ELISA TEST

Rapid ELISA test kit for the quantitation of glyphosate in durum wheat samples

Eurofins Abraxis' Glyphosate ELISA test kit

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Glyphosate or N-(phosphonomethyl) glycine (See Figure 1) is the world's most widely used broad-spectrum herbicide and crop desiccant, accounting for about 25 percent of the global herbicide market.

An organophosporous compound (phosphonate), it was first discovered to be an herbicide by Monsanto and introduced into the market in 1974 under the trade name 'Roundup'. Glyphosate is sometimes applied on barley, wheat and other crops as a pre-harvest drying agent to speed up harvesting operations.

While glyphosate formulations such as Roundup have been approved by regulatory bodies worldwide, concerns about their effects on humans and the environment persist. Contradictory findings on carcinogenic risks have thrust glyphosate into the center of dispute between EU and US politicians, regulators and researchers.

In March 2015 the World Health Organization (WHO) International Agency for Research on Cancer classified glyphosate as "probably carcinogenic in humans" (category 2A) based on epidemiological, animal and in vitro studies. In November 2015, however, the European Food Safety Authority (EFSA) published a report concluding that "glyphosate was unlikely to be genotoxic or pose a carcinogenic threat to humans".

Amidst this contradictory information, in June 2016, the European Commission could not agree on re-registration of glyphosate for another 15 years. Instead, it granted a temporary license extension pending further scientific studies. As the scientific debate continues, consumer concerns about glyphosate in the food chain grow. The EPA (40 CFR Part 180) has established tolerances for various commodities, grain cereals: oatmeal, wheat and barley at 30 ppm or 30,000 ng/gm but organic standards for some commodities are as low as 10 ppb (10 ng/gm).

Glyphosate analysis in environmental matrixes is often problematic because it is a small molecule with structural similarity to many naturally occurring plant materials such as amino acids and secondary plant compounds. Its high-water solubility, makes solvent extraction difficult, posing a serious challenge to chemists needing to remove matrix effects prior to instrumental analysis. In addition, plant and soil matrixes contain co-contaminants that increase complexity of sample preparation and make instrumental analysis costlier and more time-consuming.

Enzyme-linked immunoassay (ELISA) is an analytical

Table 1: Evaluation of Glyphosate ELISA reproducibility

Sample #	Extract 1 Mean ELISA concentration (ng/gm)	Extract 2 Mean ELISA concentration (ng/gm)	Extract 3 Mean ELISA concentration (ng/gm)	Average ELISA concentration (ng/gm)	Stdev	%CV
1	640	603	570	604	35.0	5.8
2	15	8	14	12	3.4	27.4
3	18	19	20	19	1.1	5.9
4	22	22	19	21	1.3	6.4
5	8	<7.5	<7.5	<7.5	0.7	9.2
6	26	29	29	28	1.8	6.5
7	202	182	234	206	26.3	12.7
8	18	22	20	20	2.0	9.9
9	846	656	714	739	97.4	13.2
10	787	646	731	721	71.0	9.8
11	48	46	47	47	1.1	2.3
12	14	17	19	17	2.7	16.2

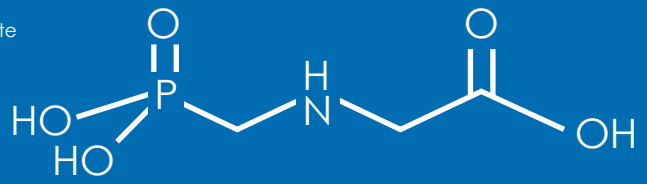
Table 2: ELISA run-to-run correlation data

Sample	Mean ELISA concentration Run 1(ppb)	Mean ELISA concentration Run 2 (ppb)
1	640	603
2	15	8
3	18	19
4	22	22
5	8	6
6	26	29
7	202	182
8	18	22
9	846	656
10	787	646
11	48	46
12	14	17

Table 3: Spike recoveries at 0.5 ng/gm glyphosate equivalent
* This is the raw concentration from the ELISA plate analysis of the sample extract prior to application of the dilution factor (100x) associated with the sample extraction procedure. Calculated values for the raw wheat sample are 100x higher

Sample #	Mean ELISA concentration of sample extract (ng/gm)*	Mean ELISA concentration of spiked sample (ng/gm)	Difference	Spiked concentration (ng/gm)	% Recovery
4	0.125	0.701	0.486	0.5	97.2%
5	<0.075	0.518	0.518	0.5	103.6%
Blank	<0.075	0.516	0.516	0.5	103.2%

Figure 1: Glyphosate structure



technique that has been widely employed for decades across a variety of clinical and industrial applications ranging from blood, water, mycotoxin, pathogens, etc., to provide near real time answers regarding contaminant concentrations in numerous sample matrices in a timely, accurate and cost-effective manner.

This study evaluates the Eurofins Abraxis Glyphosate ELISA test kit (Part #500086, see Figure 2), a commercially available ELISA test kit for the quantitative analysis of glyphosate, as a means of obtaining sample results in three-to-four hours to support on-site decision-making for agricultural testing applications.

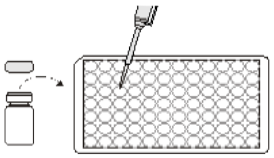
Reproducibility

Twelve raw durum wheat samples with unknown concentrations of glyphosate were obtained for this study. A 0.5 g subsample of each raw durum sample was ground and extracted in 10 mL deionized water a total of three times according to the protocol outlined in the Eurofins Abraxis Technical Bulletin, "Glyphosate in Oats, Wheat and Barley".

