KRIBIOLISA® Streptomycin ELISA

REF : KRA1001

Ver 2.0

RUO

Enzyme Immunoassay 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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REF KRA1001 Σ 96 tests

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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.
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 antigen. Streptomycin residue in the sample competes with the antigen coated on the microtiter plate for the antibody. After the addition of Streptavidin:HRP, chromogenic substrate (TMB) 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. Antigen coated Microplate (12 x 8 wells) - 1 no.
2. Standards (0 ppb, 0.2 ppb, 0.6 ppb, 1.8 ppb, 5.4 ppb, 16.2 ppb) - 1 ml each
3. Spiking Standard Solution (1 ppm) - 1 ml
4. Streptavidin:HRP - 7 ml
5. Biotinylated Antibody - 7 ml
6. (20X) Sample Extraction A - 15 ml
7. (2X) Sample Extraction B - 2 x 50 ml
8. (5X) Sample Extraction C - 10 ml
9. (20X) Sample Dilution - 15 ml
10. (20X) Wash Buffer - 25 ml
11. TMB Substrate - 12 ml
12. Stop Solution - 12 ml
13. Instruction Manual

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.

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.

Handling/Storage:

1. Store main kit components at 2-8ºC.
2. Before using, bring all components to room temperature (18-25ºC). Upon assay completion return all components to appropriate storage conditions.

Sample Preparation and Storage:

1. Ensure the samples which are pig serum should be collected with no bacteria.
2. Hemolytic or contaminated samples should not be run. Repeated freezing and thawing should be avoided. If samples are to be used for several assays, initially aliquot samples and keep at -20ºC.
3. Samples to be diluted 1:40 using Sample Diluent provided in the kit.

Preparation Before Use:

Allow samples to reach room temperature prior to assay. Take care to agitate samples gently in order to ensure homogeneity.

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. Sample Extraction A – 1 part of 20X Sample Extraction A + 19 parts of deionized water, mix it evenly.
4. Sample Extraction B – 1 part of 2X Sample Extraction B + 1 part of deionized water, mix it evenly.
5. Sample Extraction C – 1 part of 5X Sample Extraction C + 4 parts of deionized water, mix it evenly.
6. Sample Dilution – 1 part of 20X Sample Dilution + 19 parts of deionized water, mix it evenly.
7. 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 4ºC for 1 month.

Tissue/Aquatic product (Method 1):

1. Take 2 ± 0.05 g of the homogenized tissue sample into 50 ml centrifuge tube, add 3 ml Sample Extraction A, shake for 3 min.
2. Then add 600 ul 1M NaOH solution and 2.4 ml Sample Extraction B, shake for 3 min, centrifuge at above 4000 r/min at room temperature (20-25ºC) for 5 minutes.
3. Take 200 ul up-layer clear liquid, add 800 ul Sample dilution, mix it evenly.
4. Take 50 ul above mixed solution for analysis.

Fold of dilution of the sample: 20

Tissue/Aquatic product (Method 2):

1. Take 2 ± 0.05 g of the homogenized tissue sample into 50 ml centrifuge tube, add 4 ml 3% Trichloroacetic acid solution, shake for 3 min, centrifuge at above 4000 r/min at room temperature (20-25ºC) for 5 minutes.
2. Take 100 ul up-layer clear liquid, 100 ul of Sample extraction C and 300 ul Sample Dilution, mix it evenly.
3. Take 50 ul above mixed solution for analysis.

Fold of dilution of the sample: 10

Honey:

1. Take 1 ± 0.05 g of the homogenized honey sample into 10 ml centrifuge tube, add 4 ml 50% Methanol solution, shake for 3 min, centrifuge at above 4000 r/min at room temperature (20-25ºC) for 5 minutes.
2. Take 200 ul up-layer clear liquid and 800 ul Sample Dilution, mix it evenly.
3. Take 50 ul above mixed solution for analysis.

