Glyphosate in Serum
Sample Preparation

1. Intended Use
For the detection of Glyphosate in serum.

2. Sensitivity
0.25 ppb in matrix

3. Materials and Reagents Required
Millipore Amicon Ultra 0.5mL 10k centrifugal filter units
Micropipettes with disposable plastic tips
Microcentrifuge tubes
Microcentrifuge
Ethyl acetate
Vortex mixer
ABRAXIS® Glyphosate Plate ELISA Kit (PN 500205)

4. Notes and Precautions
This procedure is intended for use with human serum. Other matrices should be thoroughly validated before use with thisprocedure.

- Ethyl acetate must be handled with care. Wear appropriate protective clothing (gloves, glasses, etc.). Avoid contact withskin and mucous membranes. If contact occurs, wash with copious amounts of water and seek appropriate medical attention. When pouring off ethyl acetate for initial use, do so under a fume hood or a well ventilated area. If a spill occurs,use paper towels and place them in a Ziploc bag before disposal.
- Analysis should be performed with ABRAXIS® Glyphosate Plate ELISA Kit as soon as possible after extraction. Samples should not sit more than one day in plastic microcentrifuge tubes before being analyzed.
- This procedure is for research use only. It is not intended for diagnostic procedures.

5. Procedure
5.1 Place a filter from the Millipore Amicon Ultra kit into an appropriately labeled 2 mL microcentrifuge tube and pipette 500 µL of serum sample into the filter.

Note: If the serum sample being tested is particularly turbid/cloudy, it is advised to aliquot 500 µL of thesample into two separate filters to ensure that enough supernatant is obtained for subsequent steps.

5.2 Centrifuge the sample at 8,000 x g for 15 minutes.
5.3 Transfer 300 µL of the supernatant to an appropriately labeled microcentrifuge tube.
5.4 Add 200 µL of ethyl acetate to the microcentrifuge tube.
5.5 Vortex for 30 seconds.
5.6 Centrifuge for 3 minutes at 8000 x g.
5.7 Transfer the bottom aqueous phase into a new appropriately labeled microcentrifuge tube.
5.8 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.

Note: As 250 µL of sample may not be obtained from step 5.7 above, it is advisable to perform the derivatization with half the volume of each reagent as noted in steps 7c, 7d, and 7e of the Derivatization of

Standards, Control and Samples instructions. For example, use 125 µL of sample/standard/control, 500 µL of assay buffer and 50 µL of the diluted derivatization reagent. Step 7a remains the same; dilute the ABRAXIS® Derivatization Reagent with 3.5 mL of ABRAXIS® Derivatization Reagent Diluent.

6. Evaluation of Results

Sample extracts showing a ***concentration lower than 0.25 ppb should be reported as containing < 0.25 ppb*** of Glyphosate(please note that the limit of detection is greater than the first standard, 0.075 ppb). Samples showing a higher concentration than standard 5 (4.0 ppb) can be reported as containing > 4 ppb of Glyphosate or diluted with ABRAXIS® Glyphosate Sample Diluent (provided in the ELISA kit) and re-analyzed to obtain an accurate quantitative result.

7. Performance Data

Recovery

Serum samples were spiked with various amounts of Glyphosate, extracted as described above, and then derivatized andassayed using the ABRAXIS® Glyphosate Plate ELISA. Average recovery was 91%.

8. For ordering or technical assistance contact:

Gold Standard Diagnostics
795 Horsham Road
Horsham, PA 19044
WEB: www.abraxiskits.com

Phone: (215) 357 3911
Fax: (215) 357 5232
Ordering: info.abraxis@us.goldstandarddiagnostics.com
Technical Support: support.abraxis@us.goldstandarddiagnostics.com

Date this Technical Bulletin is effective: 05/16/2024 Version: 01