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Melamine ELISA Kit

Catalog No. CSB-E12003f

(96 tests)

- This immunoassay kit allows for the in vitro quantitative determination of Melamine concentrations in milk, food material, dairy products, pet food and feedstuff.
- **Expiration date** **six** months from the date of manufacture
- **FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.**

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INTRODUCTION

Melamine (2,4,6-triamino-1,3,5-triazine) is used in the production of plastics, in finishers for paper, in fertilizer, as a flame retardant, and in the manufacture of wrinkle-free textiles. Recent cases of adulteration with melamine have led to the need for rapid and reliable screening methods. The discovery of melamine in pet food, animal feed, milk and protein sources including wheat gluten, rice protein concentrate, and corn gluten created an urgent need for rapid methods for detecting melamine in food. The ELISA for melamine proved to be a useful alternative to more cumbersome methods.

PRINCIPLE OF THE ASSAY

This assay employs the competitive inhibition enzyme immunoassay technique. A polyclonal antibody specific for Melamine has been pre-coated onto a microplate. A competitive inhibition reaction is launched between HRP labeled Melamine and unlabeled Melamine (Standards or samples) with the pre-coated antibody specific for Melamine. The more the amount of Melamine in samples, the less the HRP labeled Melamine bound by pre-coated antibody. The substrate solutions are added to the wells, respectively. And the color develops in opposite to the amount of Melamine bound in the initial step. The color development is stopped and the intensity of the color is measured.

STANDARD

The standard curve concentrations used for the ELISA's were 2000 ng/ml，250 ng/ml，31.25 ng/ml, 3.91ng/ml, 0 ng/ml.

SPECIFICITY

This assay recognizes Melamine. No significant cross-reactivity or interference was observed.

SENSITIVITY

The minimum detectable dose of Melamine is typically less than 2 ng/ml.
The sensitivity of this assay, or Lower Limit of Detection (LLD) was defined as the lowest concentration that could be differentiated from zero.

MATERIALS PROVIDED

Reagent	Quantity
Assay plate (96 tests)	1
Standard	5 ×250μl
Sample Diluent	1 x 20 ml
HRP-Melamine	1 x 60μl
Wash Buffer	1 x 20 ml (25 x concentrate)
TMB Substrate	1 x 10 ml
Stop Solution	1 x 10 ml

Standard 1	Standard 2	Standard 3	Standard 4	Standard 5
2000 ng/ml	250 ng/ml	31.25 ng/ml	3.91 ng/ml	0 ng/ml

STORAGE

1. Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air. The test kit may be used throughout the expiration date of the kit. Refer to the package label for the expiration date.
2. Opened test kits will remain stable until the expiring date shown, provided it is stored as prescribed above.
3. A microtiter plate reader with a bandwidth of 10 nm or less and an optical density range of 0-3 OD or greater at 450nm wavelength is acceptable for use in absorbance measurement.

SAMPLE PREPARATION

1. Solid milk extraction protocol: Dilution factor: 1:10

1) Add 10ml 60% methanol / water to 1g solid milk sample. Vortex vigorously.
2) Sonicate for 1min and then shake for 1 min. Let stand for 5 min.
3) Transfer the supernatant to new tube, centrifuge at 10000rpm for 5min.
4) Filtering the supernatant with G6 glass filter paper, the filtrate is ready for the assay.

2. Liquid milk extraction protocol: Dilution factor: 1:10

1) Add 9900ul 60% methanol / water to 100ul liquid milk sample. Vortex vigorously.
2) Sonicate for 1min and then shake for 1 min. Let stand for 5 min.
3) Transfer the supernatant to new tube, centrifuge at 10000rpm for 5min.
4) Filtering the supernatant with G6 glass filter paper, the filtrate is ready for the assay.

3. Moist food extraction protocol: Dilution factor: 1:5

1) Homogenize moist food using a blender or coffee grinder.
2) Weigh 2g of homogenized sample and add 10 ml 60% methanol / water, vortex vigorously.
3) Sonicate for 1min and then shake for 1 min. Let stand for 5 min.
4) Transfer the supernatant to new tube, centrifuge at 10000rpm for 5min.
5) Filtering the supernatant with G6 glass filter paper, the filtrate is ready for the assay.

4. Dry food extraction protocol: Dilution factor: 1: 10

1) Homogenize dry food using a blender or coffee grinder.
2) Weigh 1g of homogenized sample and add 10 ml 60% methanol / water. Vortex vigorously.
3) Sonicate for 1min and then shake for 1 min. Let stand for 5 min.
4) Transfer the supernatant to new tube, centrifuge at 10000rpm for 5min.
5) Filtering the supernatant with G6 glass filter paper, the filtrate is ready for the assay.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

1. **Wash Buffer** If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 ml of Wash Buffer Concentrate into deionized or distilled water to prepare 500 ml of Wash Buffer.
2. **Standard** The **Standard** concentration is 2000 ng/ml. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making serial dilutions. The **Sample Diluent** serves as the zero standard (0 ng/ml).
3. **HRP-Melamine** Dilute to the working concentration specified on the vial label using **Sample Diluent**(1:100), respectively.

Precaution: The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all samples, standards, and controls be assayed in duplicate.

1. Add 100μl of Sample Diluent to Blank well, 50μl Standard or Sample to other wells. Add 50ul HRP-Melamine working solution immediately after adding each sample（Don't to Blank well！）. Mix with the pipette and shake the plate gently for 60 seconds.
2. Cover with the adhesive strip. Then incubate for 30 minutes at 37° C.
3. Aspirate each well and wash, repeating the process five times for a total of five washes. Wash by filling each well with Wash Buffer (200μl) using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
4. Add 90μl of **TMB Substrate** to each well. Incubate for 20 minutes at 37°C. Keeping the plate away from drafts and other temperature fluctuations in the dark.
5. Add 50μl of **Stop Solution** to each well. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.
6. Determine the optical density of each well within 15 minutes, using a microplate reader set to 450 nm.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, control, and sample and divide the average zero standard optical density. Create a standard curve by reducing the data using computer software. As an alternative, construct a standard curve by plotting the absorbance ratio for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the Melamine concentrations versus the ratio and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

LIMITATIONS OF THE PROCEDURE

- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- It is important that the Calibrator Diluent selected for the standard curve be consistent with the samples being assayed.
- If samples generate values higher than the highest standard, dilute the samples with the appropriate Calibrator Diluent and repeat the assay.
- Any variation in Standard Diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.

- This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Quantikine Immunoassay, the possibility of interference cannot be excluded.

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- When using an automated plate washer, adding a 30 second soak period following the addition of wash buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.