OTC(Oxytetracycline) ELISA Kit

Catalog No: E-FS-183

96T/96T*3/96T*5

Version Number:	V1.3
Replace version:	V1.2
Revision Date:	2026.09.16

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.

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Test principle

This kit uses Competitive-ELISA as the method for the quantitative detection. It can detect Oxytetracycline (OTC) in samples, such as tissue, 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, 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 ofthe samples to the standard curve.

Technical indicators

Reaction mode (Incubation time and temperature)**:** 25±2℃; 30 min, 15-20 min.

Detection limit: Tissue Method 1 (duck, fish, shrimp, sea cucumber), honey---6 ppb; Tissue Method 2 (pork liver, pork, beef, chicken, fish)---200 ppb.

Cross-reactivity: Oxytetracycline---100%; Tetracycline---206%;Aureomycin---300%; Doxycycline ---18.5%.

Sample recovery rate: 90%±30%.

Kit components

Item	Specifications
ELISA Microtiter plate	96 wells
Standard Liquid	1.5 mL each (ppb=ng/mL=ng/g) (0 ppb, 0.2 ppb, 0.6 ppb, 1.8 ppb, 5.4 ppb, 16.2 ppb)
HRP Conjugate	7 mL
Antibody Working Solution	7 mL
20×Concentrated Sample Diluent	50 mL
20×Concentrated Wash Buffer	25 mL
Substrate Reagent A	7 mL
Substrate Reagent B	7 mL
Stop Solution	7 mL
Plate Sealer	1 piece
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

Instrument: Microplate reader, Homogenizer, Nitrogen evaporators, Water bath, Vortex mixer, Centrifuge, Graduated pipette, Balance (sensibility 0.01 g).

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

Reagents: $Na_2HPO_4 \cdot 12H_2O$, $NaH_2PO_4 \cdot 2H_2O$, Methanol, Tween-20, Trichloroacetic acid(TCA).

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℃.
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-E183. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other E-FS-E183 with different lot numbers.**
6. Substrate Reagent should be abandoned if it turns 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 descriptio n. Please consult technical support for the applicability if other sample need to be tested.**
11. The time of each step of adding the sample solution, enzyme conjugate working solution, AB mixture and stop solution in plate well should not exceed 3 min.
12. 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℃. After opening, the kit is stable for up to 1 month.

Expiry date: expiration date is on the packing box.

Experimental preparation

Restore all reagents and samples to room temperature (25±2℃) 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**

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

   Solution 1: **Sample Diluent**

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

   Solution 2: **Wash Buffer**

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

   Solution 3: **0.02 M PB**

   Dissolve 5.74 g of **$Na_2HPO_4 \cdot 12H_2O$** and 0.59 g of **$NaH_2PO_4 \cdot 2H_2O$** to 1000 mL with deionized water, mix fully.

   Solution 4: **Honey Diluent**

   Take 900 mL of **0.02 M PB** (Solution 3), add 50 mL **Methanol** and 50 mL **Tween-20**, and mix fully (*Note: This solution is unstable. The validity period is 15 days when stored at 4℃ away from light. It is recommended to prepare it freshly before use*).

   Solution 5: **1% TCA**

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

4. **Sample pretreatment**

3.1 Pretreatment of duck, fish, shrimp, sea cucumber sample (Tissue Method 1):

(1) Weigh 1±0.05 g of homogenate sample into a 50 mL centrifuge tube. Add 9 mL of **Sample Diluent** (Solution 1), vortex for 1 min to ensure that the sample is fully dispersed.

(2) Centrifuge at 4000g for 10 min at room temperature.

(3) Take 50 μL of supernatant for analysis.

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

3.2 Pretreatment of pork liver, pork, beef, chicken, fish sample (Tissue Method 2):

(1) Weigh 1±0.05 g of homogenate sample into a 50 mL centrifuge tube. Add 4 mL of **1% TCA** (Solution 5), vortex for 1 min to ensure that the sample is fully dispersed.

(2) Centrifuge at 4000g for 5 min at room temperature.

(3) Take 40 µL of supernatant into a 4 mL centrifuge tube, add 1560 µL of **Sample Diluent** (Solution 1), vortex for 1 min.

(4) Take 50 µL for analysis.

Note: Sample dilution factor: 200 detection limit: 200 ppb

3.3 Pretreatment of honey sample:

(1) Weigh 1±0.05 g of honey sample into a 50 mL centrifuge tube. Add 9 mL of **Honey Diluent** (Solution 4), vortex for 1 min to ensure that the sample is fully dispersed.

(2) Take 50 µL for analysis.

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

Assay procedure

Restore 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℃.

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 50 µL of **Antibody Working Solution** in sequence 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 in each well. Immediately add 260 µL of **Wash Buffer** (Solution 2) to each well. Repeat 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 100 µL of **Substrate mixed solution** to each well (**Substrate Reagent A** and **Substrate Reagent B** are fully mixed at ratio 1:1 by volume, the mixture should be used within 5 min, avoid using metal containers or stirring the reagents), Gently oscillate for 10 s to mix thoroughly. Incubate at 25±2℃ for 15-20 min in shading light.
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_0×100%**

   A: Average absorbance of standard or sample

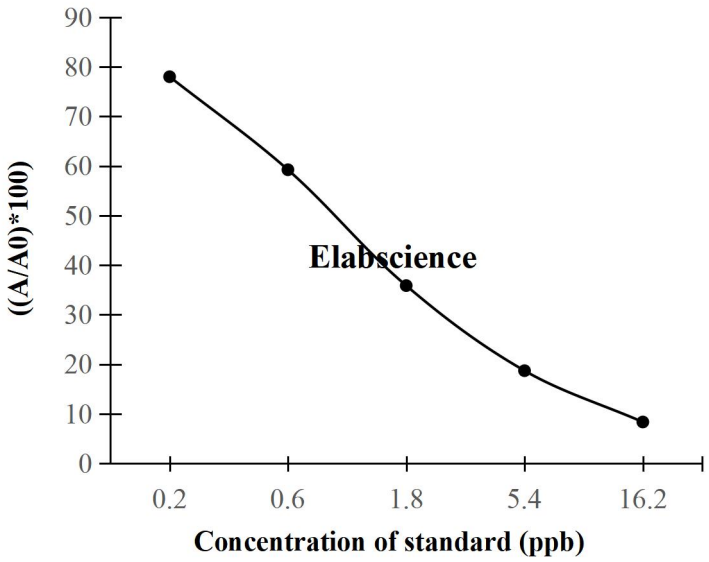
   A_0: Average absorbance of 0 ppb Standard

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 on a large number of samples.

OTC (E-FS-E183) **Standard Curve**



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