Melamine ELISA Kit

1. Background
Melamine is an organic raw material for the resin industry. It has many industrial uses, including in the production of laminates, glues and adhesives, molding compounds, coatings and flame retardants. Overdose or repetitive ingesting of melamine in human being would cause bladder and kidney damage, even stones in the urine system. Currently, melamine is determined by chromatography technology like GC-MS, LC-MS/MS, which are regarded as time-consuming and expensive. This product is based on indirect competitive ELISA, which is rapid, accurate and sensitive compared with conventional instrumental analysis. It needs only 45 min in one run, which can considerably reduce operation error and work intensity.

2. Test Principle
This kit is based on indirect-competitive ELISA. The microtiter wells are coated with coupling antigen. Melamine in the sample competes with the antigen coated on the microtiter plate for the antibody. After the addition of enzyme conjugate, chromogenic substrate is used to show the color. Absorbance of the sample is negatively related to the melamine residue, after comparing with the standard curve, multiplied by the dilution factor, melamine residue quantity in the sample can be calculated.

3. Applications
This kit can be used for qualitative and quantitative analysis of melamine in milk (raw milk, UHT milk, reconstituted milk, dairy drinks, Pasteurized milk) and skim milk powder, animal tissue (pork, chicken, fish and shrimp), feed and egg samples.

4. Cross reactions
Melamine……………………………………………… ..100%
Cyanuric acid………………………………………………..<1%
Cyanurodiamide ammeline………………………………..<1%

5. Materials Required

5.1 Equipments
----Microtiter plate spectrophotometer (450nm/630nm)
----Shaker
----Vortex mixer
----Centrifuge
----Homogenizer
----Analytical balance (inductance: 0.01g)
----Graduated pipette: 10ml
----Volumetric flask: 500ml
----Polystyrene centrifuge tubes: 2ml, 10ml, 50ml
----Micropipettes: 20μl-200μl, 100μl-1000μl, 250μl-multichanel

5.2 Reagents
----Acetonitrile(AR)
----Methanol (AR)
----Concentrated hydrochloric acid (AR)
----Acetone (AR)
----Deionized water

6. Kit components
- Microtiter plate coated with antigen (96wells)
- Standard Solution×6 bottle (1ml/bottle)
  - 0ppb, 1ppb, 3ppb, 9ppb, 27ppb, 81ppb
- Spiking Standard Control: (1ml/bottle) 10ppm
- Enzyme conjugate 7ml……………..…………red cap
- Antibody solution 7ml ………………………green cap
- Substrate solution A 7ml………………. ……white cap
- Substrate solution B 7ml………………………red cap
- Stop solution 7ml……………………………yellow cap
- 20×concentrated wash solution  40ml
  ……………………………………………transparent cap
- 2×concentrated extraction solution  50ml
  ……………………………………………blue cap

7. Reagents Preparation

Solution 1: 1M hydrochloric acid (HCl)
Dilute 41.5ml hydrochloric acid with deionized water, and dilute to 500ml.

Solution 2: Extraction solution
Dilute 2×concentrated extraction solution with deionized water.

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water in the volume ratio of 1:1 (e.g., 10ml of 2×concentrated extraction solution + 10ml of deionized water), which will be used for sample extraction. This solution can be stored for 1 month at 4°C.

**Solution 3: Wash solution**

Dilute 20×Concentrated wash solution with deionized water in the volume ratio of 1:19 (e.g., 10ml of 20×Concentrated wash solution + 190ml of deionized water), which will be used to wash the plates. This diluted solution can be stored for 1 month at 4°C.

### 8. Sample Preparation

#### 8.1 Notice and precautions before operation

(a) Please use one-off tips in the process of experiment, and change the tips when absorbing different reagent.  
(b) Make sure that all experimental instruments are clean.

#### 8.2 Milk (raw milk, UHT milk, reconstituted milk, dairy drinks, Pasteurized milk)

-----Take 400μl of fresh milk to a 2ml centrifuge tube;  
-----Add 800μl of acetonitrile, vortex for 1min;  
-----Centrifuge for separation: ambient temperature / 5min / 3000g;  
-----Take 100μl of the supernate to a 2ml centrifuge tube, add 500μl of extraction solution (solution 2), vortex for 1min;  
-----Take 50μl per well for assay.

**Dilution factor:** 18

#### 8.3 Skim Milk powder

-----Weigh 1.0±0.05g of sample into a 10ml polystyrene tube;  
-----Add 2.5ml of deionized water and vortex for 3min;  
-----Take 400μl into a 2ml centrifuge tube, add 400μl of acetonitrile, 400μl of methanol, vortex for 1min;  
-----Centrifuge for separation: ambient temperature / 5min / at least 3000g;  
-----Take 100μl of supernate mix with 500μl of extraction solution (solution 2), vortex for 1min, mix completely.  
-----Take 50μl per well for assay.

**Dilution factor:** 45

#### 8.4 Animal tissue (pork, chicken, fish and shrimp)

-----Weigh 1.0±0.02g of sample into a 50ml polystyrene tube;  
-----Add 2ml of acetone, 2ml of deionized water and vortex for 5min.  
-----Centrifuge for separation: ambient temperature / 5min / 3000g;  
-----Take 100μl of supernate to a 2ml centrifuge tube, mix with 900μl of extraction solution (solution 2), vortex completely.  
-----Take 50μl per well for assay.

