KRIBIOLISA® Tetracycline ELISA

REF : KRA1006

Ver 2.0

RUO

Enzyme Immunoassay for the Quantitative determination of Tetracycline for tissue, honey and egg samples

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:

This KRIBIOLISA® Tetracycline ELISA Kit for Quantitative determination of Tetracycline for tissue, honey and egg samples.

Principle:

KRIBIOLISA® Tetracycline ELISA kit is based on competitive ELISA. The microtiter wells are coated with coupling antigen. Tetracycline 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 TMB Substrate is used to show the color. Absorbance of the sample is negatively related to the Tetracycline residue in it, after comparing with the Standard Curve, multiplied by the dilution factor, Tetracycline residue in the sample can be calculated.

Materials Provided:

1. Antigen coated Microplate (12 x 8 wells) - 1 no.
2. Standards (0 ppb, 0.1 ppb, 0.3 ppb, 0.9 ppb, 2.7 ppb, 8.1 ppb) - 1 ml / vial
3. Enzyme conjugate - 7 ml
4. Antibody Working Solution - 7 ml
5. (20X) Sample Extract A - 15 ml
6. (2X) Sample Extract B – 50 ml x 2 vials
7. (20X) Sample Diluent - 10 ml
8. (20X) Wash Buffer - 25 ml
9. TMB Substrate - 12 ml
10. Stop Solution - 12 ml
11. 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.

Solution preparation before sample pre-treatment:

1) Sample extract A
1 part of 20× sample extract A + 19 parts of deionized water

2) Sample extract B
1 part of 2× sample extract B + 1 part of deionized water

3) Sample extract C
1 part of 20× sample extract A + 3 parts of deionized water

4) 1M NaOH solution
Weigh 4g NaOH, add deionized water to 100ml

5) Sample diluent
1 part of 20× sample diluent + 19 parts of deionized water

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 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.
4. Enzyme Conjugate preparation- take 1 part (11X) Concentrated Enzyme Conjugate, add 10 parts Enzyme Conjugate Dilution, dilute at 1:10 **(Note: Please be sure to mix and use as immediately needed).**

Tissue (fish, beef, lamb, pork, horse meat, poultry), Egg, Honey:

1. Take 2± 0.05g of the homogenized Tissue/honey/Egg sample into 50 mL centrifuge tube, add 3mL diluted Sample extract A, shake for 3min.
2. Then add 600ul 1M NaOH solution and 2.4ml diluted Sample extract B, shake for 3min; centrifuge at above 4000 r/min at room temperature (20 - 25ºC) for 5 minutes.
3. Take 50 ul up-layer clear liquid, add 450ul diluted Sample diluent, mix it evenly;
4. Take 50ul above mixed solution for analysis

Fold of dilution of the sample: 40

Milk:

1. Take 1± 0.05mL of the milk sample into 2 mL centrifuge tube.
2. Add 50uL Sample extract C, vortex for 1min; centrifuge at above 4000 r/min at room temperature (20 - 25ºC) for 5 minutes.
3. Take 50ul up-layer clear liquid, add 950ul diluted Sample diluent, mix it evenly.
3. Take 50 ul for analysis.

Fold of dilution of the sample: 20

Milk Powder:

1. Take 1± 0.05g of the milk powder sample into 50 mL centrifuge tube, add 7mL deionized water, vortex for 3min.
2. Add 1mL above mixed liquid, add 50uL Sample extract C, vortex for 1min; centrifuge at above 4000 r/min at room temperature (20 - 25ºC) for 5 minutes.
3. Take 100ul up-layer clear liquid, add 900ul diluted Sample diluent, mix it evenly.
4. Take 50 ul 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 **enzyme conjugate working solution** in each well.

4. Add **50 ul** of **Antibody solution** 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 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.

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 the comparison the average OD value of the 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, while those of the standard solutions are as the followings: 2.243 for 0 ppb, 1.816 for 0.1 ppb, 1.415 for 0.3 ppb, 0.74 for 0.9 ppb, 0.313 for 2.7 ppb and 0.155 for 8.1 ppb, accordingly the concentration range of the sample I is 2.7 to 8.1ppb, and that of the sample II is 0.3 to 0.9ppb.

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 ng/ml) standard solution

2) Standard Curve:
Draw the standard curve with the absorption percentages of the standard solutions and the semilogarithmic values of the Tetracycline Standard solutions (ng/ml) 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 Tetracycline concentration in the sample.

Performance Characteristics:

Sensitivity: 0.1 ppb
Standard Curve range: 0.1 ppb – 8.1 ppb

Precision:

Intra-Assay precision: <5%
Inter-Assay precision: <15%
The best value of B_0 Absorbance: >0.8

Limit of Detection (LOD):

Tissue (fish, beef, lamb, pork, horse meat, poultry), Egg, Honey, Milk …. 4 ppb
Milk Powder ……10 ppb

Note: ppb=ng/ml or ng/g

Recovery Rate:

Tissue, Egg, Milk, Milk powder ..... 70%～120%
Honey ..... 70%～130%

Cross – reactivity:

Doxycycline 100%
Tetracycline 150%
Minocycline 92%
Chlortetracycline..... 25%
Demeclocycline 28%
Oxytetracyline 18%

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
ENZY CONJ	Enzyme Conjugate
ANT SOLN	Antibody Working Solution
20x SAMP EXTA	(20X) Sample Extract A
2x SAMP EXTB	(2X) Sample Extract B
20x SAMP DIL	(20X) Sample Diluent
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