



Glyphosate ELISA Test Kit Validation



Validations have been completed according to AOAC protocols and guidelines to ensure that Eurofins Abraxis' Glyphosate ELISA 96 well test kits are accurate, specific, reproducible and rugged. Test kit validation provides an assurance of reliability during normal use, and is the process of providing documented evidence that the method does what it is intended to do. The performance results below demonstrate:

- Lot-to-lot reproducibility
- Overall precision
- Correlation to traditional analytical (LC-MS/MS) methods
- Sensitivity/Specificity

Glyphosate Test Method

Enzyme-Linked Immunosorbent Assay (ELISA) 96 well plate kit

Sample Matrices

For the performance validation study, extracts from various sample matrices were evaluated for glyphosate using the Eurofins Abraxis Glyphosate ELISA test kits.

- Whole grain durum wheat
- Whole grain oats
- Groats
- Dried yellow peas
- Dried red lentils

Glyphosate ELISA Test Validation

Lot-to-Lot Reproducibility

Lot-to-lot reproducibility is a frequent challenge that limits a user or laboratory’s ability to produce consistent results over time. Assuring lot-to-lot consistency is important to a successful testing program.

Study: A commercially available, incurred (naturally glyphosate positive) whole oat sample was extracted and tested alongside glyphosate standards and controls to evaluate product consistency through quantitation in three different ELISA test kit lots. All extracted samples, standards and controls were analyzed in duplicate following derivatization per kit instructions.

Results:

Product consistency results for ground incurred whole oats

Lot	N	Mean, $\mu\text{g/kg}$	s_r	95% CI ^a
C3	2	200.0	20.2	(180, 220)
C4	2	196.60	15.7	(177,216)
C5	2	210.18	15.6	(191, 230)

^a95% CI is based on a pooled s_r of 17.3.

Analysis of Variance of product consistency results

Source	DF	Adj SS	Adj MS	F-Value	P-value
Factor	2	399.2	199.6	0.67	0.537
Error	9	2697.1	299.7		
TOTAL	11	3096.3			

- 95% confidence level for the mean result from each lot overlap
- No statistically significant differences at the 95% level among lots as shown by the p-value >0.05

Precision Study

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility.

Study: Four 1 kg laboratory samples were weighed from a 6 kg primary sample. Each laboratory sample (A, B, C, D) was spread out on a tray and six 5 g analytical samples were taken from each laboratory sample with a square scoop then ground as per the ELISA method. Each analytical sample was then extracted, derivatized and run in duplicate following the test kit procedure.

Results:

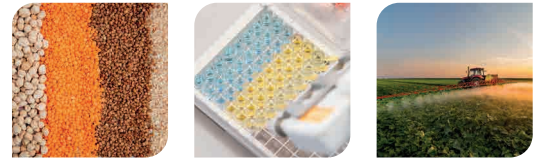
SUMMARY

Groups	Count	Sum	Average	Variance
A	12	13.948	1.162333	0.013239
B	12	14.571	1.21425	0.028859
C	12	12.79	1.065833	0.011813
D	12	14.196	1.183	0.024617

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.1475	3	0.049167	2.504431	0.071464	2.816466
Within Groups	0.863805	44	0.019632			
TOTAL	0.011305	47				

- ANOVA demonstrated no significant difference between the means of the analytical samples ($F < F_{crit}$, $p > 0.05$).



LC-MS/MS Correlation

The aim of the correlation study is to assess the closeness of agreement between results of the ELISA Kit and LC-MS/MS methods for determination of glyphosate in grains.

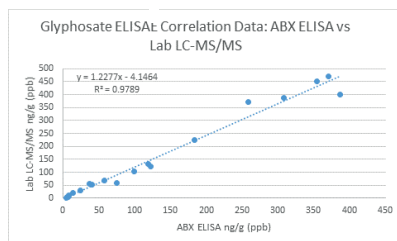
Study: To evaluate relative recovery of the Glyphosate ELISA Test Kit versus LC-MS/MS analysis, a blind test was designed and executed with an independent commercial laboratory (not linked to the Eurofins group) that included durum wheat, oats, groats, dried peas and dried lentil samples.

