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User's Manual

Gentamicin ELISA Kit

REF **DEIA047**

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

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PRODUCT INFORMATION

Intended Use

This kit can be used in quantitative and qualitative analysis of gentamicin residue in vaccine and cell culture.

General Description

Gentamicin residue in the production of biological samples may lead to severe allergic reactions in certain groups. Thus it is strictly controlled in many countries in the world. This kit is a new product for drug residual detection based on ELISA technology, which is rapid, easy-to-use, and sensitive, and can considerably minimize operation errors and work intensity.

Principles of Testing

This kit is based on indirect-competitive ELISA technology. The microtiter wells are coated with antigen. Gentamicin residue in the sample competes with the antigen coated on the plate for the antibody. After the addition of enzyme conjugate, TMB substrate is used to show the color. Absorbance of the sample is negatively related to the gentamicin residue in it, after comparing with the Standard Curve, multiplied by the dilution factor, gentamicin residue in the sample can be calculated.

Reagents And Materials Provided

1. Microtiter plate with 96 wells coated with antigen
2. Standard solutions(6×1ml/bottle) :0ng/ml,0.1ng/ml,0.3ng/ml,0.9ng/ml,2.7ng/ml,8.1ng/ml
3. High concentration Gentamicin Standard (1ml/bottle) 1μg/ml
4. Enzyme conjugate 12ml
5. Antibody solution 7ml
6. Substrate Solution: 2x7ml
7. Stop solution: 7ml
8. 20×concentrated wash solution: 40ml
9. 2×Sample diluent: 50ml

Materials Required But Not Supplied

1. Microtiter plate spectrophotometer (450nm/630nm)
2. Polystyrene centrifuge tube:2ml, 50ml
3. Micropipettes: 20μl-200μl, 200μl-1000μl
4. 250μl-multipipette
5. deionized water

Storage

2-8°C, 12 months.

Specimen Collection And Preparation

1. Dilute the 2×Sample diluent with deionized water in the volume ratio of 1:1, which will be used for sample dilution. This solution can be stored at 4°C for 1 month.
2. Dilute sample solution with diluted sample diluent to proper gentamicin concentration (0.1-8.1ng/ml) in it.
3. Take 50μl 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 for sample dilution. 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 (1 fold 20×concentrated wash solution:19 folds deionized water), which will be used for washing the plates. This solution can be stored at 4°C for 1 month

Assay Procedure

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 diluted wash solution 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 or prepared sample to corresponding wells. Add 50μl antibody solution. Mix gently by shaking the plate manually and incubate for 30min at 37°C with cover.
6. Wash: Remove the cover gently and pour the liquid out of the wells and rinse the microwells with 250μl diluted wash solution (see Reagent Preparation) 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).
7. Add enzyme conjugate: Add 100μl of enzyme conjugate to each well, Mix gently by shaking the plate manually and incubate for 30min at 37°C with cover, take out and repeat the wash step;
8. Coloration: Add 100μl substrate solution to each well. Incubate for 15 min at 37°C with cover.
9. Measure: Add 50μl the stop solution 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 5min after addition of stop solution).

Calculation

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.

Absorbance (%) = B/B_0 * 100%

B ——absorbance standard (or sample)

B/B_0 ——absorbance 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 gentamicin standards solution (ng/ml) as x-axis. The gentamicin concentration of each sample (ng/ml), 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: software has been developed for data reduction, which can be provided upon request. Dilution factor of samples: according to your operation.

Precision

Intra-assay and inter-assay of the ELISA kit is less than 5%

Detection Range

0.1-8.1ng/mL

Sensitivity

0.1ng/mL

Recovery

70-120%

Precautions

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. For sample containing aluminium adjuvant, please have a trial experiment first to evaluate the performance of the kit, due to the severe interference. In this case, please dilute the sample with diluent to try.
4. Make sure all reagents and microwells are all at room temperature (20-25°C)
5. Return all the rest reagents to 2-8°C immediately after used.
6. 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.

7. Avoid the light and cover the microwells during incubation.