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(FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !)

CPFX(Ciprofloxacin) ELISA Kit

Catalog No: E-FS-E033

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)

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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 Ciprofloxacin (CPFX) 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 CPFX antigen. During the reaction, CPFX in the samples or standard competes with CPFXantigen coated on the solid phase supporter for CPFXantibody. 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 CPFX. The concentration of CPFX 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°C,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---3ppb.

Cross-reactivity: Ciprofloxacin---100%, Oxolinic acid---28%, Levofloxacin---10%,
Lomefloxacin---4%, Marbofloxacin---4%, Sarafloxacin---2%

Sample recovery rate:Tissue, honey, milk, milk powder, eggs---85% ± 15%.

Kits components

Item	Specifications
Micro ELISA Plate	96 wells
Standard Solution	1mL each (0ppb, 0.1 ppb,0.3 ppb,0.9 ppb, 2.7 ppb, 8.1 ppb)
High Concentrated Standard (100ppb)	1mL
HRPConjugate(Red cap)	5.5mL
Antibody Working Solution (Blue cap)	5.5mL
Substrate Reagent A (White cap)	6mL
Substrate Reagent B (Black cap)	6mL
Stop Solution (Yellow cap)	6mL
20×Concentrated Wash Buffer (White cap)	40mL
5×Redissolved Buffer (Yellow cap)	50mL
Product Description	1 copy

Other supplies required

Instruments: Microplatereader, Printer, Homogenizer, Oscillators, Centrifuge, Graduated pipette, Balance (sensitivity 0.01g).

High-precision transferpette: Single-channel (20-200 μ L, 100-1000 μ L), Multi-channel (300 μ L).

Reagents: Anhydrous acetonitrile, N-hexane, Concentratedhydrochloricacid (HCl).

Experimental preparation

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: 0.15M HCl. Add 5 mL Concentrated hydrochloric acid (HCl) to 400 mL deionized water, mix fully.

Solution 2: Sample extraction solution. Add 10 mL 0.15M HCl to 90 mL anhydrous acetonitrile, mix fully.

Solution 3: Re-dissolve solution. Dilute the 5 \times Concentrated Re-dissolve solution for 5 times with deionized water (for sample re-dissolution). It can be stored at 4 $^{\circ}$ C for 1 month.

Solution 4: Wash working buffer. Dilute the 20 \times Concentrated Wash Buffer with deionized water (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 solution 2 and oscillate for 5 minutes. 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 with Nitrogen Evaporators at 50-60 $^{\circ}$ C.
- (4) Add 1 mL N-hexane and oscillate for 2 min. Then add 1 mL of re-dissolve solution (solution 3) and oscillate for 30 seconds to mix fully. Centrifuge for 5 min at 4000 r/min at room temperature.
- (5) Remove the N-hexane upper layer, take 50 μ L 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 10 min at room temperature.
- (3) Remove the supernatant and transfer 8 mL of the upper layer organic solution to a dry container. Dry with Nitrogen Evaporators at 50-60 $^{\circ}$ C.
- (4) Dissolve the dry residue with 1 mL re-dissolve solution. Add 1 mL N-hexane and oscillate for

30seconds. Centrifuge for 5 min at a speed of over 3000r/minat room temperature.

- (5) Remove the N-hexane upper layer, take 50 μ L of the lower layer solution for analysis.

Note: Sample dilution factor:2, minimum detection dose: 0.4 ppb

3.3 Pretreatmentof milk sample:

- (1) Dilute the milk with solution 3 for 20times (eg: add 25 μ L of milk into 475 μ L of solution 3), oscillatefor 1 minute to dissolve it fully.
- (2) Take 50 μ Lfor detection and analysis.

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

3.4 Pretreatmentofmilk powder sample:

- (1) Weigh 0.5 ± 0.02 g of homogenate sample into a 10 mL EP tube, add5 mLof deionized water and oscillate to dissolve it fully.
- (2) Mix100 μ L of sample solution with 400 μ L of re-dissolve solution. Oscillate for 1 minute.
- (3) Take 50 μ Lfor detection and analysis.

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

3.5 Pretreatmentof eggs sample:

- (1) Weigh 1 ± 0.02 of homogenate egg into a 10mL EP tube, add 5 mLof deionized water and oscillate to dissolve it fully.
- (2) Mix100 μ L of sample solution with 400 μ L of re-dissolve solution. Oscillate for 1 minute.
- (3) Take 50 μ Lfor 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 before use. **All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming.** Dilute 40mL of concentrated wash buffer into 800mL wash working buffer with deionized or distilled water.

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 50µL of Standard, Blank, or Sample per well, then add 50µL of HRP conjugate to each well. Add 50µL of antibody working solution. Gently oscillate for 5s to mix thoroughly and cover the plate with sealer. Incubate for 45min at 25°C .
3. **Wash:** Uncover the sealer carefully, remove the liquid in each well. Immediately add 250µL of wash working buffer to each well and wash. Repeat the wash procedure for 5 times, 30s 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. **HRP conjugate:** add 100µL HRP conjugate to each well, shading light incubation for 30min at 25°C .
5. **Wash:** repeat step 3
6. **Color Development:** Add 50µL of substrate solution A to each well, and then add 50µL of substrate solution B. Gently oscillate for 5s to mix thoroughly. Incubate for 15min at 25°C in the dark.
7. **Stop reaction:** Add 50µL of stop solution to each well, oscillate gently to mix thoroughly.
8. **OD Measurement:** Determine the optical density (OD value) of each well at 450nm 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(%) = $A/A_0 \times 100\%$**

A: Average absorbance of standard or sample

A₀: Average absorbance of Oppb 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°C.
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°C. Avoid freeze / thaw cycles.

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