Melamine (MEL) ELISA Kit

Catalog No.: abx365113

Size: 96T

Sensitivity: 0.5 ng/ml (ppb)

Detection Limit: Yogurt – 20 ng/ml; Egg – 50 ng/ml; Cheese, Cream – 200 ng/ml; Feed – 1000 ng/ml

Sample Recovery Rate: 90% ± 30%

Cross-Reactivity: Melamine – 100%, Cyanuric Acid – <0.1%

Storage: Store all components at 4°C.

Application: For quantitative detection of MEL in Yogurt, Egg, Cheese, Cream, and Feed samples.

Principle of the Assay

This kit is based on competitive enzyme-linked immuno-sorbent assay technology. An antigen is pre-coated onto a 96-well plate. Controls, test samples, HRP-conjugated reagent and biotin-conjugated reagent are added to the wells and incubated. A competitive inhibition reaction takes place between the pre-coated MEL and the MEL in the sample with the biotin-labelled antibody. Unbound conjugates are removed using wash buffer at each stage. TMB substrate is used to quantify the HRP enzymatic reaction. After TMB substrate is added, only wells that contain sufficient MEL will produce a blue-colored product, which then changes to yellow after adding the acidic stop solution. The intensity of the yellow color is inversely proportional to the MEL concentration in the sample. The OD is measured spectrophotometrically at 450 nm in a microplate reader, from which the concentration of MEL can be calculated.

Kit components

1. Pre-coated 96 well plate
2. Standard: 1 ml each (0 ng/ml, 0.5 ng/ml, 1.5 ng/ml, 4.5 ng/ml, 13.5 ng/ml, 40.5 ng/ml).
3. Detection Reagent A: 7 ml
4. Detection Reagent B: 12 ml
5. Substrate Reagent A: 6 ml
6. Substrate Reagent B: 6 ml
7. Stop Solution: 6 ml
8. Wash Buffer (20X): 25 ml
9. Egg Diluent (10X): 30 ml
10. Yogurt Diluent (4X): 120 ml
11. Cheese Diluent: 50 ml
12. Cream Diluent: 50 ml
13. Plate Sealer: 3
14. Hermetic Bag: 1

Material Required But Not Provided

1. Microplate reader (wavelength: 450 nm)
2. High-precision pipette and sterile pipette tips
3. Centrifuge or microfuge tubes
4. Distilled or deionized water
5. Absorbent filter papers
6. 100 ml and 1 L graduated cylinders
7. Nitrogen evaporator or water bath
8. Orbital shaker and vortex
9. Centrifuge

Reagents Required But Not Provided

1. Acetonitrile (100%)
2. N-Hexane
3. Methanol
4. Trichloroacetic acid

Protocol

A. Preparation of sample and reagents

1. Reagents

1X Egg Diluent

Dilute the concentrated Egg DIluent 10-fold (1/10) with distilled water (e.g. add 30 ml of concentrated reconstitution solution into 270 ml of distilled water).

1X Yogurt Diluent

Dilute the concentrated Yogurt DIluent 4-fold (1/4) with distilled water (e.g. add 120 ml of concentrated reconstitution solution into 360 ml of distilled water).

Trichloroacetic Acid Solution

Dissolve 2 g of Trichloroacetic acid with 100 ml of distilled water and mix fully.

1X Wash Buffer

Dilute the concentrated Wash Buffer 10-fold (1/20) with distilled water (e.g. add 40 ml of concentrated wash buffer into 760 ml of distilled water).

2. Samples

It is recommended to test samples in duplicate or triplicate where possible.

- **Yogurt:** Add 0.5 g of yogurt sample to a 50 ml centrifuge tube. Add 2 ml of 1X Yogurt Diluent and vortex until fully mixed. Take 50 µl of the sample for detection and analysis.
  Note: the sample dilution factor is 5.
- **Egg:** Add 1 g of homogenized egg sample to a 50 ml centrifuge tube. Add 0.5 ml of Egg Diluent and 4.5 ml of Acetonitrile Solution and vortex for 5 minutes. Centrifuge at 4000 rpm for 10 minutes at room temperature. Take 1 ml of clear liquid and add to another centrifuge tube. Dry using a nitrogen evaporator or water bath at 50°C. Dissolve the residue with 2 ml of N-hexane. Add 1 ml of Egg Diluent. Vortex for 30 seconds to mix fully. Centrifuge at 4000 rpm for 10 minutes at room temperature. Remove the upper layer (N-hexane) and middle layer supernatant. Take 50 µl of the bottom layer supernatant for detection and analysis.
  Note: the sample dilution factor is 5.
- **Cream:** Add 1 g of homogenized cream sample to a 50 ml centrifuge tube. Add 4 ml of deionized water and vortex until mixed fully. Centrifuge at 4000 rpm for 10 minutes at room temperature. Take 0.2 ml of middle layer supernatant and add 0.6 ml of Cream Diluent. Mix thoroughly with a vortex for 30 seconds. Take 50 µl of the sample for detection and analysis.
  Note: the sample dilution factor is 20.
- **Cheese:** Add 1 g of homogenized cheese sample to a 50 ml centrifuge tube. Add 1 ml of 2% Trichloroacetic acid and vortex for 2 minutes until mixed fully. Add 5 ml of Methanol and vortex for 2 minutes. Centrifuge at 4000 rpm for 10 minutes at room temperature. Dry using a nitrogen evaporator or water bath at 50°C. Dissolve the residue with 2 ml of N-hexane. Add 1 ml of Cheese Diluent. Vortex for 30 seconds to mix fully. Centrifuge at 4000 rpm for 5 minutes at room temperature. Remove the upper layer (N-hexane) and middle layer supernatant. Take 50 µl of the bottom layer supernatant for detection and analysis.
  Note: the sample dilution factor is 6.

