

7th Edition, revised in April, 2017

(FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !)

ENR(Enrofloxacin) ELISA Kit

Catalog No: E-FS-E032

96T

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.

Phone: 240-252-7368(USA)240-252-7376(USA)

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 Indirect-Competitive-ELISA as the method. It can be used to detect Enrofloxacin (ENR) in samples, such as honey, animal tissues (chicken, porcine, fish, and shrimp) milk, eggs, etc. This kit is composed of Micro ELISA Plate, HRP conjugate, antibody, standard and other supplementary reagents. The micro-plate provided in this kit has been pre-coated with ENR antigen. During the reaction, ENR in the samples or standard competes with ENR antigen coated on the solid phase supporter for ENR antibody. Then Horseradish Peroxidase (HRP) conjugate is added to each micro 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 ENR. The concentration of ENR in the samples can be calculated by comparing the OD of the samples to the standard curve.

Technical indicator

Sensitivity: 0.1 ppb (ng/mL)

Reaction mode: 25°C, 45 min ~ 15 min

Detection limit: Tissue (chicken, porcine, fish, shrimp) --- 0.3 ppb.

Honey --- 0.4 ppb, Milk --- 3 ppb, Milk powder --- 6 ppb, Eggs --- 3 ppb.

Cross-reactivity: Enrofloxacin --- 100%, Oxolinic acid --- 28%, Levofloxacin --- 10%, Lomefloxacin --- 4%, Marbofloxacin --- 4%, Sarafloxacin --- 2%

Sample recovery rate: Tissue/honey/milk/milk powder/eggs --- 85% ± 15%.

Kits components

| Item | Specifications |
|---|---|
| Micro ELISA Plate | 96 wells |
| Standard Solution | 1 mL each (0 ppb, 0.1 ppb, 0.3 ppb, 0.9 ppb, 2.7 ppb, 8.1 ppb) |
| High concentrated Standard (100 ppb) | 1 mL |
| HRP Conjugate (Red cap) | 5.5 mL |
| Antibody Working Solution (Blue cap) | 5.5 mL |
| Substrate Reagent A (White cap) | 6 mL |
| Substrate Reagent B (Black cap) | 6 mL |
| Stop Solution (Yellow cap) | 6 mL |
| 20× Concentrated Wash Buffer (White cap) | 40 mL |
| 5× Concentrated Re-dissolve Solution (Yellow cap) | 50 mL |
| Manual | 1 copy |

Other supplies required

Instrument: Microplate reader, Printer, Homogenizer, Oscillators, Centrifuge, Graduated pipette, Balance (sensitivity 0.01g),

High-precision transfer pipette: Single-channel (20-200 μ L, 100-1000 μ L), Multi-channel (300 μ L)

Reagents: Anhydrous acetonitrile, N-hexane, Concentrated hydrochloric acid (HCl)

Experimental preparation

Bring all reagents and samples to room temperature before use.

Open the micro-plate 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

Solution 1: 0.15M HCl. Add 5 mL Concentrated hydrochloric acid (HCl) to 400 mL deionized water, mix fully.

Solution 2: Sample extraction solution. Add 10 mL 0.15M HCl to 90 mL anhydrous acetonitrile, mix fully.

Solution 3: Re-dissolve solution. Dilute the 5 \times Concentrated Re-dissolve solution for 5 times with deionized water (for sample re-dissolution). It can be stored at 4 $^{\circ}$ C for 1 month.

Solution 4: Wash working buffer. Dilute the 20 \times Concentrated Wash Buffer with deionized water (1:19).

3. Sample pretreatment procedure

3.1 Pretreatment of animal tissue sample:

- (1) Weigh 2 ± 0.05 g of tissue homogenate into a 50 mL EP tube.
- (2) Add 8 mL of solution 2 and oscillate for 5 minutes. Centrifuge at 4000 r/min for 10 min at room temperature.
- (3) Remove 2 mL of the clear upper organic layer solution to a clean and dry glass tube, dry with Nitrogen Evaporators at 50-60 $^{\circ}$ C.
- (4) Add 1 mL N-hexane and oscillate for 2 min. Then add 1 mL of re-dissolve solution (solution 3) and oscillate for 30 s to mix fully. Centrifuge for 5 min at 4000 r/min at room temperature.
- (5) Remove the N-hexane upper layer, take 50 μ L of the lower layer solution for analysis.

