QUALICHEK™ Enrofloxacin ELISA

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Ver 1.0

RUO

Quantitative testing of Enrofloxacin in samples, such as honey, animal tissues, milk, eggs etc.

RUO	**For Research Use Only**	**REF**	**Catalog Number**
	**Store At**	**LOT**	**Batch Code**
	**Manufactured By**		**Biological Risk**
	**Expiry Date**		**Consult Operating Instructions**

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Introduction:

Enrofloxacin (ENR) is a fluoroquinolone antibiotic sold by the Bayer Corporation under the trade name Baytril. Antibiotics have served to protect humanity from deadly pathogenic infections since the discovery of penicillin in 1928. However, overestimating the effectiveness of antibiotics has led to their overuse and numerous unwanted side-effects. Excessive exposure to antibiotics can cause symptoms such as nausea, vomiting, diarrhea, headache, and insomnia, but the most urgent problem associated with antibiotic overuse is the emergence of antibioticresistant bacteria.

Intended Use:

This kit is for quantitative testing for Enrofloxacin in sample, such as honey, animal tissues, milk, eggs etc.

Principle:

This kit uses Indirect-Competitive-ELISA as the method. It can be used to detect Enrofloxacin (ENR) in samples, such as honey, animal tissues (chicken, porcine, fish, and shrimp) milk, eggs, etc. This kit is composed of ELISA Microtiter plate, HRP conjugate, antibody, standard and other supplementary reagents. The micro-plate provided in this kit has been pre-coated with ENR antigen. During the reaction, ENR in the samples or standard competes with ENR antigen coated on the solid phase supporter for ENR antibody. Then Horseradish Peroxidase (HRP) conjugate is added to each micro plate well, and TMB substrate 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.

Materials Provided:

1. Coated Microtiter plate - 1x 96 wells
2. Standards Liquid -1mL each (0 ppb, 0.1 ppb, 0.3 ppb, 0.9 ppb, 2.7 ppb, 8.1 ppb)
3. High Concentrated Standard (100 ppb) - 1mL
4. HRP Conjugate - 5.5 mL
5. Antibody Working Solution - 5.5 mL
6. Substrate A - 6 mL
7. Substrate B - 6 mL
8. Stop Solution - 6 mL
9. Wash Buffer (20X) - 40 mL
10. Plate Sealer - 3 pieces

Material required but not provided:

Instrument: Microtiter plate reader, Printer, Homogenizer, Nitrogen Evaporators/Water bath, Oscillators, centrifuge, Graduated pipette, Balance (sensibility 0.01 g)

High-precision transferpettor:single channel (20-200 µL, 100-1000 µL), Multichannel (300 µL) micropipettes.

Reagents: Anhydrous acetonitrile, N-hexane, Concentrated hydrochloric acid (HCl),

Storage Information:

1. Store main kit components at 2-8°C Avoid freeze / thaw cycles.
2. Before using, bring all components to room temperature (18-25°C). Upon assay completion return all components to appropriate storage conditions.

Sample pretreatment:

Bring 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:**

Solution 1: 0.15 M HCl Solution.Dilute 5 mL of concentrated hydrochloric acid (HCl) with deionized water to a final volume of 400 mL, mix fully.
Solution 2: Sample Extraction Solution.Add 10 mL of 0.15 M HCl to 90mL of anhydrous acetonitrile, mix fully.
Solution 3 Reconstitution Buffer: Dilute the 5×Reconstitution Buffer with deionized water. (5×Reconstitution Buffer :deionized water=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:**

Pretreatment of animal tissue sample:
1. Weigh 2±0.05 g of tissue homogenate into a 50 mL EP tube.
2. Add 8 mL of Sample Extraction Solution (Solution 2) and oscillate for 5 min. Centrifuge at 4000 r/min for 10 min at room temperature.
3. Remove 2 mL of the clear upper organic layer solution to a clean and dry glass tube, dry at 50-60℃ with Nitrogen Evaporators/Water bath.
4. Add 1 mL of N-hexane and oscillate for 2 min. Then add 1 mL of Reconstitution buffer (Solution 3) and oscillate for 30 sec to mix fully. Centrifuge for 5 min at 4000 r/min at room temperature.
5. Remove the N-hexane upper layer, take 50 µL of the lower water layer solution for analysis.

