ENR (Enrofloxacin) ELISA Kit

Catalog No: E-FS-E032

96T/96T*3

Version Number:	V1.2
Replace version:	V1.1
Revision Date:	2024.03.14

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 honey, 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℃, 45 min, 15min

Detection limit: Muscle---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: 85%±15%.

Kits components

Item	Specifications
ELISA Microtiter plate	96 wells
Standard Liquid	1 mL each (ppb=ng/mL=ng/g) (0 ppb, 0.1 ppb, 0.3 ppb, 0.9 ppb, 2.7 ppb, 8.1 ppb)
HRP Conjugate	5.5 mL
Antibody Working Solution	5.5 mL
Substrate Reagent A	6 mL
Substrate Reagent B	6 mL
Stop Solution	6 mL
20×Concentrated Wash Buffer	40 mL
5×Reconstitution Buffer	50 mL
Plate Sealer	3 pieces
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, Dichloromethane

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. 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-E032. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other E-FS-E032 with different lot numbers.**
6. Substrate Reagent should be abandoned if it turns blue color. When OD value of standard (concentration: 0) < 0.5 unit (A450nm < 0.5), 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℃.

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 1:0.15 M HCl Solution.

   Dilute 5 mL of **HCl** to 400 mL with deionized water, mix fully.

   Solution 2: Sample Extraction Solution *(for livestock, fish, shrimp, honey sample)*

   Add 10 mL of **0.15 M HCl Solution** (Solution 1) to 90 mL of **Acetonitrile**, mix fully.

   Solution 3: Reconstitution Buffer

   Dilute the **5 ×Reconstitution Buffer** with deionized water. (5 ×Reconstitution Buffer (V): Deionized water (V) = 1:4). The Reconstitution buffer can be store at 4℃ for a month.

   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 (livestock, fish, shrimp) sample:

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

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

(3) Add 8 mL of **Sample Extraction Solution** (Solution 2) and vortex for 5 min. Centrifuge at 4000 rpm for 10 min at room temperature.

(4) Remove 2 mL of the clear upper organic layer solution to a clean and dry glass tube, dry at 50-60℃ with nitrogen evaporators or water bath. (Please do it in a ventilated environment.)

(5) Add 1 mL of **N-hexane** and vortex for 2 min. Then add 1 mL of **Reconstitution Buffer** (Solution 3) and vortex for 30 s to mix fully. Centrifuge for 5 min at 4000 rpm at room temperature.

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

Note: Sample dilution factor: 2, detection limit: 0.3 ppb

3.2 Pretreatment of honey sample:

(1) Weigh 1±0.05 g of honey into a 50 mL centrifuge tube, add 6 mL of **Sample Extraction Solution** (Solution 2) and vortex for 5 min to ensure thoroughly dissolved.

(2) Add 3 mL of **Reconstitution Buffer** (Solution 3) and 11 mL of **Dichloromethane**, vortex for 5 min. Then centrifuge at 4000 rpm for 5 min at room temperature.

(3) Remove the supernatant and transfer 8 mL of the under layer organic solution to a dry tube. Dry at 50-60℃ with nitrogen evaporators or water bath.

(4) Dissolve the dry residue with 1 mL of **Reconstitution Buffer** (Solution 3). Add 1 mL of **N-hexane** and vortex for 30 s. Centrifuge for 5 min at 3000 rpm 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, detection limit: 0.4 ppb

3.3 Pretreatment of milk sample:

(1) Dilute the milk with **Reconstitution Buffer** (Solution 3) for 20 times (*e.g.*, add 25 μL of milk into 475 μL of Reconstitution Buffer), vortex for 1 min to dissolve it fully.

(2) Take 50 μL for analysis.

Note: Sample dilution factor: 20, detection limit: 3 ppb

3.4 Pretreatment of milk powder sample:

(1) Weigh 0.5±0.05 g of homogenate sample into a 10 mL centrifuge tube, add 5 mL of deionized water and vortex to dissolve it fully.

(2) Mix 100 μL of sample solution with 400 μL of **Reconstitution Buffer** (Solution 3). Vortex for 1 min.

(3) Take 50 μL for analysis.

Note: Sample dilution factor: 50, detection limit: 6 ppb

3.5 Pretreatment of eggs sample:

(1) Weigh 1±0.05 g of homogenate egg into a 10 mL centrifuge tube, add 5 mL of deionized water and vortex to dissolve it fully.

(2) Mix 100 μL of sample solution with 400 μL of **Reconstitution Buffer** (Solution 3). Vortex for 1 min.

(3) Take 50 μL for analysis.

Note: Sample dilution factor: 30, detection limit: 3 ppb

Assay procedure

Restore all reagents and samples to room temperature (25℃) 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**. Gently oscillate for 5 s to mix thoroughly and cover the plate with plate sealer. Incubate at 25℃ for 45 min in shading light.
3. **Wash:** uncover the sealer carefully, remove the liquid in each well. Immediately add 300 μL of **Wash Buffer** (Solution 4) to each well and wash. Repeat the wash procedure for 5 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 50 μL of **Substrate Reagent A** to each well, and then add 50 μL of **Substrate Reagent B**. Gently oscillate for 5 s to mix thoroughly. Incubate 25℃ for 15 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 10 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.