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 I00 and 900ftL
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 I0% 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%

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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 900µL
Glass vials with Teflon lined caps
Methanol
10% MeOH/20 mM PBS, pH 7.2-7.4
ABRAXIS® 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 µL of the extracted milk with 500 µL of 10% MeOH/20 mM PBS.
5.5. The sample is now ready to analyze according to the procedure described in the ABRAXIS® 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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