






KRIBIOLISA™ Streptomycin ELISA

REF : KRA1001

Ver 1.0

RUO

ELISA Set for Accurate Quantitation of Streptomycin

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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Introduction:

Streptomycin is an aminoglycoside antibiotic, which is broadly applied in animal disease treatment. For it has neurotoxicity and kidney toxicity, its residue in animal-derived food is harmful to human; it is strictly controlled in use in EU, US and China. At present, ELISA is the common approach in supervision and control of streptomycin drug.

Intended Use:

This KRIBIOLISA™ Streptomycin ELISA Kit for Accurate Quantitation of Streptomycin from the sample.

Principle:

KRIBIOLISA™ Streptomycin ELISA kit is based on indirect-competitive ELISA. The microtiter wells are coated with antigen. Streptomycin residue in the sample competes with antigen coated on the microtitre plate for the antibody. After the addition of enzyme conjugate immune complex will form. After washing, addition of Substrate A and Substrate B is used to show the color. Absorbance of the sample is negatively related to the Streptomycin residue in it, after comparing with the Standard Curve, multiplied by the dilution factor, Streptomycin residue in the sample can be calculated.

Materials Provided:

1. Microtiter Coated Plate (12 X 8 wells) – 1 no
2. Standards (0ng/ml, 0.05ng/ml, 0.15ng/ml, 0.45ng/ml, 1.35ng/ml, 4.05ng/ml) – 1 ml each
3. Spiking standard solution 1µg/ml – 1ml
4. Enzyme conjugate – 1 ml
5. Enzyme conjugate diluent – 10 ml
6. Wash Buffer (20X) – 40ml
7. Sample Diluent (2X) – 50ml
8. Substrate A – 7ml
9. Substrate B – 7ml
10. Stop Solution – 7ml
11. 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.

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 Streptomycin concentration (0.5- 40.5 ng/ml) in it.

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 aluminum 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:

1. Sample Diluent (1X):
Add 2500 µl of Sample Diluent (2X) in 2500 µl of deionized water. Mix it well
2. Wash Buffer (1X):
500 µl of Wash Buffer (20X) in 9500µl of deionized water. Mix it well
3. Concentrated conjugated enzyme:
Dilute the concentrated conjugated enzyme 1:10 (Eg: Add 0.5 ml concentrated enzyme conjugate + 5ml enzyme conjugate diluent)

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 **50µl** of **standard** solution or prepared **sample** to corresponding wells.
3. Add **50µl** of **diluted enzyme conjugate** into each well.
4. Mix gently by shaking the plate manually and incubate for **30 min** at **25°C** with cover.
5. Aspirate and wash plate 4 times with 250 µl 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 **50 µl** of Substrate A and Substrate B to each well and incubate for **15 min** at **25°C** with cover
9. Stop reaction by adding **50µl** of **Stop Solution** to each well.
10. Read absorbance at 450nm within 30 minutes of stopping reaction. (It's suggested measure with the dual-wavelength of 450/630nm. Read the result within 5min

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 :

- 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.
- The Streptomycin 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:

Streptomycin.....	100%
Dihydrostreptomycin.....	106%
Streptomycinsulphate.....	67%
Neomycin.....	<1%
Gentamycin.....	<1%
Kanamycin.....	<1%
Amikacin.....	<1%
Spectinomycin.....	<1%
Apramycin.....	<1%

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.

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