



## **Enrofloxacin ELISA Kit**

**Catalog Number. CSB-E12035f**

**This immunoassay kit allows for the in vitro quantitative determination of Enrofloxacin concentrations in tissue (chicken, pork, duck, fish, shrimp), egg, serum, honey.**

This package insert must be read in its entirety before using this product.

### **If You Have Problems**

### **Technical Service Contact information**

Phone: 86-27-87582341

Fax: 86-27-87196150

Email: [tech@cusabio.com](mailto:tech@cusabio.com)

Web: [www.cusabio.com](http://www.cusabio.com)

In order to obtain higher efficiency service, please ready to supply the lot number of the kit to us (found on the outside of the box).

## **PRINCIPLE OF THE ASSAY**

This assay employs the competitive enzyme immunoassay technique. The coupling antigen is pre-coated on the micro-well stripes. The Enrofloxacin in the testing sample competes with the coupling antigens pre-coated on the micro-well stripes for the antibodies against Enrofloxacin. After the addition of the enzyme conjugate, the TMB substrate is added for coloration. The optical density (OD) value of the testing sample has a negative correlation with the content of Enrofloxacin in it. This value is compared to the standard curve and the content of the corresponding Enrofloxacin is subsequently obtained.

## **DETECTION RANGE**

1 ppb-81 ppb.

## **SENSITIVITY**

The minimum detectable dose of the kit is typically less than 1 ppb.

The sensitivity of this assay, or Lower Limit of Detection (LLD) was defined as the lowest concentration that could be differentiated from zero.

## **Cross-reaction rate**

Enrofloxacin	100%
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## **Recovery rate**

Tissue, egg	80% ±15%
serum	80% ±15%
honey	75% ±15%

## **PRECISION**

### **Intra-assay Precision (Precision within an assay): CV%<8%**

Three samples of known concentration were tested twenty times on one plate to assess.

### **Inter-assay Precision (Precision between assays): CV%<10%**

Three samples of known concentration were tested in twenty assays to assess.

## **LIMITATIONS OF THE PROCEDURE**

- **FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.**
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Immunoassay, the possibility of interference cannot be excluded.

### **MATERIALS PROVIDED**

<b>Reagent</b>	<b>Quantity</b>
Assay plate (96 tests)	1
Standard	6 x 1 ml
HRP-conjugate	1 x 7 ml
Antibody	1 x 7 ml
Substrate A	1 x 7 ml
Substrate B	1 x 7 ml
Stop Solution	1 x 7 ml
Wash Buffer(20xconcentrate)	1 x 40 ml
Redissolving Solution(2xconcentrate)	1 x 50 ml

### **STANDARD CONCENTRATION**

Standard	S0	S1	S2	S3	S4	S5
Concentration (ppb)	0	1	3	9	27	81

### **STORAGE**

Unopened kit	Store at 2-8°C. Do not use the kit beyond the expiration date
Opened kit	May be stored for up to 1 month at 2-8° C.

**\*Provided this is within the expiration date of the kit.**

### OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm.
- An incubator which can provide stable incubation conditions up to  $37^{\circ}\text{C}\pm 0.5^{\circ}\text{C}$ .
- Squirt bottle, manifold dispenser, or automated microplate washer.
- Absorbent paper for blotting the microtiter plate.
- 100 ml and 500 ml graduated cylinders.
- Deionized or distilled water.
- Pipettes and pipette tips.
- Test tubes for dilution.
- Concentrated HCl, dichloromethane, N-hexane,  $\text{Na}_2\text{HPO}_4\cdot 12\text{H}_2\text{O}$ ,  $\text{NaH}_2\text{PO}_4\cdot 2\text{H}_2\text{O}$ , acetonitrile, heparin sodium
- Nitrogen or rotary evaporator

### PRECAUTIONS

The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

#### **Note:**

- **Kindly use graduated containers to prepare the reagent.**
- Bring all reagents to room temperature ( $18\text{-}25^{\circ}\text{C}$ ) before use for 30min.
- Distilled water is recommended to be used to make the preparation for reagents. Contaminated water or container for reagent preparation will influence the detection result.

