# ENR (Enrofloxacin) ELISA Kit

Catalog No: E-FS-E159

96T/96T*3

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|---|---|
| **Version Number:** | V1.5 |
| **Replace version:** | V1.4 |
| **Revision Date:** | 2026.06.01 |

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.

## Test principle

This kit uses Competitive-ELISA as the method for the quantitative detection. It can detect Enrofloxacin (ENR) in samples, such as muscle, milk, 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, ENR in the samples or standard competes with coupled antigen on the solid phase supporter for sites of anti-ENR 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 ENR. The concentration of ENR 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±2℃, 30 min, 15-20 min

**Detection limit:** Muscle (Fish, shrimp)---1 ppb(Method 1); Muscle(Chickens, ducks, pigs, cows, sheep)---2 ppb(Method 1); Muscle(Chickens, ducks)---5 ppb(Method 2); Muscle(pigs, cows, sheep), Raw milk, Reconstituted milk, Bovine serum, Egg---10 ppb(Method 2); Cooked food (meat products)---20 ppb; Muscle (Fish, shrimp), Pork liver, Swine urine---30 ppb(Method 3).

**Cross-reactivity:** Enrofloxacin--100%; Oxolinic acid--186%; Pefloxacin--136%; Enoxacin--101%; Lomefloxacin--123%; Danofloxacin--131%; Norfloxacin--118%; Ofloxacin--39%; Flumequine--110%; Ciprofloxacin--103%; Marbofloxacin--111%.

**Sample recovery rate:** 90%±30%.

## Kits components

| Item | Specifications |
|---|---|
| ELISA Microtiter plate | 96 wells |
| Standard Liquid | 1.5 mL each (ppb=ng/mL=ng/g)<br>(0 ppb, 0.3 ppb, 0.9 ppb, 2.7 ppb, 8.1 ppb, 24.3 ppb) |
| HRP Conjugate | 7 mL |
| Antibody Working Solution | 7 mL |
| Substrate Reagent A | 7 mL |
| Substrate Reagent B | 7 mL |
| Stop Solution | 7 mL |
| 20×Concentrated Wash Buffer | 25 mL |
| 20×Concentrated Sample Diluent | 50 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

**Instruments:** Microplate reader, Printer, 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:** Acetonitrile, N-hexane, HCl, Nacl.

## 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 the kit to strong light.
5. **Each reagent is optimized for use in the E-FS-E159. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other E-FS-E159 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 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 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 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: **0.2 M HCl Solution**

   Dilute 1.72 mL of **HCl** to 100 mL with deionized water, mix fully.

   Solution 2: **Sample Diluent A**

   Dilute **20×Concentrated Sample Diluent** with deionized water. (**20×Concentrated Sample Diluent** (V): Deionized water (V) = 1:9). The Reconstitution buffer can be store at 4℃ for a month.

   Solution 3: **Sample Diluent B**

   Dilute **20×Concentrated Wash Buffer** with deionized water. (20×Concentrated Wash Buffer (V): Deionized water (V) = 1: 9). Take 100 mL of the diluted solution, add 1 g **Nacl** solid, mix well and set aside.

   Solution 4: **Wash Buffer**

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

3. **Sample pretreatment procedure**

**3.1 Pretreatment of Muscle(Fish, shrimp, Chickens, ducks, pigs, cows, sheep) sample(Method 1):**

(1) Remove fat from sample. Homogenize the representative sample with a homogenizer and mix fully.

(2) Weigh 1±0.05 g of muscle homogenate into a 15 mL centrifuge tube.

(3) **For Fish, shrimp:**

Add 0.5 mL of **20×Concentrated Sample Diluent** and vortex for 30s.

**For Chickens, ducks, pigs, cows, sheep:**

Add 0.5 mL of **0.2 M HCl Solution** (Solution 1) and vortex for 30s.

(4) Add 4.5 mL acetonitrile, vortex immediately for 2 min until the tissue is completely dispersed. Centrifuge for 5 min at 4000 rpm at room temperature.

(5) Remove 3 mL of the clear upper organic layer solution to a new centrifuge tube, dry at 50-60℃ with nitrogen evaporators or water bath. (Please do it in a ventilated environment.)

(6) Add 2 mL of **N-hexane** and vortex for 30s. Then add 1 mL of **Sample Diluent A** (Solution 2) and low-speed vortex for 30 s. Centrifuge for 5 min at 4000 rpm at room temperature.

(7) Remove the N-hexane upper layer, take 50 µL of the lower water layer solution for analysis.