Note: Sample dilution factor: 2, minimum detection dose: 0.3 ppb

Pretreatment of honey sample:
1. Weigh 1±0.05 g of honey into a 50 mL EP tube, add 6 mL of Solution 2 and oscillate for 5 min to ensure thoroughly dissolved.
2. Add 3 mL of Solution 3 and 11 mL of dichloromethane, oscillate for 5 min. Then centrifuge at 4000 r/min for 5 min at room temperature.
3. Remove the supernatant and transfer 8 mL of the upper layer organic solution to a dry container. Dry at 50-60℃ with Nitrogen Evaporators/Water bath.
4. Dissolve the dry residue with 1 mL of Reconstitution buffer. Add 1 mL of N-hexane and oscillate for 30 sec. Centrifuge for 5 min at a speed of over 3000 r/min 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, minimum detection dose: 0.4 ppb

Pretreatment of milk sample:
1. Dilute the milk with solution 3 for 20 times (e.g., add 25 µL of milk into 475 µL of Solution 3), oscillate for 1 min to dissolve it fully.
2. Take 50 µL for detection and analysis.

Note: Sample dilution factor: 20, minimum detection dose: 3 ppb

Pretreatment of milk powder sample:
1. Weigh 0.5±0.02 g of homogenate sample into a 10 mL EP tube, add 5 mL of deionized water and oscillate to dissolve it fully.
2. Mix 100 µL of sample solution with 400 µL of Reconstitution buffer. Oscillate for 1 min.
3. Take 50 µL for detection and analysis.

Note: Sample dilution factor: 50, minimum detection dose: 6 ppb

Pretreatment of eggs sample:

1. Weigh 1±0.02 of homogenate egg into a 10 mL EP tube, add 5 mL of deionized water and oscillate to dissolve it fully.
2. Mix100 µL of sample solution with 400 µL of Reconstitution buffer. Oscillate for 1 min.
3. Take 50 µL for detection and analysis.

Note: Sample dilution factor: 30, minimum detection dose: 3 ppb

Assay procedure:

Centrifuge the sample again after thawing before the assay. Bring all reagents to room temperature for 30 min before use. **All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming.**

1. **Number:** number the sample and standard in order (multiple well), and keep a record of standard wells and sample wells.
2. **Add sample:** add 50 µL of Standard or Sample per well, then add 50µL of HRP conjugate to each well, then add 50 µL of antibody working solution, cover the plate with sealer, oscillate for 5 s gently to mix thoroughly, incubate for 45 min at 25℃.
3. **Wash:** uncover the sealer carefully; remove the liquid in each well. Immediately add 300 µL of wash buffer to each well and wash. Repeat 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 shading light for 15 min at 25℃ (The reaction time may be shortened or prolonged according to the depth of the color).
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 with a microplate reader (the 450/630 nm double wavelength is recommended). This step should be finished in 10 min after stop reaction.

Judgment of result:

Absorbance (%) =A/A0×100%

A: Average absorbance of standard or sample

A_0: Average absorbance of 0 ppb Standard

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 software for accurate and fast analysis on a large number of samples.

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. ELISA Microtiter plate should be covered by plate sealer. Avoid the reagents to strong light.
5. Do not use expired kit or reagents of different batches.
6. TMB should be abandoned if it turns color. When OD value of standard (concentration: 0)＜0.5 unit (A450nm＜0.5), it indicates the reagent may be deteriorated.
7. Stop solution is caustic; avoid contact with skin and eyes.

Technical specification:

Sensitivity: 0.1 ppb (ng/mL)
Reaction mode: 25℃, 45 min~15 min
Detection limit: Tissue (chicken, porcine, fish, shrimp) ---0.3 ppb.
Honey ---0.4 ppb, Milk---3 ppb, Milk powder---6 ppb, Eggs---3 ppb.
Reactivity: Enrofloxacin---100%, Oxolinic acid---28%, Levofloxacin--10%,
Lomefloxacin---4%, Marbofloxacin---4%, Sarafloxacin---2%
Sample recovery rate: Tissue, Honey, Milk, Milk powder, Eggs---85%±15%.

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