VERSION 1.01

ERYTHROMYCIN ELISA TEST KIT

MANUAL

ELISAKITS.ONLINE

By Immunomart

Erythromycin ELISA Test Kit

Catalogue Number. IP100028

Principle

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

Technical specifications

Sensitivity: 0.2 ppb
Incubation Temperature: 25℃
Incubation Time: 30min—15min
Detection limit: Raw milk about 10ppb, Honey (method 1) about 4ppb, Honey (method 2) about 0.2ppb Tissue about 15ppb
Note: ppb=ng/mL or ng/g
Cross-reaction rate: Erythromycin 100%
Recovery rate: 90%±30%

Components

1	Micro-well strips	12 strips with 8 removable wells each
2	standard solution (1mL each)	0ppb, 0.2ppb, 0.6ppb, 1.8ppb, 5.4ppb and 16.2ppb
3	11x concentrated Enzyme conjugate	0.7ml
4	Enzyme conjugate dilution	7ml
5	Substrate A	7ml
6	Substrate B	7ml
7	Stop solution	7ml
8	20× concentrated washing buffer	30ml
9	1x Redissolving solution	50ml

Materials required but not provided

Equipments: microplate reader (450nm, 630nm), rotary evaporator/nitrogen-drying device, homogenizer, oscillator, centrifuge (4000g and above), balance (a sensibility reciprocal of 0.01 g), measuring pipets, incubator (adjustable 25℃),timer
Micropipettors: single-channel 20~200 µL and 100~1000 µL, and eight-channel 30~300 µL;
Reagents: Deionized water, Trichloroacetic acid, NaOH, HCl, NaCl, Na_2CO_3, $NaHCO_3$, Methanol, Ethyl acetate.

Sample pre-treatment

Instructions

1) Only the disposable tips can be used for the experiments and the tips must be changed when used for absorbing 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) 2% NaCl Solution: weigh 2.0g NaCl, add 100ml deionized water to dissolve and mix it evenly;
2) Methanol-2% NaCl Solution= 2:1 : take 100ml Methanol, add into 50ml 2% NaCl Solution to dissolve and mix it evenly;
3) 1% Trichloroacetic acid solution: weigh 5g Trichloroacetic acid, add 500ml deionized water to dissovle and mix it evenly;
4) 0.1M CB Solution: weigh 4.66g Na_2CO_3 and 0.5g $NaHCO_3$, add 500ml deionized water to dissolve and mix it evenly;
5) 0.05M NaOH solution: weigh 1g NaOH, add 500ml deionized water to dissolve and mix it evenly;
6) 0.1M HCl Solution: take 100ul HCl, add 10.9ml deionized water, mix it evenly;
7) Washing buffer: 1 part 20× concentrated washing buffer + 19 parts deionized water

Samples preparation

a) Milk

1. Take 1ml milk sample into 2ml Polystyrene centrifuge tube, add 1ml 1% Trichloroacetic acid solution, vortex for 3min;
2. Centrifuge at above 3000g at room temperature (20 - 25 ℃) for 5min;
3. Take 50ul up-layer liquid into 2ml Polystyrene centrifuge tube, add 450ul redissolving solution, vortex for 1min;
4. Take 50 µL for analysis.
Fold of dilution of the sample: 20

b) Honey
Method 1

1) Take 1.0±0.05g honey sample into 50ml Polystyrene centrifuge tube;
2) Add 2ml Methanol-2% NaCl Solution, vortex for 2min until honey is dissolved completely, Centrifuge at 3000g at room temperature (20 - 25 ℃) for 5min;
3) Take 100ul bright solution into 2ml Polystyrene centrifuge tube, add 900ul redissolving solution;
4) Take 50 µL for analysis.
Fold of dilution of the sample: 20

Method 2

1) Take 2.0±0.05g honey sample into 50ml Polystyrene centrifuge tube;
2) Add 2ml 0.1M CB solution, vortex until honey sample is completely dissolved, then add 6ml Ethyl acetate, shake for 5min;
3) Centrifuge at 3000g at room temperature (20 - 25 ℃) for 5min;
4) Take 3ml up-layer organic phase into 10ml clean dry glass tube, blow to dry in 50-60℃ water bath by Nitrogen;
5) Add 0.5ml redissolving solution, vortex for 5min, mix it evenly;

6) Take 50 µL for analysis.
Fold of dilution of the sample: 0.5

c) Tissue

1) Take 1.0±0.05g tissue sample into 50ml Polystyrene centrifuge tube;
2) Add 4ml 0.05M NaOH solution, vortex for 5min until tissue sample separate;
3) Centrifuge at 4000g at room temperature (20 - 25 ℃) for 5min;
4) Take 200ul up-layer clear liquid, add 750ul redissolving solution and 0.1M HCl Solution, vortex for 1min and mix evenly;
5) Take 50 µL for analysis.
Fold of dilution of the sample: 25

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. Take out all the necessary reagents from the kit and place at the room temperature (20 to 25 ℃) for at least 30 minutes. Note that each liquid reagent must be shaken to mix evenly before use.
2. Take the required micro-well strips and plate frames. Re-sealed the unused microplate, stored at 2-8 ℃, not frozen.
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. Enzyme conjugate preparation: take 1 part 11X Concentrated Enzyme conjugate, add 10 parts Enzyme conjugate dilution, dilute at 1:10, get the ready to use Enzyme conjugate.
5. Add 50µL of the sample or standard solution to separate duplicate wells, then add enzyme conjugate, 50 µL each well. Mix gently by shaking the plate manually, seal the microplate with the cover membrane, and incubate at 25 ℃ at dark for 30 minutes.
6. Pour liquid out of microwell, add 250 µL/well of washing buffer for 15-30 seconds, repeat three to four times, then flap to dry (if there are the bubbles after flapping, cut them with the clean tips).
7. Coloration: add 100 µL mixture of the substrate A and substrate B into each well (Note: mix Substrate A and Substrate B at 1:1, the mixture should be used in 10min, never use metal container or metal to stir the solution, otherwise the substrate may be invalid.). Mix gently by shaking the plate manually, and incubate at 25 ℃ for 15 minutes at dark for coloration.
8. Determination: add 50 µL of the stop solution into each well (The substrate color from blue to yellow, it means the stop succeeds). Mix gently by shaking the plate manually. Set the wavelength of the microplate reader at 450 nm to determine the OD value (Recommend to read the OD value at the dual-wavelength 450/630 nm within 5 minutes).

Result judgment

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 semilogarithm values of the Erythromycin standard solutions (ng/mL) as Y- and X-axis, respectively. Read the corresponding concentration of the 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, thus finally obtaining Erythromycin concentration in the sample.

Precautions

1. The room temperature below 25 ℃ or the temperature of the reagents and the 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.
3. Mix every reagent and reaction mixture evenly and wash the microplate thoroughly, otherwise there will be the undesirable reproducibility.
4. The stop solution is the 2 M sulfuric acid solution, avoid contacting with the skin.
5. 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.
6. 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.
7. Discard the colouration solution with any color that indicates the degeneration of this solution. The detecting value of the 0 standard solution of less than 0.5 indicates its degeneration.
8. The optimum reaction temperature is 25 ℃, and too high or too 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.