

## Caffeine in Decaffeinated Coffee

### 1. Intended Use

For the detection of caffeine in decaffeinated coffee

### 2. Sensitivity

Dependent on dilution factor (see table 5.2.1).

### 3. Materials and Reagents Required

Deionized or distilled water  
 20 mL glass vials with Teflon-lined caps  
 Micropipettes with disposable plastic tips  
 Serological pipettes  
 Vortex mixer  
 ABRAXIS® Caffeine Plate ELISA Kit (PN 515575)

### 4. Notes and Precautions

This procedure is intended for use with brewed, decaffeinated coffee. Other matrices should be thoroughly validated before use with this procedure.

### 5. Sample Preparation Procedure

5.1 Brew a 12-oz. cup of decaffeinated coffee.

5.2 Dilute the brewed coffee into deionized or distilled water per the chart below

**NOTE:** dilutions of 1:100 and 1:1,000 are generally insufficient to bring the caffeine concentration into the range of the calibration curve; quantitative results are most likely to be found with dilutions of 1:10,000 and higher.

Dilution Factor	Range of Quantification	Volumes
1:100	17.5 - 500 ppb	100 uL of coffee into 9.9 mL of DI water; vortex well
1:1,000	175 - 5,000 ppb	1 mL of <b>1:100</b> into 9 mL of DI water; vortex well
1:10,000	1.75 - 50 ppm	1 mL of <b>1:1,000</b> into 9 mL of DI water; vortex well
1:50,000	8.75 - 250 ppm	2 mL of <b>1:10,000</b> into 8 mL of DI water; vortex well
1:100,000	17.5 - 500 ppm	5 mL of <b>1:50,000</b> into 5 mL of DI water; vortex well

5.3 Analyze samples per the ABRAXIS® Caffeine ELISA user's guide.

### 6. Evaluation of Results

The caffeine concentration in samples is determined by multiplying the ELISA results by the dilution factor (see table 5.2.1). Samples showing a concentration lower than standard 1 (0.175 ppb) should be reported as containing “< 0.175 x [dilution factor]” of caffeine. Samples showing a higher concentration than standard 5 (5.0 ppb) can be reported as containing “> 5.0 x [dilution factor]” of caffeine, or diluted further and re-

analyzed to obtain an accurate quantitative result.

**7. For ordering or technical assistance contact:**

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