KRIBIOLISA™ Tetracycline ELISA

REF : KRA1006

Ver 1.0

RUO

ELISA Set for Accurate Quantitation of Tetracycline

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 KRA1006 Σ 96 tests

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Introduction:

Tetracycline 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 Tetracycline drug.

Intended Use:

The KRIBIOLISA™ Tetracycline ELISA Kit for Accurate Quantitation of Tetracycline from the sample.

Principle:

KRIBIOLISA™ Tetracycline ELISA kit is based on indirect-competitive ELISA technology. The microtiter wells are coated with antigen. Tetracyclines residue in the sample competes with the antigen coated on the 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 the tetracyclines residue in it, after comparing with the Standard Curve, multiplied by the dilution factor, tetracyclines residue in the sample can be calculated.

Materials Provided:

1. Tetracycline antigen Coated Microtiter Plate (12 x 8 wells) – 1 no
2. Tetracycline Standards – 1.0 ml/vial (0, 0.2, 0.6, 1.8, 5.4ng/ml)
3. Spiking Standard Solution (1ug/ml) – 1 ml
4. Tetracycline:HRP Conjugate – 1 ml
5. Conjugate Diluent – 10 ml
6. Sample Diluent – 50ml
7. 20X Wash Buffer – 40 ml
8. TMB Substrate A – 7 ml
9. TMB Substrate B – 7 ml
10. Stop Solution – 7 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 25µl to 1000µl
3. Deionized (DI) water
4. Wash bottle or automated microplate washer
5. Graph paper or software for data analysis
6. Timer
7. Absorbent Paper

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 Tetracycline concentration (0.05- 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 **DI water.** Mix it well
2. Wash Buffer (1X): Add **500 µl** of **Wash Buffer (20X)** in **9500µl** of **DI water.** Mix it well
3. HRP Conjugate (1X): Dilute the concentrated HRP conjugate with Conjugate diluent in the volume of 1:10. Note: The mixture can't be stored. Please 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 **50µl** of **Standard** solution or **prepared sample** to corresponding wells.

3. Add **50µl** of **Tetracycline:HRP Conjugate** into each well.

4. Mix wells and cover the plate with a sealer and incubate for **30 min** at **25°C**.

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 **TMB Substrate A** followed by **50 µl TMB Substrate B** to each well and incubate for **15 min** at **25°C** in dark.

7. Stop reaction by adding **50µl** of **Stop Solution** to each well.

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

Calculation of Results:

(1) **Percentage absorbance:**

The mean values of the absorbance values obtained for the standards and the samples are divided by the absorbance value of the first standard (zero standard) and multiplied by 100%. The zero standard is thus made equal to 100% and the absorbance values are quoted in percentages.

$$\text{Absorbance (\%)} = \frac{B}{B0} \times 100\%$$

B ——absorbance standard or sample

B0 ——absorbance zero standard (0ng/ml)

(2) **Standard Curve** :

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 Tetracycline 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 Tetracycline 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.

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.

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.

Performance Characteristics of the kit:

Linear range: 0.2– 5.4ng/ml
Accuracy: 90±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 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.

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