

# Glyphosate in Legumes, Grains and Seeds Sample Preparation

#### 1. Intended Use

For the detection of Glyphosate in dried lentils, white beans, soybeans, corn, malt barley and sesame seeds.

## 2. Sensitivity

0.075 ppm in matrix.

## 3. Materials and Reagents Required

Analytical balance
20 mL glass vials with Teflon-lined caps
Microcentrifuge tubes
4 mL glass vials with Teflon-lined caps
Disposable pipettes
Scoopula

Micropipettes with disposable plastic tips

Vortex mixer

Microcentrifuge

Timer

Plate shaker or Micro-well plate holder with insert retainer for vortex mixer

1 N Hydrochloric Acid (HCl)

ABRAXIS® Glyphosate Sample Diluent (PN 500082)

ABRAXIS® Glyphosate Plate ELISA Kit (PN 500205)

## 4. Notes and Precautions

This procedure is intended for use with dried lentils, white beans, soybeans, corn, malt barley and sesame seeds. Othermatrices should be thoroughly validated before use with this procedure.

- Samples must be ground before extraction.
- Hydrochloric Acid must be handled 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.
- Due to the viscous nature of the sample extracts, 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. This will allowfor the appropriate mixing of all reagents in the microtiter wells.

## 5. Procedure

- 5.1 Weigh 1 g of ground sample into an appropriately labeled 20 mL glass vial.
- 5.2 Add 10 mL of 1 N HCl. Vortex for 2 minutes.
- 5.3 Allow the sample to separate for 2 minutes.
- 5.4 Pipette approximately 1 mL of the supernatant into an appropriately labeled microcentrifuge tube.
- 5.5 Centrifuge at 6000 rpm for 5 minutes.
- 5.6 Pipette the supernatant into an appropriately labeled 4 mL glass vial with a Teflon-lined cap.

Note: While most sample matrices will separate into two distinct layers, a solid bottom layer and a liquid supernatant, some sample matrices may produce a thin third layer on top of the liquid supernatant. Pipette only the liquid supernatant portion into the 4 mL glass vial.

5.7 Add 3.96 mL of ABRAXIS® Glyphosate Diluent to a clean, appropriately labeled 4 mL glass vial. Add 40 µL of the supernatant (from step 5.6) to the ABRAXIS® Glyphosate Diluent in the vial (1:100 sample dilution).

Vortex. 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.

#### 6. Evaluation of Results

The Glyphosate concentration in the samples is determined by multiplying the ELISA results by a factor of 1000. Sample extracts showing a concentration lower than standard 1 (0.075 ppb) should be reported as containing < 0.075 ppm of Glyphosate. Samples showing a higher concentration than standard 5 (4.0 ppb) can be reported ascontaining > 4 ppm of Glyphosate or diluted further and re-analyzed to obtain an accurate quantitative result.

#### 7. Performance Data

Recovery

- Lentil samples were spiked with various amounts of Glyphosate, extracted as described above, and then derivatized and assayed using the ABRAXIS® Glyphosate Plate ELISA. Average recovery was 84%.
- White beans contaminated with Glyphosate were analyzed using both instrumental analysis and the ABRAXIS® Glyphosate Plate ELISA. Instrumental analysis showed a concentration of 1.85 ppm. ELISA analysis showed a concentration of 2.45 ppm. White bean samples were also spiked with various amounts of Glyphosate, extracted as described above, and then derivatized and assayed using the ABRAXIS® Glyphosate Plate ELISA. Average recovery was 106%.
- Soybean samples were spiked with various amounts of Glyphosate, extracted as described above, and then derivatized and assayed using the ABRAXIS® Glyphosate Plate ELISA. Average recovery was 88%.
- Corn samples were spiked with various amounts of Glyphosate, extracted as described above, and then derivatized and assayed using the ABRAXIS® Glyphosate Plate ELISA. Average recovery was 119%.
- Malt Barley contaminated with Glyphosate was analyzed using both instrumental analysis and the ABRAXIS® Glyphosate Plate ELISA. Instrumental analysis showed a concentration of 0.54 ppm. ELISA analysis showed a concentration of 0.59 ppm.
- Sesame seed samples were spiked with Glyphosate, extracted as described above, and then derivatized and assayed using the ABRAXIS® Glyphosate Plate ELISA. Average recovery was 117%.

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