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User's Manual

Streptomycin ELISA Kit

REF **DEIA020**

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 vaccine and cell culture supernatant.

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. Standard solution (1 mL each): 0 ng/mL, 0.1 ng/mL, 0.4 ng/mL, 1.6 ng/mL, 6.4 ng/mL, 25.6 ng/mL
3. Enzyme conjugate (12 mL), red cap
4. Antibody working solution (7 mL), green cap
5. Substrate solution (6 mL×2), red cap
6. Stop solution (7 mL), yellow cap
7. 20× concentrated wash solution (50 mL), transparent cap
8. 2× sample diluent (50 mL), blue cap

Materials Required But Not Supplied

1. Microtiter Plate Reader able to measure absorbance at 450 nm, with the correction wavelength set at 620 nm
2. Adjustable pipettes and multichannel pipettor to measure volumes ranging from 25 ul to 1000 ul
3. Deionized (DI) water

4. Wash bottle or automated microplate washer
5. Timer
6. Absorbent Paper

Storage

Store the complete kit at 2-8°C. Under these conditions, the kit is stable until the expiration date (see label on the box).

Specimen Collection And Preparation

1. Please use one-off tips in the process of experiment, and change the tips when absorb different reagent.
2. Make sure that all experimental instruments are clean; otherwise it will affect the assay result.
3. The user should estimate the concentration of target in the test sample, and select a proper dilution factor to make the diluted target protein concentration fall in the optimal detection range of the kit. Dilute the sample with the provided dilution buffer, and several trials may be necessary. The test sample must be well mixed with the dilution buffer. And also standard curves and sample should be making in pre-experiment.

Vaccine

1. Dilute the samples with the diluted sample diluent to obtain a proper streptomycin concentration in it (0.1-25.6ng/ml). The matrix components in the vaccine sample may affect the test results, which it need to be diluted at least 1/5 with Sample Buffer before testing!
2. Take 50 µL of the prepared solution for assay.

Cell Culture Supernatant

1. Centrifuge supernatant for 10 minutes at 1000-3000rpm at 2-8°C to remove insoluble impurity and cell debris. Collect the clear supernatant and carry out the assay immediately.

Plate Preparation

Pipetting protocol									
	1	2	3	4	5	6	7	…	12
A	Std. 0ng/mL	Std. 0ng/mL	Sample3	Sample3					
B	Std. 0.1ng/mL	Std. 0.1ng/mL	Sample4	Sample4					
C	Std. 0.4ng/mL	Std. 0.4ng/mL	Etc.	Etc.					
D	Std. 1.6ng/mL	Std. 1.6ng/mL							
E	Std. 6.4ng/mL	Std. 6.4ng/mL							
F	Std. 25.6ng/mL	Std. 25.6ng/mL							
G	Sample1	Sample1							
H	Sample2	Sample2							

Reagent Preparation

Solution 1: Sample diluent

Dilute the 2× sample diluent with deionized water in the volume ratio of 1:1 (e.g. 10ml 2× Sample diluent + 10ml deionized water), which will be used to dilute sample.

Solution 2: Wash solution

Dilute the 20× concentrated wash solution with deionized water in the volume ratio of 1:19 (e.g. 10ml 20× Wash solution + 190ml deionized water), which will be used for washing the plates.

Assay Procedure

1. Take all reagents out at room temperature (20-25°C) for more than 30min, homogenize before use. Put the required micro-well strips into plate frames, re-sealed the unused microplate, stored at 2-8°C, not frozen.
2. **Number:** Number every micro-well position and all standards and samples should be run in duplicate. Record the standards and samples positions.
3. **Add standard solution/sample and antibody solution:** Add 50 µL of standard solution or prepared sample to corresponding wells. Add 50µL of antibody solution to each well, mix gently by shaking the plate manually and incubate for 30min at 37°C with cover.
4. **Wash:** Remove the cover gently and pour the liquid out of the wells and rinse the microwells with 300µL of diluted wash solution at interval of 10s for 5 times. Absorb the residual water with absorbent paper (the rest air bubble can be eliminated with unused tip).
5. **Add enzyme conjugate:** Add 100 µL of enzyme conjugate to each well, shake properly, and incubate for 30min at 37°C with cover.
6. **Wash:** Repeat wash as in step 4.
7. **Coloration:** Add 100 µL of the substrate solution 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 450/620nm 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 1 is 0.3, and that of the sample 2 is 1.0, the OD value of standard solutions is: 2.1542 for 0 ng/mL, 1.6330 for 0.1 ng/mL, 0.9459 for 0.4 ng/mL, 0.3961 for 1.6 ng/mL, 0.1680 for 6.4 ng/mL, 0.0574 for 25.6 ng/mL, accordingly the concentration range of the sample 1 is 1.6 to 6.4 ng/mL, and that of the sample 2 is 0.1 to 0.4 ng/mL.

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 (B_0) of the first standard solution (0 standard) and subsequently multiplied by 100%, that is:

Percentage of absorbance value = B/B_0 x 100%

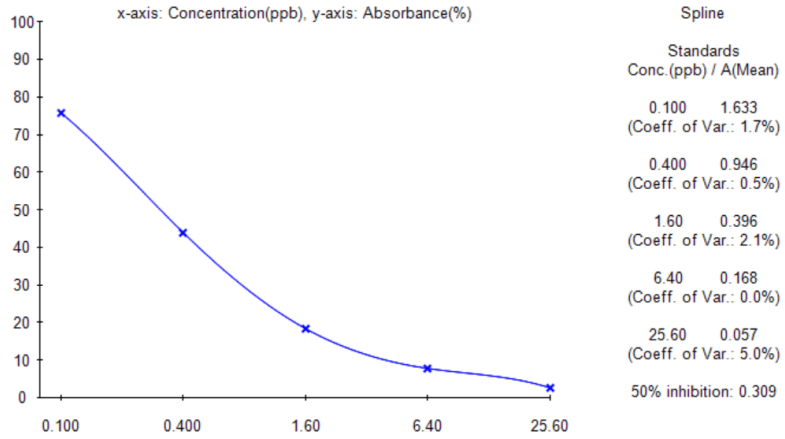
B - the average (double wells) OD value of the testing sample or the standard solution

B_0 - 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.

Typical Standard Curve



Precision

Intra-assay: < 10%

Inter-assay: < 10%

Detection Range

0-25.6ng/mL

Recovery

Vaccine:86-117%

Cell culture supernatant:82-106%

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 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 nonlinear 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 colorless color former are light sensitive, and thus they cannot be directly exposed to the light.
11. Discard the coloration solution with any color that indicates the degeneration of this solution. The detecting value of standard solution 1(0 ng/mL) of less than 0.5 indicates its degeneration.
12. Coloration time is about 15 min, if the color is light, prolong the time of coloration but don't exceed 30 min.
13. The reaction temperature is 37°C, and too high or low temperatures will result in the changes in the detecting sensitivity and OD values.