

Melamine in Egg (Chicken) Sample Preparation

1. Range of Detection

1,800-45,000 ng/mL (ppb). Samples with higher concentrations must be diluted further and re-analyzed.

2. Materials Required (Not Provided)

Microcentrifuge capable of spinning at 3,000 x g

Blender

Pipettes capable of delivering 100 and 900µL

Glass vials with Teflon lined caps

Methanol

Eurofins Abraxis Melamine ELISA Kit (PN 50005B)

3. Preparation of Solutions

3.1. 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.

4. Notes and Precautions

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

5. Procedure

- 5.1. Weigh 10 gm of a whole egg sample to a glass vial
- 5.2. Add 30 mL of methanol and mix well by shaking for 30 seconds
- 5.3. Pour into a blender and mix well for 60 seconds
- 5.4. Take a sample aliquot from 4.3 and centrifuge at 3,000 x g for 10 minutes. Sample should separate into 2 layers
- 5.5. Carefully remove a portion of the top layer
- 5.6. Dilute and aliquot of the top layer 1:30 in 10% MeOH/20 mM PBS. For example, adding 100 μL of the extracted egg with 2.9 mL of 10% MeOH/20 mM PBS.

The sample is now ready to analyze according to the procedure described in the Eurofins Abraxis Melamine Kit package insert.

6. Evaluation of Results

Results obtained for egg samples prepared as described above must be multiplied by a factor of 90 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 < 1,800 ppb Melamine detected (using the dilution factor). Results above the highest standard must be diluted and re-analyzed.

7. Performance Data

The sample preparation procedure detailed above was used with whole eggs spiked with various amounts of Melamine. Average recoveries obtained were 90%.

8. Assistance

For ordering or technical assistance contact:

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