VERSION 1.01

AZITHROMYCIN ELISA TEST KIT

MANUAL

ELISAKITS.ONLINE

By Immunomart

Azithromycin ELISA Test Kit

Catalogue Number. IP100033

Principle

This test kit is based on the competitive enzyme immunoassay for the detection of Azithromycin in the sample. The coupling antigens are pre-coated on the micro-well stripes. The Azithromycin in the sample and the coupling antigens pre-coated on the micro-well stripes compete for the anti-Azithromycin antibodies. 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 Azithromycin in it. This value is compared to the standard curve and the Azithromycin concentration is subsequently obtained.

Technical specifications

Sensitivity: 0.2ppb
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: Azithromycin 100%, Erythromycin 100%
Recovery rate: 90±30%

Components

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

Materials required but not provided

Equipments: microplate reader (450nm, 630nm), printer, homogenizer, nitrogen-drying device, vortex, shaker, centrifuge (4000g and above), measuring pipets, balance (a reciprocal sensibility of 0.01 g), incubator (25℃), timer;
Micropipettors: single-channel 20-200 µL, 100-1000 µL, and eight-channel 30～300 µl;
Reagents (AR): Deionized water, Trichloroacetic acid ($C_2HCl_3O_2$), NaOH, HCl, Na_2CO_3, $NaHCO_3$, Methanol, NaCl.

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 NaOH, add 100ml deionized water, mix it evenly.
2) Methanol-2%NaCl solution=2:1: take 100ml Methanol, add into 50ml 2%NaCl solution, mix it evenly.
3) 1%Trichloroacetic acid ($C_2HCl_3O_2$) solution: weigh 5.0g Trichloroacetic acid ($C_2HCl_3O_2$), add 500ml deionized water, mix it evenly.
4) 0.1M CB solution*:* weigh 4.66g Na_2CO_3 and 0.5g $NaHCO_3$, add 500ml deionized water, mix it evenly.
5) 0.05M NaOH solution: weigh 1.0g NaOH, add 500ml deionized water, 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 ($C_2HCl_3O_2$) 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 50ul 0.1M HCl Solution, vortex for 1min and mix evenly(If the sample is too cloudy, please centrifuge and analyze);
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 Azithromycin 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 Azithromycin concentration in the sample.

Precautions

1) Read the manual carefully before use;
2) Return all reagents in the kit to room temperature(25±2℃) (about 1 hour) before use;
3) Shake the reagent evenly before use, avoid bubbles when mixing;
4) The tips are disposable, to avoid cross-pollution, do not repeat use the tips;
5) Do not use test kits out of date, do not mix use reagents from different batch;
6) Test the sample immediately after sample preparation, otherwise it may affect results;
7) The substrate A and substrate B are both colorless and transparent liquids. If they become blue before use, or turn blue immediately after mixing, the reagents are contaminated or deteriorated.
8) Sampling process must be rapid on the premise of ensuring accuracy, so as to avoid the influence of reaction time difference on the test results.
9) The stop solution contains sulfuric acid. If you accidentally splash on your skin or clothing, rinse immediately with plenty of water. If you accidentally get into the eyes, please go to the hospital for examination after thorough cleaning.

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