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## **OTC (Oxytetracycline) ELISA Kit**

Catalog No: E-FS-E112

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](mailto:techsupport@elabscience.com)

Website: [www.elabscience.com](http://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, raw milk, 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. Then Horseradish Peroxidase (HRP) conjugate is added to each microtiter plate well, 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): 25°C; 30 min, 15 min.

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

**Cross-reactivity:** Oxytetracycline ---100%; Chlortetracycline --->100%; Tetracycline--->100%;  
Doxycycline --- 18.5%

**Sample recovery rate:** 90% ± 30%.

## Kits components

Item	Specifications
ELISA Microtiter plate	96 wells
Standard Liquid	1 mL each (ppb=ng/mL=ng/g) (0 ppb, 0.3 ppb, 0.9 ppb, 2.7 ppb, 8.1 ppb, 24.3 ppb)
Antibody Working Solution	7 mL
HRP Conjugate	7 mL
20×Concentrated Sample Diluent	50 mL
Substrate Reagent A	6 mL
Substrate Reagent B	6 mL
Stop Solution	6 mL
20×Concentrated Wash Buffer	25 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.

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**Other materials required but not supplied**

**Instrument:** Microplate reader, Homogenizer, Vortex mixer, Centrifuge, Balance (sensitivity 0.01 g).

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

**Reagents:** Trichloroacetic acid, Na<sub>2</sub>Fe (CN)<sub>5</sub>NO•2H<sub>2</sub>O, ZnSO<sub>4</sub>•7H<sub>2</sub>O.

**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 °C.
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 kit to strong light.
5. **Each reagent is optimized for use in the E-FS-E112. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other E-FS-E112 with different lot numbers.**
6. Substrate Reagent should be abandoned if it turns blue color. When OD value of standard (concentration: 0) < 0.8 unit (A450nm < 0.8), 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 description. 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 at 2-8 °C. Avoid freeze.

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

**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: 0.36 M  $\text{Na}_2\text{Fe}(\text{CN})_5\text{NO}\cdot 2\text{H}_2\text{O}$  Solution (*for raw milk sample*)

Weigh 0.54 g of  **$\text{Na}_2\text{Fe}(\text{CN})_5\text{NO}\cdot 2\text{H}_2\text{O}$**  and add 4.7 mL of deionized water, vortex until dissolved fully.

*Prepare the fresh solution before use!*

Solution 2: 1 M  $\text{ZnSO}_4$  Solution (*for raw milk sample*)

Weigh 1.44 g of  **$\text{ZnSO}_4$**  and add 4.32 mL of deionized water, vortex until dissolved fully.

Solution 3: 1% Trichloroacetic acid Solution (*for feed sample*)

Dissolve 1 g of **Trichloroacetic acid** to 100 mL with deionized water, mix fully.

Solution 4: Sample Diluent (*for livestock, egg, feed sample*)

Dilute **20×Concentrated Sample Diluent** with deionized water. (20×Concentrated Sample Diluent (V): Deionized water (V) = 1:19). Mix fully.

Solution 5: Wash Buffer

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

### 3. Sample pretreatment procedure

#### 3.1 Pretreatment of muscle (livestock) sample:

- (1) Remove fat from sample, homogenize the sample with homogenizer.
- (2) Weigh  $1 \pm 0.05$  g of homogenate muscle into the 50 mL centrifuge tube.
- (3) Add 9 mL of **Sample Diluent** (Solution 4), mix fully.
- (4) Vortex for 1 min, centrifuge at 4000 r/min for 10 min at room temperature.
- (5) Take 50  $\mu\text{L}$  of supernatant for analysis.

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

**3.2 Pretreatment of raw milk sample:**

- (1) Take 3 mL of homogenate fresh sample into a 5 mL centrifuge tube.
- (2) Centrifuge for 10 min at 4000 r/min at 4-10°C. If a refrigerated centrifuge is not available, chill sample to approx 10°C prior to centrifugation.
- (3) Remove the upper fat layer, take 2 mL of skim milk of lower layer to another 5 mL centrifuge tube.
- (4) Add 50 µL of **0.36 M Na<sub>2</sub>Fe (CN)<sub>5</sub>NO•2H<sub>2</sub>O Solution** (Solution 1), vortex for 90 s. Add 50 µL of **1 M ZnSO<sub>4</sub> Solution** (Solution 2), vortex for 1 min.
- (5) Centrifuge at 3000 r/min for 10 min at room temperature.
- (6) Take 50 µL of the supernatant to another 2 mL centrifuge tube, add 450 µL of deionized water. Vortex for 30 s.
- (7) Take 50 µL for analysis.

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

**3.3 Pretreatment of egg sample:**

- (1) Weigh  $1 \pm 0.05$  g of homogenate egg into the 50 mL centrifuge tube. Add 5 mL of deionized water, vortex for 2 min.
- (2) Centrifuge at 4000 r/min for 10 min at room temperature.
- (3) Take 1 mL of the supernatant to another centrifuge tube, and add 1 mL of **Sample Diluent** (Solution 4), vortex for 30 s.
- (4) Centrifuge at 4000 r/min for 5 min at room temperature.
- (5) Take 50 µL of supernatant for analysis.

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

**3.4 Pretreatment of feed (cattle, swine) sample:**

- (1) Homogenize the representative sample with a homogenizer and mix fully.
- (2) Weigh  $1 \pm 0.05$  g of homogenate sample into the 50 mL centrifuge tube. Add 5 mL of **1% Trichloroacetic acid Solution** (Solution 3). Vortex for 5 min.
- (3) Centrifuge at 4000 r/min for 10 min at room temperature.
- (4) Take 40 µL of the supernatant to another centrifuge tube, and add 1560 µL of **Sample Diluent** (Solution 4). Vortex for 30 s.
- (5) Take 50 µL for analysis.

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

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## Assay procedure

Restore all reagents and samples to room temperature (25°C) 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°C.

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 of **HRP Conjugate** and add 50 µL of **Antibody Working Solution** into each well. Gently oscillate for 10 s to mix thoroughly and cover the plate with plate sealer. Incubate at 25°C for 30 min in shading light.
3. **Wash:** uncover the sealer carefully, remove the liquid in each well. Immediately add 260 µL of **Wash Buffer** (Solution 5) to each well and wash. Repeat the wash procedure for 4 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. **Color Development:** add 50 µL of **Substrate Reagent A** to each well, and then add 50 µL of **Substrate Reagent B**. Gently oscillate for 10 s to mix thoroughly. Incubate at 25°C in for 15 min shading light (The reaction time can be extended according to the actual color change).
5. **Stop Reaction:** add 50 µL of **Stop Solution** to each well. Gently oscillate for 10 s 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.

## Result analysis

### 1. Absorbance(%)= $A/A_0 \times 100\%$

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 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 form for accurate and fast analysis of batch samples.

