Elabscience®

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FQNs (Fluoroquinolones) ELISA Kit

Catalog No: E-FS-E053

96T

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.

Phone: 240-252-7368(USA) 240-252-7376(USA)
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 Indirect-Competitive-ELISA as the method. It can be used to detect FQNs (Fluoroquinolones) in samples, such as honey, animal tissues (chicken, porcine, fish, and shrimp) milk, eggs, etc. This kit is composed of Micro ELISA Plate, HRP conjugate, antibody, standard and other supplementary reagents. The micro-plate provided in this kit has been pre-coated with FQNs antigen. During the reaction, FQNs in the samples or standard competes with FQNS antigen coated on the solid phase supporter for FQNs 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 FQNs. The concentration of FQNs in the samples can be calculated by comparing the OD of the samples to the standard curve.

Technical indicator

Sensitivity: 0.1 ppb (ng/mL)

Reaction mode: 25℃,45 min~15min

Detection limit: Tissue (chicken, porcine, fish, shrimp) ---0.3 ppb.

Honey ---0.4 ppb, Milk---3 ppb, Milk powder---6 ppb, Eggs---3 ppb, Urine---0.5 ppb.

Cross-reactivity:

Enrofloxacin	100%
Norfloxacin	174%
Ciprofloxacin	170%
Lomefloxacin	132%
Flumequine	126%
Peflacine	125%
Danofloxacin	110%
Sarafloxacin	107%
Difloxacin	84%
Enoxacin	66%
Ofloxacin (racemate)	58%
Oxolinic acid	28%
Levofloxacin	10%
Marbofloxacin	4%

Sample recovery rate: Tissue, Honey, Milk, Milk powder, Eggs---85%±15%.

Kits components

Item	Specifications
Micro ELISA Plate	96 wells
Standard Liquid (black cap)	1mL each (0 ppb, 0.1 ppb, 0.3 ppb, 0.9 ppb, 2.7 ppb, 8.1 ppb)
High concentrated Standard (100 ppb)	1 mL
HRP Conjugate (red cap)	5.5 mL
Antibody Working Solution (blue cap)	5.5 mL
Substrate Reagent A (white cap)	6 mL
Substrate Reagent B (black cap)	6 mL
Stop Solution (yellow cap)	6 mL
20×Concentrated Wash Buffer (white cap)	40 mL
5×Reconstitution Buffer (yellow cap)	50 mL
Plate Sealer	3 pieces
Sealed Bag	1 piece
Manual	1 copy

Other supplies required

Instrument: Microplate reader, Printer, Homogenizer, Oscillators, Centrifuge, Graduated pipette, Balance (sensibility 0.01g),

High-precision transferpettor: Single-channel (20-200 μL, 100-1000 μL), Multi-channel (300 μL)

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

Experimental preparation

Bring all reagents and samples to room temperature before use.

Open the micro-plate 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.

   Add 5 mL of Concentrated hydrochloric acid (HCl) to 400 mL of deionized water, 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

3.1 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 or air.

(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 layer solution for analysis.

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

3.2 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 or air.

(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

3.3 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

3.4 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) Mix100 μL of sample solution with 400 μL of 1×Reconstitution solution. Oscillate for 1 min.

(3) Take 50 μL for detection and analysis.

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

3.5 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

3.6 Pretreatment of urine sample:

(1) Take 1 mL of clear urine sample and add 4 mL of Reconstitution buffer. Oscillate for 30 sec.

(2) Take 50 μL for detection and analysis.

Note: Sample dilution factor: 5, minimum detection dose: 0.5 ppb

Assay procedure

Centrifuge the sample again after thawing before the assay. Bring all reagents to room temperature 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 50 μL of HRP Conjugate to each well. Add 50 μL of antibody working solution. Gently oscillate for 5 sec to mix thoroughly and cover the plate with sealer. Incubate for 45 min at 25℃.
3. **Wash:** Uncover the sealer carefully, remove the liquid in each well. Immediately add 250 μL of wash buffer to each well and wash. Repeat the wash procedure for 5 times, 30 sec 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 solution A to each well, and then add 50 μL of substrate solution B. Gently oscillate for 5 sec to mix thoroughly. Incubate for 15 min at 25℃ in the dark.
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 10min 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 software for accurate and fast analysis of batch samples.

Notes

1. The overall OD values will be lower when reagents have not been brought to room temperature before use or the 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 repeatability of this ELISA kit.
4. Micro ELISA plate should be covered with plate sealer. Prevent the reagents to strong light.
5. Do not use expired kit and reagents of different batches.
6. TMB should be abandoned if it turns color. When OD value of standard(concentration: 0)＜0.5 unit(A_{450nm}＜0.5), it indicates the reagent is deteriorated.
7. Stop solution is caustic, avoid of contacting with the skin.

Storage and valid period

Storage: Store at 2-8℃. Avoid freeze / thaw cycles.

Valid Period: 1 year, production date is on the packing box.