Glyphosate in Green Coffee Bean
Sample Extraction

1. Intended Use
For the detection of Glyphosate in green coffee beans.

2. Sensitivity
10 ppb in matrix

3. Materials and Reagents Required
Glass vials – 4 mL and 20 mL with Teflon-lined caps
Conical Centrifuge Tubes – 15 mL or 50 mL
Deionized water
Serological pipettes, 5 mL or 10 mL
Microcentrifuge tubes, 2.0 mL
Microcentrifuge capable of 10,000 rpm,
Analytical balance
Weighing boats or equivalent
Spatula
Rotator and/or shaker
Vortex mixer
Hammer and zip-lock bags (4 MIL heavy duty)
Coffee grinder, blender/food processor, or equivalent
Sieve or mesh filter, 500 microns (PN 500103) or equivalent
ABRAXIS® Glyphosate Sample Diluent (PN 500082)
ABRAXIS® Glyphosate ELISA kit (PN 500205)

4. Notes and Precautions
This procedure is intended for use with green coffee bean samples. Other matrices should be thoroughly validated before use with this procedure.

- It is highly recommended to add the appropriate amount of green coffee beans into a 4 MIL heavy-duty bag and homogenized/pulverized with hammer, and then finely ground with a coffee grinder, blender, or equivalent. The ground coffee beans should then be passed through a sieve or mesh filter of 500-micron pore size before extraction to produce accurate results.
- To minimize the potential for sample contamination due to carryover from the preparation of highly contaminated samples, the blender jar should be thoroughly washed and dried between samples.
- Due to the viscous nature of the sample extracts, if possible, the microtiter plate should be placed on a plate shaker or vortex mixer fitted with a micro-well plate holder adapter for the incubations with the antibody and conjugate solutions (Steps F.2 and F.3 in the ABRAXIS® Glyphosate ELISA user's guide). This will allow for the appropriate mixing of all reagents in the microtiter wells.
- Assay should be performed with ABRAXIS® ELISA kit as soon after extraction as possible. Samples should not sit more than one day in plastic microcentrifuge tubes before being run with the ELISA kit.
- This procedure is for research use only. It is not intended for diagnostic procedures.

5. Extraction Procedure
5.1 Grind sample using a grinder, blender or equivalent.
5.2 Pass the ground sample through a sieve or mesh filter (500-micron pore size) and collect in a disposable weighing boat. If more sample size is needed, repeat steps 5.1 and 5.2.
5.3 Weigh 0.5 g of finely ground sample into a 15 or 50 mL conical centrifuge tube, or 20 mL glass vial.

5.4 Add 10 mL of deionized water to sample (20-fold dilution).
5.5 Vortex vigorously for 10 – 15 seconds. Place sample on rotator or shaker at 40 rpm for 10 minutes.
5.6 After mixing, let sample settle for at least 2 minutes. Transfer 1.5 mL of extracted sample to a clean, appropriately labeled 2.0 mL microcentrifuge tube.
5.7 Centrifuge tube at 8100 x g for 5 minutes. Make sure the centrifuge is properly balanced.
5.8 Add 850 µL of ABRAXIS® Glyphosate Sample Diluent to an appropriately labeled 4 mL glass vial. Add 150 µLof the supernatant (from 5.8) to the ABRAXIS® Glyphosate Diluent in the vial (1:6.67 dilution) and vortex or mixfor 15 seconds.
5.9 Derivatize the sample according to Section D Test Preparation in step 7 of *Derivatization of Standards, Control and Samples* instructions of the ABRAXIS® ELISA kit.
5.10 Perform assay as noted in Section F Assay Procedure instructions provided in the kit.

6. Evaluation of Results

The ELISA results must be multiplied by a factor of 133.3 to account for the necessary dilution. Samples showing a concentration lower than Standard 1 (0.075 ppb) should be reported as < 10 ppb of Glyphosate. Highly contaminated samples (those outside of the calibration range of the assay) must be diluted and re-analyzed to obtain an accurate quantitative result.

7. For ordering or technical assistance contact:

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