Technical Manual

MEL (Melamine) ELISA Kit

- Catalogue Code: FSES0009
- Competitive ELISA Kit
- Research Use Only

Contents

1. Key features and Sample Types

Sensitivity:

2 ppb (ng/mL)

Assay Procedure:

25°C, 30 min~15 min

Detection Limit:

Milk powder - 40 ppb; Milk - 54 ppb; Milk, Milk powder (method 2) - 2 ppb;
Muscle, Liver - 4 ppb; Feed - 200 ppb; Eggs - 40 ppb; Serum - 8 ppb

Cross Reactivity:

Melamine - 100%, Cyanuric Acid - 60%, s-Triazine < 1%

Sample Recovery rate:

90±25%

Storage:

2-8°C for 6 months.

Expiry:

See Kit Label

2. Storage

Store the kit at 2~8°C. Do not freeze any test kit components.
Return any unused microwells to their original foil bag and reseal them together with the desiccant provided and further store at 2 - 8°C.

3. Test Principle

This kit uses a Competitive-ELISA method. It can detect Melamine (MEL) in samples, such as milk, muscle, feed, etc. This kit is composed of ELISA Microtiter plate, HRP conjugate, antibody working solution, standard and other supplementary reagents. The microtiter plate in this kit has been pre-coated with coupled antigen. During the reaction, MEL in the samples or standard competes with coupled antigen on the solid phase supporter for sites of anti-MEL antibody. Then Horseradish Peroxidase (HRP) conjugate is added to each microtiter plate well, and substrate reagent is added for color development. There is a negative correlation between the OD value of samples and the concentration of MEL. The concentration of MEL in the samples can be calculated by comparing the OD of the samples to the standard curve.

4. Kit Contents

Each kit contains reagents for 96 assays including:

No.	Component	96-WellKit
1	ELISA Microtiter plate	96 wells
2	Standards	1 mL each (0 ppb, 2 ppb, 6 ppb, 18 ppb, 54 ppb, 162 ppb)
3	HRP Conjugate	5.5 mL
4	Antibody Working Solution	5.5 mL
5	Substrate Reagent A	6 mL
6	Substrate Reagent B	6 mL
7	Stop Solution	6 mL
8	20×Concentrated Wash Buffer	40 mL
9	2×Reconstitution Buffer	50 mL
10	Plate Sealer	3 pieces
11	Sealed Bag	1 piece
12	Manual	1 copy

Note: All reagent bottle caps must be tightened to prevent evaporation and microbial pollution.

Additional materials required:

Other materials required but not supplied

- **Instruments:** Microplate reader, Printer, Homogenizer, Nitrogen evaporators, Water bath, Vortex mixer, Centrifuge, Graduated pipette, Balance (sensitivity 0.01 g).
- **Micropipette:** Single channel (20-200 µL, 100-1000 µL), Multichannel (30-300 µL).
- **Reagents:** N-hexane, Acetonitrile, NaOH, Concentrated HCl, Methanol.

5. Experimental Preparation

Bring all reagents and samples to room temperature before use.

Open the microplate reader in advance, preheat the instrument, and set the testing parameters.

1. Sample pre-treatment Notice:

Experimental apparatus should be clean, and the pipette should be disposable to avoid cross- contamination during the experiment.

2. Solution preparation

Solution 1: 1 M HCl Solution *(for feed sample*)
Dilute 8.6 mL of **Concentrated HCl** to 100 mL with deionized water.

Solution 2: Acetonitrile- 0.1 M NaOH Solution
(for milk, milk powder, livestock, fish, shrimp, liver, eggs, serum sample)
Mix 84 of mL **Acetonitrile** and 16 of mL **0.1M NaOH Solution** (Solution 3) fully.

Solution 3: 0.1 M NaOH Solution
Dissolve 0.4 g of **NaOH** to 100 mL with deionized water.

Solution 4: 1M NaOH Solution *(for feed sample*)
Dissolve 4 g of **NaOH** to 100 mL with deionized water.

Solution 5: Reconstitution Buffer
Dilute the 2×Reconstitution Buffer with deionized water for re-dissolution of samples. The Reconstitution Buffer can be stable for 1 month at 4°C.

Solution 6: Wash Buffer
Dilute **20×Concentrated Wash Buffer** with deionized water. (20×Concentrated Wash Buffer (V): Deionized water (V) = 1:19).

3. Sample pre-treatment procedure

Targets may be distributed unevenly, resulting in no detection. To avoid this, ensure to take sufficient samples when sampling.

