



## Technical Manual

### OTC (Oxytetracycline) ELISA Kit

- Catalogue Code: FSES0080
- Competitive ELISA Kit
- Research Use Only

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## 1. Key features and Sample Types

### Sensitivity:

0.3 ppb (ng/mL)

### Assay Procedure:

25°C, 30 min~15 min

### Detection Limit:

Muscle, Milk, Egg - 6 ppb; Feed - 400ppb

### Cross Reactivity:

Oxytetracycline - 100%; Chlortetracycline - 100%; Tetracycline - 100%;  
Doxycycline - 18.5%

### Sample Recovery rate:

90%±30%.

### Storage:

2-8°C for 6 months.

### Expiry:

See Kit Label

## 2. Storage

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.

## 3. Test Principle

This kit uses a Competitive-ELISA method. 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.

## 4. Kit Contents

Each kit contains reagents for 96 assays including:

| No. | Component                      | 96-WellKit   |
|-----|--------------------------------|--|
| 1   | ELISA Microtiter plate         | 96 wells   |
| 2   | Standards                      | 1 mL each<br>(0 ppb, 0.3 ppb, 0.9 ppb, 2.7 ppb, 8.1 ppb, 24.3 ppb) |
| 3   | Antibody Working Solution      | 7 mL   |
| 4   | HRP Conjugate                  | 7 mL   |
| 5   | 20xConcentrated Sample Diluent | 50 mL  |
| 6   | Substrate Reagent A            | 6 mL   |
| 7   | Substrate Reagent B            | 6 mL   |
| 8   | Stop Solution                  | 6 mL   |
| 9   | 20xConcentrated Wash Buffer    | 25 mL  |
| 10  | Plate Sealer                   | 3 pieces   |
| 11  | Sealed Bag                     | 1 piece  |
| 12  | Manual                         | 1 copy   |

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

### Additional materials required:

#### Other materials required but not supplied

- **Instrument:** Microplate reader, Homogenizer, Vortex mixer, Centrifuge, Balance (sensitivity 0.01 g).
- **Micropipette:** Single channel (20-200  $\mu$ L, 100-1000  $\mu$ L), Multichannel (30-300  $\mu$ L).
- **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.

## 5. Experimental Preparation

Bring 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 pre-treatment 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)

Dissolve 10.7g of  **$\text{Na}_2\text{Fe}(\text{CN})_5\text{NO}\cdot 2\text{H}_2\text{O}$**  to 100 mL with deionized water, mix fully.

*Prepare the fresh solution before use!*

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

Dilute 28.8 g of  **$\text{ZnSO}_4\cdot 7\text{H}_2\text{O}$**  to 100 mL with deionized water, mix 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 pre-treatment procedure

*Targets may be distributed unevenly, resulting in no detection. To avoid this, ensure to take sufficient samples when sampling.*

#### 3.1 Pre-treatment of muscle (livestock) sample:

1. Weigh  $1\pm 0.01$  g of homogenate muscle into the 50 mL centrifuge tube.
2. Add 9 mL of **Sample Diluent** (Solution 4), mix fully;
3. Vortex for 10 min, centrifuge at 4000 r/min for 10 min at room temperature;
4. Take 50  $\mu\text{L}$  of supernatant for detection and analysis.

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

### 3.2 Pre-treatment of raw milk sample:

1. Take 3 mL of 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 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 90s. 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 30s
7. Take 50 µL for detection and analysis.

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

### 3.3 Pre-treatment of egg sample:

1. Weigh 1±0.01 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 30s
4. Centrifuge at 4000 r/min for 5 min at room temperature;
5. Take 50 µL for detection and analysis.

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

### 3.4 Pre-treatment of feed sample:

1. Weigh 1 ± 0.01 g of homogenate egg into the 50 mL centrifuge tube. Add 5 mL of **1% Trichloroacetic acid Solution** (Solution 3), Vortex for 10 min;
2. Centrifuge at 4000 r/min for 10 min at room temperature;
3. Take 40 µL of the supernatant to another centrifuge tube, and add 1560 µL of **Sample Diluent** (Solution 4). vortex for 30s;
4. Take 50 µL for detection and analysis.

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

## 6. Assay Procedure

Bring 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 must be tested 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 vortex for 10s to mix thoroughly and cover the plate with plate sealer. Incubate at 25°C for 30 min away from direct sunlight.
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 5 times, 30s intervals/time. Invert the plate and pat it against absorbent paper (If bubbles exist in the wells, clean tips can be used to prick them).
4. **Colour Development:** add 50 µL of **Substrate Reagent A** to each well, and then add 50 µL of **Substrate Reagent B**. Gently vortex for 10s to mix thoroughly. Incubate at 25°C for 15 min away from direct sunlight (The reaction time can be extended according to the actual colour change).
5. **Stop Reaction:** add 50 µL of **Stop Solution** to each well. Gently vortex for 10s 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.

## 7. Data 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.

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## 8. 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 FSES0080. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other FSES0080 with different lot numbers.**
6. Substrate Reagent should be abandoned if it turns blue colour. When OD value of standard (concentration: 0) < 0.8 unit (A450nm < 0.8), it indicates the reagents are 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.



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Notes:

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### **Assay Genie 100% money-back guarantee!**

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

### **Contact Details**



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