Melamine (MEL) ELISA Kit

Catalog No.: abx364793

Size: 96 tests

Storage: Store at 4°C in the dark.

Application: The quantitative detection of melamine in Milk, Milk Powder, Eggs, Muscle and Liver tissue, Serum, and Feed.

Sensitivity: 2 ng/ml (ppb)

Detection Limits: Milk -- 54 ng/ml; Milk powder -- 40 ng/ml; Milk and Milk Powder (using preparation Method 2) -- 2 ng/ml; Eggs -- 40 ng/ml; Muscle and Liver -- 4 ng/ml; Serum -- 8 ng/ml; Feed -- 200 ng/ml

Cross-Reactivity: Cyanuric Acid -- 60%; s-Triazine -- < 1%

Sample Recovery Rate: 90 ± 25%

Introduction: Melamine (MEL) is a white nitrogenous solid widely used in industry for the production of melamine resins and foams. These composites are valued for their high durability and fire-retardant properties, and are used to manufacture decorative laminates, insulation, and concretes. In a biological context, melamine was once used as a source of non-protein nitrogen in livestock feed; however, it is now known to be a mammalian nephrotoxin, and ingestion can lead to breakdown in kidney function. Melamine has been implicated in several instances of severe renal failure in livestock, pets, and humans.

Principle of the Assay: This kit is based on competitive enzyme-linked immuno-sorbent assay technology. Melamine antigen is precoated onto a 96-well plate. The standards and samples and a biotin-conjugated antibody specific to melamine are added to the wells and incubated. After washing away the unbound conjugates, avidin conjugated to Horseradish Peroxidase is added to each microplate well. After TMB substrate solution is added only wells that contain bound melamine antibody will produce a blue color product that changes into yellow after adding the stop solution. The intensity of the yellow color is inversely proportional to the melamine amount present in the sample. The O.D. absorbance is measured spectrophotometrically at 450 nm in a microplate reader, and then the concentration of melamine can be calculated.

Kit Components

1. 96-well microplate
2. Standards (1 ml each): 0 ng/ml, 2 ng/ml, 6 ng/ml, 18 ng/ml, 54 ng/ml, 162 ng/ml
3. HRP Conjugate Reagent: 5.5 ml
4. Antibody Solution: 5.5 ml
5. Substrate Reagent A: 6 ml
6. Substrate Reagent B: 6 ml
7. Stop Solution: 6 ml
8. Wash Buffer (20X): 40 ml
9. Reconstitution Buffer (2X): 50 ml
10. Plate sealer: 3
11. Hermetic bag: 1

Materials Required But Not Provided

1. Incubator
2. Microplate reader (450 nm)
3. High-precision pipette and sterile pipette tips
4. Automated plate washer
5. ELISA shaker
6. 50 ml centrifuge tubes
7. Absorbent filter papers
8. 100 ml and 1 L graduated cylinders
9. Nitrogen evaporator or water bath
10. Homogenizer
11. Deionized water

Reagents Required But Not Provided

1. Hydrochloric acid (HCl; 37% w/w)
2. Acetonitrile (CH_3CN)
3. Sodium hydroxide (NaOH)
4. N-hexane (C_6H_{14})
5. Methanol (CH_3OH)

Protocol

Bring all reagents and samples to room temperature before use.

A. Preparation of samples and reagents

1.Samples

- **Solution 1 – 1 M HCl solution** (for **Feed** samples)

Dilute 8.6 ml of Concentrated HCl with 91.4 ml Deionized water. Mix thoroughly.

- **Solution 2 – Acetonitrile x 0.1 M NaOH Solution** (for **Milk**, **Milk Powder**, **Muscle and Liver tissue**, **eggs**, and **serum** samples)

Thoroughly mix 84 ml Acetonitrile with 16 ml 0.1 M NaOH Working Solution (see Solution 3 for preparation instructions).

- **Solution 3 – 0.1 M NaOH Working Solution**

Dissolve 0.4 g Sodium hydroxide in 100 ml Deionized water.

