

## **Melamine in Whole Milk (Liquid) Sample Preparation**

### **Procedure 1 (To be used with defatted milk)**

#### **1. Intended Use**

For the detection of Melamine in pasteurized whole milk (liquid). For powdered infant formula or powdered milk/milk solids please see the appropriate application bulletin.

#### **2. Range of Detection**

200 - 5,000 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

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 whole milk (liquid) to be tested for the presence of Melamine, samples must be diluted in 10% MeOH/20 mM PBS.

#### **6. Procedure**

- 6.1. Milk samples should be diluted 1:10 in 10% MeOH/20 mM PBS. For example, adding 100 µL of milk to 900 µL of 10% MeOH/20 mM PBS.
- 6.2. 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 whole milk samples (liquid form) prepared as described above must be multiplied by a factor of IO 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 < 200ppb 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 whole milk spiked with various amounts of Melamine. Recoveries were between 70 - 92%

## **Procedure 2 (To be used with defatted or non-defatted milk)**

### **1. Range of Detection**

60 - 1,500 ng/mL (ppb). Samples with higher concentrations must be diluted further and re-analyzed.

### **2. Materials Required (Not Provided)**

Microcentrifuge capable of spinning at 10,000 - 13,000 x g

Microcentrifuge tubes

Pipettes capable of delivering 100 and 900 $\mu$ L

Glass vials with Teflon lined caps

Methanol

10% MeOH/20 mM PBS, pH 7.2-7.4

ABRAXIS<sup>®</sup> ELISA Kit (PN 50005B)

### **3. Notes and Precautions**

To eliminate matrix interference from whole milk (liquid) to be tested for the presence of Melamine, samples must be diluted in 10% MeOH/20 mM PBS.

### **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. Procedure**

5.1. Add approximately 1 mL of milk samples to a microcentrifuge tube

5.2. Centrifuge at 10,000 - 13,000 x g for 5 minutes. Sample should separate into 3 layers

5.3. Carefully remove a portion of the middle layer

5.4. Dilute and aliquot of the middle layer 1:3 in 10% MeOH/20 mM PBS. For example, adding 250  $\mu$ L of the extracted milk with 500  $\mu$ L of 10% MeOH/20 mM PBS.

5.5. The sample is now ready to analyze according to the procedure described in the ABRAXIS<sup>®</sup> Melamine ELISA Kit package insert.

### **6. Evaluation of Results**

Results obtained for whole milk samples (liquid form) prepared as described above must be multiplied by a factor of 3 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 < 60 ppb Melamine detected. Results above the highest standard must be diluted and re-analyzed.

### **7. Performance Data**

The sample preparation procedure detailed above was used with whole milk spiked with various amounts of Melamine. Recoveries were between 96 - 120%

### **For ordering or technical assistance contact**

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