KRIBIOLISA® Kanamycin ELISA

REF : KRA1004

Ver 1.3

RUO

Enzyme Immunoassay for the Quantitative Determination of Kanamycin in cell culture supernatant and other purified systems including vaccines

RUO	**For Research Use Only**	**REF**	**Catalog Number**
	**Store At**	**LOT**	**Batch Code**
	**Manufactured By**		**Biological Risk**
	**Expiry Date**		**Consult Operating Instructions**

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REF **KRA1004** **Σ 96 tests**

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KRIBIOLISA® Kanamycin ELISA

Introduction:

Kanamycin or kanamycin A is an aminoglycoside bacteriocidal 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.

Intended Use:

The KRIBIOLISA® Kanamycin ELISA Kit for Accurate Quantitation of Kanamycin in cell culture supernatant and other purified systems including vaccines.

Principle:

KRIBIOLISA® Kanamycin ELISA kit is based on indirect-competitive ELISA. The microtiter wells are coated with antigen. Kanamycin residue in the sample competes with antigen coated on the microtitre plate for the antibody. After the addition of biotinylated antibody and enzyme conjugate, an immune complex will form. After washing, addition of TMB Substrate is used to show the color. Absorbance of the sample is inversely proportional to the Kanamycin residue in the samples. Color development is stopped by addition of stop solution. Absorbance is measured at 450 nm.

Materials Provided:

1. Kanamycin Coated Microtiter Plate (12x8 wells) - 1 no
2. Kanamycin Standards (1 ml) - 0, 0.5, 1.5, 4.5, 13.5, 40.5 ng/ml
3. Biotinylated Kanamycin Antibody - 10 ml
4. Streptavidin:HRP Conjugate - 7 ml
5. (20X) Wash Buffer - 2 x 25 ml
6. (2X) Sample Diluent - 50 ml
7. TMB Substrate - 12 ml
8. Stop Solution - 12 ml
9. Instruction Manual

Materials to be provided by the End-User:

1. Microplate Reader able to measure absorbance at 450 nm.
2. Adjustable pipettes to measure volumes ranging from 5 ul to 1000 ul.
3. Deionized (DI) water.
4. Wash bottle or automated microplate washer.
5. Graph paper or software for data analysis.
6. Polystyrene centrifuge tube：2ml, 50ml.
7. Timer.

Storage Information:

1. Store main kit components at 2-8°C.
2. Before using, bring all components to room temperature (18-25°C). Upon assay completion return all components to appropriate storage conditions.

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin. Refer to the MSDS online for details.

2. To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum and/or plasma in accordance with NCCLS regulations.

Specimen Collection and Handling:

Dilute the samples with (1X) Sample Diluent for achieving optimal Kanamycin concentration.

Reagent Preparation (all reagents should be diluted immediately prior to use):

1. Label any aliquots made with the kit Lot No and Expiration date and store it at appropriate conditions mentioned.
2. Bring all reagents to Room temperature before use.
3. To make Wash Buffer (1X); dilute 50 ml of 20X Wash Buffer in 950 ml of DI water.
4. To make Sample Diluent (1X); add 2.5 ml of Sample Diluent (2X) in 2.5 ml of DI water.

Procedural Notes:

1. In order to achieve good assay reproducibility and sensitivity, proper washing of the plates to remove excess un-reacted reagents is essential.
2. Avoid assay of Samples containing sodium azide (NaN_3), as it could destroy the HRP activity resulting in under-estimation of the amount of Kanamycin.
3. It is recommended that all Standards and Samples be assayed in duplicates.
4. Maintain a repetitive timing sequence from well to well for all the steps to ensure that the incubation timings are same for each well.
5. If the Substrate has a distinct blue color prior to use it may have been contaminated and use of such substrate can lead to erroneous results in the assay.
6. The plates should be read within 30 minutes after adding the Stop Solution.
7. Make a work list in order to identify the location of Standards and Samples.

Assay Procedure:

1. Bring all reagents to room temperature prior to use. It is strongly recommended that all standards and samples be run in duplicate or triplicate. A standard curve is required for each assay.

