KRIBIOLISA™ Streptomycin ELISA

REF : KRA1001

Ver 1.1

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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KRISHGEN BioSystems

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.
This kit is a new product for drug residual detection based on ELISA technology, which only costs 45 min in each operation and can considerably minimize operation errors and work intensity.

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 coupling antibody. Streptomycin residue in the sample competes with the antigen coated on the microtiter plate for the antibody. After the addition of enzyme conjugate, chromogenic substrate 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 quantity in the sample can be calculated.

Materials Provided:

1. Microtiter Coated Plate (8 X 12 wells) – 1 no
2. Standards (0 ng/ml, 0.05 ng/ml, 0.15 ng/ml, 0.45 ng/ml, 1.35 ng/ml, 4.05 ng/ml) – 1 ml each
3. Spiking standard solution 1ml/bottle – 1 ug/ml
4. Concentrated Enzyme conjugate – 1 ml
5. Enzyme conjugate diluent - 10ml
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.

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 (0.05- 40.5 ng/ml) in it.

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.
5. Enzyme conjugate working solution: Dilute the concentrated enzyme conjugate with the enzyme conjugate diluent in the volume ratio of 1:10 (e.g. 0.5 ml of concentrated enzyme conjugate + 5 ml of enzyme conjugate diluent), mix complete. (this mixture can't be conserved use immediately).

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 **enzyme conjugate working solution** in each well.

4. Mix gently by shaking the plate manually and incubate for **30 min** at **25^{0}C** with cover.

5. 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.

6. Add **100 ul** of TMB Substrate to each well and incubate for **15 min** at **25^{0}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:

1) <u>Percentage absorbance:</u>

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%.

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

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 (ppb) as x-axis.

2. 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%
Streptomycin sulphate-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

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