## REAGENT PREPARATION

1. **Sample Diluent:** The **Redissolving Solution (2x)** is diluted with deionized water at 1:1 (eg: 1 mL **Redissolving Solution (2x)** + 1 mL deionized water, shake well).
2. **Wash Buffer:** If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals have completely dissolved. Dilute 40 mL of **Wash Buffer (20x)** with deionized or distilled water to prepare 800 mL of **Wash Buffer (1x)**. Keep it at 4°C for one month.
3. **Solution 1 (0.1M HCl solution):** Dissolve 860 µl of **concentrated HCl** to 100 mL of deionized water, shake well.
4. **Solution 2 (acetonitrile- dichloromethane mixed solution):**  
 $V_{\text{acetonitrile}}: V_{\text{dichloromethane}} = 1:4$   
(eg: 1 mL **acetonitrile** + 4 mL **dichloromethane**, shake well).
5. **Solution 3(pH=7.2 0.02M PB buffer):**  
Dissolve 5.16g  $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ , 0.87g  $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$  into deionized water to 1000 mL.
6. **Solution 4 (acetonitrile- dichloromethane- 0.1M HCl mixed solution):**  
Take 100 mL of acetonitrile- dichloromethane mixed solution ( $V_{\text{acetonitrile}}: V_{\text{dichloromethane}} = 4:1$ ), add 5 mL 0.1M HCl solution, shake well.

### **Note:**

1. CUSABIO is only responsible for the kit itself, but not for the samples consumed during the assay. The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient samples in advance.
2. If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.
3. Please predict the concentration before assaying. If values for these are not within the range of the standard curve, users must determine the

- optimal sample dilutions for their particular experiments.
4. Tissue or cell extraction samples prepared by chemical lysis buffer may cause unexpected ELISA results due to the impacts of certain chemicals.
  5. Owing to the possibility of mismatching between antigen from other resource and antibody used in our kits (e.g., antibody targets conformational epitope rather than linear epitope), some native or recombinant proteins from other manufacturers may not be recognized by our products.

## **SAMPLE COLLECTION AND STORAGE**

### **A: Tissue (pork, chicken, porcine liver, duck, shrimp, fish, etc)**

- (1) Weigh  $2.0 \pm 0.05$  g of the homogenized sample, put into 50 mL centrifugal tube.
- (2) Add 8 mL of acetonitrile-dichloromethane mixed solution (**Solution 2**), vortex for 5min. Centrifuge at more than 4000 r/min for 10 min at 15°C.
- (3) Transfer 4 mL of organic layer into a new centrifugal tube, evaporated to dryness at 56°C by nitrogen or rotary evaporator.
- (4) Add 1 mL of **Sample Diluent** and 1 mL of **N-hexane**, shake for 30s. Centrifuge at more than 4000 r/min for 5 min at 15°C.
- (5) Take 50  $\mu$ L of under layer for further analysis.  
Dilution factor of the samples: 1

### **B: Chicken blood**

- (1) Collect chicken blood using heparin sodium as an anticoagulant (20-30 unit/mL). Stand for one hour at room temperature. Centrifuge at more than 4000 r/min for 10 min at 15°C. Collect 1ml plasma.
- (2) Add 4 mL of **Acetonitrile**, mix well for 5 min. Centrifuge at more than 4000 r/min for 10 min at 15°C.
- (3) Transfer supernatant into a new centrifugal tube, add 2 mL of 0.02M PB

buffer (**Solution 3**). Mix well.

- (4) Add 5 mL of **Dichloromethane**, mix well for 5 min. Centrifuge at 4000 r/min for 10 min at 15°C.
  - (5) Remove supernatant. Transfer lower organic phase into a drying bottle, evaporated to dryness at 50°C by nitrogen or rotary evaporator.
  - (6) Add 1 mL of **Sample Diluent** and 1 mL of **N-hexane**. Shake for 30s. Centrifuge at more than 4000 r/min for 5 min at 15°C.
  - (7) Take 100 µL lower phase, add 100 µL of **Sample Diluent**. Shake for 30s.
  - (8) Take 50 µL for further analysis.
- Dilution factor of the samples: 2

### **C: Honey**

- (1) Weight  $2.0 \pm 0.05$ g honey into 50 mL centrifugal tube, then add 8 mL of **acetonitrile-dichloromethane-0.1M HCl** mixed solution (**Solution 4**), mix well for 3 min. Centrifuge at more than 4000 r/min for 10 min at 15°C.
  - (2) Transfer 2 mL supernatant into a new centrifugal tube, evaporated to dryness at 56°C by nitrogen or rotary evaporator.
  - (3) Add 1 mL of **Sample Diluent**, shake for 1 min.
  - (4) Take 50µL for further analysis.
- Dilution factor of the samples: 2



## ASSAY PROCEDURE

**Bring all reagents and samples to room temperature before use. Centrifuge the sample again after thawing before the assay. It is recommended that all samples and standards be assayed in duplicate.**

