Glyphosate in Milk
Sample Preparation

1. Intended Use
For the detection of Glyphosate in milk (whole, reduced fat, and soy)

2. Sensitivity
0.075 ppm in matrix

3. Materials and Reagents Required
Microcentrifuge tubes
4 mL glass vials with Teflon-lined caps
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 milk samples (whole, reduced fat, and soy). Other matrices should be thoroughlyvalidated before use with this procedure.

- 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.
- The microtiter plate should be placed on a plate shaker or vortex mixer fitted with a micro-well plate holder adapter for theincubations with the antibody and conjugate solutions. This will allow for the appropriate mixing of all reagents in the microtiter wells.

5. Procedure
5.1 Add 100 µL of milk sample to an appropriately labeled microcentrifuge tube.
5.2 Add 900 µL of 1 N HCl. Vortex for 2 minutes.
5.3 Centrifuge at 6000 rpm for 5 minutes. The sample will separate into two or three layers, depending on the fat content of the sample: a white precipitate (bottom layer), a clear liquid supernatant, and possibly a thinwhite film (on top of the liquid layer).
5.4 Pipette the clear liquid supernatant into a clean, appropriately labeled 4 mL glass vial. Avoid pipetting any of the white film layer into the vial.
5.5 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 5.4) 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. Sampleextracts 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 as containing > 4 ppm of Glyphosate or diluted further and re-analyzed to obtain an accurate quantitative result.

7. Performance Data

Recovery

Whole, reduced fat, and soymilk 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 121.7%.

8. For ordering or technical assistance contact:

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Date this Technical Bulletin is effective: 28FEB2022 Version: 01