods*^{SM} License Number, 072104 this method is distributed by:
NONE

Under this AOAC *Performance Tested Methods*^{SM} License Number, 072104 this method is distributed as:
NONE

PRINCIPLE OF THE METHOD (1)
The test is a direct competitive ELISA based on the recognition of glyphosate by polyclonal antibodies. The test portion is solubilized, and the extract is derivatized and added to microtiter wells coated with goat anti-rabbit antibodies. A rabbit anti-glyphosate antibody solution is added to the wells with the derivatized extracts and allowed to incubate for 30 minutes. The glyphosate enzyme conjugate is then added, and a competitive reaction occurs between the derivatized glyphosate, if present, and the enzyme-labeled glyphosate for binding to the rabbit anti-glyphosate antibodies bound by the goat anti-rabbit antibodies immobilized on the microtiter plate. The reaction is allowed to continue for 60 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 glyphosate in the test portion. The color reaction is stopped after 25 minutes, and the color intensity is measured using an ELISA reader. The glyphosate concentration is determined by interpolation using the standard curve constructed with each assay.

DISCUSSION OF THE VALIDATION STUDY (1)

Method developer studies demonstrated no practical matrix effects for the matrixes claimed, supporting the use of solution standards. The ELISA method showed good correlation to the LC-MS/MS reference method performed by the independent laboratory, with a few random instances of repeatability or recovery outside the draft SMPR criteria, indicating no systematic error. The method developer estimates the test LOD as the concentration at 90% B/B_o corresponding to 5 µg/kg; using the measured respective background sample to approximate the LOQ, the estimated LOQ was 13 to 23 µg/kg. The validated LOQ were 12 to 23 µg/kg. The glyphosate in the ground matrix materials was shown to be stable for at least 14 days at 2–8°C or at room temperature (22-28°C) and glyphosate in matrix extracts was shown to be stable for at least 14 days at 2–8°C and at least 5 days at room temperature (22–29°C). Selectivity testing showed no effect from a variety of related and/or potentially interfering compounds.

The independent laboratory showed very strong positive correlation between the Abraxis ELISA method and reference LC-MS/MS method and very similar ELISA results to the method developer. There was a negative bias for the ELISA method in all detectable materials; however, recovery relative to the LC-MS/MS method was acceptable by draft SMPR standards. The high recovery bias of the LC-MS/MS method may also be due to undetected low level incurred glyphosate residue or interference in the rice control which ranged from 0 to 3.3 µg/ kg, depending on the batch. Repeatability between ELISA test portions from laboratory samples was generally good for all matrixes and at all detectable levels. There were a few materials with RSD_r values outside the draft SMPR acceptable limits; however, there was no observable trend by matrix or concentration level to suggest a systematic error in the ELISA method. The correlation between the method developer and independent laboratory results indicated that the method is well written and transferrable to a new user. The independent laboratory commented that the calibration standard curve and control point provided as part of the ELISA kit performed reliably across all analyses and that the user guide was well laid out with detailed and accurate instructions. Overall, the ELISA method showed recovery of 66-128% and repeatability of 2.9-30% among matrixes and laboratories.

Table 4. Matrix study results (1)