Fold of dilution of the sample: 20

Milk:

1. Take 1ml of the milk sample into 10 ml centrifuge tube, add 4ml 0.01M HCI solution, shake for 3 min, centrifuge at above 4000 r/min at room temperature (20-25ºC) for 5 minutes.
2. Take 200 ul up-layer clear liquid and 600 ul Sample Dilution, mix it evenly.
3. Take 50 ul above mixed solution for analysis.

Fold of dilution of the sample: 20

Milk Powder:

1. Take 1 ± 0.05 g of the milk powder sample into 10 ml centrifuge tube, add 5ml deionized water, shake for 3 min, centrifuge at above 4000 r/min at room temperature (20-25ºC) for 5 minutes.
2. Take 1 ml of the up-layer clear liquid, add 50 ul 1M HCI solution, shake for 3 min, centrifuge at above 4000 r/min at room temperature (20-25ºC) for 5 minutes.
3. Take 100 ul up-layer clear liquid and 1500 ul Sample Dilution, mix it evenly.
4. Take 50 ul above mixed solution for analysis.

Fold of dilution of the sample: 80

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 **Streptavidin:HRP** in each well.

4. Add **50 ul** of **Biotinylated Antibody** in each well.

5. Mix gently by shaking the plate manually and incubate for **30 min** at **25ºC** with cover.

6. Aspirate and wash plate 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.

7. Add **100 ul** of TMB Substrate to each well and incubate for **15 min** at **25ºC** with cover.

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

9. Read the absorbance at 450 nm with a microplate reader.

Calculation of Results:

Qualitative Determination:

The concentration range (ng/ml) can be obtained from comparing the average OD value of the testing sample with that of the standard solution. Assuming that the OD value of the sample I is 0.3, and that of the sample II is 1.0, the OD value of the standard solutions is: 2.243 for 0 ppb, 1.816 for 0.2 ppb, 1.415 for 0.6 ppb, 0.74 for 1.8 ppb, 0.313 for 5.4 ppb, 0.155 for 16.2 ppb, accordingly the concentration range of the sample I is 5.4 to 16.2 ppb, and that of the sample II is 1.8 to 5.4 ppb.

Quantitative Determination:

1) Percentage absorbance:

The mean values of the absorbance values is equivalent to the percentage of the average OD value (B) of the testing sample and the standard solution divided by the OD value (B_0) of the first standard solution (zero standard) and multiplied by 100%.

$$\text{Percentage of Absorbance value} = \frac{B}{B_0} \times 100\%$$

B – the average (double wells) OD value of the testing sample or the standard solution
B_0 – the average OD value of the (0 ug/l) standard solution

2) Standard Curve:
Draw the standard curve with the absorption percentages of the standard solutions and the semilogarithmic values of the Streptomycin Standard solutions (ug/l) as Y- and X-axis respectively. Read the corresponding concentration of the testing sample from the standard curve by incorporating its absorption percentage into the standard curve. The resulting value is subsequently multiplied by the corresponding dilution fold, finally obtaining the Streptomycin concentration in the sample.

Performance Characteristics:

Sensitivity: 0.2 ppb

Limit of Detection (LOD):
Tissue/Aquatic product (Method 1), honey, milk – 4 ppb
Tissue/Aquatic product (Method 2) – 2 ppb
Milk Powder – 20 ppb

Recovery Rate:
Tissue/Aquatic product – 70%~120%
Honey, Milk, milk powder – 80%~120%

Cross – reactivity:
Streptomycin-100%

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 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 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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SYMBOLS KEY

MTP	Coated Microtiter Plate (12 x 8 wells)
STD	Standard
SPK STD	Spiking Standard Solution
STREP HRP	Streptavidin:HRP
BIOTIN AB	Biotinylated Antibody
20X SAMP EXTA	(20X) Sample Extraction A
2X SAMP EXTB	(2X) Sample Extraction B
5X SAMP EXTC	(5X) Sample Extraction C
20X SAMP DIL	(20X) Sample Dilution
20X WASH BUF	(20X) Wash Buffer
SUB TMB	TMB Substrate
SOLN STOP	Stop Solution
	Consult Instructions for Use
REF	Catalog Number
	Expiration Date
	Storage Temperature