Caffeine in Soda Beverages
Sample Preparation

1. Intended Use
For the detection of Caffeine in soda beverages

2. Sensitivity
1.75 ppb (ng/mL) in soda beverages

3. Materials and Reagents Required
40 mL glass vials with Teflon-lined caps
4 mL glass vials with Teflon-lined caps
Disposable pipettes
Micropipettes with disposable plastic tips
Vortex mixer
Caffeine Sample Diluent (provided in ELISA kit, additional also available for purchase)
ABRAXIS® Caffeine Plate ELISA Kit (PN 515575)

4. Notes and Precautions
This procedure is intended for use with soda beverages. Other matrices should be thoroughly validated before use with this procedure. The following sample dilution procedure is recommended for caffeinated soda beverages to obtain results within the standard calibration curve. Non-caffeinated soda samples have shown no matrix interference at a 1:10 dilution (Sample Dilution Procedure, Step 5.3).

5. Sample Dilution Procedure
5.1 Pour about 20 mL of sample into an appropriately labeled 40 mL glass vial.

5.2 After the carbonation escapes and sample settles, cap tightly and shake briefly. Loosen cap and release pressure. Repeat a few times until no more pressure builds up and soda is "flat".

5.3 Add 900 µL of Sample Diluent to an appropriately labeled 4 mL glass vial. Add 100 µL of "flat" soda sample and vortex. This is a 1:10 dilution (dilution factor = 10). (Non-caffeinated soda samples have shown no matrix interference at this dilution.)

5.4 Add 990 µL of Sample Diluent to a different appropriately labeled 4 mL glass vial. Add 10 µL of the diluted sample from Step 5.3 and vortex. The sample is now diluted 1:1000 (dilution factor = 1000).

5.5 Depending on the assumed Caffeine concentration of the sample, the following dilutions are recommended:

5.5.1 Add 950 µL of Sample Diluent to a third appropriately labeled 4 mL glass vial. Add 50 µL of the diluted sample from Step 5.4 and vortex. Final dilution factor = 20,000.

5.5.2 Add 980 µL of Sample Diluent to a third appropriately labeled 4 mL glass vial. Add 20 µL of the diluted sample from Step 5.4 and vortex. Final dilution factor = 50,000.

5.5.3 Add 990 µL of Sample Diluent to a third appropriately labeled 4 mL glass vial. Add 10 µL of the diluted sample from Step 5.4 and vortex. Final dilution factor = 100,000. The diluted samples are now ready to be analyzed in the Caffeine ELISA Plate Kit (see Assay Procedure, Section F of the user's guide).

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6. Evaluation of Results

The Caffeine concentration in the samples is determined by multiplying the ELISA results by the appropriate dilution factorfor results in ppb (ng/mL). The minimum dilution required to eliminate matrix interference is 1:10 (dilution factor = 10). Samples (diluted 1:10) showing a concentration lower than standard 1 (0.175 ppb) should be reported as containing < 1.75ppb of Caffeine. Samples (diluted > 1:10) showing a concentration lower than standard 1 (0.175 ppb) should be re-analyzed at a lesser dilution. Samples showing a higher concentration than standard 5 (5.0 ppb) should be diluted further and re- analyzed to obtain an accurate quantitative result.

7. For ordering or technical assistance contact:

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