**Note: Sample dilution factor: 2, detection limit: 1 ppb (Fish, shrimp); 2 ppb (Chickens, ducks, pigs, cows, sheep)**

**3.2 Pretreatment of Muscle(Chickens, ducks, pigs, cows, sheep) sample(Method 2):**

(1) Remove fat from sample. Homogenize the representative sample with a homogenizer and mix fully.

(2) Weigh 1 ± 0.05 g of muscle homogenate into a 50 mL centrifuge tube.

(3) Add 9.5mL deionized water and 0.5mL **20×Concentrated Sample Diluent** successively, vortex immediately for 1 min until the tissue is completely dispersed.

(4) Then centrifuge at 4000 rpm for 10 min at room temperature.

(5) Immediately take 50μL of the supernatant for analysis.

**Note: Sample dilution factor: 10, detection limit: 5 ppb (Chickens, ducks); 10 ppb (pigs, cows, sheep)**

**3.3 Pretreatment of Raw milk, Reconstituted milk, Bovine serum, Egg sample:**

(1) **For Reconstituted milk:**

Weigh 1±0.05 g of milk powder sample into 10 mL deionized water, vortex for 1 min; Immediately transfer 100 μL to a new centrifuge tube.

**For Raw milk:**

Take 100 μL of the homogenized sample into a centrifuge tube.

**For Egg:**

Homogenate the egg sample with homogenizer to mix fully. Take 100 μL of the homogenized sample into a centrifuge tube.

**For Bovine serum:**

Take 100 μL of the homogenized sample into a centrifuge tube.

(2) Add 900 μL **Sample Diluent B (**Solution 3**),** vortex for 30s.

(3) Take 50 μL for analysis.

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

**3.4 Pretreatment of Swine urine sample:**

(1) Take 100 μL of urine into a centrifuge tube.

*If the urine is cloudy, it can be filtered or centrifuged at 4000 rpm for 5 min.*

(2) Add 900 μL **Sample Diluent A (**Solution 2**),** vortex for 30s.

(3) Take 50 μL for analysis.

**Note: Sample dilution factor: 10, detection limit: 30 ppb**

**3.5 Pretreatment of Muscle(Fish, shrimp), Pork liver sample(Method 3):**

(1) Remove fat from sample. Homogenize the representative sample with a homogenizer and mix fully.

(2) Weigh 1 ± 0.05 g of the homogenized sample into a 50 mL centrifuge tube.

(3) Add 9.5 mL deionized water and 0.5 mL of **0.2 M HCl Solution** (Solution 1) successively, vortex immediately for 1 min until the tissue is completely dispersed. Centrifuge for 5 min at 4000 rpm at room temperature.

(4) Remove 200 µL of the clear upper layer solution to a new centrifuge tube, add 200 µL **Sample Diluent A (**Solution 2**)** and 200 µL of deionized water, vortex for 30s.

(5) Take 50 µL for analysis.

**Note: Sample dilution factor: 30, detection limit: 30 ppb**

**3.6 Pretreatment of Cooked food (Meat products) sample:**

(1) Remove fat from sample. Homogenize the representative sample with a homogenizer and mix fully.

(2) Weigh 0.5 ± 0.05 g of the homogenized sample into a 50 mL centrifuge tube.

(3) Add 9.5 mL deionized water and 0.5 mL of **20×Concentrated Sample Diluent**, vortex immediately for 1 min until the tissue is completely dispersed. Centrifuge for 5 min at 4000 rpm at room temperature.

(4) Immediately take 50µL of the supernatant for analysis.

**Note: Sample dilution factor: 20, detection limit: 20 ppb**

## Assay procedure

Restore all reagents and samples to room temperature (25±2℃) 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** to each well. Add 50 μL of **Antibody Working Solution**. (Ensure that the reagents are added in the correct order.) Gently oscillate for 10 s to mix thoroughly and cover the plate with plate sealer. 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 4) to each well and wash. Repeat the wash procedure for 4 times, 15-30 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 Reagent A** and **Substrate Reagent B** mixture. (**Substrate Reagent A** and **Substrate Reagent B** are mixed 1:1 according to volume, must be fully mixed, the mixture is used within 5 minutes, avoid the use of metal container, avoid stirring reagents.)Gently oscillate for 10 s to mix thoroughly. Incubate 25±2℃ for 15-20 min at in shading light.
5. **Stop Reaction:** add 50 μL of **Stop Solution** to each well, oscillate gently 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 of batch samples.

**Enrofloxacin** (E-FS-E159) **Standard Curve**