TCs (Tetracyclines) ELISA Kit

Catalog No: E-FS-E041

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

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Test principle

This kit uses Indirect-Competitive-ELISA as the method. It can detect Tetracyclines (TCs) in samples, such as tissues, honey, eggs, urine and other samples. This kit is composed of ELISA Microtiter plate pre-coated with coupled antigen, HRP conjugate, antibody, standard and other supplementary reagents. During the detection, after adding standard or sample solution, TCs in the samples competes with pre-coated coupled antigen on the ELISA Microtiter plate for anti-CAP antibody. Then Horseradish Peroxidase (HRP) conjugate is added to each well, and TMB substrate is added for color development. There is a negative correlation between the OD value of samples and the concentration of TCs. The concentration of TCs in the samples can be calculated by comparing the OD of the samples to the standard curve.

Technical indicator

Sensitivity: 0.3 ppb (ng/mL)

Reaction mode: 37℃, 30 min~30 min~ 15min

Detection limit: Tissue/Liver/Egg---2.4 ppb, Honey---12 ppb, Urine---3 ppb.

Cross-reactivity: Tetracycline---100%, Chlortetracycline ---340%, Terramycin ---51%, Deoxytetracycline ---8.5%.

Sample recovery rate: Tissue/Liver/Eggs---85%±20%, Honey---75%±20%, Urine---80%±20%.

Kits components

Item	Specifications
ELISA Microtiter plate	96 wells
High Concentrated Standard (1.0 ppm)	1 mL
Standard (empty bottle)	(0 ppb, 0.3 ppb, 0.9 ppb, 2.7 ppb, 8.1 ppb, 24.3 ppb)
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, Oscillators, Centrifuge, Graduated pipette, Balance (sensibility 0.01 g).

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

Reagents: N, N-Dimethylformamide (DMF)

Experimental preparation

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: Reconstitution Buffer

Dilute the 5×Reconstitution Buffer with deionized water. (5×Reconstitution Buffer : deionized water=1:4) .The Reconstitution buffer can be store at 4℃ for a month.

Solution 2: Wash Buffer

Dilute the 20×Concentrated Wash Buffer with deionized water. (20×Concentrated Wash Buffer: Deionized water=1:19)

3. Sample pretreatment procedure

3.1 Pretreatment of tissue, liver, egg:

(1) Homogenize tissue, liver and egg samples with homogenizer.

(2) Accurately weigh 2±0.05 g of homogenate sample into a 50 mL centrifuge tube, add 4 mL of DMF. Oscillate for 2 min, centrifuge at 4000 rpm for 10 min at room temperature.

(3) Take 250 μL of the supernatant to another centrifuge tube, add 750 μL of Reconstitution buffer and mix fully.

(4) Take 50 μL for analysis.

Note: Sample dilution factor: 8, minimum detection dose: 2.4 ppb.

3.2 Pretreatment of honey

(1) Accurately weigh 1±0.05 g of honey into the centrifuge tube, add 2 mL of DMF. Oscillate for 2 min, centrifuge at 4000 rpm for 10 min at room temperature.

(2) Take100 μL of the supernatant to another centrifuge tube, add 1900 μL of Reconstitution buffer and mix fully for 30 sec.

(3) Take 50 μL for analysis.

Note: Sample dilution factor: 40, minimum detection dose: 12 ppb.

3.3 Pretreatment of urine

(1) Dilute the urine sample with Reconstitution buffer for 10 times (if the urine sample is muddy, it must be filtered or centrifuged at 4000 rpm for 10 min). Samples can be stored at -20℃ if not detected in the short time.

(2) Take 50 μL for analysis.

Note: Sample dilution factor: 10, minimum detection dose: 3 ppb.

Assay procedure

Restore all reagents and samples to room temperature before use. All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming.

(1) **Prepare the standard working solution. Standard solution of low concentration is unstable, prepare fresh solution before use.** The recommended process is as follows:

Add 3 mL of Reconstitution buffer into 0 ppb standard tube, 2 mL of Reconstitution buffer for 0.3 ppb, 0.9 ppb, 2.7 ppb, and 8.1 ppb standard tube, and 2.93mL of Reconstitution buffer for 24.3 ppb standard tube.

Standard solution 6: Take 73 μL of high concentrated Standard (1.0 ppm) into 2.93 mL of Reconstitution buffer and mix fully. The concentration of Standard Solution 6 is 24.3 ppb.

Standard solution 5: Take 1 mL of standard solution 6 into 2 mL of Reconstitution buffer and mix fully. The concentration of Standard Solution 5 is 8.1 ppb.

Standard solution 4: Take 1 mL of standard solution 5 into 2 mL of Reconstitution buffer and mix fully. The concentration of Standard Solution 4 is 2.7 ppb.

Standard solution 3: Take 1 mL of standard solution 4 into 2 mL of Reconstitution buffer and mix fully. The concentration of Standard Solution 3 is 0.9 ppb.

Standard solution 2: Take 1 mL of standard solution 3 into 2 mL of Reconstitution buffer and mix fully. The concentration of Standard Solution 2 is 0.3 ppb.

Standard solution 1: Use the Reconstitution buffer directly. The concentration of Standard Solution 1 is 0 ppb.

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.**
2. **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 s gently to mix thoroughly, incubate at 37℃ for 30 min with shading light.
3. **Wash:** uncover the sealer carefully, remove the liquid of each well. Immediately add 300 μL of **wash buffer** to each well and immerse for 30 s each time. 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 100 μL of **HRP conjugate** to each well. Cover the ELISA plate with sealer and incubate for 30 min at 37℃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 at 37℃ for 15 min with shading light (The reaction time can be extended according to the actual color change).
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.

Result analysis

1. **Absorbance% =A/A_0×100%**

   A: Average absorbance of standard or sample

   A_0: Average absorbance of 0 ppb 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 the average absorbance value 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. 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. Micro ELISA plate should be covered by plate sealer. Avoid the reagents to strong light.
5. **Do not use expired kit, reagents of different batches and reagents that do not belong to this kit**.
6. TMB should be abandoned if it turns color. When OD value of standard (concentration: 0)＜0.5 unit($A_{450\ nm}$＜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.

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.

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