OTC (Oxytetracycline) ELISA Kit

Catalog No: E-FS-E167

96T/96T*3

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.

Toll-free: 1-888-852-8623 Tel: 1-832-243-6086 Fax: 1-832-243-6017

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 for the quantitative detection. It can detect Oxytetracycline (OTC) in samples, such as muscle, milk, egg, feed etc. This kit is composed of ELISA Microtiter plate, HRP conjugate, antibody working solution, standard and other supplementary reagents. The microtiter plate in this kit has been pre-coated with coupled antigen. During the reaction, OTC in the samples or standard competes with coupled antigen on the solid phase supporter for sites of anti-OTC antibody, an antigen-antibody- HRP Conjugate compound is formed, and substrate reagent is added for color development. There is a negative correlation between the OD value of samples and the concentration of OTC. The concentration of OTC in the samples can be calculated by comparing the OD of the samples to the standard curve.

Technical indicator

Reaction mode (Incubation time and temperature)**:** 37℃; 30 min, 15min.

Detection limit: Muscle, Milk, Egg---6 ppb; Feed---400 ppb.

Cross-reactivity: Oxytetracycline---100%; Tetracycline---206%; Chlortetracycline ---300%; Deoxytetracycline ---18.5%.

Sample recovery rate: 90%±30%.

Kits components

Item	Specifications
ELISA Microtiter plate	96 wells
Standard Liquid	1.5 mL each (ppb=ng/mL=ng/g) (0 ppb, 0.3 ppb, 0.9 ppb, 2.7 ppb, 8.1 ppb, 24.3 ppb)
HRP Conjugate	7 mL
Antibody Working Solution	7 mL
Substrate Reagent A	7 mL
Substrate Reagent B	7 mL
Stop Solution	7 mL
20×Concentrated Wash Buffer	25 mL
20×Sample Extraction 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 materials required but not supplied

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

Micropipette: Single channel (20-200 μL, 100-1000 μL), Multichannel (30-300 μL).

Reagents: $Na_2[Fe(CN)_5NO] \cdot 2H_2O$, $ZnSO_4 \cdot 7H_2O$, Trichloroacetic acid.

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. FOR RESEARCH USE ONLY. ELISA Microtiter plate should be covered by plate sealer. Avoid th e kit to strong light.
5. **Each reagent is optimized for use in the E-FS-E167. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other E-FS-E167 with different lot numbers.**
6. Substrate Reagent should be abandoned if it turns blue 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.
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. **For mentioned sample fast and efficient extraction methods are included in the kit descriptio n. Please consult technical support for the applicability if other sample need to be tested.**
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 expiry date

Store the kit at 2-8℃. Do not freeze any test kit components.

Return any unused microwells to their original foil bag and reseal them together with the desiccant provided and further store at 2-8℃.

Expiry date: expiration date is on the packing box.

Experimental preparation

Restore 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**

   Please prepare solution according to the number of samples. Don't use up all components in the kit at once!

   Solution 1: 0.36M $Na_2[Fe(CN)_5NO] \cdot 2H_2O$

   Weigh 0.54 g of $Na_4Fe(CN)_6 \cdot 2H_2O$ and add 4.7 mL of deionized water, vortex thoroughly to dissolve completely (for raw milk method).

   Solution 2: 1M $ZnSO_4 \cdot 7H_2O$

   Weigh 1.44g of $ZnSO_4 \cdot 7H_2O$ and dd 4.32 mL of deionized water, vortex thoroughly to dissolve completely (for raw milk method).

   Solution 3: 1% Trichloroacetic acid

   Weigh 1 g of trichloroacetic acid, add 100 mL of deionized water, and vortex thoroughly to dissolve completely.

   Solution 4: Wash Buffer

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

   Solution 5: Sample Extraction Buffer

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

3. **Sample pretreatment procedure**

3.1 Pretreatment of muscle (livestock) sample:

(1) Weigh 1±0.05 g of homogenate sample into the 50 mL centrifuge tube, add 9 mL of Solution 5. Vortex for 1 min, the sample is fully mixed with the liquid. Centrifuge at 4000 g for 10 min at room temperature.

(2) Take 50 μL for analysis.

Note: Sample dilution factor: 10, detection limit: 6 ppb.

3.2 Pretreatment of milk sample:

(1) Take 3mL of milk into the 5 mL centrifuge tube, centrifuge at 3000 g for 10 min at 4-10℃.

(2) Discard the upper layer of fat and take 2mL of the lower layer of skim milk into a new 5 mL centrifuge tube.

(3) Add 50 μL of Solution 1, Vortex for 90s, then add 50 μL of Solution 2, Vortex for 1min, centrifuge at 3000 g for 10 min at room temperature (25±2℃).

(3) Take 50 μL of the supernatant to another 2 mL centrifuge tube, add 450 μL of deionized water, mix

fully for 10 s.

(4) Take 50 μL for analysis.

Note: Sample dilution factor: 10, detection limit: 6 ppb.

3.3 Pretreatment of egg sample:

(1) Weigh 1±0.05 g of homogenate egg sample into the 50 mL centrifuge tube, add 5 mL of deionized water. Vortex for 1 min, the sample is fully mixed with the liquid. Centrifuge at 4000 g for 10 min at room temperature.

(2) Transfer 1 mL of the supernatant to a new centrifuge tube, add 1 mL of solution 5 , and vortex thoroughly for 30 s, centrifuge at 4000 g for 5 min at room temperature.

(3) Take 50 μL for analysis.

Note: Sample dilution factor: 12, detection limit: 6 ppb.

3.4 Pretreatment of feed sample:

(1) Weigh 1±0.05 g of feed sample into the 50 mL centrifuge tube, add 5 mL of Solution 3. Vortex for 2 min, until the sample is fully dispersion in the liquid. Centrifuge at 4000 g for 10 min at room temperature.

(2) Take 40 μL of the supernatant to a new centrifuge tube, add 1560 μL of solution 5, and vortex thoroughly for 30 s.

(3) Take 50 μL for analysis.

Note: Sample dilution factor: 200, detection limit: 400 ppb.

4. Assay procedure

Restore all reagents and samples to room temperature (25℃) before use. All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming. The unused ELISA Microtiter plate should be sealed as soon as possible and stored at 2-8℃.

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 **HRP Conjugate and Antibody Working Solution** to each well, cover the plate with plate sealer, oscillate for 10 s gently to mix thoroughly, incubate at 25±2℃ for 30 min in shading light.
3. **Wash:** uncover the sealer carefully, remove the liquid of each well. Immediately add 260 μL of **Wash Buffer** (Solution 4) to each well and wash. Repeat wash procedure for 4 times, 15-30 s intervals/time. Invert the plate and pat it against thick clean with absorbent paper.
4. **Color Development:** add 100 μL of **Substrate Reagent A and Substrate Reagent B mixture** to each well, incubate at 25±2℃ for 15-20 min in shading light

   Note: Substrate A solution and substrate B solution were mixed 1:1 by volume. The mixture must be thoroughly mixed, and the mixture should be used within 5 min. Avoid using metal packaging or stir the reagent!
5. **Stop Reaction:** add 50 μL of **Stop Solution** to each well, oscillate gently to mix thoroughly.

6. **OD Measurement:** determine the optical density (OD value) of each well at 450 nm (reference wavelength 630 nm) with a microplate reader. This step should be finished in 5 min after stop reaction.

5. 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 form for accurate and fast analysis on a large number of samples.

Oxytetracycline (E-FS-E167) **Standard Curve**