Version 18-04/24

User's Manual

Enrofloxacin ELISA Kit

REF **DEIA029**

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This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

This kit can be used in quantitative analysis of enrofloxacin in animal tissues, milk and eggs, etc. **For research use only, not for use in diagnostic procedures.**

General Description

Enrofloxacin (EN) drug is one of the most important anti-infective drugs, which is widely used for treatment, prevention and growth promotion. However, drug resistance and potential carcinogenicity of the substance have aroused widespread concern.

CD's enrofloxacin enzyme-linked immunosorbent assay kit is developed using the latest biotechnology, with the characteristics of convenience, rapidity and sensitivity.

Principles of Testing

This kit is based on indirect-competitive ELISA technology. The microtiter wells are coated with coupling antigen. Enrofloxacin residue in the sample competes with the antigen coated on the microtiter plate for the antibody. After the addition of Enzyme conjugate antibody, substrate A and B are used to show the color.

Reagents And Materials Provided

1. Standard solution: 0 ppb, 0.02ppb, 0.06 ppb, 0.18 ppb, 0.54 ppb, 1.62 ppb, 1 mL/bottle.

2. Microtiter plate coated with antigen: 1 piece (96 wells)

3. 11×Enzyme conjugate antibody: 1 bottle (0.7mL/bottle)

4. Enzyme conjugate diluent: 1 bottle (7mL/bottle)

5. 10×Sample buffer: 1 bottle (10 mL/bottle)

6. 20×Wash solution: 1 bottle (30 mL/bottle)

7. Substrate A solution: 1 bottle (7 mL/bottle)

8. Substrate B solution: 1 bottle (7 mL/bottle)

9. Stop solution: 1 bottle (7mL)

10. Plate sealer

11. Ziplock bag

Materials Required But Not Supplied

Microtiter plate spectrophotometer (450nm/630nm)

Analytical balance (inductance: 0.01 g)

Centrifuge (4000 g and above)

Incubator (adjustable 25°C)

Shaker

Vortex

Micropipette: (20µl-200µl, 100µl-1000µl, 30-300µl-multipipette)

Timer

Deionized water

NaCl (AR)

HCl (AR)

Storage

2-8°C, do not freeze. Unused microtiter plates must be sealed and stored at 2-8°C.

Specimen Collection And Preparation

1. Tissue method 1 (dilution factor: 10 times)

Weigh 1.0±0.05g sample into a 10mL tube. Add 4 ml of 1.5% NaCl. Vortex at high speed for 1 min.

Centrifuge for separation: room temperature, 5min, at 3000g (>3000g);

Pipete 200 µl supernatant into a 2ml tube. Add 200 µl 1× Sample buffer (see Reagent Preparation), and vortex for 10s with a vortexer;

Take 50 µl for analysis.

2. Milk method 1 (dilution factor: 10 times)

Weigh 1.0±0.05g sample into a 10ml tube. Add 4 ml deionized water. Add 40 µl 1 M HCl (see Reagent Preparation), vortex at high speed for 1min. Centrifuge for separation: room temperature, 5min, at 3000g (>3000g);

Pipete 200 µl supernatant into a 2ml tube. Add 200 µl 1× Sample buffer (see Reagent Preparation), and vortex for 10s with a vortexer;

Take 50 µl for analysis.

3. Egg method 1 (dilution factor: 10 times)

Weigh 1.0±0.05g sample into a 10ml tube. Add 4 ml deionized water. Vortex at high speed for 1min. Centrifuge for separation: room temperature, 5min, at 3000g (>3000g);

Pipete 200 µl supernatant into a 2ml tube. Add 200 µl 1× Sample buffer (see Reagent Preparation), and vortex for 10s with a vortexer;

Take 50 µl for analysis.

4. Tissue method 2 (dilution factor: 100 times)

Weigh 1.0±0.05g sample into a 10mL tube. Add 4 ml of 1.5% NaCl. Vortex at high speed for 1 min. Centrifuge for separation: room temperature, 10min, at 3000g (>3000g);

Pipete 50 µl supernatant into a 2ml tube. Add 950 µl 1× Sample buffer (see Reagent Preparation), and vortex for 10s with a vortexer;

Take 50 µl for analysis.

5. Milk method 2 (dilution factor: 100 times)

Weigh 1.0±0.05g sample into a 10ml tube. Add 4 ml deionized water. Add 40 µl 1 M HCl (see Reagent Preparation), vortex at high speed for 1min. Centrifuge for separation: room temperature, 5min, at 3000g (>3000g);

Pipete 50 µl supernatant into a 2ml tube. Add 950 µl 1× Sample buffer (see Reagent Preparation), and vortex for 10s with a vortexer;

Take 50 µl for analysis.

6. Egg method 2 (dilution factor: 100 times)

Weigh 1.0±0.05g sample into a 10ml tube. Add 4 ml deionized water. Vortex at high speed for 1min. Centrifuge for separation: room temperature, 5min, at 3000g (>3000g);

Pipete 50 µl supernatant into a 2ml tube. Add 950 µl 1× Sample buffer (see Reagent Preparation), and vortex for 10s with a vortexer;

Take 50 µl for analysis.

