VERSION 1.01

KANAMYCIN ELISA TEST KIT

MANUAL

ELISAKITS.ONLINE

By Immunomart

Kanamycin ELISA Test Kit

Catalogue Number. IP100034

Principle

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

Technical specifications

Sensitivity: 0.2ppb
Incubator temperature: 25℃
Incubator time: 20min～10min
Detection limit: Liquid milk (method 2) about 10ppb, Pork, Chicken about 6ppb, Liquid milk (method 1), Honey, Vaccine about 5ppb
Note: ppb= ng/ml or ng/g
Cross-reaction rate: Kanamycin 100%, Spectinomycin,Streptomycin,Tilmicosin <0.1%
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	Enzyme conjugate	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	20X concentrated tissue redissolving solution	10ml
10	10X concentrated honey redissolving solution	10ml

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): NaOH, NaCl, KCl, KH_2PO_4, Na_2HPO_4▪$12H_2O$(The above reagents are of analytical grade), Deionized water

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) *Tissue redissolving solution:* 1 part of 20X concentrated tissue redissolving solution, add 19 parts of deionized water, mix it evenly.
2) *Honey redissolving solution:* 1 part of 10X concentrated honey redissolving solution, add 9 parts of deionized water, mix it evenly.
3) Buffer: 0.1M PBS solution(PH=10-11): weigh 13.4g Na_2HPO_4▪$12H_2O$, 20g NaCl, 0.32g NaOH, 0.5g KH_2PO_4, 0.5gKCl, add deionized water to 500ml.
4) 1M HCl: take 1ml HCl, add 11ml deionized water, mix it evenly.
5) Washing buffer: 1 part 20× concentrated washing buffer + 19 parts deionized water.

Samples preparation

a) Milk
Method 1

1) Take the collected raw milk , thaw and return to room temperature for above 30min;
2) Put tips into down-layer of raw milk, take out 1ml sample into 2ml centrifuge tube(Note: do not take up-layer cream);
3) Add 50ul 1M HCl, shake strongly for 1min (or vortex for 30s);
4) Centrifuge at above 4000 g at room temperature for 10min;
5) Take 50ul up-layer clear liquid into another centrifuge tube (do not take up-layer cream), then add 450ul Tissue Redissolving solution,shake strongly for 1min (or vortex for 30s);
6) Take 50ul liquid to test.
Fold of dilution of the sample: 10

Method 2

1) Take 50ul liquid milk sample, add 1950ul Tissue Redissolving solution, vortex for 1min, mix it evenly;
2) Take 50 µL for analysis.
Fold of dilution of the sample: 20

b) Tissue (Chicken, Pork)

1) Take 1.0±0.05g homogenized tissue sample into 50ml Polystyrene centrifuge tube;
2) Add 3ml Buffer: 0.1M PBS solution(PH=10-11), shake strongly for 5min, then put it at 60℃ water bath for 60min, then take out and centrifuge at above 4000 r/min at room temperature (20 - 25 ℃) for 10min;
3) Take 100ul up-layer clear liquid, add 400ul Tissue redissolving solution, vortex for 20S;
5) Take 50 µL for analysis.
Fold of dilution of the sample: 15

c) Honey

1) Take 1.0±0.05g honey sample into 50ml Polystyrene centrifuge tube;
2) Add 2ml deionized water, shake strongly for 2minl(or vortex for 1min)
3) Take 100ul up-layer clear liquid, add 400ul Honey redissolving solution, vortex for 30S;
4) Take 50 µL for analysis.
Fold of dilution of the sample: 10

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. Add 50µL of the sample or standard solution to separate duplicate wells, then add enzyme conjugate, 50µL each well, then add antibody working solution, 50ul each well. Mix gently by shaking the plate manually, seal the microplate with the cover membrane, and incubate at 25 ℃ at dark for 20 minutes.
5. 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).
6. 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 10 minutes at 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 (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 Kanamycin 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 Kanamycin 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.