Glyphosate in Matrix
ELISA General Validation Procedure for Solids or Liquids

1. Intended Use

For the detection of Glyphosate in matrix.

2. Materials and Reagents Required

Analytical balance
40 mL glass vials with Teflon-lined caps
4 mL glass vials with Teflon-lined caps
25mL graduated cylinder or disposable pipettes
Micropipettes with disposable plastic tips
Vortex mixer
Shaker or vial holder with insert retainer for vortex mixer
Centrifuge
Timer
ABRAXIS® Glyphosate Sample Diluent (PN 500082)
ABRAXIS® Glyphosate Spiking Solution, 10 ppm; 1 mL (PN 301358)
ABRAXIS® Glyphosate Plate ELISA Kit (PN 500205)

3. Notes and Precautions

- Some matrices may need extraction buffers such as Sodium hydroxide, Hydrochloric acid or Deionized water. If using sodium hydroxide or Hydrochloric acid, handle with care. Wear appropriate protective clothing (gloves, glasses, etc.). Avoid contact with skin and mucous membranes. If contact occurs, wash with copious amounts of water and seek appropriate medical attention.
- It is optional to use a plate shaker or vortex mixer fitted with a micro-well plate holder adapter for the incubations with the antibody and conjugate solutions. This will allow for the appropriate mixing of all reagents in the microtiter wells.
- For testing it is recommended that 20 wells (10 replicates) be used for known Blank and Low spiked samples, 10 wells (5 replicates) for Medium and High spiked samples.
- This procedure is for research use only. It is not intended for diagnostic procedures.

4. Procedure for Solids

4.1. Weigh 1 gram of ground material (flour-like consistency, using food processor, Vitamix blender, IKA grinder, or mortar and pestle) – Optionally, this is where you can spike glyphosate
4.2. Add 20 mL diH2O (20-fold dilution)
4.3. Vortex to mix and then store on rotator for 10 minutes around 45 rpm
4.4. Let sample settle for at least two minutes to separate the particles
4.5. Remove 2 mL of extract to 2 mL microcentrifuge tube. Alternatively, syringe filter with Environmental Express 0.2 µm PES (PN SF020E).
4.6. Centrifuge for 5 minutes at 8100xg (if not using syringe filter)
4.7. Transfer the supernatant into a clean vial
4.8. Dilute 5-fold in Sample Diluent – 400 uL extract + 1600 uL Diluent – mix well – proceed to Derivatization (total dilution 100-fold – 7.5 ppb LOQ).

NOTE: To determine any matrix interferences, it is recommended that a 1:1 serial dilution in Glyphosate Sample Diluent (remove 1 mL of 100-fold extract to 1 mL of sample diluent = 200-fold; remove 1 mL of 200-fold extract to 1 mL of sample diluent = 400-fold, et al).

5. Procedure for Liquids

5.1. Pipette 400 µL of the liquid sample to appropriately labeled test tube and dilute with 1600 µL of ABRAXIS® Glyphosate Sample Diluent. Vortex 30 seconds to mix.
5.2. If particles are present, remove 2 mL of extract to 2 mL microcentrifuge tube. Alternatively, syringe filter with Environmental Express 0.2 µm PES (PN SF020E).
5.3. Centrifuge for 5 minutes at 8100xg (if not using syringe filter).
5.4. Transfer the supernatant into a clean vial.
5.5. This will then be analyzed as sample, see Derivatization of Standards, Control and Samples in the Test Preparation section of the ABRAXIS® Glyphosate Plate ELISA Kit user's guide. (total dilution 5-fold – 0.375 ppb LOQ).

NOTE: To determine any matrix interferences, it is recommended that a 1:1 serial dilution in Glyphosate Sample Diluent (remove 1 mL of 5-fold extract to 1 mL of sample diluent = 10-fold; remove 1 mL of 10-fold extract to 1 mL of sample diluent = 20-fold, et al).

6. Evaluation of Results

The Glyphosate concentration in the samples is determined by multiplying the ELISA results by the dilution factor. Sample extracts showing a concentration lower than standard 1 (0.075 ppb) should be reported as containing less than the concentration multiplied by the dilution factor of Glyphosate. Samples showing a higher concentration than standard 5 (4.0 ppb) can be reported as containing greater than the concentration multiplied by the dilution factor of Glyphosate or diluted further and re-analyzed to obtain an accurate quantitative result.

7. For ordering or technical assistance contact:

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