- **Solution 4 – 1 M NaOH Solution** (for **Feed** samples)

Dissolve 4 g Sodium hydroxide in 100 ml Deionized Water.

- **Solution 5 – Diluted Reconstitution Buffer**

Dilute the provided 2X Reconstitution Buffer with Deionized Water for sample treatment (described in Section A.2).

- **Solution 6 – Wash Buffer**

Dilute the provided 20X Wash Buffer 20-fold with Deionized Water (i.e. in a 1:19 ratio of 20X Wash Buffer to water).

2. Sample Pretreatment

- **Milk (method 1):** Introduce 600 µl of Milk sample and 1 ml Acetonitrile into a centrifuge tube. Vortex until mixed fully. Centrifuge at 4000 rpm for 5 minutes. Take 100 µl of supernatant and mix thoroughly with 900 µl Diluted Reconstitution Buffer (prepared as Solution 5). Aliquot 50 µl for analysis.

  Note: Sample dilution factor: 27; Detection limit: 54 ng/ml.

- **Milk Powder (method 1):** Weigh 2 g Milk Powder into a centrifuge tube. Add 4 ml Methanol, then vortex until mixed fully. Centrifuge at 4000 rpm for 10 minutes. Take 100 µl of supernatant and mix thoroughly with 900 µl Diluted Reconstitution Buffer (prepared as Solution 5). Aliquot 50 µl for analysis.

  Note: Sample dilution factor: 20; Detection limit: 40 ng/ml.

- **Milk and Milk powder (method 2):** Add 2 ml of Milk or 2 g of Milk Powder into a centrifuge tube. Add 8 ml Acetonitrile x 0.1 M NaOH Solution (prepared as Solution 2) and vortex fully for 2 minutes. Centrifuge at 4000 rpm for 10 minutes. Remove 4 ml of the uppermost liquid layer and dry at 50 - 60°C with nitrogen evaporators or a water bath. Add 1 ml N-hexane to dissolve the dry material, and then add 1 ml Diluted Reconstitution Buffer (Solution 5). Vortex strongly for 30 seconds and centrifuge until an upper N-hexane phase layer forms. Discard the upper layer. Aliquot 50 µl of the lower liquid layer for analysis.

  Note: Sample dilution factor: 1; Detection limit: 2 ng/ml.

- **Muscle or Liver:** Remove as much fat as possible from the sample, then homogenize and mix fully. Weigh 2 g of homogenate into 8 ml of Acetonitrile x 0.1 M NaOH Solution (Solution 2) in a centrifuge tube, and vortex fully for 2 minutes. Centrifuge at 4000 rpm for 10 minutes. Remove 2 ml of the uppermost liquid layer and dry at 50 - 60°C with nitrogen evaporators or a water bath. Add 1 ml N-hexane to dissolve the dry material, and then add 1 ml Diluted Reconstitution Buffer (Solution 5). Vortex strongly for 30 seconds and centrifuge until an upper N-hexane phase layer forms. Discard the upper layer. Aliquot 50 µl of the lower liquid layer for analysis.

  Note: Sample dilution factor: 2; Detection limit: 4 ng/ml.

- **Eggs**: Homogenize the Egg sample. Weigh 2 g of homogenate into 8 ml of Acetonitrile x 0.1 M NaOH Solution (Solution 2) in a centrifuge tube, and vortex fully for 2 minutes. Centrifuge at 4000 rpm for 10 minutes. Remove 1 ml of the uppermost liquid layer and dry at 50 - 60°C with nitrogen evaporators or a water bath. Add 1 ml N-hexane to dissolve the dry material, and then add 1 ml Diluted Reconstitution Buffer (Solution 5). Vortex strongly for 30 seconds and centrifuge until an upper N-hexane phase layer forms. Discard the upper layer. Aliquot 50 µl of the lower liquid layer for analysis.

  Note: Sample dilution factor: 20; Detection limit: 40 ng/ml.

  The sample may have a jelly-like consistency when first mixed with the Acetonitrile x 0.1 M NaOH Solution (Solution 2). This is normal and will not affect sample quality.

