

Melamine in Meat (Chicken, Fish) Sample Preparation

1. Intended Use

For the detection of Melamine in chicken and fish meat. For other matrices, please see the appropriate application bulletin.

2. Range of Detection

1,344 -33,600 ng/mL (ppb). Samples with higher concentrations must be diluted further and re-analyzed.

3. Materials Required (Not Provided)

Blender

Overhead tube rotator

Pipettes capable of delivering 100 and 900 μ L

Glass vials with Teflon lined caps

Methanol

ABRAXIS® Melamine ELISA Kit (PN 50005B)

4. Notes and Precautions

To eliminate matrix interference from chicken meat for the presence of Melamine, samples must be diluted in 10% MeOH/20 mM PBS.

5. Preparation of Solutions

- 5.1. **3% Trichloro acetic acid (TCA):** Add 3.0 g of TCA (Sigma cat# T-6399) to 100 mL of distilled water, mix to dissolve.
- 5.2. **1 M Phosphate Buffer:** Add 14.2 g of sodium phosphate dibasic anhydrous to 100 mL of distilled or deionized water, mix to dissolve.
- 5.3. **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, bring to 1 L volume with distilled or deionized water, pH 7.2-7.4.
- 5.4. **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.

6. Procedure

- 6.1. 20 mL of 20 mM PBS (solution 4.3) is added to 10 g of chicken muscle sample or fish filet and homogenized for 5 minutes using a blender.
- 6.2. Weigh 1 g of the homogenized sample into a plastic tube with cap, and add 3 mL of 3% TCA (solution 4.1).
- 6.3. Mix using an overhead tube rotator for 30 minutes.
- 6.4. Centrifuge for 10 minutes at 2500 +/- 200 g.
- 6.5. Carefully remove 500 μ L of the supernatant (upper layer) into a glass tube and add 200 μ L of 1 M phosphate buffer solution (solution 4.2).
- 6.6. Dilute 1:8 in Sample Diluent (10% MeOH/20 mM PBS solution 4.4) i.e. 100 μ L of supernatant (step 5) and 700 μ L of Sample Diluent (10% MeOH/20 mM PBS).
- 6.7. The sample is now ready to analyze according to the procedure described in the ABRAXIS® Melamine Kit package insert.

For highly contaminated samples (outside the range of the curve), we recommend further dilutions of 1:10 or 1: 100 with sample dilution buffer (10% MeOH/20 mM PBS).

7. Evaluation of Results

Results obtained for chicken/fish meat samples prepared as described above must be multiplied by a factor of 67.2 to account for the sample dilution (this is the dilution factor introduced by the extraction/dilution procedure). 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 < 1,344 ppb Melamine detected. Results above the highest standard must be diluted and re-analyzed. If further dilutions are performed on the samples, this dilution factor needs to be multiplied by the sample value to obtain the final Melamine concentration on the sample.

8. Performance Data

The sample preparation procedure detailed above was used with chicken meat spiked with various amounts of Melamine. Average recoveries were 87 % for chicken and 85% for fish.

9. For ordering or technical assistance contact

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