Melamine in Milk Chocolate
Sample Preparation

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

2. Range of Detection
3,200-80,000 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 900µL
Glass vials with Teflon lined caps
Methanol
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
- This procedure was developed using milk chocolate candy bars only. Candy bars containing additional ingredients such as nuts, caramel, etc. has not been evaluated.
- To eliminate matrix interference from milk chocolate to be tested for the presence of Melamine, samples must be diluted in 10% MeOH/20 mM PBS.

6. Procedure
6.1 Milk 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. Mix for about 30 seconds by shaking.
6.4 Centrifuge at 3000 RPM for 10 minutes. Sample should separate into three layers
6.5 Carefully remove a portion of the middle layer.
6.6 Dilute an aliquot of the middle layer 1:80 in 10% MeOH/20 mM PBS. For example, 50 µL of sample extract to 4.0 mL of 10% MeOH/20 mM PBS. Mix by vortexing or shaking for 15-30 seconds.
6.7 The sample is now ready to analyze according to the procedure described in the ABRAXIS® Melamine Kit package insert.

7. Evaluation of Results
Results obtained for milk chocolate samples prepared as described above must be multiplied by a factor of 160 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 < 3,200 ppb Melamine detected. Results above the highest standard must be diluted and re-analyzed.

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8. Performance Data

The sample preparation procedure detailed above was used with milk chocolate spiked with various amounts of Melamine. Recoveries were between 83-105%.

9. For ordering or technical assistance contact

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