

**MEL (Melamine) ELISA Kit**

Catalog No: E-FS-E109

96T/96T\*3

<b>Version Number:</b>	V1.1
<b>Replace version:</b>	V1.0
<b>Revision Date:</b>	2024.3.7

This manual must be read attentively and completely before using this product.

If you have any problems, please contact our Technical Service Center for help.

Toll-free: 1-888-852-8623 Tel: 1-832-243-6086 Fax: 1-832-243-6017

Email: [techsupport@elabscience.com](mailto:techsupport@elabscience.com)

Website: [www.elabscience.com](http://www.elabscience.com)

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

## Test principle

This kit uses Competitive-ELISA as the method for the quantitative detection. It can detect Melamine (MEL) in samples, such as yogurt, 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.

## Technical indicator

**Reaction mode** (Incubation time and temperature): 25°C; 20 min, 20 min, 15 min

**Detection limit:** Raw milk---10 ppb; Yogurt---20 ppb; Cheese ---200 ppb; Feed---1000 ppb

**Cross-reactivity:** Melamine ---100%, Ammelide, Ammeline, Cyanuric Acid, Sulfanilamide--- < 0.1%

**Sample recovery rate:** 90 ± 30%

## Kits components

Item	Specifications
ELISA Microtiter plate	96 wells
Standard Liquid	1 mL each (ppb=ng/mL=ng/g) (0 ppb, 0.5 ppb, 1.5 ppb, 4.5 ppb, 13.5 ppb, 40.5 ppb)
HRP Conjugate	12 mL
Antibody Working Solution	7 mL
Substrate Reagent A	6 mL
Substrate Reagent B	6 mL
Stop Solution	6 mL
20×Concentrated Wash Buffer	25 mL
Sample Diluent A	30 mL
Sample Diluent B	60 mL
10×Yogurt Diluent	10 mL
Plate Sealer	3 pieces
Sealed Bag	1 piece
Manual	1 copy

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

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**Other materials required but not supplied**

**Instruments:** Microplate reader, Printer, Homogenizer, Vortex mixer, Centrifuge, Graduated pipette, Balance (sensitivity 0.01 g).

**Micropipette:** Single channel (20-200 µL, 100-1000 µL), Multichannel (30-300 µL).

**Reagents:** Methanol anhydrous, Trichloroacetic acid.

**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. FOR RESEARCH USE ONLY. ELISA Microtiter plate should be covered by plate sealer. Avoid the kit to strong light.
5. **Each reagent is optimized for use in the E-FS-E109. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other E-FS-E109 with different lot numbers.**
6. Substrate Reagent should be abandoned if it turns blue color. When OD value of standard (concentration: 0) < 0.8 unit (A450nm < 0.8), it indicates the reagent be 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. **For mentioned sample fast and efficient extraction methods are included in the kit description. Please consult technical support for the applicability if other sample need to be tested.**
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.

**Storage and expiry date**

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.

**Expiry date:** expiration date is on the packing box.

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## Experimental preparation

Restore 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 pretreatment Notice:

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

### 2. Reagent preparation

*Please prepare solution according to the number of samples. Don't use up all components in the kit at once!*

Solution 1: Yogurt Diluent (*for yogurt sample*)

Dilute **10×Yogurt Diluent** with deionized water. (10×Yogurt Diluent (V): Deionized water (V) = 1:9).

Solution 2: 2% Trichloroacetic acid Solution (*for raw milk, cheese, feed, sample*)

Dissolve 2 g of **Trichloroacetic acid** to 100 mL with deionized water, mix fully.

Solution 3: 0.25% Trichloroacetic acid Solution (*for yogurt sample*)

Take 10 mL **2% Trichloroacetic acid** and add 70 mL deionized water, mix fully.