3.1 Pre-treatment of milk sample:

1. Take 600 µL of milk sample into 2 mL centrifuge tube and add 1 mL **Acetonitrile**, vortex until it mixed fully. Centrifuge at 4000 r/min for 5 min.
2. Take 100 µL of supernatant and add 900 µL of **Reconstitution Buffer** (Solution 5). Mix fully.
3. Take 50 µL for analysis.
   Note: Sample dilution factor: 27, detection limit: 54 ppb.

3.2 Pre-treatment of milk powder sample:

1. Weigh 2±0.05 g of milk powder sample into a 50 mL centrifuge tube, add 4 mL of **Methanol**, vortex until it mixed fully. Centrifuge at 4000 r/min for 10 min.
2. Take 100 µL of supernatant and add 900 µL of **Reconstitution Buffer** (Solution 5). Mix fully.
3. Take 50 µL for analysis.
   Note: Sample dilution factor: 20, detection limit: 40 ppb.

3.2 Pre-treatment of milk, milk powder sample (method 2) sample:

1. Take 2 mL of milk sample or 2 g of milk powder sample into a centrifuge tube.
2. Add 8 mL of **Acetonitrile- 0.1 M NaOH Solution** (Solution 2) and vortex fully for 2 min. Centrifuge at 4000 r/min for 10 min. Take 4 mL of the upper layer liquid and dry at 50-60°C with nitrogen evaporators or water bath.
3. Add 1 mL of **N-hexane** to dissolve the remaining dry material, then add 1 mL of **Reconstitution Buffer** (Solution 5). Vortex strongly for 30s and centrifuge to remove the upper layer n-hexane phase.
4. Take 50 µL of the lower layer liquid for analysis.
   Note: Sample dilution factor: 1, detection limit: 2 ppb.

3.3 Pre-treatment of muscle (livestock, fish, shrimp), liver sample:

1. Weigh 2±0.05 g of homogenate muscle sample into a 50 mL centrifuge tube.
2. Add 8 mL of **Acetonitrile- 0.1 M NaOH Solution** (Solution 2) and vortex fully for 2 min. Centrifuge at 4000 r/min for 10 min. Take 2 mL of the upper layer liquid and dry at 50-60°C with nitrogen evaporators or water bath.
3. Add 1 mL **N-hexane** to dissolve the remaining dry material, then add 1 mL of **Reconstitution Buffer** (Solution 5). Vortex strongly for 30s and centrifuge to remove the upper layer n-hexane phase.
4. Take 50 µL of the lower layer liquid for analysis.
   Note: Sample dilution factor: 2, detection limit: 4 ppb.

3.4 Pre-treatment for feed sample:

1. Weigh 2±0.05 g of crushed feed sample into a centrifuge tube. Add 2 mL of **1 M HCl Solution** (Solution 1) and 16 mL of deionized water, then homogenate the sample.
2. Vortex for 5 min to mix fully.
3. Centrifuge at 4000 r/min for 15 min. Take 10 mL of the supernatant and adjust the pH to 6~8 with **1M NaOH Solution** (Solution 4). (The added amount of 1M NaOH Solution is different according to the feed sample. The needed amount is generally between 0.5 mL~1 mL).
4. Centrifuge at 4000 r/min for 15 min. Take the supernatant to another centrifuge tube (It is recommended to increase the centrifuge speed or filter the supernatant with filter paper if the supernatant is muddy).
5. Dilute the supernatant for 10 times with the **Reconstitution Buffer** (Solution 5) (Take 100 µL of supernatant and add 900 µL of Reconstitution Buffer. Mix fully.)
6. Take 50 µL for analysis.
   Note: Sample dilution factor: 100, detection limit: 200 ppb.

3.5 Pre-treatment for eggs sample:

1. Homogenate the egg sample with homogenizer to mix fully.

2. Weigh 2±0.05 g of homogenate egg sample into centrifuge tube. Add 8 mL of

Acetonitrile- 0.1M NaOH Solution (Solution 2) and vortex fully for 2 min. (After adding the Acetonitrile-0.1M NaOH Solution to vortex, it may be jelly, which is normal).

3. Centrifuge at 4000 r/min for 10 min at room temperature. Take 1 mL of the upper layer liquid and dry at 50-60°C with nitrogen evaporators or water bath.
4. Add 1 mL of **N-hexane** to dissolve the remaining dry material, and then add 1 mL of **Reconstitution Buffer** (Solution 5). Vortex strongly for 30s and centrifuge to remove the upper layer n-hexane phase.
5. Take 50 µL for analysis.
   Note: Sample dilution factor: 20, detection limit: 40 ppb.

