# Enrofloxacin (ENR) ELISA Kit

**Catalog No.:** abx364837.

**Size:** 96 tests.

**Storage:** Store at 4°C for up to 12 months.

**Application:** The quantitative detection of Enrofloxacin in Muscle tissue, Honey, Milk, Milk powder, and Eggs.

**Sensitivity:** 0.1 ng/ml.

**Detection Limits:** Muscle tissue – 0.3 ng/ml; Honey -- 0.4 ng/ml; Milk -- 3 ng/ml; Milk powder -- 6 ng/ml; Eggs – 3 ng/ml.

**Sample Recovery Rate:** 85 ± 15%

**Principle of the Assay:** This kit is based on competitive enzyme-linked immunosorbent assay technology. Enrofloxacin (ENR) antigen is precoated onto a 96-well plate. The standards and samples and a Biotin-conjugated antibody specific to Enrofloxacin are added to wells and incubated. ENR antigen coated on the plate and free ENR antigen in samples compete for anti-ENR antibody binding. After washing away unbound conjugates, avidin conjugated to Horseradish Peroxidase is added to each well. After TMB substrate solution is added only wells that contain bound Enrofloxacin antibody will produce a blue color product that changes into yellow after addition of the stop solution. The intensity of the yellow color is inversely proportional to the Enrofloxacin amount present in the sample. The O.D. absorbance is measured spectrophotometrically at 450 nm using a microplate reader, and then the concentration of Enrofloxacin can be calculated.



**Kit Components**

1. 96-well microplate
2. Standards (1 ml each): 0 ng/ml, 0.1 ng/ml, 0.3 ng/ml, 0.9 ng/ml, 2.7 ng/ml, 8.1 ng/ml
3. HRP Conjugate Reagent: 5.5 ml
4. Antibody Solution: 5.5 ml
5. Substrate Reagent A: 6 ml
6. Substrate Reagent B: 6 ml
7. Stop Solution: 6 ml
8. Wash Buffer (20X): 40 ml
9. Reconstitution Buffer (5X): 50 ml
10. Plate sealer: 3
11. Hermetic bag: 1

**Materials Required But Not Provided**

1. Incubator
2. Microplate reader (450 nm)
3. High-precision pipette and sterile pipette tips
4. Automated plate washer
5. ELISA shaker
6. 50 ml centrifuge tubes
7. Absorbent filter papers
8. 100 ml and 1 L graduated cylinders
9. Nitrogen evaporator or water bath
10. Homogenizer

**Reagents Required But Not Provided**

1. Hydrochloric acid (HCl)
2. Acetonitrile ($CH_3CN$)
3. N-hexane ($C_6H_{14}$)
4. Dichloromethane ($CH_2Cl_2$)
5. Deionized water

## Protocol

Bring all reagents and samples to room temperature before use.

**A. Preparation of Samples and Reagents**

**1. Solutions**

- **Solution 1 – 0.15 M HCl solution.**

  Dilute 5 ml Concentrated HCl to 400 ml with deionized water, mix thoroughly.

- **Solution 2 – Sample extraction solution (for livestock, fish, shrimp, honey samples)**

  Add 10 ml of Solution 1 to 90 ml of Acetonitrile, mix thoroughly.

- **Solution 3 – Reconstitution buffer.**

  Dilute the 5X Reconstitution buffer with deionized water to 1:4 dilution. Solution 3 may be stored at 4 ºC for up to 1 month.

- **Solution 4 – Wash Buffer**

  Dilute the 20X Wash Buffer with deionized water to 1:19 dilution.

**2. Sample Pretreatment**

- **Muscle tissue (livestock, fish, shrimp) sample:** Remove as much fat as possible from the sample, then homogenize and mix fully. Weigh 2 g of muscle tissue homogenate into a centrifuge tube. Add 8 ml of Sample Extraction Solution (Solution 2) and vortex mix for 5 minutes, then centrifuge at 4000 rpm for 10 minutes. Collect 2 ml of the clear upper organic later and dry at 50 – 60 ºC via nitrogen evaporator or water bath. Add 1 ml of N-Hexane and vortex mix for 2 minutes, then add 1 ml of Reconstitution Buffer (Solution 3) and vortex mix for 30 seconds. Centrifuge at 4000 rpm for 5 minutes. Remove the upper N-Hexane layer and collect 50 µl of the lower water layer solution for analysis.