2. Add **20 ul** of **Standards and Samples** to corresponding wells.

3. Add **80 ul Biotinylated Kanamycin Antibody** to each well.

4. Add **50 ul Streptavidin:HRP Conjugate** to each well.

5. Mix gently by shaking the plate manually and **incubate** for **40 mins** at **room temperature (25°C)**.

6. Aspirate and **wash plate 4 times** with 250 ul of **Wash Buffer (1X)** and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step.

7. Add **100 ul** of **TMB Substrate** to each well.

8. **Incubate** the plate for **15 minutes** at **room temperature (25°C)**. DO NOT SHAKE or else it may result in higher backgrounds and worse precision.

9. Stop reaction by adding **100 ul** of **Stop Solution** to each well.

10. Read absorbance at 450 nm within 30 minutes of stopping reaction.

Interpretation of Results:

Determine the Mean Absorbance for each set of duplicate Standards and Samples. Using standard graph paper, plot the average value (absorbance 450nm) of each standard on the Y-axis versus the corresponding concentration of the standards on the X-axis. Draw the best fit curve through the standard points.

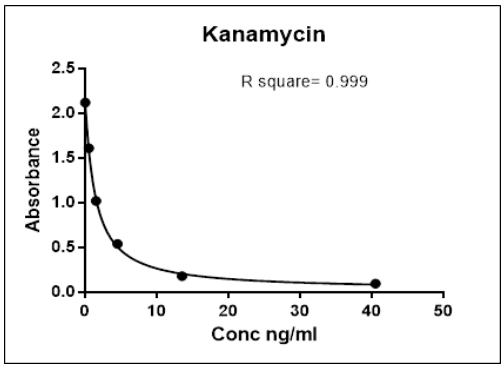
To determine the unknown Kanamycin concentrations, find the unknown's Mean Absorbance value on the Y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the Kanamycin Concentration. If samples were diluted, multiply by the appropriate dilution factor.

Software which is able to generate a polynomial regression (2nd order) or a cubic spline curve-fit is best recommended for automated results.

Typical Data

Concentration ng/ml	Mean Absorbance	Interpolated Concentration	% Recovery
0	2.133	0.00	100%
0.5	1.474	0.49	97.6
1.5	0.866	1.56	104.2
4.5	0.429	4.25	94.5
13.5	0.169	13.36	98.9
40.5	0.066	48.27	119.2

Typical Graph



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.

Performance Characteristics of the Kit:

Sensitivity:
Limit Of Detection: It is defined as the lowest detectable concentration corresponding to a signal of Mean of '0' standard plus 2* SD.
10 replicates of '0' standards were evaluated and the LOD was found to be less than 0.35 ng/ml.

Specificity:
The kit is specific for Kanamycin. It has cross-reactivity to other antibiotics as under -

Kanamycin	- 100%
Streptomycin	- <1%
Dihydrostreptomycin	- <1%
Neomycin	- <1%

Linearity:
Standards provided in the kit are used for measuring the linearity range of Kanamycin present in matrix. A Positive Control is included as optional to dilute it to the desired concentration for using as a control in data interpretation or for Quality Control to observe recovery of the samples.

The linear graph range is 0.5 - 40.5 ng/ml.

Accuracy:
95+30%

Precision:
Precision is defined as the percent coefficient of variation (%CV) i.e. standard deviation divided by the mean and multiplied by 100. Assay precision was determined by both intra (n=5 assays) and inter assay (n=5 assays) reproducibility on two pools with low, medium and high concentrations.

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.

Pool	Intra Assay %CV	Inter Assay %CV
Low	<12%	<15%
Medium	<12%	<12%
High	<10%	<10%

Safety Precautions:

- **This kit is for research use only**. Follow the working instructions carefully.
- The expiration dates stated on the kit are to be observed. The same relates to the stability stated for reagents.
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept at 2 - 8°C before use in the original shipping container.
- Some of the reagents contain small amounts (< 0.1 % w/w) sodium azide as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Source materials maybe derived from body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.
- Since the kit contains potentially hazardous materials, the following precautions should be observed.
  - Do not smoke, eat or drink while handling kit material.
  - Always use protective gloves.
  - Never pipette material by mouth.
  - Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.
- In any case GLP should be applied with all general and individual regulations to the use of this kit.

LIMITED WARRANTY

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