Elabscience®

7th Edition, revised inApril, 2017

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Melamine(MEL)ELISA Kit

Catalog No: E-FS-E010

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)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 Micro ELISA 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:2ppb(ng/mL)

Reaction mode: 25℃, 30min~15min

Detection limit: Milk powder---40 ppb, Milk---54 ppb,Milk/ Milk powder (method 2)---4 ppb,
Tissue (chicken, porcine, duck, fish, shrimp, liver)---4 ppb,
Feed---200 ppb, Eggs---40 ppb, Serum---8 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---85±20%

Kits components

Item	Specifications
ELISA Micro-plate	96 wells
Standard Liquid	1 mL each (0 ppb,2 ppb,6 ppb,18 ppb,54 ppb, 162 ppb)
High ConcentratedStandard (1ppm) cap)	1mL
HRPConjugate(red cap)	5.5mL
Antibody Working Solution(blue cap)	5.5mL
Substrate Reagent A(white cap)	6mL
Substrate Reagent B(black cap)	6mL
Stop Solution(yellow cap)	6mL
20×Concentrated Wash Buffer(white cap)	40mL
2×Redissolve Buffer(yellow lid)	50mL
Product Description	1 copy

Other supplies required

Instruments: Micro-plate reader, Printer, Homogenizer, Nitrogen Evaporators, Oscillators, Centrifuge, Graduated pipette, Balance (sensibility 0.01g).

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

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

Experimental preparation

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**

Reagent 1: 1M HCl

Dilute 8.6 mLConcentrated HCl with deionized water to 100 mL

Dissolve 25.8 g $Na_2HPO_4 \cdot 12H_2O$ and 4.4 g $NaH_2PO_4 \cdot 2H_2O$ with1000 mL deionized water.

Reagent 2:Acetonitrile-0.1M NaOH solution

Mix 84 mL Acetonitrile and 16 mL 0.1M NaOHsolution fully.

Reagent 3:0.1M NaOH

Dissolve0.4 g NaOHwith100 mL deionized water.

Reagent 4: 1M NaOH

Dissolve 4 g NaOHwith100 mL deionized water.

Reagent 5: Re-dissolve solution.

Dilute the 2×Re-dissolve solutionwith deionized water for re-dissolution of samples. The re-dissolve solution can be stable for 1 month at 4℃.

Reagent 6: Working wash buffer

Dilute the 20×Concentrated wash bufferwith deionized water (V/V=1:19).

3. **Sample pretreatmentprocedure**

3.1 Pretreatment ofmilk sample

(1) Take 600 μL of milk sample into 2 mL EP tube and add 1 mLacetonitrile,oscillateuntil it mixed fully. Centrifuge at 4000r/min for 5 min.

(2) Take 100 μL of supernatant and add 900 μL re-dissolvesolution. Mix fully.

(3) Take 50μL for analysis.

Note: Sample dilution factor: 27, minimum detection dose: 54ppb.

3.2 Pretreatment ofmilk powder sample

(1) Weigh2±0.05g of milk powder sample into a 50 mL centrifuge tube, add 4 mL of methanol, oscillateuntil it mixed fully.

(2) Centrifuge at 4000r/min for 5 min.Take 100 μL of supernatant and add 900 μL re-dissolvesolution. Mix fully.

(3) Take 50μL for analysis.

Note: Sample dilution factor: 20, minimum detection dose: 40ppb.

3.3 Pretreatment ofmilk/milk powder sample (method 2)

(1) Take 2 mL of milk sample or 2 g milk powder sample into a centrifuge tube.

(2) Add8 mL ofAcetonitrile-0.1M NaOH solution and oscillate fully for 2 min.Centrifuge at 4000r/min for 10 min.Take 4mL of the upper layer liquid and dry with Nitrogen Evaporators at 50-60℃ or air.

(3) Add1mLn-hexane to dissolve the remaining dry material, then add 1mL re-dissolvesolution.Oscillate stronglyfor 30s and centrifuge to remove the upper layer n-hexane phase.

(4) Take 50μL of the lower layer liquidfor analysis.

Note: Sample dilution factor: 1,minimum detection dose: 2ppb.

3.4 Pretreatment oftissue (chicken, porcine, duck, fish, shrimp, liver):

(1) Weigh2±0.05g of homogenatetissue sample into a 50 mL centrifuge tube.

(2) Add 8 mL ofAcetonitrile-0.1M NaOH solution and oscillate fully for 2 min.Centrifuge at 4000r/min for 10 min.Take 2mL of the upper layer liquid and dry with Nitrogen Evaporators at 50-60℃ or air.

