MEL (Melamine) ELISA Kit
Catalog No: E-FS-E019
96T

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.

Phone: 240-252-7368(USA)    Fax: 240-252-7376(USA)
Email: techsupport@elabscience.com
Website: 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 Indirect-Competitive-ELISA as the method. It can detect Melamine (MEL) in samples, such as milk powder, tissue, feed, eggs, serum, etc. This kit is composed of ELISA Microtiter plate, HRP conjugate, antibody, standard and other supplementary reagents. The microplate provided in this kit has been pre-coated with MEL antigen. During the reaction, MEL in the samples or standard competes with MEL on the solid phase supporter for sites of MEL antibody. Then Horseradish Peroxidase (HRP) conjugate is added to each micro plate well, and TMB substrate is 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
Sensitivity: 1 ppb (ng/mL)
Reaction mode: 25℃, 30 min~15 min
Detection limit: Milk powder---20 ppb, Milk---27 ppb, Milk/ Milk powder (method 2) ---1 ppb,
Tissue (chicken, porcine, duck, fish, shrimp, liver) ---2 ppb,
Feed---100 ppb, Eggs---20 ppb, Serum---4 ppb
Cross-reactivity: Melamine (MEL) ---100%, Cyanuric Acid---60%, Trizine <1%
Sample recovery rate: Milk powder, Milk---90±20%, Tissue---85±20%
Feed---85±20%, Eggs---80±20%

Kits components

<table>
<thead>
<tr>
<th>Item</th>
<th>Specifications</th>
</tr>
</thead>
<tbody>
<tr>
<td>ELISA Microtiter plate</td>
<td>96 wells</td>
</tr>
<tr>
<td>Standard Liquid</td>
<td>1 mL each</td>
</tr>
<tr>
<td></td>
<td>(0 ppb, 1 ppb, 3 ppb, 9 ppb, 27 ppb, 81 ppb)</td>
</tr>
<tr>
<td>HRP Conjugate</td>
<td>5.5 mL</td>
</tr>
<tr>
<td>Antibody Working Solution</td>
<td>5.5 mL</td>
</tr>
<tr>
<td>Substrate Reagent A</td>
<td>6 mL</td>
</tr>
<tr>
<td>Substrate Reagent B</td>
<td>6 mL</td>
</tr>
<tr>
<td>Stop Solution</td>
<td>6 mL</td>
</tr>
<tr>
<td>20×Concentrated Wash Buffer</td>
<td>40 mL</td>
</tr>
<tr>
<td>2×Reconstitution Buffer</td>
<td>50 mL</td>
</tr>
<tr>
<td>Plate Sealer</td>
<td>3 pieces</td>
</tr>
<tr>
<td>Sealed Bag</td>
<td>1 piece</td>
</tr>
<tr>
<td>Manual</td>
<td>1 copy</td>
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</tbody>
</table>
Other supplies required

**Instruments:** Microtiter plate reader, Printer, Homogenizer, Nitrogen Evaporators/Water bath, Oscillators, Centrifuge, Graduated pipette, Balance (sensibility 0.01 g).

**High-precision transferpettor:** Single channel (20-200 μL, 100-1000 μL), Multichannel (300 μL).

**Reagents:** N-hexane, Acetonitrile, NaOH, Concentrated HCl, Methanol.

Experimental preparation

1. **Notes for sample pretreatment:**
   Experimental apparatus should be clean and the pipette should be disposable to avoid cross-contamination during the experiment.

2. **Reagent preparation**

   **Solution 1:** 1 M HCl
   Dilute 8.6 mL of Concentrated HCl with deionized water to 100 mL

   **Solution 2:** Acetonitrile-0.1M NaOH Solution
   Mix 84 mL of Acetonitrile and 16 mL of 0.1 M NaOH solution fully.

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

   **Solution 4:** 1 M NaOH Solution
   Dissolve 4 g of NaOH with 100 mL of deionized water.

   **Solution 5:** Reconstitution Buffer
   Dilute the 2×Reconstitution Buffer with deionized water. (2×Reconstitution Buffer (V): Deionized water (V)=1:1) The Reconstitution buffer can be store at 4°C for a month.

   **Solution 6:** 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 milk sample:**
   (1) Take 600 μL of milk sample into 2 mL EP tube and add 1 mL of acetonitrile, oscillate 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. Mix fully.
   (3) Take 50 μL for analysis.

   **Note:** Sample dilution factor: 27, minimum detection dose: 27 ppb.
3.2 Pretreatment 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, oscillate until it mixed fully.
(2) Centrifuge at 4000 r/min for 5 min. Take 100 μL of supernatant and add 900 μL of Reconstitution buffer. Mix fully.
(3) Take 50 μL for analysis.
   Note: Sample dilution factor: 20, minimum detection dose: 20 ppb.

