Version 01-06/20

User's Manual

Kanamycin ELISA Kit

REF **DEIA048**

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 ELISA is intended for the quantitative and qualitative analysis of kanamycin residue in biological samples. For in vitro diagnostic use only.

General Description

Kanamycin or kanamycin A is an aminoglycoside bactericidal antibiotic, used to treat a wide variety of infections and tuberculosis. Kanamycin is isolated from the bacterium Streptomyces kanamyceticus and its most commonly used form is kanamycin sulfate. Kanamycin is commonly used as an antibiotic during the cell culture process. Regulatory authorities across the world have restricted and sought to quantify the Kanamycin residue in view of its potent action on human beings in the in-process and finished pharmaceutical products. 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.

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 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.

Reagents And Materials Provided

Label	Kit components	Quantity
PLATE	Microtiter plate, pre-coated	12 x 8 wells
WASHBUF	Wash buffer concentrate, 20 x	1 x 40 mL
SAMPLE DILUENT	Sample diluent concentrate, 2x	1 x 50 mL
AB	Antibody solution, anti-kanamycin, ready-to-use	1 x 10 mL
STD 1–6	Standards, ready-to-use 0, 0.5, 1.5, 4.5, 13.5, 40.5 ng/mL	6 x 1 mL
SPIKING STD	Spiking standard solution, 1 μg/mL	1 x 1 mL
ENZCONJ	Enzyme conjugate, monoclonal peroxidase- labeled antibody, ready-to-use	1 x 7 mL
SUB A	Substrate A (hydrogen peroxide), ready-to-use	1 x 7 mL
SUB B	Substrate B (tetramethylbenzidine), ready-to-use	1 x 7 mL
STOP	Stop solution, ready-to-use	1 x 7 mL

Materials Required But Not Supplied

1. Microtiter plate spectrophotometer (450nm/630nm)
2. Polystyrene centrifuge tube: 2 mL, 50mL
3. Micropipettes: 20 μL-200 μL, 100 μL-1000 μL, 250 μL-multipipette
4. Deionized water

Storage

Unopened kits can be stored stably for 12 months at 2-

8°C. 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 affect the assay result.

Sample preparation

1. Dilute the test sample with the prepared sample diluent to get a final concentration of 0.5-40.5 ng/mL (kanamycin).
2. Take 20 μL of the prepared solution for assay.

Reagent Preparation

1. To run the assay more than once, ensure that reagents are stored at the conditions stated on the label. **Prepare only the appropriate amount necessary for each run.**

2. **Preparation of the wash buffer**: 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.
3. **Preparation of the 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.
4. **Microtiter strips**: After opening the sealed aluminum packaging, unused strips have to be covered with adhesive foil and stored in the closed aluminum packaging together with desiccant at 2–8 ° C. We recommend not assembling wells of different microtiter plates for analysis, even if they are of the same batch. Opened microtiter plates are exposed to different conditions than sealed ones.
5. All other test reagents are ready-to-use. Test reagents are stable until the expiry date (see label) when stored at 2–8 °C.

Assay Procedure

Notices before test:

(1) Make sure all reagents and microwells are all at room temperature (20-25°C).

(2) 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.

(3) Avoid the light and cover the microwells during incubation.

Test procedure:

We recommend carrying out the tests in duplicate.

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 every microwell position and all standards and samples should be run in duplicate. Record the standards and samples positions.
5. Add 20 μL of standard solution or prepared sample to corresponding wells.
6. Add 50 μL of enzyme conjugate solution, 80 μL of antibody solution to each well, mix gently by shaking the plate manually and incubate for 40 min at 25°C with cover.
7. Remove the cover gently and pour the liquid out of the wells and rinse the microwells with 250 μL 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).
8. Add 50 μL of solution A and 50 μL of solution B to each well. Mix gently by shaking the plate manually and incubate for 15 min at 25°C with cover.
9. Add 50 μL of the stop solution to each well. Mix gently by shaking the plate manually.
10. Measure the absorbance at 450nm against an air blank within 5min after addition of stop solution. (It's suggested measure with the dual-wavelength of 450/630nm.) (We can also measure by sight without stop solution in short of the ELISA reader).

Quality Control

It is recommended that for each laboratory assay appropriate quality control samples in each run to be used to ensure that all reagents and procedures are correct.

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%.

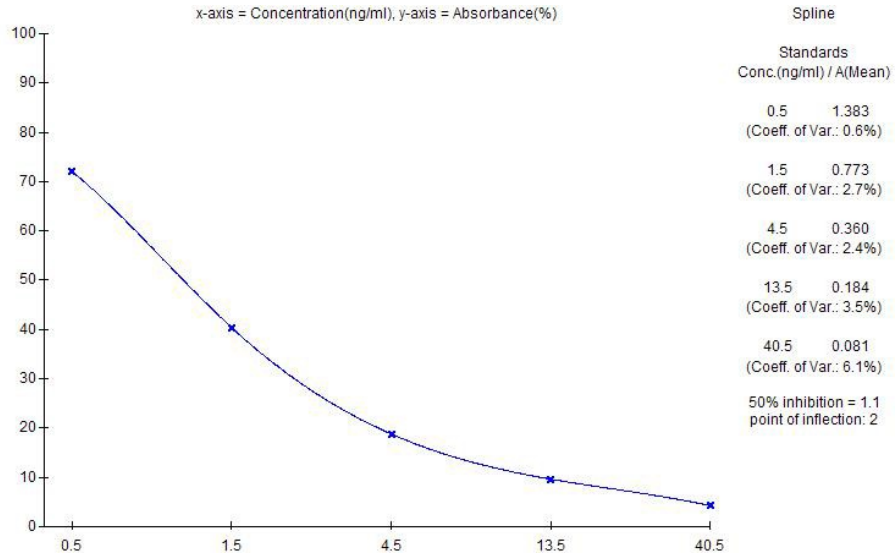
Absorbance (%) = (B/B0)*100%

B - absorbance of standards or samples

B0 - absorbance of zero standard (0 ng/mL)

Typical Standard Curve

This curve cannot be used for the assay calculations and is listed for quality control purpose only. New standard curve must be generated each time the assay is run.



Detection Range

The standards / linear graph range is 0.5-40.5 ng/mL.

Sensitivity

The analytical sensitivity of the Kanamycin ELISA was found to be 0.5 ng/mL.

Sample	Sensitivity
Vaccine	0.5 ng/mL

Recovery

The recovery of Kanamycin in Vaccine was 81.2% to 102%.

Precision

Assay precision was determined by both intra (n=5 assays) and inter assay (n=5 assays), CV of the ELISA kit all less than 10%. While actual precision may vary from laboratory to laboratory and technician to technician, it is recommended that all operators achieve precision below these design goals before reporting results.

Sample	Intra Assay %CV	Inter Assay %CV
5	< 10%	< 10%

Specificity

The specificity was tested by measuring the cross-reactivity against streptomycin, dihydrostreptomycin and neomycin. The result indicates that the kit is specific for kanamycin.

Components	Cross-Reactivity
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 needs 20-30min after the addition of solution A and solution B; but you can prolong the incubation time.