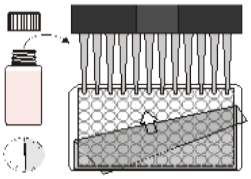
Duplicates of all sample extracts, standards and controls were subsequently derivatized and analyzed per test kit instructions on a single lot of the Glyphosate ELISA plates (Part #500086) for a total of 72 determinations. Data is presented in Table 1.

Figure 2: Glyphosate ELISA test procedure

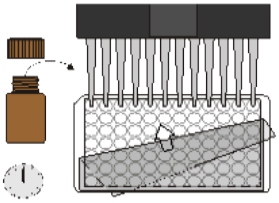
1: Addition of Standards, Samples
Add 50uL of the derivatised standard solutions, control, or samples.



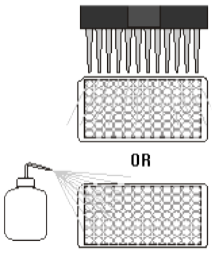
2: Addition of Antibody Solution
Add 50uL of the anti-Glyphosate Antibody solution. Cover and mix for 30 seconds. Incubate for 30 minutes at room temperature.

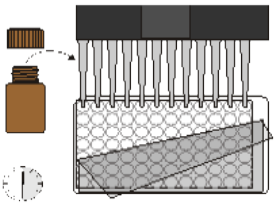


3: Addition of Enzyme Conjugate
Add 50uL of the enzyme conjugate. Cover and mix for 30 seconds. Incubate for 60 minutes at room temperature.

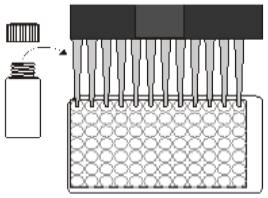


4: Washing of Plates
Wash the plates three times with 250uL of 1x washing buffer.

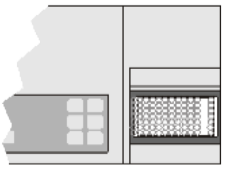




5: Addition of Substrate/Colour Solution
Add 150uL of substrate/colour solution. Incubate the strips for 20-30 minutes at room temperature and away from direct sunlight.



6: Addition of Stopping Solution
Add 100uL of stop solution.

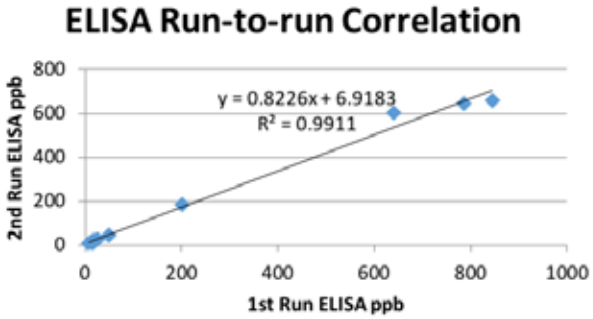


7: Measurement of Colour
Read the absorbance at 450nm using a microplate ELISA reader. Calculate the results.

Only one sample, #5, was determined to have glyphosate concentration below detectable assay limits (<7.5 ng/gm). All other samples were determined to have glyphosate concentrations ranging between 12 and 739 ng/gm. The %CV between the three extracts for each sample ranged from 2.3 percent to 27.4 percent. Only one of the twelve samples displayed a %CV above 20 percent across the three extracts, indicating excellent assay reproducibility.

Data plotted between two separate ELISA analyses of durum wheat sample extracts from the same raw durum sample (see Table 2), demonstrated excellent correlation with one another as indicated in Figure 3, the r2 value between the two analyses was 0.991.

Figure 3: Glyphosate ELISA run-to-run correlation graph



Spike recovery

Duplicates of durum wheat sample extracts previously determined by ELISA to have a glyphosate concentration equivalent below detectable assay limits (<7.5 ng/gm) were spiked with a known quantity of glyphosate (0.5 ng/gm equivalent).

Duplicates of another raw durum wheat sample previously determined to have a glyphosate concentration equivalent of 21.5 ng/gm by ELISA were also spiked with 0.5 ng/gm of glyphosate. A 0.5 ng/mL spike check was also prepared with deionized water (LOQ in water = 0.05 ng/mL) to evaluate any potential matrix effects on sample recovery.

All spiked sample extracts, deionized water spike checks, standards and controls were subsequently derivatized and analyzed in duplicate as described in the Eurofins Abraxis Technical Bulletin, "Glyphosate in Oats, Wheat and Barley" and Glyphosate Test Kit User's Guide.

As indicated in Table 3, the mean concentration of the spiked extracts was determined to be 0.486 and 0.518 ng/gm respectively. Comparing the concentration of the ground and extracted sample to the spiked concentration of 0.5 ng/gm glyphosate equivalent, sample recovery was determined to be 97-103 percent.

Conclusion

The Eurofins Abraxis Glyphosate ELISA test kit is capable of analyzing durum wheat samples for glyphosate concentrations. Evaluation of the test kits for this application suggests that data is both reproducible and accurate. The %CV was less than 20 percent, across three sample extracts of the same durum sample prepared and analyzed separately according to kit instructions. Correlation between ELISA runs were greater than 0.990. Likewise, the assay demonstrated good recovery of spiked samples with percent recovery ranging from 97 percent to 103 percent.

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