3.3 Pretreatment for milk/milk powder sample (method 2):
(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 and oscillate 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 under a gentle stream of nitrogen or water bath.
(3) Add 1 mL of n-hexane to dissolve the remaining dry material, then add 1 mL of Reconstitution buffer. Oscillate strongly for 30 sec 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, minimum detection dose: 1 ppb.

3.4 Pretreatment for tissue (chicken, porcine, duck, fish, shrimp, liver):
(1) Weigh 2 ± 0.05 g of homogenate tissue sample into a 50 mL centrifuge tube.
(2) Add 8 mL of Acetonitrile-0.1 M NaOH solution and oscillate 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 under a gentle stream of nitrogen or water bath.
(3) Add 1 mL of n-hexane to dissolve the remaining dry material, then add 1 mL of Reconstitution buffer. Oscillate strongly for 30 sec and centrifuge to remove the upper layer n-hexane phase.
(3) Take 50 μL of the lower layer liquid for analysis.
   Note: Sample dilution factor: 2, minimum detection dose: 2 ppb.
3.5 Pretreatment 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 and 16 mL of deionized water, then homogenate the sample.
(2) Swirl for 1 min and oscillate for 2 min with vortex.
(3) Centrifuge at 4000 r/min for 15 min. Take 10 mL of the supernatant and adjust the pH to 6~8 with 1 M NaOH. (The added amount of 1 M NaOH 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. (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 (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, minimum detection dose: 100 ppb.

3.6 Pretreatment for eggs sample:
(1) Homogenate the egg sample with homogenizer to mix the egg whites and yolks fully.
(2) Weigh 2±0.05 g of homogenate egg sample into a centrifuge tube. Add 8 mL of Acetonitrile-0.1M NaOH solution and oscillate fully for 2 min.
(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 under a gentle stream of nitrogen or water bath.
(4) Add 1 mL of n-hexane to dissolve the remaining dry material, then add 1 mL of Reconstitution buffer. Oscillate strongly for 30 sec and centrifuge to remove the upper layer n-hexane phase.
(5) Take the lower layer phase solution and dilute it with Reconstitution buffer for 4 times (50 μL of sample + 50 μL of 1×Reconstitution Buffer), mix fully.
(6) Take 50 μL for analysis.
Note: Sample dilution factor: 20, minimum detection dose: 20 ppb.

3.7 Pretreatment for serum:
(1) Take 0.5 mL of serum sample into a 50 mL of centrifuge tube.
(2) Add 2 mL of Acetonitrile-0.1M NaOH solution and oscillate fully for 2 min. Centrifuge at 4000 r/min for 10 min. Take 1 mL of the supernatant and dry at 50-60°C under a gentle stream of nitrogen or water bath.
(3) Add 1 mL of n-hexane to dissolve the remaining dry material, then add 1 mL of Reconstitution buffer. Oscillate strongly for 30 sec and centrifuge to remove the upper layer n-hexane phase.
(4) Take 50 μL for analysis.
Note: Sample dilution factor: 4, minimum detection dose: 4 ppb.
Assay procedure

Centrifuge the sample again after thawing before the assay. Bring all reagents to room temperature before use. All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming.

1. **Number**: number the sample and standard in order (multiple well), and keep a record of standard wells and sample wells.

2. **Add sample**: add 50 μL of Standard or Sample per well, then add 50 μL HRP Conjugate to each well. Add 50 μL antibody working solution, cover the plate with sealer we provided, oscillate for 5 sec gently to mix thoroughly, shading light incubation for 30 min at 25°C.

3. **Wash**: uncover the sealer carefully, remove the liquid in each well. Immediately add 300 μL of wash buffer to each well and wash. Repeat wash procedure for 5 times, 30 sec 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. **Color Development**: add 50 μL of substrate solution A to each well, and then add 50 μL of substrate solution B. Gently oscillate for 5 sec to mix thoroughly. Incubate shading light for 15 min at 25°C.

5. **Stop reaction**: add 50 μL of stop solution to each well, gently oscillate and mix fully to stop the reaction.

6. **OD Measurement**: determine the optical density (OD value) of each well at 450 nm with a microplate reader (the 450/630 nm double wavelength is recommended). This step should be finished in 10 min after stop reaction.

Result analysis

1. **Absorbance % = A/A₀ × 100%**
   
   A: Average absorbance of standard solution or sample
   
   A₀: 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 software for accurate and fast analysis on a large number of samples.
Notes
1. The overall OD values will be lower when reagents have not been brought to room temperature before use or the room temperature is below 25℃.
2. During the washing procedure, if the wells turn dry, it will lead to bad linear standard curve and poor repeatability, move on to 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 reagents to strong light.
5. Do not use expired kit and reagents of different batches.
6. TMB should be abandoned if it turns color. When OD value of standard(concentration: 0)<0.5 unit(A450 nm<0.5), it indicates the reagent may be deteriorated.
7. Stop solution is caustic, avoid contact with skin and eyes.

Storage and valid period
Valid period: 1 year, expiration date is on the packing box.