Fluoroquinolones ELISA Kit

rev 11/20

(Catalog # K4205-100, 100 assays, Store at 4°C)

I. Introduction:

Fluoroquinolones drugs belong to chemical synthesis antibacterial and are clinically used to treat urinary tract infections, intestinal tract infections, respiratory tract infections, skin soft tissue infections, peritoneal infections, osteoarticular infections. But in recent years, adverse drug reaction is increasingly prominent as a result of Fluoroquinolones drugs' wide application, and resistance of human pathogenic bacterial is produced by long-term consumption of food containing low Fluoroquinolone drugs residues, thus resulting in a bad effect on the treatment of human disease. This kit is based on Competitive ELISA principle, with operation time as short as 50 min and a sensitivity of 1 ppb, and linear range from 1 ppb to 81 ppb.

II. Application:

This ELISA kit is used for *in vitro* quantitative determination of Fluoroquinolones including Ofloxacin, Ciprofloxacin, Enrofloxacin
Detection Range: 1 – 81 ppb
Sensitivity: < 1 ppb
Recovery Rate: 80 ± 15% for tissues, 75 ± 15% for honey

III. Sample Type:

Tissues and honey

IV. Kit Contents:

Components	K4205-100	Part No.
Micro ELISA Plate	8 X 12 Strips	K4205-100-1
Standard	1 ml X 6	K4205-100-2
HRP-conjugate	7 ml	K4205-100-3
Antibody	7 ml	K4205-100-4
Substrate A	7 ml	K4205-100-5
Substrate B	7 ml	K4205-100-6
Stop Solution	7 ml	K4205-100-7
Wash Buffer (20X)	40 ml	K4205-100-8
Sample Redissolving Solution (2X)	50 ml	K4205-100-9
Plate sealers	4	K4205-100-10

V. User Supplied Reagents and Equipment:

- Concentrated HCl, Acetonitrile, Dichloromethane, N-hexane
- Microplate reader capable of measuring absorbance at 450 nm
- 25°C incubator
- Precision pipettes with disposable tips
- Distilled or deionized water
- Clean eppendorf tubes for preparing standards or sample dilutions
- Absorbent paper

VI. Storage and Handling:

The entire kit may be stored at 4°C for up to 12 months from the date of shipment. Opened kit may be stable for 1 month at 4°C.

VII. Reagent and Sample Preparation:

Note: Bring all reagents to room temperature (20-25°C) 30 minutes before use.
Before using the kit, spin tubes and bring down all components to the bottom of tubes.

1. **Redissolving Solution:** Dilute **Sample Redissolving Solution (2X)** into deionized H_2O at 1:1 ratio (e.g.: 1 ml of Sample Redissolving Solution + 1 ml deionized water). Mix well.
2. **Solution 1 (0.1 M HCl solution):** Dissolve 860 µl of **Concentrated HCl** in 100 ml ddH_2O. