VERSION 1.01

MELAMINE ELISA TEST KIT

MANUAL

ELISAKITS.ONLINE

By Immunomart

Melamine ELISA Test Kit

Catalogue Number. IP100048

Principle

This test kit is based on the indirect 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.

Technical specifications

Sensitivity: 0.5ppb
Incubator temperature: 25℃
Incubator time: 30min～15min
Detection limit:
Fresh milk, pure milk 5ppb
Yogurt, milk powder 10ppb
Cross-reaction rate:
Melamine 100%
Recovery rate:
Fresh milk, pure milk 90±25%
Yogurt, milk powder 80±25%

Components

1	Micro-well strips	12 strips with 8 removable wells each	
2	6× standard solution (1mL each)	0 ppb, 0.5 ppb, 1.5 ppb, 4.5 ppb, 13.5 ppb, 40.5 ppb	
3	Enzyme conjugate	7ml	red cap
4	Antibody working solution	7ml	blue cap
5	Substrate A	7ml	white cap
6	Substrate B	7ml	black cap
7	Stop solution	7ml	yellow cap
8	20× concentrated washing buffer	15ml	white cap
9	20x sample extracting solution	50ml	transparent cap
10	10x sample dilution	50ml	Blue cap

Materials required but not provided

1) Equipments: microplate reader, printer, 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 30～300 µl;

Sample pre-treatment

Instructions

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.

Samples preparation

A. Sample extracting solution
Dilute 20x sample extracting solution with deionized water at 1:19;
B. Sample dilution
Dilute 10x sample dilution with deionized water at 1:9.

5.1 Milk(pure milk, fresh milk)

1. Take 50ul fresh milk for analysis

Fold of dilution of sample: 1

5.2 Milk powder

1. Take 1 g fresh milk powder into 50 ml centrifuge tube, add 9ml Sample extracting solution, vortex and shake for 3min, centrifuge at above 4000 r/min at 15 ℃ for 10 min;
2. Leave aside the top layer of condensed protein fat, take 50ul of the middle layer liquid for analysis
Fold of dilution of sample: 10
5.3 Yogurt
1. Take 1 g fresh yogurt sample into 10ml/15ml/50 ml centrifuge tube, add 4.5ml Sample dilution, vortex and shake for 2min;
2. Take 50μL upper-layer liquid for analysis

Fold of dilution of sample: 5

ELISA procedures

Instructions

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.

Operation 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 15mL of the 20Xconcentrated washing buffer with the deionized water to 300mL 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 50μL of the sample/standard solution to separate duplicate wells, then add 50μL enzyme conjugate, then add 50μL antibody working solution to each well, shake properly, seal the microplate with the cover membrane, and incubate at 25 ℃ for 30 min;
5. Pour the liquid out of the wells, wash the microplate with the diluted washing buffer at 250 μL/well for 5-6 times. Each time soak the well with the diluted washing buffer for 15-30 seconds and then flap to dry on absorbent paper (if there are the bubbles after flapping, cut them with the clean tips).
6. Coloration: add 50μL of the substrate A, then 50 μL of the substrate B 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 50μL of the stop solution into each well. Mix gently by shaking the plate manually. Set the wavelength of the microplate reader at 450nm to determine the OD value of every well. (Recommend to read the OD value at the dual-wavelength 450/630nm within 5 min).

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.

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: 2.043 for 0ppb, 1.604 for 0.5ppb, 1.101 for 1.5ppb, 0.512 for 4.5ppb, 0.161 for 13.5ppb, 0.055 for 40.5ppb, accordingly the concentration range of the sample Ⅰ is 4.5 to 13.5ppb, and that of the sample Ⅱ is 1.5 to 4.5ppb.

Quantitative determination

The mean values of the absorbance values obtained for the percentage of the average OD value (B) of the 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,

Percentage of absorbance value = $(B / B_0) \times 100\%$

B—the average OD value of the sample or the standard solution
B_0—the average OD value of the 0 ng/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.

Precautions

1 The room temperature below 25℃ 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.

2 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.
3 Mix evenly, otherwise there will be the undesirable reproducibility.
4 The stop solution is the 2 M sulfu ric acid solution, avoid contacting with the skin.
5 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.
6 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.
7 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.
8 The optimum reaction temperature is 25 ℃, and too high or low temperatures will result in the changes in the detecting sensitivity and OD values.

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