Kanamycin ELISA Kit

Cat.No: DEIA048
Lot. No. (See product label)

Size

96T

Intended use

This kit can be used in quantitative and qualitative analysis of kanamycin residue in biological samples.

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 kanamycin residues which is sensitive, accurate and time-saving. It can considerably reduce the operation errors in the assay.

Principle Of The Test

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 x 6 bottles: 0ng/ml, 0.5ng/ml,1.5ng/ml,4.5ng/ml,13.5ng/ml, 40.5ng/ml (1ml/bottle)
3. Spiking standard solution: 1ml, 1μg/ml
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. 20x Concentrated wash solution (40ml): transparent cap
10. 2x Sample Dilute (50ml): blue cap

Materials Required But Not Supplied

_ Microtiter plate spectrophotometer (450nm/630nm)
_ polystyrene centrifuge tube: 2ml, 50ml
_ Micropipettes: 20μl-200μl, 100μl-1000μl, 250μl-multipipette

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_ Deionized water

Storage

Unopened kit: Store at 2-8°C.
Storage period: 12 months.
Do not use the kit beyond the expiration date.

Specimen Collection And Preparation

Notice and precautions for the users before operation:
a. Please use one-off tips in the process of experiment, and change the tips when absorb different reagent.
b. Make sure that all experimental instruments are clean, otherwise it will effect the assay result.

Sample Preparation:
- Dilute the test sample with the prepared sample diluent to get a final concentration of **0.5-40.5** ng/ml (kanamycin).
- Take 20µl of the prepared solution 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, which will be used for washing the plates. This solution can be stored at 4°C for 1 month.

Assay Procedure

1. Notice before assay:
1.1 Make sure all reagents and microwells are all at room temperature (20-25°C).
1.2 Return all the rest reagents to 2-8°C immediately after used.
1.3 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.
1.4 Avoid the light and cover the microwells during incubation.

2. Assay Steps:
2.1 Take all reagents out at room temperature (20-25°C) for more than 30min, homogenize before use.
2.2 Get the microwells needed out and return the rest into the zip-lock bag at 2-8°C immediately.
2.3 The wash solution should be brought to room temperature (20-25°C) before use.
2.4 Number: Number every microwell position and all standards and samples should be run in duplicate. Record the standards and samples positions.
2.5 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.
2.6 Wash: Remove the cover gently and pour the liquid out of the wells and rinse the microwells with 250µl 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

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with unused tip).
2.7 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 .
2.8 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

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 (%) = (B/B0)*100%

B - absorbance of standards or samples
B0 - absorbance of zero standard (0ng/ml)

Performance Characteristics

Linear range: 0.5-40.5ng/ml
Accuracy: 85±10%
Precision: CV of the ELISA kit all less than 10%.

Specificity

Cross-reactions:
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 H2SO4.
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

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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.

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