Melamine ELISA Test Kit

Catalog #: LSY-10026

1. Principle

This test kit is based on the competitive enzyme immunoassay for the detection of Melamine in the sample. The coupling antigen is pre-coated on the micro-well stripes. The Melamine in the sample and the coupling antigens pre-coated on the micro-well stripes compete for anti-Melamine antibodies. After the addition of the enzyme conjugate, the TMB substrate is added for coloration.
The optical density (OD) value of the testing sample has a negative correlation with the Melamine concentration in the sample. This value is compared to the standard curve and the Melamine concentration is subsequently obtained.

2. Technical specifications

Sensitivity : 1 ppb
Detection limit:
Milk Powder.....20 ppb
Milk..... 16 ppb
Milk/Milk Powder(Second pre-treatment).....1 ppb
Tissue(chicken, pork, duck, fish, shrimp and liver).....2 ppb
Feed..... 100 ppb
Egg..... 20 ppb
Serum.....4 ppb
Recovery rate:
Milk/Milk Powder..... 90±15%
Tissue..... 85±10%
Feed.....85±10%
Egg.....80±10%
Cross-reaction rate:
Melamine..... 100%
Cyanuric acid..... 60%
Triazine..... <1%
Diamino atrazine.....<1%

Gentaur Molecular Products
Marienbongard 20
52062 Aachen Deutschland

3. Components

1） Micro-well strips: 12 strips with 8 removable wells each
2） 6× standard solution (1 mL each): 0 ppb,1 ppb, 3 ppb, 9 ppb, 27 ppb, 81 ppb
3） Enzyme conjugate (7 mL).....red cap
4） Antibody working solution (7 mL).....blue cap
5） Substrate A solution (7 mL).....white cap
6） Substrate B solution (7 mL)..... white cap
7） Stop solution (7 mL)..... yellow cap
8） 20× concentrated washing buffer (40 mL)..... white cap
9） 2× concentrated redissolving solution (50 mL).....transparent cap

4. Materials required but not provided

1) **Equipments:** microplate reader, printer, homogeniser, nitrogen-drying device, vortex, centrifuge, measuring pipets, balance(a reciprocal sensibility of 0.01 g).
2) **Micropipettors:** single-channel 20~200 µL, 100~1000 µL; and multi-channel 250 µL;
3) **Reagents:** HCl, NaOH, Acetonitrile(CH3CN), N-hexane, Methanol.

5. Sample pre-treatment

Instructions (The following points must be dealt with before the pre-treatment)
1) Only the disposable tips can be used for the experiments and the tips must be changed when used for different reagents;
2) Before the experiment, each experimental equipment must be clean and should be re-cleaned if necessary, in order to avoid the contamination that interferes with the experimental results.

Solution preparation before sample pre-treatment:
1) The 2×concentrated redissolving solution is diluted with deionized water at 1:1 (1 mL 2×concentrated redissolving solution + 1 mL deionized water)
2) 1 M HCl: dissolve 8.6 mL HCl(approx 36.5%) in the deionized water to 100 mL.
3) 0.1 M NaOH solution: dissolve 0.4 g NaOH in the deionized water to 100 mL.
4) 1 M NaOH solution: dissolve 4 g NaOH in the deionized water to 100 mL.
5) Acetonitrile-0.1 M NaOH solution: Mix 84 mL Acetonitrile with 16 mL 0.1 M NaOH solution.

5.1 Milk sample

1 Take 600 µL sample to 2 mL tube, add 1 mL Acetonitrile(CH3CN) solution. Mix evenly.
2 Take 100 µL supernatant, then add 500 µL diluted redissolving solution. Mix evenly.
3 Take 50 µL for analysis.
Fold of dilution of sample :20

5.2 Milk Powder

1 Take 2±0.05 g homogenized sample to 50 mL tube, add 4 mL Methanol. Mix evenly.
2 Centrifuge at above 4000 r/min at 15 ℃for 10 min.
3 Take 100 µL supernatant, then add 900 µL diluted redissolving solution. Mix evenly.
4 Take 50 µL for analysis.
Fold of dilution of sample :20

