QUALICHEK™ Chlorotetracycline ELISA

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RUO

Quantitative testing of Chlorotetracycline in samples, such as tissue, honey, urine, etc.

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:

Chlortetracycline (trade name Aureomycin, Lederle Laboratories) is a tetracycline antibiotic, the first tetracycline to be identified. In veterinary medicine, chlortetracycline is commonly used to treat conjunctivitis in cats,[3] dogs and horses. It is also used to treat infected wounds in cattle, sheep and pigs, and respiratory tract infections in calves, pigs and chickens.

Intended Use:

This kit is for quantitative testing for Chlorotetracycline in sample, such as tissue, honey, urine, etc.

Principle:

This kit uses Indirect-Competitive-ELISA as the method. The micro-plate provided in this kit has been pre-coated with coupled antigen. When samples are added into the ELISA Microtiter plate wells, the residual chlorotetracycline in the samples will compete with coupled antigen on the Microplate for the anti-chlorotetracycline antibody. Then Horseradish Peroxidase (HRP) conjugate is added to each micro plate well, and TMB substrate is added for color development. There is a negative correlation between the OD value of samples and the concentration of chlorotetracycline. The concentration of chlorotetracycline in the samples can be calculated by comparing the OD of the samples to the standard curve.

Materials Provided:

1. Coated Microtiter plate - 1x 96 wells
2. Standards Liquid -1mL each (0 ppb, 0.1 ppb, 0.3 ppb, 0.9 ppb, 2.7 ppb, 8.1 ppb)
3. High Concentrated Standard (1 ppm) - 1mL
4. HRP Conjugate - 11 mL
5. Antibody Working Solution - 5.5 mL
6. Substrate A - 6 mL
7. Substrate B - 6 mL
8. Stop Solution - 6 mL
9. Wash Buffer (20X) - 40 mL
10. Plate Sealer - 3 pieces

Material required but not provided:

Instrument: Microtiter plate reader, Printer, Homogenizer, Nitrogen Evaporators/Water bath, Oscillators, centrifuge, Graduated pipette, Balance (sensibility 0.01 g)

High-precision transferpettor:single channel (20-200 µL, 100-1000 µL), Multichannel (300 µL) micropipettes.

Reagents: Methanol, Trichloroacetic acid.

Storage Information:

1. Store main kit components at 2-8°C Avoid freeze / thaw cycles.
2. Before using, bring all components to room temperature (18-25°C). Upon assay completion return all components to appropriate storage conditions.

Sample pretreatment:

Bring all reagents and samples to room temperature before use.

Open the microplate reader in advance, preheat the instrument, and set the testing parameters.

1. Sample pretreatment Notice:

Experimental apparatus should be clean, and the pipette should be disposable to avoid cross-contamination during the experiment.

2. Solution preparation:

Solution 1: 1% Trichloroacetic Acid Solution: Dissolve 1 g of trichloroacetic acid with 100 mL of deionized water**.**
Solution 2: Reconstitution Buffer: Dilute the 5×Reconstitution Buffer with deionized water. (1×Reconstitution Buffer (V): Deionized water (V)=1:4) .The Reconstitution buffer can be store at 4℃ for a month.
Solution 3: Wash Buffer: Dilute 20×Concentrated Wash Buffer with deionized water. (20×Concentrated Wash Buffer (V): Deionized water (V) = 1:19).

3. Sample pretreatment procedure:

Pretreatment of tissue (chicken, duck, porcine meat/liver, shrimp, fish) or eggs samples:
1. Weigh 2±0.05 g of homogenate samples into EP tube. Then add 4 mL of 1% Trichloroacetic acid solution to EP tube. Oscillate strongly for 2 min, centrifuge at a speed over 4000 r/min for 10 min at room temperature
2. Take 250 µL of the supernatant to another tube, then add 750 µL of Reconstitution buffer to dissolve it.
3. Take 50 µL for analysis

Note: Sample dilution factor: 8, Detection limited: 0.8 ppb

Pretreatment of honey samples:
1. Weigh 1±0.05 g of honey samples into a EP tube. Then add 2 mL of 1% Trichloroacetic acid solution. Oscillate strongly for 2 min, centrifuge at a speed over 4000 r/min for 10 min at room temperature.
2. Take 100 µL of the supernatant to another tube. Add 1900 µL of Reconstitution buffer. Mix for 30 sec.
3. Take 50 µL for analysis.

Note: Sample dilution factor: 40, Detection limited: 4 ppb

Pretreatment of urine samples:
1. Take urine samples centrifuge at a speed over 4000 r/min for 10 min at room temperature.
2. Dilute clear urine samples with Reconstitution buffer for 10 times. (urine: Reconstitution Buffer (V) = 1:9).
3. Take 50 µL for analysis.

