Cylindrospermopsin in Human Urine
Sample Preparation

1. **Intended Use:**
   For the detection of Cylindrospermopsin in human urine

2. **Range of Detection:**
   The range of detection is 0.05 ppb to 2 ppb in matrix. If samples exceed calibration, are known to contain higher analyte levels, or ahigher detection range is necessary, samples should be diluted prior to analysis.

3. **Materials Required:**
   15 mL polypropylene tubes
   0.1 M Sodium Hydroxide
   Centrifuge capable of 1200 x g
   0.1 M Phosphate buffer (pH 7)
   Bulk graphitized carbon black (UCT PN UCT-CUCARB00X)
   5.5 mL bed area capacity empty column with frit (Environmental Express PN R1010)
   Vacuum manifold (no pump required; elution by gravity only)
   Disposable flow control valve liners for vacuum manifold
   Methanol
   Deionized (DI) distilled water
   Trifluoroacetic acid
   Nitrogen evaporator
   Nitrogen gas
   3 mL transfer pipettes
   20 200 µL micropipette and tips
   1-5 mL pipette and tips
   pH paper or meter
   Weigh boats
   Scale capable of three decimal places
   60 mL glass collection vials with caps
   20 mL vials for collecting eluted sample
   ABRAXIS® Cylindrospermopsin plate ELISA kit (PN 522011)

4. **Notes and Precautions**
   4.1 Before dispensing any volume of liquid, condition each pipette tip by drawing the liquid in and out of the tip 3 times beforethe final dispense. This will ensure that an accurate volume is transferred.
   4.2 Trifluoroacetic acid is extremely hazardous and great care must be taken while handling it. Use proper PPE and dispenseunder a hood or well-ventilated area.
   4.3 When adding liquid to the prepared column, allow the graphitized carbon black to settle back into the bottom of the columnbefore allowing liquid to drain through column.
   4.4 All column steps use gravity flow at approximately 1 drop per second.

5. **Column Preparation**
   5.1 Weigh out 150 mg of graphitized carbon black.
   5.2 Carefully pour graphitized carbon black into empty column.
   5.3 To condition, add 5 mL of 100% methanol. Note: allow carbon to settle at the bottom of the

column before allowing ethanol to drain through column (see Note 4.3 above).

5.4 Drain and discard methanol from column.
5.5 Repeat steps 5.3 to 5.4.
5.6 Add 5 mL DI water to column. Note: allow carbon to settle at the bottom of the column before allowing methanol to drainthrough column (see Note 4.3 above).
5.7 Drain and discard the water.
5.8 Repeat Steps 5.6 to 5.7.
5.9 Column is conditioned and ready to use. Continue to Section 6: Procedure.

6. Procedure

6.1 Pipette 2 mL of urine sample into a 15 mL polypropylene tube.
6.2 Adjust to sample > pH 10 with 0.1 M sodium hydroxide, dropwise, with a transfer pipette.
6.3 Centrifuge sample at 1200 x *g* for 10 minutes at room temperature.
6.4 Transfer the supernatant to a clean 15 mL polypropylene tube
6.5 Dilute sample to 3 mL with 0.1 M phosphate buffer solution.
6.6 Load sample onto column prepared and conditioned as described in Section 5 above. Discard flow through.
6.7 Rinse column with 2 mL of DI water. Discard water.
6.8 Elute cylindrospermopsin with 8 mL 0.1 % TFA in methanol (collect in a new elution vial).
6.9 Evaporate extract down to dryness with nitrogen at 60 °C.
6.10 Reconstitute dried extraction by adding 2 mL DI water and vortex thoroughly.
6.11 Analyze samples, as described in the ABRAXIS® Cylindrospermopsin ELISA user's guide.

7. Evaluation of Results

Sample extraction does not produce a net concentration or dilution of original sample, so no correction factor is required whendetermining final sample results in the assay.
Samples showing a concentration lower than standard 1 (0.05 ppb) should be reported as containing < 0.05 ppb of cylindrospermopsin. Samples showing a higher concentration than standard 5 (2.0 ppb) can be reported as containing > 2 ppb of cylindrospermopsin ordiluted further and re-analyzed to obtain an accurate quantitative result.

8. Performance Data

Recovery Urine samples were spiked with various amounts of cylindrospermopsin, prepared as described above, and then assayed using theCylindrospermopsin Plate Assay. Average recovery was 85%.

9. For ordering or technical assistance contact:

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