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User's Manual

Neomycin ELISA Kit

REF **DEIA-XY34**

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 chicken and milk, egg.

General Description

Neomycin is aminoglycosides antibiotics and is widely used in treatment of animal diseases. Because it has neurovirulence and nephrotoxicity, it will influence the human's health. The Europe and America and our country all ask for limited use. Recently, immunoassay is still the most common method in monitoring aminoglycoside antibiotic residues.

The kit is a new generation of medicine residue test product based on the ELISA technology. It only needs 45min. It can reduce the operation error and the work strength.

Principles of Testing

This ELISA kit is designed to detect neomycin based on the principle of indirect-competitive enzyme immunoassay. The microtiter wells are coated with capture BSA-linked antigen. Neomycin in sample competes with the antigen coated on the microtiter plate for the antibody added. After the addition of enzyme conjugate, chromogenic substrate is used and the signal is measured by a spectrophotometer. The absorption is inversely proportional to neomycin concentration in the sample.

Reagents And Materials Provided

1. Microtiter plate with 96 wells coated with antigen
2. Standard solutions(6 *1ml/bottles)
   0ppb, 0.5ppb,1.5ppb,4.5ppb,13.5ppb,40.5ppb
3. Spiking standard solution: (1ml/bottle), 10ppm
4. Antibody solution, 7ml, green cap
5. Enzyme conjugate 7ml, red 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×concentrated extraction solution, 50ml, blue cap

Materials Required But Not Supplied

Equipments

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

2. Homogenizer
3. Shaker
4. Vortex mixer
5. Centrifuge
6. Analytical balance (inductance: 0.01g)
7. Rubber pipette bulb
8. Polystyrene centrifuge tube: 2ml, 50ml
9. Micropipettes: 20ul-200ul, 100ul-1000ul, 250ul-multipipette

Reagents

1. NaOH (AR)
2. Na_2HPO_4. $12H_2O$ (AR)
3. NaH_2PO_4. $2H_2O$ (AR)
4. H_2SO_4 (AR)
5. NaCl (AR)
6. Methanol (AR)
7. Deionized water

Storage

Storage condition: 2-8°C.

Storage period: 12 months

Specimen Collection And Preparation

1. Notice and precautions before operation:

a. Please use one-off tips in the process of experiment, and change the tips when absorbing different reagent.

b. Make sure that all experimental instruments are clean.

c. Treated samples should be used immediately.

2. Chicken sample

a. Homogenize the samples with homogenizer;

b. Weigh 2.0±0.05g of the homogenate into a 50ml polystyrene centrifuge tube.

c. Add 6ml 0.1M PBS (solution 2) and shake for 5min.

d. Centrifuge: at 4000r/min /5min/room temperature (20-25°C).

e. Take 100µl supernatant into 2ml polystyrene centrifuge tube. Add 400µl extraction solution (solution 4). Mix completely.

f. Take 50µl for assay.

3. Milk (raw milk, finished milk)

a. Take 1ml±0.05ml of milk sample into 2ml polystyrene centrifuge tube.

b. Add 30μl of 2M H_2SO_4 (solution 1) and vortex for 2min. Centrifuge for separation: room temperature (20-25°C)/5min/ 4000r/min;

c. Take 50μl supernatant and add 100ul of 5% NaCl (solution 3) and add 600μl extraction solution (solution 4). Vortex it for 2min and mix completely.

d. Take 50μl for assay.

4. Egg

a. Weigh 1.0±0.05g of the blended egg into a 10ml polystyrene centrifuge tube.

b. Add 4ml extraction buffer (solution 6) and vortex for 1min.

c. Centrifuge: at 4000r/min /5min/room temperature (20-25°C).

d. Take 200μl supernatant into 2ml polystyrene centrifuge tube. Add 130μl of 0.2M NaOH solution (solution 7) and 470μl extraction solution (solution 4). Vortex for 30s.

e. Take 50μl for assay.

Reagent Preparation

Solution 1: 2M H_2SO_4

Add 55.5ml of 98% H2SO4 into deionized water, till the volume is 500ml and mix them.

Solution 2: 0.1M PBS

Take 16.96g of Na_2HPO_4. $12H_2O$, 0.41g of NaH_2PO_4. $2H_2O$, add 500ml of deionized water, mix completely.

Solution 3: 5% NaCl solution

Take 5.0g NaCl into 100ml of deionized water and mix them.

Solution 4: 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). This solution can be stored at 4°C for 1 month.

Solution 5: Wash solution

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

Solution 6: Extraction buffer (for egg)

Take 44ml of deionized water, 3ml of 2M H_2SO_4 (solution 1), 13ml of methanol and mix them.

Solution 7: 0.2M NaOH solution (for egg)

Take 0.8g NaOH into 100ml of deionized water and mix them.

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 concentrated extraction solution should be rewarmed to room temperature before use.
4. Number: Numbered every microwell positions and all standards and samples should be run in duplicate. Record the standards and samples positions.
5. Add standard solution / sample and enzyme conjugate and antibody solution: add 50µl of standard solution or prepared sample to corresponding wells. Add 50ul of enzyme conjugate to each well. And add 50ul of antibody solution into each well. Shake gently. incubate for 30min at 25°C with cover (or in dark place)
6. Wash: Remove the cover gently and pour the liquid out of the wells and rinse the microwells with 250µl of diluted wash solution (solution 5) 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. Coloration: Add 50µl of substrate solution A and 50µl of substrate solution B into each well. Mix gently by rocking the plate manually and incubate for 15min at 25°C with cover.
8. Measure: Add 50µl the stop solution to each well. Mix gently by rocking the plate manually and measure the absorbance at 450nm (It is suggested measure with the dual-wavelength of 450/630nm. Read the result within 5min 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 standard (or sample)

B_0 ——absorbance zero standard

Standard Curve

To draw a standard curve: Take the absorbance value of standards as y-axis, semi logarithmic of the concentration of the AMOZ standard 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 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.

Dilute ratio:

Chicken: 20

Milk: 15

Egg:20

Performance Characteristics

Accuracy:

Chicken: 100±30%

Milk: 100±30%

Egg: 100±20%

Precision

CV of the ELISA kit is less than 10%

Detection Limit

Chicken: 10ppb

Milk: 10ppb

Egg: 10ppb

Sensitivity

0.5ppb

Specificity

Neomycin: 100%

Streptomycin: <0.1%

Kanamycin: <0.1%

Apramycin: <0.1%

Gentamycin: <0.7%

Tobramycin: <0.1%

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 H_2SO_4 solution.
5. Don't use the kits out of date. Don't exchange the reagents of different batches, or else it will drop the sensitivity.
6. Keep the ELISA kit at 2-8°C,do not freeze. Seal rest microwell plates, Avoid straight sunlight during all incubations. Covering the microtiter plates is recommended.
7. Substrate solution should be abandoned if it turns colors. The reagents may be deteriorated if the absorbance value (450/630nm) of the zero standard is less than 0.5 (A450nm<0.5).
8. The coloration reaction needs 15min after the addition of solution A and solution B; But you can prolong the incubation time to 20min or more 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.