KRIBIOLISA™ GENTAMICIN ELISA

REF : KRA1009

Ver 2.0

RUO

Enzyme Immunoassay for the Quantitative Estimation of Gentamicin in cell culture supernatants and other biological preparations.

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 KRA1009 Σ 96 tests

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:

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 and the analyte needs to be measured in biological preparations before release.

Intended Use:

The KRIBIOLISA™ Gentamycin ELISA Kit for Quantitative Estimation of Gentamycin in cell culture supernatants and other biological preparations. Note the kit is not to be used for estimation of Gentamicin in food and other sample matrices. Kindly refer our website or contact us for other sample matrices.

Principle:

The method employs sandwich ELISA technique. Monoclonal antibodies to Gentamicin are pre-coated onto microwells. Samples and standards are pipetted into microwells and Gentamicin present in the sample are bound by the antibodies competing with Gentamicin labeled HRP. Post incubation a complex is formed. After washing the microwells in order to remove any non-specific binding, the substrate solution (TMB) is added to microwells and color develops proportionally to the amount of Gentamicin in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

Materials Provided:

1. Anti-Gentamicin Coated Microtiter Plate (12 x 8 wells) – 1 no
2. Gentamicin Standard (concentrated, 32 ng/ml) – 0.5 ml
3. Gentamicin:HRP Conjugate – 6 ml
4. Standard Diluent – 6 ml
5. 25X Wash Buffer – 20 ml
6. TMB Substrate A – 6 ml
7. TMB Substrate B – 6 ml
8. Stop Solution – 6 ml

Materials to be provided by the End-User:

1. Microtiter Plate Reader able to measure absorbance at 450 nm.
2. Adjustable pipettes and multichannel pipettor to measure volumes ranging from 25 ul to 1000 ul
3. Deionized (DI) water
4. Wash bottle or automated microplate washer
5. Graph paper or software for data analysis
6. Timer
7. Absorbent Paper

Handling/Storage:

1. Store main kit components at recommended storage temperature indicated on the component label.
2. Before using, bring all components to room temperature (18-25°C). Upon assay completion return all components to appropriate storage conditions.
3. The Substrate is light-sensitive and should be protected from direct sunlight or UV sources.

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin.

Sample Preparation and Storage:

It is recommended to dilute the sample with Standard Diluent (1X) for achieving proper Gentamicin concentration within the detectable range of the graph of the kit.

Notice and precautions for 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.
c. For sample containing aluminium adjuvant, please have a trial experiment first to evaluate the performance of the kit, due to the severe interference. In this case, please dilute the sample with diluent to try.

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 **20 ml of 25X Wash Buffer in 480 ml of DI water**.
4. **Standards Preparation**: Dilute 120 ul of original **Standard (32 ng/ml)** with 120 ul of Standard Diluent to generate a **16 ng/ml Standard stock solution**. Keep the standard for 15 mins with gentle agitation before making further dilutions. Prepare the **Standards** by serially diluting the standard stock solution as per the below table.

Standard Concentration	Standard Vial	Dilution Particulars
32 ng/ml	Original Standard	Original Standard provided in the Kit
16 ng/ml	Standard No.6	120 ul Standard Provided (32 ng/ml) + 120 ul Standard Diluent
8 ng/ml	Standard No.5	120 ul Standard No.6 + 120 ul Standard Diluent
4 ng/ml	Standard No.4	120 ul Standard No.5 + 120 ul Standard Diluent
2 ng/ml	Standard No.3	120 ul Standard No.4 + 120 ul Standard Diluent
1 ng/ml	Standard No.2	120 ul Standard No.3 + 120 ul Standard Diluent
0.5 ng/ml	Standard No.1	120 ul Standard No.2 + 120 ul Standard Diluent
0 ng/ml	Standard No. 0	Standard Diluent

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. High Dose Hook Effect may be observed in samples with very high concentrations of Gentamicin. High Dose Hook Effect is due to excess of concentrations of Gentamicin present in the sample. High Dose Hook effect is most likely encountered from samples early in the purification process. If Hook Effect is possible, the samples to be assayed should be diluted with the diluent further.
3. Gentamicin concentration of the undiluted sample is less than the diluted sample, this may be indicative of the Hook Effect.
4. Avoid assay of Samples containing sodium azide (NaN_3), as it could destroy the HRP activity resulting in under-estimation of the amount of Gentamicin.
5. It is recommended that all Standards and Samples be assayed in duplicates or triplicates.
6. Maintain a repetitive timing sequence from well to well for all the steps to ensure that the incubation timings are same for each well.
7. 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.
8. The plates should be read within 30 minutes after adding the Stop Solution.
9. Make a work list in order to identify the location of Standards and Samples.

