Version 22-04/24

User's Manual

Kanamycin ELISA Kit

REF **DEIA-004H**

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 kanamycin residue in animal tissues (muscle and liver, etc.) and milk etc **For research use only, not for use in diagnostic procedures.**

General Description

Kanamycin residue in the production of biological products may lead to abnormal reactions of human beings, thus strict MRLs have been established. This kit is a rapid test product for the determination of tetracycline residues which is sensitive, accurate and time-saving. It can considerably reduce the operation errors in the assay.

Principles of Testing

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

Reagents And Materials Provided

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

Materials Required But Not Supplied

Equipments:

1. Microtiter plate spectrophotometer (450nm/630nm)
2. Homogenizer or Stomacher

3. Electric-heated thermostatic water bath
4. Shaker
5. Vortex mixer
6. Centrifuge
7. Analytical balance (inductance: 0.01g)
8. Graduated pipette: 10ml
9. Rubber pipette bulb
10. Polystyrene centrifuge tube: 2ml, 50ml
11. Micropipettes: 20μl-200μl, 200μl-1000μl, 250μl-multipipette

Reagents

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

Storage

Storage condition: 2-8°C.

Storage period: 12 months.

Specimen Collection And Preparation

1. Tissue

a. Weigh 2.0±0.05g of comminuted sample (after removing the fat) into a 50ml polystyrene centrifuge tube, add 6ml of 0.1M PBS (PH=10-11) buffer solution (solution 1), mix completely for 10min;

b. Incubate in 60°C water bath for 60min, take out and cool to room temperature;

c. Centrifuge for separation: room temperature, 10min, at 3000g;

d. Take 100μl of the supernate, dilute with 400μl of extraction solution (Solution 2), vortex for 1min.

e. Take 20μl of the prepared solution for assay.

f. **Dilution factor: 20**

2. Milk

a. Take 50μl of raw milk sample;

b. Add 200μl of extraction solution(see solution 2), vortex for 1min to mix completely;

c. Take 20μl of the prepared solution for assay.

d. **Dilution factor: 5**

Reagent Preparation

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

Weigh 13.4g of sodium phosphate dibasic dodecahydrate ($Na_2HPO_4 \cdot 12H_2O$), 0.5g of potassium dihydrogen phosphate(KH_2PO_4), 20g of Sodium chloride(NaCl), 0.5g of potassium chloride(KCl), 0.32g of Sodium hydrate(NaOH), dissolve with deionized water and dilute to 500ml;

2. Solution 2: 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 extraction, this solution can be stored at 4°C for 1 month.

3. Solution 3: 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

1. Take all reagents out at room temperature (20-25°C) for more than 30min, homogenize before use.
2. Get the microwells needed out and return the rest into the zip-lock bag at 2-8°C immediately.
3. The wash solution should be brought to room temperature (20-25°C) 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, enzyme conjugate and antibody:** Add 20µl of standard solution or prepared sample to corresponding wells. Add 50µl of enzyme conjugate solution, 80µl of antibody solution to each well, mix gently by shaking the plate manually and incubate for 40min at 25°C with cover.
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 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. **Coloration:** Add 50µl of solution A and 50µl of solution B to each well. Mix gently by shaking the plate manually and incubate for 15 min at 25°C with cover (see **Precautions 8**)
8. **Measure:** Add 50µl of the stop solution to each well. Mix gently by shaking the plate manually and measure the absorbance at 450nm against an air blank (It' s suggested measure with the dual-wavelength of 450/630nm. Read the result within 5min after addition of stop solution.) (We can also measure by sight without stop solution in short of the ELISA reader).

Calculation

1. Percentage absorbance

The mean values of the absorbance values obtained from the standards and the samples are divided by the absorbance value of the first standard (zero standard) and multiplied by 100%.

Absorbance(%)=100%×B/B_0

B ——absorbance of standards or samples

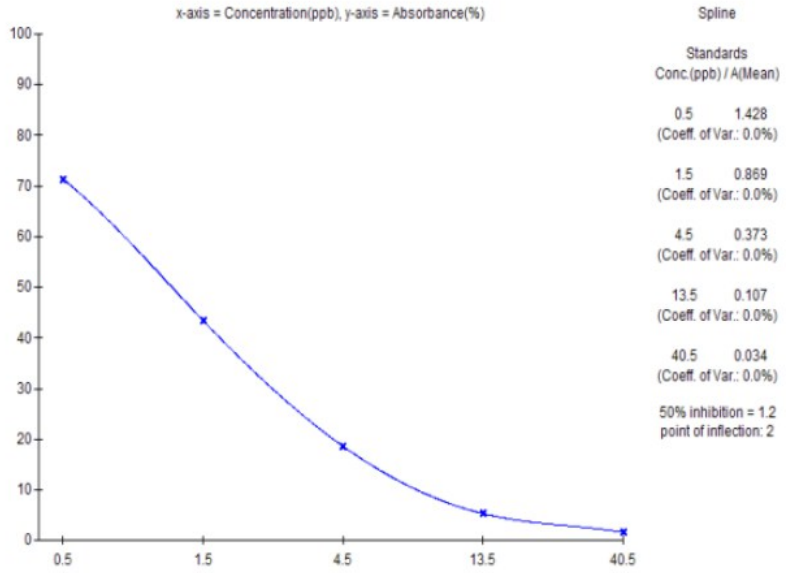
B_0 ——absorbance of zero standard (0ng/ml)

2. Standard Curve

a. To draw a standard curve: The absobance value of standards as y-axis, semilogarithmic of the concentration of the standards (ng/ml) as x-axis.

b. The kanamycin concentration of each sample (ng/ml), which can be read from the calibration curve, is multiplied by the corresponding dilution rate of each sample followed, and the actual concentration of sample is obtained.

Typical Standard Curve



Performance Characteristics

Accuracy:

Tissue: 90±15%

Milk: 85±25%

Precision

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

Detection Limit

Tissue: 10ppb

Milk: 2.5ppb

Sensitivity

0.5ppb

Specificity

Kanamycin: 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 reagents and samples have not been regulated to room temperature (20-25°C).
2. Do not allow microwells to be dry between steps to avoid unsuccessful repetitiveness and operate the next step immediately after tap the microwells holder.
3. Mix the homogenate and elute the plate adequately.
4. Avoid the stop solution touching skin for the 2M H_2SO_4.
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. Storage constitution: Keep the ELISA kits at 2-8°C without frozen. Avoid direct sunlight during all incubations. Covering the microtiter plates is recommended.
7. The reagents go bad: Substrate solution should be abandoned if its color has changed. 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 need 20-30min after the addition of solution A and solution B; But you can prolong the incubation time ranges from 35min to 40min if the color is too light to be determined. On the contrary, shorten the incubation time properly.
9. The best reaction temperature is 25°C, temperature too high or too low both will lead to the changes of sensitivity and absorbance values.