



Technical Manual

OTC (Oxytetracycline) ELISA Kit

- Catalogue Code: FSES0081
- Competitive ELISA Kit
- Research Use Only

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1. Key features and Sample Types

Sensitivity:

0.05 ppb (ng/mL)

Assay Procedure:

37°C, 30 min~30 min~ 15min

Detection Limit:

Muscle, Liver, Eggs - 0.4 ppb; Honey - 2 ppb; Urine - 0.5 ppb.

Cross Reactivity:

Tetracycline - 100%; Oxytetracycline - 100%; Chlortetracycline - 16.7%
Deoxytetracycline - 4.2%.

Sample Recovery rate:

Muscle, Liver, Eggs - 85%±20%, Honey - 75%±20%, Urine - 80%±20%.

Storage:

2-8°C for 6 months.

Expiry:

See Kit Label

2. Storage

Store the kit at 2~8°C. 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°C.

3. Test Principle

This kit uses a Competitive-ELISA method. It can detect Tetracyclines (TC_S) in samples, such as muscle, honey, 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, TC_S in the samples or standard competes with coupled antigen on the solid phase supporter for sites of anti-TC_S antibody. Then Horseradish Peroxidase (HRP) conjugate is added to each microtiter 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 TC_S. The concentration of TC_S 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	High Concentrated Standard (1.0 ppm)	1 mL
3	Standards (empty bottle)	(0 ppb, 0.05 ppb, 0.15 ppb, 0.45 ppb, 1.35 ppb, 4.05 ppb)
4	HRP Conjugate	11 mL
5	Antibody Working Solution	5.5 mL
6	Substrate Reagent A	6 mL
7	Substrate Reagent B	6 mL
8	Stop Solution	6 mL
9	20xConcentrated Wash Buffer	40 mL
10	5xReconstitution Buffer	50 mL
11	Plate Sealer	3 pieces
12	Sealed Bag	1 piece
13	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

- **Instruments:** Microplate reader, Printer, Homogenizer, Vortex mixer, Centrifuge, Graduated pipette, Balance (sensitivity 0.01 g).
- **Micropipette:** Single channel (20-200 µL, 100-1000 µL), Multichannel (30-300 µL).
- **Reagents:** N, N-Dimethylformamide (DMF)

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: 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 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, fish, shrimp), liver, egg sample:

1. Homogenize muscle, liver and egg samples with homogenizer.
2. Weigh 2 ± 0.05 g of homogenate sample into the a 50 mL centrifuge tube, add 4 mL of **DMF**. Vortex for 5 min, the sample is fully mixed with the liquid. Centrifuge at 4000 r/min for 10 min at room temperature.
3. Take 250 μ L supernatant, add 750 μ L of **Reconstitution Buffer** (Solution 1) and mix fully.
4. Take 50 μ L for analysis.

Note: Sample dilution factor: 8, detection limit: 0.4 ppb.

3.2 Pre-treatment of honey sample:

1. Weigh 1 ± 0.05 g of honey into the centrifuge tube, add 2 mL of **DMF**. Vortex for 2 min, centrifuge at 4000 r/min for 10 min at room temperature.
2. Take 100 μ L of the supernatant to another centrifuge tube, add 1900 μ L of **Reconstitution Buffer** (Solution 1) for diluting, mix fully for 30s.
3. Take 50 μ L for analysis.

Note: Sample dilution factor: 40, detection limit: 2 ppb.

3.3 Pre-treatment of urine (swine)* sample:

(*Data validated in swine urine but pre-treatment can be applied for urine samples of multiple species.)

1. Dilute the urine sample with **Reconstitution Buffer** (Solution 1) for 10 times (if the urine sample is epinephelos, 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 μ L for analysis.

Note: Sample dilution factor: 10, detection limit: 0.5 ppb.

6. Assay Procedure

Bring all reagents and samples to room temperature 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.

Prepare the Standard Liquid. Standard Liquid of low concentration is unstable, prepare fresh solution before use.

Take 3 mL of **Reconstitution Buffer** (Solution 1) into 0 ppb **bottle**. Take 2 mL of **Reconstitution Buffer** (Solution 1) into **0.05 ppb bottle, 0.15ppb bottle, 0.45 ppb bottle, 1.35ppb bottle** respectively. Take 3 mL of **Reconstitution Buffer** (Solution 1) into **4.05 ppb bottle**.

- (1) **Standard Liquid 6:** Take 12 μ L of **High Concentrated Standard (1.0 ppm)** into 4.05 ppb bottle, then mix fully. The concentration of Standard Liquid 6 is 4.05 ppb.
 - (2) **Standard Liquid 5:** Take 1 mL of Standard Liquid 6 into 1.35 ppb bottle, then mix fully. The concentration of Standard Liquid 5 is 1.35 ppb.
 - (3) **Standard Liquid 4:** Take 1 mL of Standard Liquid 5 into 0.45 ppb bottle, then mix fully. The concentration of Standard Liquid 4 is 0.45 ppb.
 - (4) **Standard Liquid 3:** Take 1 mL of Standard Liquid 4 into 0.15 ppb bottle, then mix fully. The concentration of Standard Liquid 3 is 0.15 ppb.
 - (5) **Standard Liquid 2:** Take 1 mL of Standard Liquid 3 into 0.05 ppb bottle, then mix fully. The concentration of Standard Liquid 2 is 0.05 ppb.
 - (6) **Standard Liquid 1:** Use Reconstitution Buffer directly. The concentration of Standard Liquid 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 must be tested in duplicate.**
 2. **Add Sample:** add 50 μ L of **Standard or Sample** per well, then add 50 μ L **Antibody Working Solution** to each well, cover the plate with plate sealer, vortex for 5s gently to mix thoroughly, incubate at 37°C for 30 min away from direct sunlight.
 3. **Wash:** uncover the sealer carefully, remove the liquid of each well. Immediately add 300 μ L of **Wash Buffer** (Solution 2) to each well and wash. Repeat wash procedure for 5 times, 30s intervals/time. Invert the plate and pat it against with absorbent paper (If bubbles exist in the wells, clean tips can be used to prick them).
 4. **HRP Conjugate:** add 100 μ L of **HRP Conjugate** to each well, incubate at 37°C for 30 min away from direct sunlight.
 5. **Wash:** repeat step 3.
 6. **Colour Development:** add 50 μ L of **Substrate Reagent A** to each well, and then add 50 μ L of **Substrate Reagent B**. Gently vortex for 5s to mix thoroughly. Incubate at 37°C for 15 min away from direct sunlight (The reaction time can be extended according to the actual colour change).
 7. **Stop Reaction:** add 50 μ L of **Stop Solution** to each well, vortex gently to mix

thoroughly.

8. **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 10 min after stop reaction.

7. Data Analysis

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

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.

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 FSES0081. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other FSES0081 with different lot numbers.**
6. Substrate Reagent should be abandoned if it turns blue colour. When OD value of standard (concentration: 0) < 0.5 unit ($A_{450nm} < 0.5$), 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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