8th Edition, revised in February, 2018

CTC (Chlorotetracycline) ELISA Kit

Catalog No: E-FS-E024

96T

This manual must be read attentively and completely before using this product.

If you have any problems, please contact our Technical Service Center for help.

Phone: 240-252-7368(USA) Fax: 240-252-7376(USA)
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

Test principle

This kit uses 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.

Technical indicator

Sensitivity: 0.1 ppb

Reaction model: 37 ℃, 30 min~30 min~15 min.

Detection limit: Tissue, eggs ---0.8 ppb, Honey ---4 ppb, Urine ---1 ppb

Cross-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%

Kits components

Item	Specifications
ELISA Microtiter plate	96 wells
Standard Liquid (empty bottle)	0 ppb, 0.1 ppb, 0.3 ppb, 0.9 ppb, 2.7 ppb, 8.1 ppb
High Concentration Standard (1 ppm)	1 mL
HRP Conjugate	11 mL
Antibody Working Solution	5.5 mL
Substrate Reagent A	6 mL
Substrate Reagent B	6 mL
Stop Solution	6 mL
20×Concentrated Wash Buffer	40 mL
5×Reconstitution Buffer	50 mL
Plate Sealer	3 pieces
Sealed Bag	1 piece
Manual	1 copy

Note: All reagent bottle caps must be tightened to prevent evaporation and microbial pollution.

Other supplies required

Instruments: Microplate reader, Printer, Homogenizer, Centrifuge, Graduated pipette, Balance (sensibility 0.01 g).

High-precision pipettor: Single-channel (20-200 μL, 100-1000 μL), Multi-channel (30~300 μL).

Reagents: Methanol, Trichloroacetic acid.

Experimental preparation

Bring all reagents and samples to room temperature before use.

Open the micro-plate 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

3.1 Pretreatment of tissue (chicken, duck, porcine, 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

3.2 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

3.3 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 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. **Standard and Samples need test in duplicate**.
1. **Add sample:** Add 50 μL of **Standard or Sample** per well, then add 50 μL **antibody working solution**, cover the plate with sealer. Oscillate for 5 sec gently to mix thoroughly. Incubate for 30 min at 37℃in the dark.
2. **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 sec 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).
3. **HRP Conjugate:** Add 100 μL of **HRP conjugate** to each well. Incubate for 30 min at 37℃ in the dark.
4. **Wash:** Repeat step 3.
5. **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 sec to mix thoroughly. Incubate for 15 min at 37℃ in the dark.
6. **Stop reaction:** Add 50 μL of **stop solution** to each well, gently oscillate for 5 sec.
7. **OD Measurement:** Determine the optical density (OD value) of each well at 450 nm with a micro-plate reader (the 450/630 nm double wavelength is recommended). This step should be finished in 10 min after stop reaction.

Result analysis

1. **Absorbance (%) =A/A_0 ×100%**

   A: Average absorbance of standard or sample

   A_0: Average absorbance of 0ppb Standard

2. **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 of batch samples.

Notes

1. Overall OD value will be lower when reagents is not brought to room temperature before use or room temperature is below 25℃.
2. During the washing procedure, if the wells turn dry, it will lead to bad linear standard curve and poor repeatability, move on to 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 and reagents of different batches.
6. TMB (Substrate Reagent A or Substrate Reagent B) should be abandoned if it turns blue color. When OD value of standard(concentration: 0)＜0.5 unit(A_{450nm}＜0.5), it indicates reagent is deteriorated.
7. Stop solution is caustic, avoid contact with skin and eyes.
8. As the OD values of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique, washing technique or temperature effects), the operator should establish a standard curve for each test.
9. Even the same operator might get different results in two separate experiments. In order to get reproducible results, the operation of every step in the assay should be controlled.
10. If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.
11. The kit is used for rapid screening of actual samples. If the test result is positive, the instrument method such as HPLC, LC/MS, etc. can be used for quantitative confirmation.

Storage and valid period

Store at 2~8℃ for 1 year. Avoid freeze.

Please store the opened kit at 2~8℃, protect from light and moisture. The valid period is 2 months.

Expiry date: expiration date is on the packing box.