5.3 Feed

1 Take 2±0.05 g homogenized sample; Add 2 mL 1 M HCl and 16 mL deionized water.
2 Mix for 1 minutes, and then vortex for 2 minutes.
3 Centrifuge at above 4000 r/min at 15 ℃for 15 min. Take 10 mL supernatant and adjust pH to 6-8 with 1 M NaOH.
4 Centrifuge at above 4000 r/min at 15 ℃for 15 min. Take the supernatant(if it's still not clear, should centrifuge at more speed).
5 Dilute the supernatant with the diluted redissolving solution at 1:9(100µL supernatant + 900µL diluted redissolving solution, mix for 30 s).
6 Take 50 µL for analysis.
Fold of dilution of sample :100

5.4 Milk/Milk Powder

1 Take 2 mL milk sample or 2 g milk powder to tube;
2 Add 8 mL Acetonitrile-0.1 M NaOH solution, mix throughly for 2 min. Centrifuge at above 4000 r/min at 15 ℃for 10 min.
3 Take 4 mL supernatant and blow to dry by nitrogen at 56 ℃.
4 Dissolve the dry residue in 1 mL N-hexane, then add 1mL of the diluted redissolving solution. Mix properly for 30 seconds, centrifuge and remove the N-hexane layer(top layer)
5 Take 50 µL for analysis
Fold of dilution of sample :1

5.5 Tissue(meat, liver, chicken, shrimp, fish, duck)

1 Take 2±0.05 g homogenized sample to 50 mL tube.
2 Add 8 mL Acetonitrile-0.1 M NaOH solution and mix for 5 min, Centrifuge at above 4000 r/min at 15 ℃for 10 min. Take 2 mL supernatant and blow to dry by nitrogen at 56 ℃.
3 Dissolve the dry residue in 1 mL N-hexane, then add 1mL of the diluted redissolving solution. Mix properly for 30 seconds, centrifuge and remove the N-hexane layer(top layer)
4 Take 50 µL for analysis
Fold of dilution of sample :2

5.6 Egg

1. Take 2±0.05 g homogenized sample(Egg white, egg yolk or whole egg) into 50 mLcentrifugal, add 8mL Acetonitrile-0.1 M NaOH solution, mix evenly for 5min.
2. Centrifuge at above 4000 r/min at 15 ℃for 10 min, transfer 1 mL supernatant into a new centrifugal tube and evaporate to dryness by nitrogen at 56 ℃.

3. Dissolve the dry residues in 1 mL N-hexane, add 1mL of the diluted redissolving solution, mix properly for 30s, centrifuge and remove the N-hexane layer(top layer).
4. Dilute sample with the diluted redissolving solution at 1:3 (50 µL sample + 150µL diluted redissolving solution, mix for 30 s).
5. Take 50 µL for further analysis.
Fold of dilution of sample :20

5.7 Serum

1 Take 0.5 mL sample to 50 mL tube.
2 Add 2 mL Acetonitrile-0.1 M NaOH solution and mix for 2 min, Centrifuge at above 4000 r/min at 15 ℃for 10 min. Take 1 mL supernatant and blow to dry by nitrogen at 56 ℃.
3 Dissolve the dry residue in 1 mL N-hexane, then add 1mL of the diluted redissolving solution. Mix properly for 30 seconds, centrifuge and remove the N-hexane layer(top layer)
4 Take 50 µL for analysis
Fold of dilution of sample :4

6. ELISA procedures

1 Bring test kit to the room temperature (20-25 ℃) for at least 30 min, note that each reagent must be shaken evenly before use; put the required micro-well strips into plate frames. Re-sealed the unused microplate, stored at 2-8 ℃, not frozen.
2 Solution preparation: dilute 40 mL of the concentrated washing buffer (20×concentrated) with the distilled or deionized water to 800 mL (or just to the required volume) for use;
3 Numbering: number the micro-wells according to samples and standard solution; each sample and standard solution should be performed in duplicate; record their positions.
4 Add 50 µL of the sample or the standard solution into separate duplicate wells, then add enzyme conjugate, 50 µL/well; and antibody working solution, 50 µL/well. Mix gently by shaking the plate manually, seal the microplate with the cover membrane, incubate at 25 ℃for 30 min.
5 Pour liquid out of microwell, flap to dry on absorbent paper; add 250 µL/well of washing buffer for 15-30 seconds, then take out and flap to dry with absorbent paper, repeat 5 times.
6 Coloration: add 50 µL of the substrate A solution, 50 µL of the B solution into each well. Mix gently by shaking the plate manually, and incubate at 25 ℃for 15 min in the dark for coloration;
7 Determination: add 50 µL of the stop solution into each well. Mix gently by shaking the plate manually. Set the wavelength of the microplate reader at 450 nm to determine the OD value of every well. (Recommend to read the OD value at the dual-wavelength 450/630nm within 5 min).