Assay Procedure:

1. It is strongly recommended that all Standards and Samples be run in duplicates or triplicates. A standard curve is required for each assay.
2. Add **50 ul prepared Standards** to respective standard wells.
3. Add **50 ul diluted Sample** to respective sample wells.
4. Pipette **50 ul Gentamicin:HRP conjugate** to all wells. Mix well.
5. Cover the plate with a sealer and incubate for **60 minutes** at **37°C.**
6. Aspirate and wash plate 4 times with diluted **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. Pipette **50 ul TMB Substrate A** followed by **50 ul TMB Substrate B** in all the wells.
8. Incubate the plate at **37°C** for **10 minutes**. DO NOT SHAKE or else it may result in higher backgrounds and worse precision. Positive wells should turn bluish in color.
9. Pipette **50 ul** of **Stop Solution** in all wells. The wells should turn from blue to yellow in color.
10. Read the absorbance at 450 nm with a microplate within 30 minutes after addition of Stop solution.

Calculation of Results:

Determine the Mean Absorbance for each set of duplicate or triplicate Standards and Samples. Using 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 Gentamicin 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 Gentamicin Concentration.

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

Note:
It is recommended to repeat the assay at a different dilution factor in the following cases:
- If the sample absorbance value is below the first standard.

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:
This kit has been validated. Please view the details herein below.

Standard Calibration Range:
0.5 ng/ml – 16 ng/ml

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 0.2 ng/ml.

Specificity:
The antibodies used in this kit are monoclonal antibodies specific for Gentamicin.

Precision:
Intra-Assay Precision: 3 samples (n=3) with low, middle and high concentration of Gentamicin were tested in triplicate respectively. The Intra-Assay was found to be <15%
Inter-Assay Precision: 3 samples (n=3) with low, middle and high concentration of Gentamicin were tested in triplicate on two plates respectively on two consecutive days. The Inter-Assay was found to be <18%.
The Cumulative Variance % was calculated as CV (%) = SD/mean x 100 [SD=standard deviation]

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 in the original shipping container.
- Some of the reagents contain small amount of sodium azide (< 0.1 % w/w) as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Source materials maybe derived from Mouse 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.

SCHEMATIC ASSAY PROCEDURE

1. Remove all components, 30 minutes before adding into the assay plate.

Components (2-8ºC) → 30 mins RT Before Use → 

Thaw at Room Temperature (18-24ºC) → USE NOW

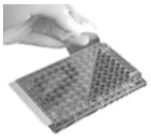
2. Avoid repeated cool-thaw of the components as there will be a loss of activity and this can affect the results.

Kit Components → AVOID → COOL (2-8ºC) → THAW (22±4ºC) → AVOID → THAW (22±4ºC) → COOL (2-8ºC) → AVOID → NO CHANGE IN RESULTS

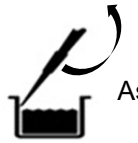
3.  Pipette **50 ul prepared Standards** into respective Standard wells.

4.  Pipette **50 ul diluted Samples** into the respective wells.

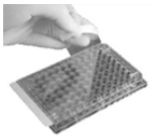
5.  Pipette **50 ul Gentamicin:HRP Conjugate** to all wells.

6. Cover plate  and incubate for 

at 37°C.

7.  Aspirate and wash wells 4 times with **Wash Buffer (1X).**

8.  Pipette **50 ul TMB Substrate A followed by 50 ul TMB Substrate B** to all wells.

9. Cover plate  and incubate for 

at 37°C.

10.  Pipette **50 ul Stop Solution** in all wells.

11. Read absorbance at 450nm with a  microplate reader within 

of stopping reaction.

Typical Example of a Work List

Well #	Contents	Absorbance at 450nm	Mean Absorbance	ng/ml Gentamicin equivalent
1A 2A	zero std zero std			
1B 2B	0.5 ng/ml 0.5 ng/ml			
1C 2C	1 ng/ml 1 ng/ml			
1D 2D	2 ng/ml 2 ng/ml			
1E 2E	4 ng/ml 4 ng/ml			
1F 2F	8 ng/ml 8 ng/ml			
1G 2G	16 ng/ml 16 ng/ml			
1H 2H	Sample			
3A 4A	Sample			
3B 4B	Sample			

LIMITED WARRANTY

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

THANK YOU FOR USING KRISHGEN PRODUCT!

SYMBOLS KEY

MTP	Anti-Gentamicin Microtiter Plate (12X8 wells)
STD	Gentamicin Standard
HRP CONJ	Conjugate Horseradish Peroxidase
STD DIL	Standard Diluent
25X WASH BUF	(25X) Wash Buffer
SUB TMB	TMB Substrate
SOLN STOP	Stop Solution
	Consult Instructions for Use
REF	Catalogue Number
	Expiration Date
	Storage Temperature