  *Note: Sample dilution factor: 2; detection limit: 0.3 ng/ml.*

- **Honey sample:** Weigh 1 g of Honey homogenate into a centrifuge tube. Add 6 ml of Sample Extraction Solution (Solution 2). Vortex mix for 5 minutes, then add 11 ml of Dichloromethane. Vortex mix for 5 minutes, then centrifuge at 4000 rpm for 5 minutes. Remove the supernatant and collect 8 ml of the lower layer organic solution to a dry tube. Dry at 50 – 60 ºC via nitrogen evaporator or water bath. Dissolve the dry residue in 1 ml of Reconstitution Buffer (Solution 3) then add 1 ml of N-Hexane and vortex mix for 30 seconds. Centrifuge at 3000 rpm for 5 minutes. Remove the upper N-Hexane layer and collect 50 µl of the lower layer solution for analysis.

  *Note: Sample dilution factor: 2; detection limit: 0.4 ng/ml.*

- **Milk sample:** Dilute the milk with Reconstitution Buffer (Solution 3) to 1:19 dilution (e.g., 25 µl milk into 475 µl Solution 3), vortex mix for 1 minute, then take 50 µl for analysis.

  *Note: Sample dilution factor: 20; detection limit: 3 ng/ml.*

- **Milk powder sample:** Weigh 0.5 g of homogenized sample into a centrifuge tube, add 5 ml deionized water and vortex mix to dissolve. Mix 100 µl sample solution with 400 µl Reconstitution Buffer (Solution 3) and vortex mix for 1 minute. Collect 50 µl for analysis.

  *Note: Sample dilution factor: 50; detection limit: 6 ng/ml.*

- **Eggs:** Weigh 1 g of homogenized egg sample into centrifuge tube, add 5 ml deionized water and vortex mix. Mix 100 µl sample solution with 400 µl Reconstitution Buffer (Solution 3) and vortex mix for 1 minute. Collect 50 µl for analysis.

  *Note: Sample dilution factor: 30, detection limit: 3 ng/ml.*

### B. Assay Procedure

Bring all reagents to room temperature prior to use.

1. Number the sample and ordered standard wells and record their positions. *All samples and standards should be tested in duplicate.*
2. Add 50 µl of standard or sample into the respective standard and sample wells.
3. Add 50 µl HRP Conjugate Reagent to each well,
4. Add 50 µl Antibody Working Solution to each well.
5. Cover the plate with a plate sealer, shake gently for 5 seconds to mix, and incubate at 25°C for 45 minutes in the dark.
6. Carefully remove the plate sealer and remove the liquid in each well. Immediately add 300 µl Wash Buffer (Solution 4) and wash.
7. Repeat this wash procedure 5 times, with 30 second intervals. Invert the plate and pat onto absorbent towels. Prick any bubbles remaining in the wells with a clean pipette tip.
8. Add 50 µl of Substrate Reagent A to each well.
9. Add 50 µl of Substrate Reagent B to each well. Shake gently for 5 seconds to ensure thorough mixing and incubate at 25°C for 15 minutes in the dark.
10. Add 50 µl of Stop Solution to each well and shake gently to ensure thorough mixing.
11. Within 10 minutes of introducing the Stop Solution, determine the optical density of each well with a microplate reader at 450 nm.

### C. Analysis of Results

**Calculating Sample Concentration from Standard Curve**

This assay is competitive, therefore there is an inverse correlation between Enrofloxacin concentration in the sample and the absorbance measured. Create a graph with the log of the standard concentration on the y-axis, and absorbance measured on the x-axis (a semi-logarithmic plot). Apply a best fit trendline through the standard points. Use this graph to calculate sample concentrations based on their OD values.

*Note: If the samples measured were diluted, multiply the dilution factor by the concentrations from interpolation to obtain the concentration before dilution.*

### D. Precautions

1. Bring all reagents to room temperature prior to use.
2. Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results. It is recommended to store samples to be used within 5 days at 4°C, within 1 month at -20°C and within 2 months at -80°C.
3. Do not allow the wells to dry fully during the washing procedure. Ensure the Wash Buffer is thoroughly mixed, and the wells are washed completely.
4. Do not use the Substrate Reagent if it has begun to turn blue without being added to the wells.
5. Do not use any reagents that are expired or use reagents from other kits with this assay.
6. The Stop Solution is caustic. Avoid contact with the skin or eyes, and thoroughly wash any spilled solution with water.
7. It is recommended that the same operator perform the experiment throughout, to avoid inconsistencies between operator procedures.