Version 10-09/22

User's Manual

Streptomycin ELISA Kit

REF **DEIA-XY2272**

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.

Creative Diagnostics

Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe) **Fax: 1-631-938-8221**

Email: info@creative-diagnostics.com **Web: www.creative-diagnostics.com**

PRODUCT INFORMATION

Intended Use

This kit can be used in quantitative and qualitative analysis of streptomycin residue in vaccine etc.

General Description

Streptomycin is an aminoglycoside antibiotic, which is broadly applied in animal disease treatment. For it has neurotoxicity and kidney toxicity, its residue in animal-derived food is harmful to human; it is strictly controlled in use in EU, US and China. At present, ELISA is the common approach in supervision and control of streptomycin drug.

This kit is a new product for drug residual detection based on ELISA technology, which only costs 45 min in each operation and can considerably minimize operation errors and work intensity.

Principles of Testing

This kit is based on direct-competitive ELISA. The microtiter wells are coated with coupling antibody. Streptomycin residue in the sample competes with the conjugate for the antibody. After the addition of enzyme conjugate, chromegenic substrate is used to show the color. Absorbance of the sample is negatively related to the streptomycin residue in it, after comparing with the Standard Curve, multiplied by the dilution factor, Streptomycin residual quantity in the sample can be calculated.

Reagents And Materials Provided

1. Microtiter plate with 96 wells coated with antibody
2. Standard solutions (6 bottles×1ml/bottle)

0, 0.05, 0.15, 0.45, 1.35, 4.05ng/ml

3. Spiking standard solution: (1ml/bottle) 1μg/ml
4. Concentrated Enzyme conjugate 1ml…….red cap
5. Enzyme conjugate diluent 10ml……. green cap
6. Substrate solution A 7ml……white cap
7. Substrate solution B 7ml……red cap
8. Stop solution 7ml……..……yellow cap
9. 20×Concentrated wash solution 40ml……transparent cap
10. 2×Sample diluent 50ml……blue cap

Materials Required But Not Supplied

Equipments

1. Microtiter plate spectrophotometer (450nm/630nm)

2. Polystyrene centrifuge tube: 2ml
3. Micropipettes: 20μl-200μl, 100μl-1000μl, 250μl-multipipette

Reagents

1. Deionized water

Storage

Storage condition: 2-8°C .

Storage period: 12months.

Specimen Collection And Preparation

Notice and precautions before operation

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.

Vaccine

1. concentration of 0.05-4.05 ng/ml (streptomycin).
2. Take 50 ml of the prepared solution for assay.

Reagent Preparation

Solution 1: Sample diluent

Dilute the 2×sample diluent with deionized water in the volume ratio of 1:1, which will be used to dilute sample; this solution can be stored at 4°C for 1 month.

Solution 2: Wash solution

Dilute the 20×concentrated wash solution with deionized water in the volume ratio of 1:19, which will be used for washing the plates. This solution can be stored at 4°C for 1 month.

Assay Procedure

Notice before assay

1. Make sure all reagents and microwells are all at room temperature (20-25°C).
2. Return all the rest reagents to 2-8°C immediately after used.
3. Washing the microwells correctly is an important step in the process of assay; it is the vital factor to the reproducibility of the ELISA analysis.
4. Avoid the light and cover the microwells during incubation.

Assay Steps

1. Take all reagents out at room temperature (20-25°C) for more than 30min, shake gently before use.
2. Get the microwells needed out and return the rest into the zip-lock bag at 2-8°C immediately.
3. The concentrated wash solution and sample diluent should be rewarmed before use;

4. **Number:** Number every microwell position and all standards and samples should be run in duplicate. Record the standards and samples positions.
5. **Add standard solution/sample:** Add 50μl of standard solution (Kit component) or prepared sample to corresponding wells.
6. **Dilute the concentrated enzyme conjugate:** Dilute the concentrated enzyme conjugate(Kit component) with the enzyme conjugate diluent(Kit component) in the volume ratio of 1:10(e.g. 0.5ml of concentrated enzyme conjugate + 5ml of enzyme conjugate diluent), mix completely, (**this mixture can't be conserved, use immediately**).
7. **Add the diluted enzyme conjugate:** Add 50μl of the diluted enzyme conjugate to each well, shake the plate manually to mix and then incubate for 30min at 25°C with cover.
8. **Wash:** Remove the cover gently and pour the liquid out of the wells and rinse the microwells with 250μl diluted wash solution (solution 2) at interval of 10s for 4-5 times. Absorb the residual water with absorbent paper (the rest air bubble can be eliminated with unused tip).
9. **Coloration:** Add 50μl of solution A (Kit component) and 50μl of solution B (Kit component) to each well. Mix gently by shaking the plate manually and incubate for 15 min at 25°C with cover (see step 8)
10. **Measure:** Add 50μl of the stop solution (Kit component) to each well. Mix gently by shaking the plate manually and measure the absorbance at 450nm (It's suggested measure with the dual-wavelength of 450/630nm. Read the result within 5 min after addition of stop solution.).

Calculation

Percentage absorbance

The mean values of the absorbance values obtained for the standards and the samples are divided by the absorbance value of the first standard (zero standard) and multiplied by 100%. The zero standard is thus made equal to 100% and the absorbance values are quoted in percentages.

$$\text{Absorbance}(\%) = \frac{B}{B_0} \times 100\%$$

B ——absorbance of standard (or sample)

B_0 ——absorbance of zero standard

Typical Standard Curve

To draw a standard curve: Take the absorbance value of standards as y-axis, semi logarithmic of the concentration of the streptomycin standards solution (ppb) as x-axis.

The streptomycin concentration of each sample (ppb), which can be read from the calibration curve, is multiplied by the corresponding dilution factor of each sample followed, and the actual concentration of sample is obtained.

Please notice:

Special software has been developed for all data analysis, which can be provided on request.

Precision

CV of the ELISA kit all less than 10%.

Accuracy

85± 10%

Detection Range

0.05-4.05ng/ml

Specificity

Streptomycin……..100%

Dihydrostreptomycin……106%

Streptomycin sulphate……67%

Neomycin……..……..……<1%

Gentamycin……..……<1%

Kanamycin……<1%

Amikacin……..……<1%

Spectinomycin……..……..……<1%

Apramycin……..……<1%

Precautions

1. The mean values of the absorbance values obtained for the standards and the samples will be reduced if the reagents and samples have not been regulated to room temperature (20-25°C).
2. Do not allow microwells to dry between steps to avoid unsuccessful reproducibility and operate the next step immediately after tap the microwells holder.
3. Shake each reagent gently before use.
4. Keep your skin away from the stop solution for it is the 0.5M H_2SO_4 solution.
5. Don't use the kits out of date. Don't exchange the reagents of different batches, for it will drop the sensitivity.
6. Keep the ELISA kits at 2-8°C,do not freeze. Seal rest microwell plates Avoid straight sunlight for the standard sample and the colorless chromogenic reagent are sensitive to light.
7. Substrate solution should be abandoned if it turns colors.The reagents may be turn bad if the absorbance value (450/630nm) of the zero standard is less than 0.5 (A450nm<0.5).
8. The coloration reaction needs 10-15 min after adding Solution A and Solution B. And you can prolong the incubation time to 20min if the color is too light to be determined. Never exceed 25min, on the contrary, shorten the incubation time properly.
9. The optimal reaction temperature is 25°C. Higher or lower temperature will lead to the changes of sensitivity and absorbance values.