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User's Manual

Neomycin ELISA Kit

REF **DEIA043**

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

This kit can be used in quantitative and qualitative analysis of neomycin residue in cell culture supernatant, vaccine and milk.

General Description

Neomycin is an aminoglycoside antibiotic, which is broadly applied in animal disease treatment and vaccine production. 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 aminoglycoside drug.

This kit is a new product for drug residual detection based on ELISA technology, which only costs 45min in each operation 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 coupling antigen. Neomycin residue in the sample competes with the antigen coated on the microtiter 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 neomycin reside in it, after comparing with the Standard Curve, multiplied by the dilution multiple, Neomycin residue quantity in the sample can be calculated.

Reagents And Materials Provided

1. Microtiter plate with 96 wells coated with antigen
2. Neomycin standard solutions(6×1ml/bottle)
   0ppb, 0.5ppb, 1.5ppb, 4.5ppb, 13.5ppb, 40.5ppb
3. Spiking standard solution: 1ml, 1ppm
4. Antibody solution (7ml)
5. Enzyme conjugate (7ml)
6. Substrate solution (2×6ml)
7. Stop solution (7ml)
8. 20×concentrated wash solution (50ml)
9. 2×concentrated extraction solution (50ml)

Materials Required But Not Supplied

1. Microtiter plate spectrophotometer (450nm/630nm)
2. Homogenizer
3. Shaker

4. Vortex mixer
5. Graduated pipette: 10ml
6. Rubber pipette bulb
7. Polystyrene centrifuge tube: 2ml
8. Micropipettes: 20ul-200ul, 100ul-1000ul,250ul-multipipette

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.
3. Cell culture supernatant/Vaccine: Take 50ul of the specimen for assay directly.
4. Milk: Take 100ul of milk sample into a 2ml polystyrene centrifuge tube. Add 1400ul of extraction solution (solution 1), vortex for 1min. Take 50ul of the prepared solution for assay.

   Dilution factor of sample: 15

Reagent Preparation

Solution 1: Extraction solution Dilute the 2× concentrated extraction solution with deionized water in the volume ratio of 1:1(e.g. 10ml of 2× concentrated extraction solution + 10ml of deionized water), 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(e.g. 10ml of 20×concentrated wash solution + 190ml of deionized water), 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. **Number:** Number every microwell position and all standards and samples should be run in duplicate. Record the standards and samples positions.
4. **Add standard /sample, enzyme conjugate and antibody:** Add 50ul of standard solution (kit components) or prepared sample to corresponding wells. Add 50ul of enzyme conjugate (kit components), 50ul of antibody solution (kit components). Mix gently by shaking the plate manually and incubate for 30min at 25°C with cover.
5. **Wash:** Remove the cover gently and pure the liquid out of the wells and rinse the microwells with 250ul 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).
6. **Coloration:** Add 100ul of substrate solution (kit components) to each well. Incubate for 15 min at 25 °C with cover.
7. **Measure:** Add 50ul 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 (%) = $(B/B_0)\times 100\%$

B ——absorbance standard (or sample)

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 Neomycin standards solution (ppb) as x-axis.

The neomycin concentration of each sample (ppb), which can be read from the calibration curve, is multiplied by the corresponding dilution multiple 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

Variation coefficient of the ELISA kit is less than 10%.

Detection Limit

Cell culture supernatant/Vaccine, 0.5ppb

Milk, 7.5ppb

Sensitivity

0.5ppb

Recovery

70-120%

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 2M H2SO4 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 15 min. And you can prolong the incubation time if the color is too light to be determined. Never exceed 30min, 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.