Elabscience®

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TCs (Tetracycline) ELISA Kit

Catalog No: E-FS-E046

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) 240-252-7376(USA)
Email: techsupport@elabscience.com
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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 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 Micro ELISA 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 Micro ELISA Plate for TCs 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 TCs. The residual quantity of TCs in the samples can be calculated by comparing the OD of the samples to the standard curve.

Technical indicator

Sensitivity: 0.05 ppb (ng/mL)

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

Detection limit: Tissue/Liver/Egg---0.3 ppb, Honey---2 ppb, Urine---0.5 ppb.

Cross-reactivity: Tetracyclines---100%, Terramycin ---107%,
Chlortetracycline ---16.7%,Deoxytetracycline ---4.2%.

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

Kits components

Item	Specifications
Micro ELISA Plate	96 wells
High Concentrated Standard (1.0 ppm)	1 mL
Standard Liquid (black cap)	1 mL each (0 ppb,0.05 ppb,0.15 ppb, 0.045 ppb, 1.35 ppb, 4.05 ppb)
HRP Conjugate (red cap)	11 mL
Antibody Working Solution (blue cap)	5.5 mL
Substrate Reagent A (white cap)	6 mL
Substrate Reagent B (black cap)	6 mL
Stop Solution (yellow cap)	6 mL
20×Concentrated Wash Buffer (white cap)	40 mL
5×Reconstitution Buffer (yellow cap)	50 mL
Plate Sealer	3 pieces
Sealed Bag	1 piece
Manual	1 copy

Other supplies required

Instruments: Micro-plate reader, Printer, Homogenizer, Nitrogen Evaporators, 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: Trichloroacetic acid, Methanol.

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

Solution 3: 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) Accurate weigh 2±0.05 g of crushed homogenate into the a 50 mL centrifuge tube, add 6 mL of 1% trichloroacetic acid. Oscillate for 2 min, centrifuge at a speed of over 4000 r/min for 10 min at room temperature.

(3) Take 1 mL of the supernatant to another centrifuge tube, add 1 mL of methanol and oscillate for 1 min, centrifuge at a speed of over 4000 r/min for 10 min at room temperature.

(4) Take 1 mL of the upper liquid and dry at 50-60℃ with nitrogen or air. Add 1 mL of Reconstitution buffer to dissolve the remaining dry material.

(5) Take 50 μL for analysis.

Note: Sample dilution factor: 6, minimum detection dose: 0.3 ppb.

3.2 Pretreatment of honey

(1) Accurate weigh1±0.05 g of honey into the centrifuge tube, add 2 mL of 1% trichloroacetic acid. Oscillate for 2 min, centrifuge at a speed of over 4000 r/min for 10 min at room temperature.

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

(3) Take 50 μL for analysis.

Note: Sample dilution factor: 40, minimum detection dose: 2 ppb.

3.3 Pretreatment of urine sample

(1) Dilute the urine sample with Reconstitution buffer for 10 times (if the urine sample is muddy, it should be filtered or centrifuged at 4000 r/min for 10 min until the urine sample become clear). Samples temporarily not used should be frozen.

(2) Take 50 μL for analysis.

Note: Sample dilution factor: 10, minimum detection dose: 0.5 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.

(1) **Prepare the standard working solution. Standard solution of low concentration is unstable, prepare fresh solution before use.**

Add 2 mL of Reconstitution buffer into 0 ppb standard tube, 2 mL of Reconstitution buffer for 0.05 ppb, 0.15 ppb, 0.45 ppb, and 1.35 ppb standard tube, and 3mL of Reconstitution buffer for 4.05 ppb standard tube.

Standard solution 6: Take 12 μL of high concentrated Standard (1.0 ppm) into 3 mL of Reconstitution buffer, cover tightly and mix fully, then the concentration will be 4.05 ppb.

Standard solution 5: Take 1 mL of standard solution 6 into 2 mL of Reconstitution buffer, cover tightly and mix fully, then the concentration will be 1.35 ppb.

Standard solution 4: Take 1 mL of standard solution 5 into 2 mL of Reconstitution buffer, cover tightly and mix fully, then the concentration will be 0.45 ppb.

Standard solution 3: Take 1 mL of standard solution 4 into 2 mL of Reconstitution buffer, cover tightly and mix fully, then the concentration will be 0.15 ppb.

Standard solution 2: Take 1 mL of standard solution 3 into 2 mL of Reconstitution buffer, cover tightly and mix fully, then the concentration will be 0.05 ppb.

Standard solution 1: Use Reconstitution buffer directly, then the concentration will be 0 ppb.

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 antibody working solution, cover the plate with sealer we provided, oscillate for 5 sec gently to mix thoroughly, shading light incubation for 30 min at 37℃.

3. **Wash:** uncover the sealer carefully, remove the liquid in each well. Immediately add 250 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).
4. **HRP Conjugate:** add 100 μL of HRP conjugate to each well, incubate for 30 min at 37℃in the dark.
5. **Wash:** repeat step 3.
6. **Color Development:** add 50 μL of substrate solution A to each well, and then add 50 μL of substrate solution B. Gently oscillate for 5 sec to mix thoroughly. Incubate shading light for 15 min at 37℃.
7. **Stop Reaction:** add 50 μL of stop solution to each well, gently oscillate and mix fully to stop the reaction.
8. **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 solution or sample

   A_0: Average absorbance of 0 ppb Standard solution

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. The overall OD values will be lower when reagents have not been brought to room temperature before use or the 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. Micro ELISA 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 should be abandoned if it turns color. When OD value of standard(concentration: 0)＜0.5 unit(A_{450nm}＜0.5), it indicates the reagent may be deteriorated.
7. Stop solution is caustic, avoid contact with skin and eyes.

Storage and valid period

Storage: Store at 2-8℃. Avoid freeze / thaw cycles.

Valid Period: 1 year, production date is on the packing box.