(3) Add1mLn-hexane to dissolve the remaining dry material, then add 1mL re-dissolvesolution.Oscillate stronglyfor 30s and centrifuge to remove the upper layer n-hexane phase.

(4) Take 50μL of the lower layer liquidfor analysis.

Note: Sample dilution factor: 2,minimum detection dose: 4ppb.

3.5 Pretreatment for feed sample

(1) Weigh2±0.05g of crushed feedsampleinto a centrifuge tube. Add 2 mLof 1M HCl solution and 16 mLdeionized water, then homogenate the sample.

(2) Swirl for 1 min and oscillate for 2 min with vortex.

(3) Centrifuge at 4000r/min for 15 min.Take 10mL of the supernatant and adjust the pH to 6~8 with 1M NaOH. (The added amount of 1M NaOH is different according to the feed sample. The needed amount is generally between 0.5 mL~ 1 Ml.)

(4) Centrifuge at 4000r/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 re-dissolve solution (Take 100 μL of supernatant and add 900 μL re-dissolvesolution. Mix fully.)

(6) Take 50μLfor analysis.

Note: Sample dilution factor: 100,minimum detection dose: 200ppb.

3.6 Pretreatment for eggs sample

(1) Homogenate the egg sample with homogenizer to mix the egg whites and yolks fully.

(2) Weigh2±0.05g of homogenateegg sampleinto a centrifuge tube. Add 8 mL ofAcetonitrile-0.1M NaOH solution and oscillate fully for 2 min.

(3) Centrifuge at 4000r/min for 10min at room temperature.Take 1mL of the upper layer liquid and dry with Nitrogen Evaporators at 50-60℃ or air.

(4) Add1mLn-hexane to dissolve the remaining dry material, then add 1mL re-dissolvesolution.Oscillate stronglyfor 30s and centrifuge to remove the upper layer n-hexane phase.

(5) Take 50μLfor analysis.

Note: Sample dilution factor: 20,minimum detection dose: 40ppb.

3.7 Pretreatment for serum

(1) Take 0.5mL of serum sample into a 50mL centrifuge tube.

(2) Add 2 mL ofAcetonitrile-0.1M NaOH solution and oscillate fully for 2 min.Centrifuge at 4000r/min for 10 min.Take 1mL of the supernatant and dry with Nitrogen Evaporators at 50-60℃ or air.

(3) Add1mLn-hexane to dissolve the remaining dry material, then add 1mL re-dissolvesolution.Oscillate stronglyfor 30s and centrifuge to remove the upper layer n-hexane phase.

(4) Take 50μLfor analysis.

Note: Sample dilution factor: 4, minimum detection dose: 8ppb.

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.**Dilute 40mL of concentrated wash buffer into 800mLwash working buffer with deionized or distilled water.

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 50μL of Standard, Blank, or Sample per well, then add 50μL antibody working solution, cover the plate with sealer we provided, oscillate for 5s gently to mix thoroughly, shading light incubation for 30min at25℃.
3. **Wash:**Uncover the sealer carefully, remove the liquid in each well. Immediately add 250μLof wash working buffer to each well and wash.Repeat wash procedure for 5 times, 30s 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. **HRP conjugate:**add 100μL HRP conjugate to each well, shading light incubation for 30min at 25℃.
5. **Wash:** repeat step 3
6. **Color Development:** Add 50μLof substrate solution A to each well, and then add 50μLof substrate solution B.Gently oscillate for 5s to mix thoroughly. Incubate shading light for 15min at 25℃.

7. **Stop reaction:**Add 50μLof stop solution to each well,gently oscillate and mix fully to stop the reaction.
8. **OD Measurement:**Determine the optical density (OD value) of each wellwith a micro-plate reader set to 450nm (the 450/630 nm double wavelength is recommended). This step should be finished in 10min after stop reaction.

Result analysis

1. Absorbance% =$A/A_0\times100\%$
   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 software for accurate and fast analysis on a large number of samples.

Notes

1. The overall OD values will be lower when reagents havenot been brought to room temperature before use or the room temperature ＜25℃.
2. During the washing procedure, if the wells turn dry, it will lead to bad linear standard curveand 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. Micro ELISA plate should be covered by plate sealer.Avoid the reagents to strong light.
5. Do not useexpired 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(A_{450nm}＜0.5), it indicates the reagent may bedeteriorated.
7. Stop solution is caustic, avoid contact with skin and eyes.

Storage and valid period

Storage: Store at 2-8℃. Avoid freeze/ thaw cycles.

Valid period:1 year. The production date is on the packing box.

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