- **Feed**: Homogenize the sample. Weigh 2 g of crushed Feed into 2 ml 1 M HCl Solution (prepared as Solution 1) and 16 ml Deionized Water in a centrifuge tube. Homogenize the mixture. Vortex fully for 5 minutes, then centrifuge at 4000 rpm for 15 minutes. Take 10 ml supernatant, and neutralize with 1 M NaOH Solution (prepared as Solution 4). Generally, 0.5 ml - 1 ml of Solution 4 is enough to neutralize the sample. Centrifuge at 4000 rpm for 15 minutes, and transfer the supernatant to a fresh tube. Take 100 µl of supernatant, and dilute 10-fold with 900 µl Diluted Reconstitution Buffer (Solution 5). Aliquot 50 µl for analysis.

  Note: Sample dilution factor: 100; Detection limit: 200 ng/ml.

- **Serum**: Aliquot 0.5 ml of Serum into 2 ml of Acetonitrile x 0.1 M NaOH Solution (Solution 2) in a centrifuge tube. Vortex fully for 2 minutes, then centrifuge 4000 rpm for 10 minutes. Remove 1 ml of the uppermost liquid layer and dry at 50 - 60°C with nitrogen evaporators or a water bath. Add 1 ml N-hexane so any remaining dry material dissolves, and then add 1 ml Diluted Reconstitution Buffer (Solution 5). Vortex strongly for 30 seconds and centrifuge until the upper N-hexane phase layer can be removed. Aliquot 50 µl of the lower liquid layer for analysis.

  Note: Sample dilution factor: 4; Detection limit: 8 ng/ml.

B. Assay Procedure

Bring all reagents to room temperature prior to use.

1. Number the sample and ordered standard wells, and record their positions. It is recommended that all samples and standards are tested in duplicate.
2. Add 50 µl of standard or sample into the respective standard and sample wells.
3. Add 50 µl HRP Conjugate Reagent to each well, and then 50 µl Antibody Working Solution.
4. Cover the plate with a plate sealer, oscillate gently for 5 seconds to mix, and incubate at 25°C for 30 minutes in the dark.
5. **C**arefully remove the plate sealer, and remove the liquid in each well. Immediately add 300 µl Diluted Wash Buffer (prepared as Solution 6) and wash. Repeat this wash procedure 5 times, with 30 second intervals. Invert the plate and pat onto absorbent towels. Prick any bubbles remaining in the wells with a clean pipette.
6. Add 50 µl of Substrate Reagent A to each well.
7. Add 50 µl of Substrate Reagent B to each well. Oscillate gently for 5 seconds to ensure thorough mixing, and incubate at 25°C for 15 minutes in the dark.
8. Add 50 µl of Stop Solution to each well, and oscillate gently to ensure thorough mixing.
9. Within 10 minutes of introducing the Stop Solution, determine the optical density of each well with a **microplate reader at 450 nm.**

This assay is competitive, therefore there is an inverse correlation between melamine concentration in the sample and the absorbance measured. Create a graph with the log of the standard concentration on the y-axis, and absorbance measured on the x-axis (a semi-logarithmic plot). Apply a best fit trendline through the standard points. Use this graph calculate sample concentrations based on their OD values. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Note: If the samples measured were diluted, multiply the dilution factor by the concentrations from interpolation to obtain the concentration before dilution.

C. Precautions

Bring all reagents to room temperature prior to use.

1. Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results. It is recommended to store samples to be used within 5 days at 4°C, within 1 month at -20°C and within 2 months at -80°C.
2. Do not allow the wells to dry fully during the washing procedure. Ensure the Wash Buffer is thoroughly mixed, and the wells are washed completely.
3. Do not use the Substrate Reagent if it has begun to turn blue without being added to the wells.
4. Do not use any reagents that are expired, or use reagents from other kits with this assay.
5. The Stop Solution is caustic. Avoid contact with the skin or eyes, and thoroughly wash any spilled solution with water.
6. It is recommended that the same operator perform the experiment throughout, to avoid inconsistencies between operator procedures.