

## **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 $\mu$ 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  $\mu$ L of the supernatant (upper layer) into a glass tube and add 200  $\mu$ 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  $\mu$ L of supernatant (step 5) and 700  $\mu$ 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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