Solution 4: Wash Buffer

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

### 3. Sample pretreatment procedure

#### 3.1 Pretreatment of raw milk sample:

- (1) Take 2 mL of fresh milk sample into a 10 mL centrifuge tube, add 1 mL of **Methanol anhydrous**, and add 1 mL of **2% Trichloroacetic acid Solution** (Solution 2), vortex fully for 3 min.
- (2) Centrifuge at 4000 r/min for 15 min. Take 0.05 mL of the supernatant liquid into a new centrifuge tube.
- (3) Add 0.25 mL of **Sample Diluent A**. Vortex fully for 1 min.
- (4) Take 50 µL for analysis.

**Note: Sample dilution factor: 12, detection limit: 10 ppb.**

#### 3.2 Pretreatment of yogurt sample:

- (1) Take 1 mL or 1±0.05g of yogurt sample into a 10 mL centrifuge tube, add 4 mL of **0.25% Trichloroacetic acid Solution** (Solution 3), vortex fully for 3 min.
- (2) Centrifuge at 4000 r/min for 10 min. Take 0.1 mL of the supernatant liquid into a new centrifuge tube.
- (3) Add 0.3 mL of **Yogurt Diluent** (Solution 1). Vortex fully for 1 min.
- (4) Take 50 µL for analysis.

**Note: Sample dilution factor: 20, detection limit: 20 ppb.**

**3.3 Pretreatment of cheese sample:**

- (1) Weigh  $1 \pm 0.05$ g of homogenate sample into a 10 mL centrifuge tube. Add 5 mL of **2% Trichloroacetic acid Solution** (Solution 2) and vortex fully for 3 min.
- (2) Centrifuge at 4000 r/min for 15 min. Take 0.05 mL of the supernatant liquid into a new centrifuge tube.
- (3) Add 0.95 mL of **Sample Diluent B**, vortex fully for 1 min.
- (4) Take 50  $\mu$ L for analysis.

**Note: Sample dilution factor: 100, detection limit: 200 ppb.**

**3.3 Pretreatment of feed sample:**

- (1) Weigh  $1 \pm 0.05$ g of homogenate sample into a 10 mL centrifuge tube, add 5 mL of **2% Trichloroacetic acid Solution** (Solution 2) and vortex fully for 3 min.
- (2) Centrifuge at 4000 r/min for 15 min.
- (3) Take 0.99 mL of **Sample Diluent B** into another 2 mL centrifuge tube, then add 0.01 mL of the supernatant liquid after centrifugation, vortex fully for 1 min.
- (4) Take 50  $\mu$ L for analysis.

**Note: Sample dilution factor: 500, detection limit: 1000 ppb.**

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**Assay procedure**

Restore 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 samples and standard in order (multiple well), and keep a record of standard wells and sample wells. **Standard and Samples need test in duplicate**
2. **Add Sample:** add 50 µL of **Standard** or **Samples** per well, then add 50 µL of **Antibody Working Solution**, cover the plate with plate sealer, oscillate for 10 s gently to mix thoroughly. Incubate at 25°C for 20 min in shading light.
3. **Wash:** uncover the sealer carefully, remove the liquid in each well. Immediately add 260 µL of **Wash Buffer** (Solution 4) to each well and wash. Repeat wash procedure for 4 times, 30 s intervals/time. Invert the plate and pat it against thick clean absorbent paper (If bubbles exist in the wells, clean tips can be used to prick them).
4. **Add HRP Conjugate:** add 100 µL of **HRP Conjugate** to each well, gently oscillate for 10 s to mix thoroughly. Incubate at 25°C for 20 min in shading light.
5. **Wash:** Repeat step 3
6. **Color Development:** add 50 µL of **Substrate Reagent A** to each well, and then add 50 µL of **Substrate Reagent B**. Gently oscillate for 10 s to mix thoroughly. Incubate at 25°C for 15 min in shading light (The reaction time can be extended according to the actual color change).
7. **Stop Reaction:** add 50 µL of **Stop Solution** to each well, gently oscillate for 10 s to mix thoroughly.
8. **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 5 min after stop reaction.

## Result 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.

**Melamine (E-FS-E109) Standard Curve**