**Dilution factor:** 40

#### 8.5 Feed

-----Weigh 1.0±0.05g of sample into a 50ml polystyrene tube;  
-----Add 5ml of acetone, 4ml of methanol, and 0.5ml of 1M hydrochloric acid (solution 1), 0.5ml of deionized water and shake for 5min;  
-----Centrifuge for separation: ambient temperature / 10min / 3000g;  
-----Take 100μl of supernate to a 2ml centrifuge tube, mix with 900μl of extraction solution (solution 2), vortex for 1min.  
-----Take 50μl per well for assay.

**Dilution factor:** 100

#### 8.6 Egg

-----Weigh 1.0±0.05g of sample into a 50ml polystyrene tube;  
-----Add 4.5ml of deionized water, 0.5ml of methanol, and then shake for 5min;  
-----Centrifuge for separation: ambient temperature / 10min / at least 3000g;  
-----Take 100μl of supernate into a 2ml centrifuge tube, mix with 300μl extraction solution (solution 2), mix completely.  
-----Take 50μl per well for assay.

**Dilution factor:** 20

### 9. Assay process

#### 9.1 Notice before assay

9.1.1 Make sure all reagents and microwells are all at...
room temperature (20-25°C).

9.1.2 Return all the rest reagents to 2-8°C immediately after used.

9.1.3 Washing the microwells correctly is an important step in the process of assay; it is the vital factor to the reproducibility of the ELISA analysis.

9.1.4 Avoid the light and cover the microwells during incubation.

9.2 Assay Steps

9.2.1 Take all reagents out at room temperature (20-25°C) for more than 30min, shake gently before use.

9.2.2 Get the microwells needed out and return the rest into the zip-lock bag at 2-8°C immediately.

9.2.3 The concentrated wash solution and concentrated extraction solution should be rewarmed to room temperature before use.

9.2.4 Number: number every microwell position and all standards and samples should be run in duplicate. Record the standards and samples positions.

9.2.5 Add standard solution/sample, enzyme conjugate and antibody solution: Add 50µl of standard solution (Kit component) or prepared sample to corresponding wells. Add 50µl of enzyme conjugate (Kit component), 50µl of antibody solution (Kit component), mix gently by shaking the plate manually and incubate for 30min at 25°C with cover.

9.2.6 Wash: remove the cover gently and pour the liquid out of the wells and rinse the microwells with 250µl of wash solution (solution 3) at interval of 10s for 4-5 times. Absorb the residual water with absorbent paper (the rest air bubble can be eliminated with unused tip).

9.2.7 Coloration: add 50µl of solution A (Kit component) and 50µl of solution B (Kit component) to each well. Mix gently by shaking the plate manually and incubate for 15min at 25°C with cover (see 12.8).

9.2.8 Measure: add 50µl the stop solution to each well. Mix gently by shaking the plate manually and measure the absorbance at 450nm (It’s suggested measure with the dual-wavelength of 450/630nm. Read the result within 5min after adding stop solution).

10. Results

10.1 Percentage absorbance
The mean values of the absorbance values obtained from the standards and the samples are divided by the absorbance value of the first standard (zero standard) and multiplied by 100%.

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\text{Absorbance (\%) = } \frac{B}{B_0} \times 100\%
\]

B ——absorbance of standards or samples
B0 ——absorbance of zero standard

10.2 Standard Curve
To draw a standard curve: The absorbance value of standards as y-axis, semi-logarithmic of the concentration of the standards (ppb) as x-axis.

---The melamine concentration of each sample (ppb), which can be read from the calibration curve, is multiplied by the corresponding dilution factor of each sample followed, and the actual concentration of sample is obtained.

Please notice:
Special software has been developed for all data interpretation, which can be provided on request.

11. Sensitivity, accuracy and precision

Test Sensitivity: 1ppb

Detection limit

Milk………………………………………………………18ppb
Skim Milk powder……………………………………45ppb
Animal tissue…………………………………………..50ppb
Feed……………………………………………………100ppb
Egg……………………………………………………….20ppb

Accuracy

Milk……………………………………………………100±30%
Skim Milk powder…………………………………….100±30%
Animal tissue…………………………………………100±30%
Feed……………………………………………………100±30%
Egg……………………………………………………….100±30%

Precision
Variation coefficient of the ELISA kit is less than 10%.

12. Notice

12.1 The mean values of the absorbance values obtained for the standards and the samples will be reduced if the reagents and samples have not been regulated to room temperature (20-25°C).

12.2 Do not allow microwells to dry between steps to avoid unsuccessful reproducibility and operate the next step immediately after tap the microwells holder.

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12.3 Shake each reagent gently before using.
12.4 Keep your skin away from the stop solution for it is the 0.5M H₂SO₄ solution.
12.5 Don’t use the kits out of date. Don’t exchange the reagents of different batches, or else it will drop the sensitivity.
12.6 Keep the ELISA kits at 2-8°C, do not freeze. Seal rest microwell plates, Avoid straight sunlight during all incubations. Covering the microtiter plates is recommended.
12.7 Substrate solution should be abandoned if it turns colors. The reagents may turn bad if the absorbance value (450/630nm) of the zero standard is less than 0.5 (A450nm<0.5).
12.8 The coloration reaction need 10-15min after the addition of solution A and solution B; But you can prolong the incubation time ranges to 20min or more if the color is too light to be determined, never exceed 25min, On the contrary, shorten the incubation time properly.
12.9 The optimal reaction temperature is 25°C. Higher or lower temperature will lead to the changes of sensitivity and absorbance values.

13. Storage condition and storage period

   Storage condition: 2-8°C.
   Storage period: 12 months