KRIBIOLISA™ Neomycin ELISA

REF : KRA1010

Ver 1.1

RUO

ELISA Set for Accurate Quantitation of Neomycin in biological samples

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 For US/Europe Customers: toll free +1(888)-970-0827 | tel +1(562)-568-5005
For Asia/India Customers: tel +91(22)-49198700
Email: sales@krishgen.com | http://www.krishgen.com

Introduction:

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

Intended Use:

This KRIBIOLISA™ Neomycin ELISA Kit for quantitative and qualitative analysis of neomycin residue in biological samples.

Principle:

KRIBIOLISA™ This kit is based on indirect-competitive ELISA technology. The microtiter wells are coated with coupling antigen. Neomycin 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 neomycin reside in it, after comparing with the Standard Curve, multiplied by the dilution factor, neomycin residue quantity in the sample can be calculated.

Materials Provided:

1. Neomycin Microtiter Coated Plate (8 X 12 wells) – 1 no
2. Neomycin Standards - 0 ng/ml, 0.5 ng/ml, 1.5 ng/ml, 4.5 ng/ml, 13.5 ng/ml, 40.5 ng/ml - 1 ml/vial
3. Spiking standard solution: 1µg/ml -1ml
4. Streptavidin:HRP Conjugate - 7 ml
5. Biotinylated Anti-Neomycin Detection Antibody - 7ml
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. Microtiter plate spectrophotometer 450 nm
2. Polystyrene centrifuge tube: 2 ml
3. Micropipettes: 20 ul-200 ul, 100 ul-1000 ul, 250 ul-multipipette

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 sample with sample diluent (1X) for achieving proper Neomycin concentration (0.5- 40.5 ng/ml) in it.

Notice and precautions for before operation

1. Please use one-off tips in the process of experiment, and change the tips when absorb different reagent.
2. Make sure that all experimental instruments are clean; otherwise it will affect the assay result.

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.
4. Sample Diluent (1X): Add 10 ml of Sample Diluent (2X) in 10 ml of DI water. Mix well

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 Nenomycin
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 compromisation of the sensitivity of 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 **50 ul** of **standard** solution or prepared **sample** to corresponding wells.

3. Add **50 ul** of **Biotinylated Anti-Neomycin Detection Antibody**.

4. Add **50 ul of Streptavidin:HRP Conjugate** in each well. Mix gently

5. Incubate for **30 min** at **25**℃ with cover.

6. Aspirate and wash plate 4 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.

7. Incubate for **30 min** at **37**℃

8. Repeat Step No. (5).

9. Add 100 ul of TMB Substrate to each well.

10. Incubate for **15 min** at **25**℃.

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

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

Calculation of Results:

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%.

$$\text{Absorbance (\%)} = \frac{B}{B_0} * 100\%$$

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

(2) Standard Curve:

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

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.

Cross – reactivity:

Neomycin -100%
Streptomycin -<0.1%
Kanamycin -<0.1%
Apramycin -<0.1%
Gentamycin -<0.7%
Tobramycin -<0.1%

Sensitivity:
Limit Of Quantification:
It is defined as the lowest detectable concentration that can be determined with an acceptable repeatability and the LOQ was found to be 0.08 ng/ml

Linear range: 0.5-40.5 ng/ml

Accuracy: 85±10%

Precision: CV of the ELISA kit all less than 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 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. 2022

THANK YOU FOR USING KRISHGEN PRODUCT!