3.6 Pre-treatment for serum (swine)* sample:

(*Data validated in swine urine, but pre-treatment can be applied for urine samples of multiple species.)

1. Take 0.5 mL of sample into a 50 mL centrifuge tube.
2. Add 2 mL of **Acetonitrile- 0.1M NaOH Solution** (Solution 2) and vortex fully for 2 min. Centrifuge at 4000 r/min for 10 min. Take 1 mL of the upper layer liquid and dry at 50-60°C with nitrogen evaporators or water bath.
3. Add 1 mL of **N-hexane** to dissolve the remaining dry material, then add 1 mL of **Reconstitution Buffer** (Solution 5). Vortex strongly for 30s and centrifuge to remove the upper layer n-hexane phase.
4. Take 50 µL for analysis.
   Note: Sample dilution factor: 4, detection limit: 8 ppb.

6. Assay Procedure

Bring all reagents and samples to room temperature (25°C) before use. All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming. The unused ELISA Microtiter plate should be sealed as soon as possible and stored at 2~8°C.

1. **Number:** number the sample and standard in order (multiple well), and keep a record of standard wells and sample wells. **Standard and Samples must be tested in duplicate.**
2. **Add Sample:** add 50 µL of **Standard or Sample** per well, then add 50 µL of **HRP Conjugate** to each well, then add 50 µL of **Antibody Working Solution**, cover the plate with plate sealer, vortex for 5s gently to mix thoroughly, incubate at 25°C for 30 min in the dark.
3. **Wash:** uncover the sealer carefully, remove the liquid in each well. Immediately add 300 µL of **Wash Buffer** (Solution 6) to each well and wash. Repeat wash procedure for 5 times, 30s intervals/time. Invert the plate and pat it against absorbent paper (If bubbles exist in the wells, clean tips can be used to prick them).
4. **Colour Development:** add 50 µL of **Substrate Reagent A** to each well, and then add 50 µL of **Substrate Reagent B**. Gently vortex for 5s to mix thoroughly. Incubate at 25°C for 15 min in the dark (If the blue is too shallow, the reaction time can be prolonged appropriately).
5. **Stop Reaction:** add 50 µL of **Stop Solution** to each well, vortex gently to mix thoroughly.
6. **OD Measurement:** determine the optical density (OD value) of each well at 450 nm (reference wavelength 630 nm) with a microplate reader. This step should be finished in 10min after stop reaction.

7. Data Analysis

1. **Absorbance% = $A/A_0 \times 100\%$**

   A: Average absorbance of standard solution or sample

   A_0: Average absorbance of 0 ppb Standard solution

2. **Drawing and calculation of standard curve**

Create a standard curve by plotting the absorbance percentage of each standard on the y-axis against the log concentration on the x-axis to draw a semi-logarithmic plot. Add the average absorbance value to standard curve to get corresponding concentration. **If samples have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor.**

For this kit, it is more convenient to use professional analysis form for accurate and fast analysis on a large number of samples.

8. Notes

1. The overall OD value will be lower when reagents have not been brought to room temperature before use or room temperature is below 25°C.
2. If the wells turn dry during the washing procedure, it will lead to bad linear standard curve and poor repeatability. Operate the next step immediately after wash.
3. Mix thoroughly and wash the plate completely. The consistency of wash procedure can strongly affect the reproducibility of this ELISA kit.
4. ELISA Microtiter plate should be covered by plate sealer. Avoid the kit to strong light.
5. **Each reagent is optimized for use in the FSES0009. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other FSES0009 with different lot numbers.**
6. Substrate Reagent should be abandoned if it turns blue colour. When OD value of standard (concentration: 0) < 0.5 unit (A450nm < 0.5), it indicates the reagents are deteriorated.
7. Stop solution is caustic, avoid contact with skin and eyes.
8. As the OD values of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique, washing technique or temperature effects), the operator should establish a standard curve for each test.
9. Even the same operator might get different results in two separate experiments. In order to get reproducible results, the operation of every step in the assay should be controlled.
10. If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.
11. The kit is used for rapid screening of actual samples. If the test result is positive, the instrument method such as HPLC, LC/MS, etc. can be used for quantitative confirmation.

Notes:

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

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