Note: Sample dilution factor: 10, Detection limited: 1 ppb

Assay procedure:

Centrifuge the sample again after thawing before the assay. Bring all reagents to room temperature for 30 min before use. **All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming.** Before the experiment to be prepared standard solution. As low concentration of the standard solution is instable, it need be use right after it was ready. Then make serial dilution as follows:
1. Take 3 mL of Reconstitution buffer into **0 ppb bottle**. Take 2 mL of Reconstitution buffer **into 0.1 ppb bottle, 0.3 ppb bottle, 0.9 ppb bottle, 2.7 ppb bottle** respectively. Take 3 mL of Reconstitution buffer into **8.1 ppb bottle.**
2. **Standard Solution 6:** Take 24.3 µL of 1.0 ppm high concentration standard into 8.1 ppb bottle, then mix fully. The concentration of Standard Solution 6 is 8.1 ppb.
3. **Standard Solution 5**: Take 1 mL of Standard Solution 6 into 2.7 ppb bottle, then mix fully. The concentration of Standard Solution 5 is 2.7 ppb.
4. **Standard Solution 4**: Take 1 mL of Standard Solution 5 into 0.9 ppb bottle, then mix fully. The concentration of Standard Solution 4 is 0.9 ppb.
5. **Standard Solution 3:** Take 1 mL of Standard Solution 4 into 0.3 ppb bottle, then mix fully. The concentration of Standard Solution 3 is 0.3 ppb.

6. **Standard Solution 2:** Take 1 mL of Standard Solution 3 into 0.3 ppb bottle, then mix fully. The concentration of Standard Solution 2 is 0.1 ppb.
7. **Standard Solution 1**: Reconstitution buffer is as Standard Solution 1. The concentration of Standard Solution 1 is 0ppb.

1. **Number:** number the sample and standard in order (multiple well), and keep a record of standard wells and sample wells.
2. **Add sample:** add 50 µL of Standard or Sample per well, then add 50 µL of antibody working solution, cover the plate with sealer, oscillate for 5 s gently to mix thoroughly, incubate for30min at 37℃ in the dark.
3. **Wash:** uncover the sealer carefully; remove the liquid in each well. Immediately add 300 µL of wash buffer to each well and wash. Repeat wash procedure for 5 times, 30 s intervals/time. Invert the plate and pat it against thick clean absorbent paper (If bubbles exist in the wells, clean tips can be used to prick them).
4. **HRP Conjugate**: add 100µl of HRP conjugate to each well, incubate shading light for 30 min at 37^0C in the dark.
5. **Wash**: repeat step 3.
6. **Color Development:** add 50 µL of Substrate Reagent A to each well, and then add 50 µL of Substrate Reagent B. Gently oscillate for 5 s to mix thoroughly. Incubate shading light for 15 min at 37℃ (The reaction time may be shortened or prolonged according to the depth of the color).
7. **Stop reaction:** add 50 µL of stop solution to each well, oscillate gently to mix thoroughly.
8. **OD Measurement:** determine the optical density (OD value) of each well at 450 nm with a microplate reader (the 450/630 nm double wavelength is recommended). This step should be finished in 10 min after stop reaction.

Judgment of result:
Absorbance (%) =A/A0×100%

A: Average absorbance of standard or sample

A_0: Average absorbance of 0 ppb Standard

Drawing and calculation of standard curve

Create a standard curve by plotting the absorbance percentage of each standard on the y-axis against the log concentration on the x-axis to draw a semi-logarithmic plot. Add average absorbance value of sample to standard curve to get corresponding concentration. If samples have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor.

For this kit, it is more convenient to use professional analysis software for accurate and fast analysis on a large number of samples.

Notes:

1. The overall OD value will be lower when reagents have not been brought to room temperature before use or room temperature is below 25℃.
2. If the wells turn dry during the washing procedure, it will lead to bad linear standard curve and poor repeatability. Operate the next step immediately after wash.
3. Mix thoroughly and wash the plate completely. The consistency of wash procedure can strongly affect the reproducibility of this ELISA kit.
4. ELISA Microtiter plate should be covered by plate sealer. Avoid the reagents to strong light.
5. Do not use expired kit or reagents of different batches.
6. TMB should be abandoned if it turns color. When OD value of standard (concentration: 0)＜0.5 unit (A450nm＜0.5), it indicates the reagent may be deteriorated.

7. Stop solution is caustic; avoid contact with skin and eyes.

Technical specification:

Sensitivity: 0.1 ppb (ng/mL)
Reaction mode: 37 ℃, 30 min~30 min~15 min.
Detection limit: Tissue, liver, eggs ---0.8 ppb, Honey ---4 ppb, Urine ---1 ppb.
Reactivity: Chlorotetracycline ---100%, Tetracycline ---29%,
Terramycin ---15%, Doxycycline ---2.5%
Sample recovery rate: Tissue (chicken, duck, porcine meat/liver, shrimp, fish) ---90%±20%,
Honey ---75%±20%, Urine ---80±20%

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