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User's Manual

Streptomycin ELISA Kit

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Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

The Streptomycin ELISA Kit is based on the competitive enzyme immunoassay for the detection of Streptomycin in the sample.

General Description

Streptomycin is an antibiotic (antimycobacterial) drug, the first of a class of drugs called aminoglycosides to be discovered, and it was the first cure for tuberculosis. It is derived from the actinobacterium Streptomyces griseus. Streptomycin is a bactericidal antibiotic. Adverse effects of this medicine are ototoxicity, nephrotoxicity, fetal auditory toxicity, and neuromuscular paralysis.

It is on the World Health Organization's List of Essential Medicines, a list of the most important medication needed in a basic health system.

Principles of Testing

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

Reagents And Materials Provided

1. Micro-well strips: 12 strips with 8 removable wells each
2. 6x standard solution (1 mL each): 0 ppb, 0.1 ppb, 0.4 ppb, 1.6 ppb, 6.4 ppb, 25.6 ppb
3. Enzyme conjugate (12 mL) , red cap
4. Antibody working solution (7 mL), blue cap
5. Substrate A solution (7 mL), white cap
6. Substrate B solution (7 mL), black cap
7. Stop solution (7 mL), yellow cap
8. 20x concentrated washing buffer (40 mL), white cap
9. 10x concentrated redissolving solution (50 mL), transparent cap

Materials Required But Not Supplied

1. Equipments: microplate reader, printer, homogeniser, nitrogen-drying device, vortex, centrifuge, measuring pipets, balance(a reciprocal sensibility of 0.01 g).

2. Micropipettors: single-channel 20~200 µL, 200~1000 µL; and multi-channel 250 µL.

3. Reagents: H3PO4, NaOH(for honey sample), Acetonitrile(CH3CN), N-hexane.

Storage

Store the kit at 4°C upon receipt. For more detailed information, please download the following document on our website.

Specimen Collection And Preparation

Instructions (The following points must be dealt with before the pre-treatment)

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.

Solution preparation before sample pre-treatment:

1. The 10x concentrated redissolving solution is diluted with deionized water at 1:9 (1 mL concentrated redissolving solution + 9 mL deionized water)
2. 0.5% Trichloroacetic acid(for chicken and liver sample): dissolve 0.5 g Trichloroacetic acid in the deionized water to 100 mL.
3. 0.1 M H3PO4 (for honey sample): dissolve 680μL H3PO4 in the deionized water to 100 mL.
4. 1 M NaOH(for honey sample): dissolve 4 g NaOH in the deionized water to 100 mL.

1. milk:

1. Take 1 mL milk sample, diluted at 1:39 (1950 μL diluted redissolving solution+ 50 μL milk). Mix for 30 seconds.
2. Take 50 μL for analysis.

Fold of dilution of sample: 40

2. meat, liver (chicken)

1. Take 2±0.05 g homogenized sample(remove fat), add 6 mL 0.5% Trichloroacetic acid and 2 mL Acetonitrile(CH3CN). Mix for 5 min.
2. Centrifuge at above 4000 r/min at room temperature for 10 min.
3. Transfer 2 mL supernatant into a new vessel, then add 2 mL N-hexane. Mix and then still for 3 min. Take 0.5 mL clear solution on bottom layer. Centrifuge at above 4000 r/min at room temperature for 5 min.
4. Take 50 μL clear solution on bottom layer(if the layer is clear, should remove the upper layer), then add 450 μL the diluted redissolving solution, mix properly for 30 seconds.
5. Take 50 μL for analysis.

Fold of dilution of sample: 40

3. honey, Royal jelly:

1. Weigh 2±0.05 g honey sample, add 4 mL of 0.1 M H3PO4, shake properly for 10 min.
2. Centrifuge at above 4000 r/min at room temperature (20-25°C) for 5 min, until liquid is clear.

3. Add 900 μL 1 M NaOH, adjust PH to 7-9(For Royal jelly, Transfer the Supernatant to a new vessel).
4. Centrifuge at above 4000 r/min at room temperature (20-25°C) for 5 min, until liquid is clear.
5. Take 50 μL suppernatant, add 350 μL of the diluted redissolving solution, mix evenly.
6. Take 50 μL for further analysis.

Fold of dilution of sample: 20

Assay Procedure

1. Bring test kit to the room temperature (20-25°C) 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°C, not frozen.
2. Solution preparation: dilute 40 mL of the concentrated washing buffer (20x concentrated) with the distilled or deionized water to 800 mL (or just to the required volume) 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 50 μL of the sample and standard solution to separate duplicate wells, then add 50 μL of antibody working solution to each well, shake properly, seal the microplate with the cover membrane, and incubate at 37°C for 30 min.
5. Pour liquid out of microwell, flap to dry on absorbent paper; add 250 μL/well of washing buffer for 15-30 seconds, then take out and flap to dry with absorbent paper, repeat 5 times.
6. Add 100 μL of enzyme conjugate to each well, shake properly, seal the microplate with the cover membrane, and incubate at 37°C for 30 min; continue as step 5 for washing.
7. Coloration: add 50 μL of the substrate A solution, 50 μL of the B solution into each well. Mix gently by shaking the plate manually, and incubate at 37°C for 15 min in the dark for coloration.
8. 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 of every well. (Recommend to read the OD value at the dual-wavelength 450/630nm within 5 min).

Calculation

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 Streptomycin.

1. 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: 2.243 for 0 ppb, 1.816 for 0.1 ppb, 1.415 for 0.4 ppb, 0.74 for 1.6 ppb, 0.313 for 6.4 ppb, 0.155 for 25.6 ppb, accordingly the concentration range of the sample I is 6.4 to 25.6 ppb, and that of the sample II is 0.4 to 1.6 ppb.

2. Quantitative determination:

The mean values of the absorbance values is equivalent to the percentage of the average OD value (B) of the testing 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/B0 x 100%

B - the average (double wells) OD value of the testing sample or the standard solution

B0 - the average OD value of the 0ng/mL standard solution

Draw the standard curve with the absorption percentages of the standard solutions and the semilogarithmic values of the Streptomycin 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 Streptomycin concentration in the sample.

Using the professional analyzing software of this kit will be more convenient for the accurate and rapid analysis of a large amount of samples. (Please contact us for this software)

Detection Limit

Chicken, Chicken liver, Milk: 4 ppb

Honey, Royal jelly: 2 ppb

Sensitivity

0.1 ppb

Recovery

Recovery rate:

Milk: 95±15%

Chicken: 85±10%

Honey, Royal jelly: 75±15%

Precautions

1. Bring all reagents and micro-well strips to the room temperature (20-25°C).
2. Return all reagents to 2-8°C 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.
5. The room temperature below 20°C or the temperature of the reagents and the testing samples being not returned to the room temperature (20-25°C) will lead to a lower standard OD value.
6. 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.

7. Mix evenly, otherwise there will be the undesirable reproducibility.
8. The stop solution is the 2 M sulfuric acid solution, avoid contacting with the skin.
9. 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.
10. 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.
11. 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.
12. Colouration time is about 20 min, if the color is light, prolong the time of colouration but don't exceed 30 min.
13. The optimum reaction temperature is 37°C, and too high or low temperatures will result in the changes in the detecting sensitivity and OD values.