7. Result judgment

There are two methods to judge the results; the first one is the rough judgment, while the second is the quantitative determination. Note that the OD value of the sample has a negative correlation with the content of Melamine.

7.1 Qualitative determination

The concentration range (ng/mL) can be obtained from comparing the average OD value of the testing sample with that of the standard solution. Assuming that the OD value of the sample Ⅰ is 0.3, and that of the sample Ⅱ is 1.0, the OD value of standard solutions is: 2.243 for 0 ppb, 1.816 for 1 ppb, 1.415 for 3 ppb, 0.74 for 9 ppb, 0.313 for 27 ppb, 0.155 for 81 ppb, accordingly the concentration range of the sample Ⅰ is 27 to 81 ppb, and that of the sample Ⅱ is 3 to 9 ppb.

7.2 Quantitative determination

The mean values of the absorbance values is equivalent to the percentage of the average OD value (B) of the testing sample and the standard solution divided by the OD value (B0) of the first standard solution (0 standard) and subsequently multiplied by 100%, that is

$$\text{Percentage of absorbance value} = \frac{B}{B_0} \times 100\%$$

B—the average (double wells) OD value of the testing sample or the standard solution
B0—the average OD value of the 0ng/mL standard solution

Draw the standard curve with the absorption percentages of the standard solutions and the semilogarithmic values of the Melamine standard solutions (ng/mL) as Y- and X-axis, respectively. Read the corresponding concentration of the testing sample from the standard curve by incorporating its absorption percentage into the standard curve. The resulting value is subsequently multiplied by the corresponding dilution fold, finally obtaining the Melamine concentration in the sample.
Using the professional analyzing software of this kit will be more convenient for the accurate and rapid analysis of a large amount of samples. (Please contact us for this software)

8. Precautions

1 Bring all reagents and micro-well strips to the room temperature (20-25℃).
2 Return all reagents to 2-8℃ immediately after use.
3 The reproducibility of the ELISA analysis, to a large degree, depends on the consistency of plate washing. The correct operation of plate washing is the key point in the procedures of ELISA.
4 For the incubation at constant temperatures, all the samples and reagents must avoid light exposure, and each microplate should be sealed by the cover membrane.
5 The room temperature below 20℃ or the temperature of the reagents and the testing samples being not returned to the room temperature (20-25℃) will lead to a lower standard OD value.

6 Dryness of the microplate in the washing process will be accompanied by the situations including the non-linear standard curves and the undesirable reproducibility; So continue to next step immediately after washing.
7 Mix evenly, otherwise there will be the undesirable reproducibility.
8 The stop solution is the 2 M sulfuric acid solution, avoid contacting with the skin.
9 Do not use the kit exceeding its expiry date. The use of diluted or adulterated reagents from the kits will lead to the changes in the sensitivity and the detecting OD values. Do not exchange the reagents from the kits of different lot numbers to use.
10 Put the unused microplate into an auto-sealing bag to re-seal it. The standard substance and the colourless color former is light sensitive, and thus they cannot be directly exposed to the light.
11 Discard the colouration solution with any color that indicates the degeneration of this solution. The detecting value of standard solution 1（0 ppb）of less than 0.5 indicates its degeneration.
12 Colouration time is about 15 min, if the color is light, prolong the time of colouration but don't exceed 30 min.
13 The optimum reaction temperature is 25 ℃, and too high or low temperatures will result in the changes in the detecting sensitivity and OD values.

9. Storage and expiry date

Storage: stored at 2-8 ℃, not frozen.
Expiry date: 12 months; date of production is on the box

Gentaur Molecular Products
Marienbongard 20
52062 Aachen Deutschland