Note: Sample dilution factor: 2, minimum detection dose: 0.3 ppb

3.2 Pretreatment of honey sample:

- (1) Weigh 1 ± 0.05 g of honey into a 50 mL EP tube, add 6 mL of solution 2 and oscillate for 5 minutes to ensure thoroughly dissolved.
- (2) Add 3 mL of solution 3 and 11 mL of dichloromethane, oscillate for 5 min. Then centrifuge at 4000 r/min for 10 min at room temperature.
- (3) Remove the supernatant and transfer 8 mL of the upper layer organic solution to a dry container.

Dry with Nitrogen Evaporators at 50-60°C.

- (4) Dissolve the dry residue with 1 mL re-dissolve solution. Add 1 mL N-hexane and oscillate for 30 seconds. Centrifuge for 5 min at a speed of over 3000 r/min at room temperature.
- (5) Remove the N-hexane upper layer, take 50 µL of the lower layer solution for analysis.

Note: Sample dilution factor: 2, minimum detection dose: 0.4 ppb

3.3 Pretreatment of milk sample:

- (1) Dilute the milk with solution 3 for 20 times (eg: add 25 µL of milk into 475 µL of solution 3), oscillate for 1 minute to dissolve it fully.
- (2) Take 50 µL for detection and analysis.

Note: Sample dilution factor: 20, minimum detection dose: 3 ppb

3.4 Pretreatment of milk powder sample:

- (1) Weigh 0.5 ± 0.02 g of homogenate sample into a 10 mL EP tube, add 5 mL of deionized water and oscillate to dissolve it fully.
- (2) Mix 100 µL of sample solution with 400 µL of re-dissolve solution. Oscillate for 1 minute.
- (3) Take 50 µL for detection and analysis.

Note: Sample dilution factor: 50, minimum detection dose: 6 ppb

3.5 Pretreatment of eggs sample:

- (1) Weigh 1 ± 0.02 g of homogenate egg into a 10 mL EP tube, add 5 mL of deionized water and oscillate to dissolve it fully.
- (2) Mix 100 µL of sample solution with 400 µL of re-dissolve solution. Oscillate for 1 minute.
- (3) Take 50 µL for detection and analysis.

Note: Sample dilution factor: 30, minimum detection dose: 3 ppb

Assay procedure

Centrifuge the sample again after thawing before the assay. Bring all reagents to room temperature before use. **All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming.** Dilute 40mL of concentrated wash buffer into 800mL wash working buffer with deionized or distilled water.

1. **Number:** Number the sample and standard in order (multiple well), and keep a record of standard wells and sample wells.
2. **Add sample:** Add 50µL of Standard, Blank, or Sample per well, then add 50µL of HRP conjugate to each well. Add 50µL of antibody working solution. Gently oscillate for 5s to mix thoroughly and cover the plate with sealer. Incubate for 45min at 25°C .
3. **Wash:** Uncover the sealer carefully, remove the liquid in each well. Immediately add 250µL of wash working buffer to each well and wash. Repeat the wash procedure for 5 times, 30s 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. **HRP conjugate:** add 100µL HRP conjugate to each well, shading light incubation for 30min at 25°C .
5. **Wash:** repeat step 3
6. **Color Development:** Add 50µL of substrate solution A to each well, and then add 50µL of substrate solution B. Gently oscillate for 5s to mix thoroughly. Incubate for 15min at 25°C in the dark.
7. **Stop reaction:** Add 50µL of stop solution to each well, oscillate gently to mix thoroughly.
8. **OD Measurement:** Determine the optical density (OD value) of each well at 450nm with a microplatereader (the 450/630 nm double wavelength is recommended). This step should be finished in 10min after stop reaction.

Result analysis

1. **Absorbance(%)=A/A₀×100%**

A: Average absorbance of standard or sample

A₀: Average absorbance of Oppb 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 software for accurate and fast analysis of batch samples.

Notes

1. The overall OD values will be lower when reagents have not been brought to room temperature before use or the room temperature $<25^{\circ}\text{C}$.
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 repeatability of this ELISA kit.
4. Micro ELISA plate should be covered with plate sealer. Prevent the reagents to strong light.
5. Do not use expired kit and reagents of different batches.
6. TMB should be abandoned if it turns color. When OD value of standard (concentration: 0) <0.5 unit ($A_{450\text{nm}} < 0.5$), it indicates the reagent is deteriorated.
7. Stop solution is caustic, avoid of contacting with the skin.

Storage and valid period

Storage: Store at $2-8^{\circ}\text{C}$. Avoid freeze / thaw cycles.

Valid Period: 1 year, production date is on the packing box.