1. Prepare all reagents and samples as directed in the previous sections.
2. Determine the number of wells to be used and put any remaining wells and the desiccant back into the pouch and seal the ziploc, store unused wells at 2-8°C.
3. Add 50  $\mu$ L of **Standard** or **Sample** per well. Add 50  $\mu$ L of **HRP-conjugate** to each well.
4. Then add 50  $\mu$ L of **Antibody** to each well. Cover the microtiter plate with a new adhesive strip and mix well, then incubate for 30 min at 25°C.
5. Aspirate each well and wash, repeating the process 4~5 times. Wash by filling each well with 250  $\mu$ L of **Wash Buffer (1x)** using a squirt bottle, multi-channel pipette, manifold dispenser, or autowasher, and let it stand for 15~30 seconds, complete removal of liquid at each step is essential to good performance.
6. Add 50  $\mu$ L of **Substrate A** and 50  $\mu$ L of **Substrate B** to each well, mix well. Incubate for 15 minutes at 25°C. Protect from light.
7. Add 50  $\mu$ L of **Stop Solution** to each well, gently tap the plate to ensure thorough mixing.
8. Determine the optical density of each well within 5 min, using a microplate reader set to 450 nm (Recommend to read the OD value at the dual-wavelength: 450/630 nm within 5 min).

Note:

1. The final experimental results will be closely related to validity of the products, operation skills of the end users and the experimental environments.
2. Samples or reagents addition: Please carefully add samples to wells and mix gently to avoid foaming. Do not touch the well wall as possible. For each step in the procedure, total dispensing time for addition of reagents or samples to the assay plate should not exceed 10 min. This will ensure equal elapsed time for each pipetting step, without interruption. Duplication of all standards and specimens, although not required, is recommended. To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
3. Incubation: To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered for extended periods between incubation steps. Once reagents have been added to the well strips, DO NOT let the strips DRY at any time during the assay. Incubation time and temperature must be observed.
4. Washing: The wash procedure is critical. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Solution by aspirating or decanting and remove any drop of water and fingerprint on the bottom of the plate. Insufficient washing will result in poor precision and falsely elevated absorbance reading. When using an automated plate washer, adding a 15–30 second soak period following the addition of wash buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
5. Controlling of reaction time: Observe the change of color after adding Substrates (e.g. observation once every 10 min). Substrates should change from colorless or light blue to gradations of blue. If the color is too deep, add Stop Solution in advance to avoid excessively strong reaction which will result in inaccurate absorbance reading.
6. Substrates are easily contaminated. Substrates should remain colorless or light blue until added to the plate. Please protect it from light.
7. Stop Solution should be added to the plate in the same order as the Substrates. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrates.

## CALCULATION OF RESULTS

There are two methods to judge the results: the first one (A) is the rough judgment, while the second (B) is the quantitative determination. Note that the OD value of the sample has a negative correlation with Enrofloxacin in the sample.

A:

Compare the sample average absorbance values with standards values, the Enrofloxacin concentration in the samples can be concluded. For example, the absorbance value of sample 1 is 0.238, the absorbance value of sample 2 is 0.946; absorbance values of standard are: 1.845, 1.542, 1.130, 0.635, 0.326, 0.156 and the corresponding concentrations are: 0 ppb, 1 ppb, 3 ppb, 9 ppb, 27 ppb, 81 ppb; then the Enrofloxacin in sample 1 and sample 2 are 27 ppb-81 ppb and 3 ppb-9 ppb; Lastly the reader is multiplied by the corresponding dilution factor of each sample and the actual concentration of sample is obtained.

B:

The mean values of the absorbance values obtained for the standards and the samples are divided by the absorbance value of the first standard (zero standard) and multiplied by 100%. The zero standard is thus made equal to 100% and the absorbance values are quoted in percentages.

$$\text{Absorbency value (\%)} = \frac{B}{B_0} \times 100\%$$

B —the average absorbance value of the sample or standard

B<sub>0</sub> —the average absorbance value of the 0 ppb standard

To draw a standard curve: Take the absorbency value of standards as y-axis, semi-logarithmic of the concentration of the Enrofloxacin standards solution (ppb) as x-axis.

The Enrofloxacin concentration of each sample (ppb), which can be read from the calibration curve, is multiplied by the corresponding dilution factor of each

sample followed, and the actual concentration of sample is obtained. (The software offered together will facilitate the calculation process, it's suitable for accurate and fast analysis of large scale samples, please contact us)

**Note:**

- Discard the substrate with any color that indicates the degeneration of this solution; when the absorbance value of standard solution 0 less than 0.5 indicates its degeneration.
- The optimum reaction temperature is 25°C, and too high or too low will result in the changes in the absorbance value and detecting sensitivity.