Melamine in White Chocolate
Sample Preparation

1. Intended Use
For the detection of Melamine in white chocolate. For yogurt, milk chocolate, powdered infant formula, powdered milk/milk solids or whole milk, please see the appropriate application bulletin.

2. Range of Detection
1,600-40,000 ng/mL (ppb). Samples with higher concentrations must be diluted further and re-analyzed.

3. Materials Required (Not Provided)
Pipettes capable of delivering 100 and 900 µL
Glass vials with Teflon lined caps
Sonicator or vortexer
10% MeOH/20 mM PBS, pH 7.2-7.4
ABRAXIS® Melamine ELISA Kit (PN 50005B)

4. Preparation of Solutions
10% MeOH/20 mM Phosphate Buffered Saline (PBS), pH 7.4: To 800 mL of distilled or deionized water, add: Sodium phosphate dibasic anhydrous 2.277 g; Sodium phosphate monobasic monohydrate 0.548 g; Sodium chloride 18.0 g, add 100 mL of methanol and then bring to 1 L with distilled or deionized water, pH 7.2-7.4.

5. Notes and Precautions
To eliminate matrix interference from white chocolate to be tested for the presence of Melamine, samples must be diluted in 10% MeOH/20 mM PBS. If sonicator or homogenizer is available, they should be used in place of vortex mixer.

6. Procedure
6.1. White chocolate sample (5 gm) should be weighed into a glass vial.
6.2. Add 10 mL of distilled water.
6.3. Vial is then warmed in a 46-60 ºC water bath to dissolve the chocolate.
6.4. Mix for about 30 seconds by shaking or vortexing.
6.5. Centrifuge at 3000 RPM for 10 minutes. Sample should separate into three layers.
6.6. Carefully remove a portion of the middle layer.
6.7. Dilute an aliquot of the middle layer (1:40) in 10% MeOH/20 mM PBS. For example, 50 µL of sample extract to 1.95 mL of 10% MeOH/20 mM PBS. Mix by vortexing or shaking for 15-30 seconds.
6.8. The sample is now ready to analyze according to the procedure described in the ABRAXIS® Melamine ELISA Kit package insert.

7. Evaluation of Results
Results obtained for white chocolate samples prepared as described above must be multiplied by a factor of 80 to account for the sample dilution. Only use results within the analytical range of the assay (20-500 ppb). Results lower than the lowest standard (20 ppb) should not be multiplied by a dilution factor and should not be reported as negative, but should be reported as < 1,600 ppb Melamine detected. Results above the highest standard must be diluted and re-analyzed.

8. Performance Data
The sample preparation procedure detailed above was used with white chocolate spiked with various amounts of Melamine. Recoveries were between 90-96%.

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9. **For ordering or technical assistance contact**

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Date this Technical Bulletin is effective: 26APR2022 Version: 01