B. Assay Procedure

Bring all samples and reagents to room temperature (25°C) prior to use. A room temperature or reagent temperature lower than 25°C may result in lower OD value readings.

1. Any strips that are not being used should be kept dry and stored at 4°C. Set standard, test sample and control (zero) wells on the pre-coated plate and record their positions. It is recommended to measure each standard and sample in duplicate.
2. Add 50 µl of the prepared standards solutions into the standard wells.
3. Add 50 µl of the 0 ng/ml standard into the control (zero) wells.
4. Add 50 µl of appropriately prepared samples into the test sample wells.
5. Immediately add 50 µl of Detection Reagent A into each well.
6. Cover the plate with the plate sealer. Gently tap the plate to mix thoroughly. Incubate at 25°C for 20 minutes in the dark.
7. Remove the cover and discard the solution. Wash the plate 5 times with 1X Wash Buffer. *Fill each well completely with Wash buffer (350 µl) using a multi-channel Pipette or automated washer (1-2 mins soaking period is recommended). Complete removal of liquid at each step is essential for good performance. After the final wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean absorbent paper towels.*
8. Immediately add 100 µl of Detection Reagent B into each well. Gently tap the plate to mix thoroughly. Incubate at 25°C for 20 minutes in the dark.
9. Remove the cover and discard the solution. Wash the plate according to the procedure in step 7, 5 times.
10. Add 50 µl of Substrate Reagent A to each well, and then 50 µl of Substrate Reagent B into each well. Cover the plate and gently tap the plate to mix thoroughly. Incubate at 25°C in dark conditions for 15 minutes. The incubation time is for reference only, the optimal time should be determined by end user.
11. Add 50 µl of Stop Solution into each well. There should be a color change to yellow. Gently tap the plate to ensure thorough mixing. It is important that the Stop Solution is mixed quickly and uniformly throughout the microplate to inactivate the enzyme completely.
12. Ensure that there are no fingerprints or water on the bottom of the plate, and that the fluid in the wells is free of bubbles. Measure the absorbance at 450 nm in a microplate reader within 5 min of adding the stop solution.

    To calculate the absorbance, use the following equation: Absorbance (%) = A / A_0 × 100% (where A: average absorbance of standard or sample, A_0: average absorbance of 0 ng/ml of the standard). The standard curve can be plotted as the absorbance of each standard solution vs. the respective concentration of the standard solution. The MEL concentration of the samples can be determined from the standard curve.

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

C. Notes and Precautions

- Before using the kit, centrifuge the tubes to bring down the contents trapped in the lid.
- Do not leave the wells uncovered for extended periods between incubations. The addition of reagents for each step should not
- exceed 10 mins.
- Ensure that the plate is properly sealed or covered during the incubation steps, and that the time and temperature are controlled.
- Do not reuse pipette tips and tubes.
- Do not use expired components, or components from a different kit.
- The TMB substrate reagent solutions should be used under sterile conditions, and light exposure should be minimized. Unused substrate should be colorless, or a very light yellow in appearance. Do not discard any residual solution back into the vial. If the stock substrate reagent solutions turn a blue color and/or the OD value of the 0 ng/ml standard (blank) is < 0.5, it is likely that the substrate reagent solutions have been contaminated or exposed to excessive light.
- Please note that this kit is optimized for detection of native samples, rather than recombinant proteins or synthetic chemicals. We are unable to guarantee detection of recombinant proteins, as they may have different sequences or tertiary structures to the native protein.
- This kit is for qualitative detection of MEL in serum, liquid milk, and milk powder samples. Suitability for other sample types would need to be determined by the end user.