			ELISA			LC-MS/MS[b]			
Matrix[a]	Level	n	Mean, µg/kg	s_r, µg/kg	RSD_r, %	Mean, µg/kg	s_r, µg/kg	RSD_r, %	Relative Recovery, %
Durum wheat (MD)	Bkgd[e]	5	6.26	1.27	20	5.25	0.293	5.6	ND[c]
	Low	5	41.1	3.46	8.4	52.9	6.89	13	78
	Med	5	119	14.8	12	133	7.99	6.0	89
	High	5	308	37.4	12	388	12.1	3.1	79
Durum wheat (IL)	Bkgd	5	6.57	1.79	27	5.25	0.293	5.6	ND
	Low1	5	58.8[d]	26.7	45	52.9	6.89	13	111
	Low2	5	51.5	11.4	22	52.9	6.89	13	97
	Med	5	133	17.2	13	133	7.99	6.0	100
	High	5	339	45.8	13	388	12.1	3.1	87
Whole oats (MD)	Bkgd	5	8.55	1.79	21	9.93	0.723	7.3	ND
	Low	5	14.1	2.01	14	21.3	1.47	6.9	66
	Med	5	184	26.2	14	223	12.7	5.7	82
	High	5	259	19.7	7.6	371	10.5	2.8	70
Whole oats (IL)	Bkgd	5	8.88	1.62	18	9.93	0.723	7.3	ND
	Low	5	16.8	5.13	30	21.3	1.47	6.9	79
	Med	5	205	23.1	11	223	12.7	5.7	92
	High	5	353	30.9	8.8	371	10.5	2.8	95
Groats (MD)	Bkgd	5	7.96	1.34	17	6.51	0.284	4.4	ND
	Low	5	75.2	10.7	14	58.6	6.02	10	128
	Med	5	123	4.77	3.9	122	4.36	3.6	101
	High	5	387	19.5	5.1	400	58.1	15	97
Groats (IL)	Bkgd	5	5.62	2.27	40	6.51	0.284	4.4	ND
	Low	5	49.9	4.28	8.6	58.6	6.02	10	85
	Med	5	110	20.4	19	122	4.36	3.6	90
	High	5	361	17.4	4.8	400	58.1	15	90
Dried yellow peas (MD)	Bkgd	5	6.72	1.11	17	4.00	0.370	9.3	ND
	Low	5	37.4	9.19	25	53.7	3.84	7.1	70
	Med	5	99.5	2.87	2.9	103	4.11	4.0	97
	High	5	354	21.6	6.1	451	21.4	4.7	79
Dried yellow peas (IL)	Bkgd	5	7.20	1.97	27	4.00	0.370	9.3	ND
	Low	5	43.7	6.24	14	53.7	3.84	7.1	81
	Med	5	84.5	11.3	13	103	4.11	4.0	82
	High	5	360	28.3	7.9	451	21.4	4.7	80
Dried red lentils (MD)	Bkgd	5	4.68	2.51	54	0.795	0.294	37	ND
	Low	5	24.0	0.84	3.5	28.3	0.922	3.3	85
	Med	5	57.6	1.98	3.4	67.3	3.84	5.7	86
	High	5	371	15.9	4.3	472	11.0	2.3	79
Dried red lentils (IL)	Bkgd	5	4.07	1.52	37	0.795	0.294	37	ND
	Low	5	24.0	2.74	11	28.3	0.922	3.3	85
	Med	5	56.3	3.98	7.1	67.3	3.84	5.7	84
	High	5	338	19.4	5.8	472	11.0	2.3	72

[a]MD indicates data from the method developer laboratory and IL indicates data from the independent laboratory.

[b]LC-MS/MS was performed only by the independent laboratory.

[c]ND = Not determined. These results are below the limit of quantitation.

[d]ELISA data set Low1 included a statistical outlier by the Grubbs test. The outlier was replaced with a new extracted test portion and reported as Low2. There was no assignable cause to remove the outlier, so both sets of data are presented.

[e]Bkgd = Incurred matrixes with very low background glyphosate or other interference.

Table 5. LOQ validation using low concentration incurred residue matrixes (1)

Matrix	LOQ_{est}, µg/kg	n	Mean Result, µg/kg	s_r, µg/kg	RSD_r, %
Durum wheat	14	10	14.3	2.31	16.1
Whole oats	18	20/20[a]	20.0	7.66	38.3
Whole oats	18	19/20[a]	18.4	2.96	16.1
Oat groats	14	10	23.0	3.90	17.0
Dried yellow peas	13	10	12.1	1.88	15.5
Dried red lentils	23	10	14.9	2.92	19.6

[a]outlier detected; data reported with and without outlier

REFERENCES CITED

1. Yearwood, G., Validation of the Eurofins Abraxis Enzyme-Linked Immunosorbent Assay for the Determination of Glyphosate in Grains and Pulses, AOAC *Performance Tested Methods*^{SM} certification number 072104.
2. *Standard Method Performance Requirements (SMPRs) for Glyphosate, its Metabolites, and Trimesium in Fruits and Vegetables, Cereals, Food of Animal Origin, Pet Food and Baby Food* (Draft, 2021) https://www.aoac.org/wp-content/uploads/2021/03/SMPR_Gylphosate_USP_v11.pdf, accessed April 29, 2021.