7. Rabbit serum sample preparation method

Take 50µL rabbit serum, add 950 µl 1× Sample buffer, and vortex for 1min with a vortexer;

Take 50 µL for analysis.

Plate Preparation

Get the microwells needed out and return the rest into the zip-lock bag at 2-8°C immediately.

Number every microwell position and all standards and samples should be run in duplicate. Record the standards and samples positions.

Reagent Preparation

1. 1×Wash solution: Dilute the 20×Wash solution with deionized water in the volume ratio of 1:19.
2. 1×Sample buffer: Dilute the 10× Sample buffer with deionized water in the volume ratio of 1:9.
3. 1 M HCl: Dilute the 8.6 mL of HCl with deionized water to make up to 100 mL.
4. 1.5% NaCl solution: Solute the 1.5g of NaCl with 100mL of deionized water.
5. Enzyme conjugate solution: Dilute the 11×Enzyme conjugate antibody with Enzyme conjugate diluent in the volume ratio of 1:10.

Assay Procedure

1. Adding samples: Add 50 µL of standard working solution/sample solution to the corresponding microwells. Add 50 µL **Enzyme conjugate solution** to each well.

2. Incubation: Gently shake the ELISA plate for 10s to fully mix the liquid in the well. Cover the plate sealer and react in the dark at 25°C for 30 min.
3. Washing: After taking out the microtiter plate, carefully peel off the plate sealer. Pour off the liquid in the plate well. Add 250 µL of 1×Wash solution to each well. Soak for 15-30s. Pour off the wash solution. Then add the 1×Wash solution and repeat washing for 3-4 times, then put the microplate plate upside down on the absorbent paper, and remove remaining water.
4. Reaction: Add 100 µL of the mixture of Substrate A solution and Substrate B solution to each well. (**Note: The substrate A solution and the substrate B solution must be fully mixed according to the volume 1:1. The mixed solution should be used within 10 minutes. Do not use metal containers to hold or stir the reagents to avoid substrate deterioration and failure**)
5. Incubation: Gently shake the plate for 10s to fully mix the liquid in the well. Cover the plate sealer and react in the dark at 25°C for 15 minutes
6. Stop: Add 50 µL/well of Stop solution to the microwells after the reaction. The substrate solution changed from blue to yellow indicating successful termination.
7. Measure: It' s suggested measure with dual-wavelength of 450/630nm. Read the result within 5min after addition of stop solution

Calculation

The mean values of the absorbance values obtained from the standards and the samples are divided by the absorbance value of the first standard (zero standard) and multiplied by 100%.

$$\text{Absorbance}(\%)=100\%\times B/B_0$$

B ——absorbance of standards or samples

B_0 ——absorbance of zero standard (0ng/ml)

Use the semi-logarithmic system to substitute the percentage of absorbance corresponding to the standard, and fit the standard curve with the concentration of the standard.

Substitute the percentage of absorbance of the sample to be tested into the fitted standard curve equation to obtain the corresponding concentration of the sample. Finally, multiply by the corresponding dilution factor of the sample to obtain the content of the detected substance in the sample.

Performance Characteristics

Best value of B_0 absorbance: >0.8

Precision

Intra-Assay: CV<5%

Inter-Assay: CV<15%

Detection Range

0.02ppb-1.62 ppb

Detection Limit

Tissue, egg, milk method 1: about 1.0ppb

Tissue, egg, milk method 2: about 10 ppb

Rabbit serum sample: 1ppb

Sensitivity

0.02 ppb

Specificity

Enrofloxacin: 100%

Recovery

90%±15%

Rabbit serum sample: 100%±20%

Precautions

1. Please read the instructions carefully before using the kit.
2. Make sure all reagents and microwells are all at room temperature (20-25°C) before using the kit.
3. Shake the reagent well before use. Avoid creating air bubbles when adding samples.
4. The tips of the pipette is a one-time product. In order to prevent cross-contamination of the reagents, the pipette tips must not be reused.
5. Do not use expired kits. Reagents in kits with different batch numbers must not be mixed.
6. Please analyze the sample immediately after processing, otherwise the test result may be affected.
7. Substrate A and Substrate B are both colorless and transparent liquids. If they turn blue before use, or turn blue immediately after mixing, it means that the reagents have been contaminated or deteriorated.
8. The sample addition process must be fast under the premise of ensuring accuracy, so as to avoid the influence of the reaction time difference on the test results.
9. The stop solution contains sulfuric acid. If it accidentally splashes on the skin or clothing, please rinse it with plenty of water immediately. If it gets into your eyes accidentally, please go to the hospital for examination after washing thoroughly.

Limitations

This kit is a quantitative or semi-quantitative kit. Recommended for bulk sample screening analysis. If the test result is positive, it is recommended to use instrumental methods to carry out confirmatory experiments (such as GC/MS, LC-MS/MS, etc.)