KRIBIOLISA™ Gentamicin ELISA

REF : KRA1009

Ver 3.1

RUO

ELISA Set for Accurate Quantitation of Gentamicin

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

For Research Purposes Only Purchase does not include or carry the right to resell or transfer this product either as a stand-alone product or as a component of another product. Any use of this product other than the permitted use without the express written authorization of KRISHGEN BioSystems is strictly prohibited.

KRISHGEN BioSystems Unit Nos#318/319, Shah & Nahar, Off Dr E Moses Road, Worli, Mumbai 400 018.
Tel: 022) 49198700 Email: sales1@krishgen.com

KRIBIOLISA™ Gentamicin ELISA

KRISHGEN BioSystems
OUR REAGENTS, YOUR RESEARCH

Introduction:

Gentamycin residue in the production of biological samples may lead to severe allergic reactions in certain groups. Thus it is strictly controlled in many countries in the world.
This kit is a new product for drug residual detection based on ELISA technology, which is rapid, easy-to-use, and sensitive, and can considerably minimize operation errors and work intensity.

Intended Use:

This KRIBIOLISA™ Gentamicin ELISA Kit for accurate quantitation of Gentamicin from the sample.

Principle:

KRIBIOLISA™ Gentamicin ELISA kit is based on indirect-competitive ELISA. The microtiter wells are coated with antigen. Gentamicin residue in the sample competes with the antigen coated on the microtiter plate for the antibody. After the addition of enzyme conjugate, TMB substrate is used to show the color. Absorbance of the sample is negatively related to the Gentamicin residue in it, after comparing with the Standard Curve, multiplied by the dilution factor, Gentamicin residue in the sample can be calculated.

Materials Provided:

1. Microtiter Coated Plate (8 X 12 wells) – 1 no
2. Standards (0 ng/ml, 0.1 ng/ml, 0.3 ng/ml, 0.9 ng/ml, 2.7 ng/ml, 8.1 ng/ml) – 1 ml each
3. Spiking standard solution 1ml/bottle – 1 ug/ml
4. Detection Antibody – 7 ml
5. Enzyme conjugate - 12ml
6. (20X) Wash Buffer – 2 x 25 ml
7. (2X) Sample Diluent – 50 ml
8. TMB Substrate – 12 ml
9. Stop Solution – 12 ml
10. Instruction Manual

Materials to be provided by the End-User:

1. Microplate Reader able to measure absorbance at 450nm/630nm
2. Adjustable pipettes to measure volumes ranging from 5µl to 1000µl.
3. Deionized (DI) water.
4. Wash bottle or automated microplate washer.
5. Semi log 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.

KRIBIOLISA™ Gentamicin ELISA

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.

Reagent Preparation:

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 25 ml of (20X) Wash Buffer in 475 ml of DI water.
   Dilute the 20X 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.
4. Sample Diluent (1X): Add 10 ml of Sample Diluent (2X) in 10 ml of DI water. Mix well
   Dilute the 2X concentrated sample buffer with deionized water in the volume ratio of 1:1, which will be used for sample extraction, this solution can be stored at 4°C for 1 month.

Specimen Collection and Handling:
Dilute the sample with sample diluent (1X) for achieving proper Gentamicin concentration (0.1- 8.1 ng/ml) in it.

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 **50 ul** of **standard** solution or prepared **sample** to corresponding wells.

3. Add **50 ul** of **detection antibody** in each well.

4. Mix gently by shaking the plate manually and incubate for **30 min** at **37°C** with cover.

5. Aspirate and wash plate 4-5 times with 250 ul of **Wash Buffer (1X)** at interval of 10s and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe off any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step. All the washes should be performed similarly.

6. Add **100 ul** of **enzyme conjugate working solution** in each well.

7. Mix gently by shaking the plate manually and incubate for **30 min** at **37°C** with cover.

8. Aspirate and wash plate 4-5 times with 250 ul of **Wash Buffer (1X)** at interval of 10s and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe off any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step. All the washes should be performed similarly.

9. Add **100 ul** of TMB Substrate to each well and incubate for **15 min** at **37°C** with cover.

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

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

Calculation of Results:

Determine the mean absorbance for each set of duplicate or triplicate standards and samples. Subtract the mean absorbance of the zero standards (background) from each well. Plot the standard curve on semi log graph paper, with Gentamycin concentration on the x-axis and absorbance on the y-axis. Draw the best fit straight line through the standard points. To determine the unknown Gentamycin concentrations, find the unknowns 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 Gentamycin concentration.

If samples were diluted, multiply by the appropriate dilution factor. Software which is able to generate a cubic spline curve-fit or 4-PL is best recommended for automated results.

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.

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 human 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

Krishgen Biosystems does not warrant against damages or defects arising in shipping or handling, or out of accident or improper or abnormal use of the product; against defects in products or components not manufactured by Krishgen Biosystems, or against damages resulting from such non-Krishgen Biosystems made products or components. Krishgen Biosystems passes on to customer the warranty it received (if any) from the maker thereof of such non-Krishgen made products or components. This warranty also does not apply to product to which changes or modifications have been made or attempted by persons other than pursuant to written authorization by Krishgen Biosystems.

THIS WARRANTY IS EXCLUSIVE. The sole and exclusive obligation of Krishgen Biosystems shall be to repair or replace the defective product in the manner and for the period provided above. Krishgen Biosystems shall not have any other obligation with respect to the products or any part thereof, whether based on contract, tort, and

strict liability or otherwise. Under no circumstances, whether based on this Limited Warranty or otherwise, shall Krishgen Biosystems be liable for incidental, special, or consequential damages.

This Limited Warranty states the entire obligation of Krishgen Biosystems with respect to the product. If any part of this Limited Warranty is determined to be void or illegal, the remainder shall remain in full force and effect.

Krishgen Biosystems. 2024

THANK YOU FOR USING KRISHGEN PRODUCT!