Version 12-11/25

User's Manual

High Sensitivity Gentamicin ELISA Test Kit

REF **DEIA-WZ6884**

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 gentamicin residue in animal tissues (chicken and chicken liver), milk (raw milk, UHT milk, acidified milk, reconstituted milk, pasteurized milk), milk powder (skim milk powder, whole milk powder), etc.

General Description

Gentamicin 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 this drug.

This kit is a new product for drug residual detection based on ELISA technology, which only costs 1.5h 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. Gentamicin 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 Gentamicin reside in it, after comparing with the Standard Curve, multiplied by the dilution factor, gentamicin residue quantity in the sample can be calculated.

Reagents And Materials Provided

1. Microtiter plate with 96 wells coated with coupling antigen
2. Gentamicin Standard solutions(6 bottles×1ml/bottle), 0ppb, 0.1ppb, 0.3ppb, 0.9ppb, 2.7ppb, 8.1ppb
3. Spiking standard solution: (1ml/bottle), 1ppm
4. Enzyme conjugate, 12ml
5. Antibody solution, 7ml
6. Solution A, 7ml
7. Solution B, 7ml
8. Stop solution, 7ml
9. 20×concentrated wash solution, 40ml
10. 2×concentrated extraction solution, 50ml

Materials Required But Not Supplied

Equipments

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

2. Homogenizer
3. Water Bath
4. Shaker
5. Centrifuge
6. Analytical balance (inductance: 0.01g)
7. Polystyrene centrifuge tubes: 2ml, 10ml
8. Micropipettes: 20ml-200ml, 100ml-1000ml 250ml-multi pipette

Reagents

1. $Na_2HPO_4 \cdot 12H_2O$ (AR)
2. Sodium chloride (NaCl, AR)
3. Sodium hydroxide (NaOH, AR)
4. KH_2PO_4 (AR)
5. Potassium chloride (KCl, AR)
6. Deionized water

Storage

Storage condition: 2-8°C.

Storage period: 12 months.

Specimen Collection And Preparation

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) Keep untreated samples in freeze.

1. Chicken, chicken liver Dilution factor: 40

a. Take sample without fat for analysis, homogenize the sample.

b. Take 2.0±0.05g homogenized sample into a 50ml polystyrene centrifuge tube, add 6ml of 0.1M PBS (pH=10-11, solution 1), vortex for 5min.

c. Incubate at 60°C with water bath for 60min, take out and cool down to room temperature and shake well.

d. Centrifuge for separation: 4000r/min / 5min/ ambient temperature.

e. Dilute the supernatant with the extraction solution (solution 2) in the volume ratio of 1:9 (50ul of supernatant + 450ul of extraction solution).

f. Take 50ul per well for assay.

2. Milk (raw milk, UHT milk, acidified milk, reconstituted milk, pasteurized milk) Dilution factor: 40

a. Take 20ul of milk sample into 2ml polystyrene centrifuge tube. Add 780ul extraction solution (solution 2), mix completely.

b. Take 50ul per well for assay.

3. Milk powder Dilution factor: 100

a. Take 1.0±0.05g of milk powder into 10ml polystyrene centrifuge tube.

b. Add 5ml of deionized water; vortex till the powder dissolved.

c. Then take out 50ul of the prepared solution, mix with 950ul of extraction solution (solution 2), and mix completely.

d. Take 50ul per well for assay.

Reagent Preparation

Solution 1: 0.1M PBS (pH=10-11)

Dissolve 13.4g of $Na_2HPO_4 \cdot 12H_2O$, 20.0g of NaCl, 0.32g of NaOH, 0.5g of KH_2PO_4, 0.5g of KCl with 500ml of deionized water.

Solution 2: Extraction solution

Dilute 2×concentrated extraction solution with deionized water in the volume ration of 1:1(e.g. 10ml of 2×concentrated extraction solution + 10ml of deionized water), which will be used for sample extraction. This solution can be stored for 1 month at 4°C.

Solution 3: Wash solution

Dilute the 20×concentrated wash solution with deionized water in the volume ratio of 1:19(e.g. 5ml of 20×concentrated wash solution + 95ml 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 recover to 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 extraction solution and concentrated wash solution recover to room temperature 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 solution and antibody: Add 50µl of standard solution or prepared sample solution to corresponding wells. Add 50µl of antibody solution (kit component). 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 (solution 3) 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 solution (kit component) 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 50µl of solution A(kit component), add 50µl of solution B(kit component) to each well. Mix gently by shaking the plate manually and incubate for 15 min at 37°C with cover.
9. Measure: Add 50µl the stop solution (kit component) to each well. Mix gently by shaking the plate manually and measure the absorbance at 450/630nm (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.

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

B ——absorbance standard (or sample)

B_0 ——absorbance zero standard

Standard Curve

1. To draw a standard curve: Take the absorbance value of standards as y-axis, semi logarithmic of the concentration of the gentamicin standards solution (ppb) as x-axis.
2. The gentamicin 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: for data reduction of the ELISA kits, special software has been developed, which can be ordered on request.

Performance Characteristics

Accuracy:

Animal tissue(Chicken, chicken liver): 90±20%

Milk / milk powder: 100±30%

Precision

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

Detection Limit

Animal tissue(Chicken, chicken liver): 4ppb

Milk: 4ppb

Milk powder: 10ppb

Sensitivity

0.1ppb

Specificity

Gentamicin: 100%

Streptomycin: <1%

Dihydrostreptomycin: <1%

Neomycin: <1%

Precautions

1. The mean values of the absorbance values obtained for the standards and the samples will be reduced if the room temperature is lower than 20°C or 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. Don't use the kits out of date. Don't exchange the reagents of different batches, for it will drop the sensitivity.
5. 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.
6. 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).
7. The coloration reaction needs 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 37°C. Higher or lower temperature will lead to the changes of sensitivity and absorbance values.