VERSION 1.01

SPECTINOMYCIN ELISA TEST KIT

MANUAL

ELISAKITS.ONLINE

By Immunomart

Spectinomycin ELISA Test Kit

Catalogue Number. IP100035

Principle

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

Technical specifications

Sensitivity: 0.3ppb
Incubation Temperature: 25℃
Incubation Time: 20min～10min
Detection limit: Liquid milk about 5ppb, Tissue about 2ppb, Honey about 1ppb
Note: ppb= ng/ml or ng/g
Cross-reaction rate: Spectinomycin 100%, Tilmicosin <0.1%, Lincomycin <0.1%, Kanamycin <0.1%, Gentamicin <0.1%, Apramycin <0.1%, Neomycin <0.1%, Streptomycin <0.1%
Recovery rate: Tissue 90±30%

Components

1	Micro-well strips	12 strips with 8 removable wells each
2	6× standard solution (1mL each)	0ppb, 0.3ppb, 0.9ppb, 2.7ppb,8.1 ppb
3	Enzyme conjugate	6ml
4	Antibody working solution	6ml
5	Substrate A	7ml
6	Substrate B	7ml
7	Stop solution	6ml
8	20× concentrated washing buffer	30ml
9	10X concentrated tissue redissolving solution	20ml

Materials required but not provided

Equipments: microplate reader, printer, homogenizer, nitrogen-drying device, vortex, centrifuge, measuring pipets, balance (a reciprocal sensibility of 0.01 g), incubator, water bath;
Micropipettors: single-channel 20-200µL, 100-1000µL, and multi-channel 30～300µl;
Reagents: Deionized water, HCl

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) Redissolving solution: dissolve 1 part of 10x concentrated redissoving solution in 9 part of deionized water at the ratio of 1: 9.
2) Washing buffer: dissolve 1 part of 20x concentrated washing buffer with 19 part of deionized water at the ratio of 1:19.
3) 1M HCl: take 1ml HCl, add 11ml deionized water, mix it evenly.

Samples preparation

a) Milk

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*

b) Tissue (chicken, pork)

1) Weigh 1± 0.05g of the homogenized tissue sample, add into 50mL of the plastic centrifuge tube.
2) Add 2mL Redissolving solution, shake strongly for 2min(or vortex for 1min), then Centrifuge at above 4000r/min at room temperature (20-25 ℃) for 10min.
3) Take 50 µL of the up-layer clean liquid for analysis.
Fold of dilution of the sample:2

c) Honey

1) Weigh 1± 0.05g honey sample, add into 50mL of the plastic centrifuge tube;
2) Add 2mL deionized water, shake strongly for 2 min(or vortex for 2min), or use centrifuge for 2 min.
3) Add 100µL supernatant, add 400ul 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 ℃) before use;
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 ELISA the procedures;
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 to mix evenly before use, put the required micro-well strips into plate frames. Re-sealed the unused microplate, store at 2-8 ℃, not frozen.
2. Numbering: number the micro-wells according to samples and standard solution; each sample and standard solution should be performed in duplicate, record their positions.
3. Add 50μL of the sample or standard solution into separate duplicate wells; add 50ul enzyme conjugate, then 50μL of the antibody working solution into each well, mix gently by shaking the plate manually. Seal the microplate with the cover membrane, and incubate at 25 ℃ for 20min.
4. Pour liquid out of microwell, flap to dry on absorbent paper, add 250 μL/well of washing buffer to wash microplate for 15-30s, then take out and flap to dry with absorbent paper, repeat 3-4 times. (If there are the bubbles after flapping, cut them with the clean tips).
5. Coloration: 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.
6. 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 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 solution and the semilogarithm values of the Spectinomycin standard solution (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, finally obtaining the Spectinomycin 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; 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 sulfuric 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 lots to use.
6. Put the unused microplate into an auto-sealing bag to re-seal it. The standard solution 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 the standard solution 1(0 ppb) 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.