Competitive Enzyme Immunoassay Kit for Kanamycin

Quantitative Analysis of Kanamycin

1. Background
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.

2. Test Principle
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 microtiter 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.

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

4. Cross-reactions
Kanamycin…………………………………………100%
Streptomycin……………………………………..<1%
Dihydrostreptomycin…………………………….<1%
Neomycin………………………………………….<1%

5. Materials Required
5.1 Equipments:
----- Microtiter plate spectrophotometer (450nm/630nm)
----- polystyrene centrifuge tube: 2ml, 50ml
----- Micropipettes: 20μl, 200μl, 200μl-1000μl
          250μl-multipipette

5.2 Reagents:
----- Deionized water

6. Kit Components
- Microtiter plate with 96 wells coated with antigen
- Kanamycin standard solutions×6 bottles: 1ml/bottle
- 0ng/ml, 0.5ng/ml, 1.5ng/ml, 4.5ng/ml, 13.5ng/ml, 40.5ng/ml
- Spiking standard solution: 1ml, 1μg/ml
- Enzyme conjugate (7ml)……………………red cap
- Antibody solution (10ml)……………………green cap
- Solution A (7ml)……………………white cap
- Solution B (7ml)……………………red cap
- Stop solution (7ml)……………………yellow cap
- 20×Concentrated wash solution(40ml)
  ………………………………………………………………transparent cap
- 2×Sample diluent(50ml)
  ………………………………………………………………blue cap

7. Reagents Preparation:
Solution 1: wash solution
Dilute the 20×concentrated wash solution with deionized water in the volume ratio of 1:19 (1 fold 20×concentrated wash solution : 19 folds deionized water), which will be used for washing the plates. This solution can be stored at 4°C for 1 month.

8. Sample Preparations
8.1 Notice and precautions 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 affect the assay result.

8.2 Sample Preparation:
---- Dilute the 2×sample diluents with deionized water in the volume ratio of 1:1 for use.
---- Dilute the test sample with the prepared sample diluent to get a final concentration of 0.5 - 40.5 ng/ml (kanamycin).
---- Take 20 μl of the prepared solution for assay.

9. Assay process
9.1 Notice before assay:
9.1.1 Make sure all reagents and microwells are all at room temperature (20-25°C).
9.1.2 Return all the rest reagents to 2-8°C immediately after used.
9.1.3 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.
9.1.4 Avoid the light and cover the microwells during incubation.

9.2 Assay Steps:
9.2.1 Take all reagents out at room temperature (20-25°C) for

Beijing Kwinbon Biotech Co., Ltd
No.8, High Ave 4, Huilongguan International Information Industry Base, Changping District, Beijing 102206, P. R. China
Tel: +86-10-80700522/1 2 3 4, ext 623  Fax: +86-10-62711547  Email: product@kwinbon.com  rev. 2010-11  P 1 / 2
more than 30min, shake gently before use.

9.2.2 Get the microwells needed out and return the rest into the zip-lock bag at 2-8°C immediately.

9.2.3 The wash solution should be brought to room temperature (20-25°C) before use.

9.2.4 Number: Number every microwell position and all standards and samples should be run in duplicate. Record the standards and samples positions.

9.2.5 Add standard solution/sample: Add 20μl of standard solution or prepared sample to corresponding wells. Add 50μl enzyme conjugate solution, 80μl antibody solution to each well, mix gently by shaking the plate manually and incubate for 40min at 25°C with cover.

9.2.6 Wash: Remove the cover gently and pour the liquid out of the wells and rinse the microwells with 250μl diluted wash solution 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).

9.2.7 Coloration: Add 50μl solution A and 50μl solution B to each well. Mix gently by shaking the plate manually and incubate for 15 min at 25°C with cover (see 12.8)

9.2.8 Measure: Add 50μl 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. We can also measure by sight without stop solution in short of the ELISA reader).

10. Results

10.1 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%.

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\text{Absorbance} = \frac{B}{B_0} \times 100\%
\]

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

10.2 Standard Curve

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

---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.

Please notice: software has been developed for data reduction, which can be provided upon request.

11. Sensitivity, accuracy and precision

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

12. Notice

12.1 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).

12.2 Do not allow microwells to be dry between steps to avoid unsuccessful repetitiveness and operate the next step immediately after tap the microwells holder.

12.3 Mix the homogenate and elute the plate adequately.

12.4 Avoid the stop solution touching skin for the 2M H2SO4.

12.5 Don’t use the kits out of date. Don’t exchange the reagents of different batches; otherwise it will drop the sensitivity.

12.6 Keep the ELISA kits at 2-8°C without frozen. Avoid direct sunlight during all incubations. Covering the microtiter plate is recommended.

12.7 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).

12.8 The coloration reaction need 10-15min after the addition of solution A and solution B; but you can prolong the incubation time ranges to 20min or more if the color is too light to be determined, never exceed 30min. On the contrary, shorten the incubation time properly.

12.9 The optimum reaction temperature is 25°C, higher or lower temperature will lead to the changes of sensitivity and absorbance values.

13. Storage

- Storage condition: 2-8°C
- Storage period: 12months

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