Matrix study results

Matrix ^a	Level	n	ELISA			LC-MS/MS			
			Mean (µg/kg)	s_n µg/kg	RSD _n %	Mean (µg/kg)	s_n µg/kg	RSD _n %	Relative Recovery, %
Durum wheat (MD)	Bkgd	5	6.26	1.27	20	5.25	0.293	5.6	ND ^a
	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
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
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
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 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

^aLC-MS/MS was performed only by the independent laboratory.

^aND = Not determined. These results are below the limit of quantification.



Results: The data showed good correlation with the LC/MS/MS (n = 100) performance. The relative recoveries % (the % of the ELISA mean results vs the LC-MS/MS mean results) shown in the data table above for all samples above the test limit of quantification (LOQ) meet our performance acceptance criteria.

Glyphosate ELISA Test Validation

Using the Correlation study data, validated LOQ values were then determined by mixing incurred residue matrix with blank background matrix to approximate these concentrations. Incurred and blank background whole grains and pulses were mixed well and ≥ 500 g was ground according to the candidate method. Ten replicate test portions (20 replicate test portions for whole oats) were extracted, derivatized, and analyzed in duplicate by the ELISA method. For the whole oats, there was one obvious outlier that was removed.

LOQ validation using low concentration incurred residue matrixes

Matrix	LOQ _{est} , µg/kg	n	Mean Result, (µg/kg)	s _r , ng/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 Red Lentils	13	10	14.9	1.88	15.5
Dried Yellow Peas	13	10	12.1	2.92	19.6

^aoutlier detected; data reported with and without outlier

Test Sensitivity / Specificity

Eurofins Abraxis defines limit of detection (LOD) as the concentration at 90% B/B0 converted to a matrix concentration. For this ELISA kit, that LOD is calculated at 5 ppb for these matrixes. The estimated limit of quantification (LOQ_{est}), the concentration at which a sample can be consistently quantitated at the lower limit, under AOAC guidelines for 20% precision, is calculated as $LOD + 7s_r$ where s_r is the standard deviation of the background matrix so that the signal to noise ratio is approximately 10:1. This ELISA test range is determined by the lowest and highest standard concentrations within the kit taking into the dilution factor for the matrixes.

Matrix	Test Range (ppb)	Limits of Quantification (ppb)
Durum Wheat	7.5 - 400	14 - 400
Whole oats	7.5 - 400	18 - 400
Oat groats	7.5 - 400	23 - 400
Dried Red Lentils	7.5 - 400	15 - 400
Dried Yellow Peas	7.5 - 400	12 - 400

Specificity: The cross-reactivity of the Glyphosate ELISA to potentially interfering compounds is as follows. Relative recovery and bias were calculated for each spiked test portion in comparison to the unspiked test portion (110 µg/kg). Relative recovery ranged from 90 to 113% and bias ranged from -11 to 15 µg/kg.

Potential Interferent	Concentration µg/kg	ELISA result, µg/kg	Relative Recovery, %	Bias, µg/kg
None ^a	-	110	-	-
Aminomethyl phosphonic acid (AMPA)	100	121	110	11
Dicamba	10,000	103	94	-6.6
Glufosinate	100	113	103	3.0
Glyphosine	50	105	96	-4.8
Glycine	100	99.2	90	-11
Atrazine	10,000	112	102	2.4
2,4-Dichlorophenoxyacetic acid	10,000	116	105	5.9
CaCl ₂	20,000,000	125	113	15
FeSO ₄	500,000	114	104	4.2

^aincurred material with no interferent added.

Manual / Automated Test Procedure

Glyphosate ELISA tests can be run manually with laboratory equipment that includes pipettors and a microplate reader. The test method can also be automated with the BOLT or ThunderBolt automated ELISA analyzers. These analyzers will add and dispense reagents, wash, incubate, shake and interpret results with a built-in microplate reader displaying them on an on-board computer. Contact Eurofins Abraxis for information on automated analyzers.

Part Number	Product Description
500089	Glyphosate ELISA AOAC Test Kit for Durum Wheat, Whole Oats, Groats, Yellow Peas & Red Lentils (includes the Derivation Kit and extraction supplies)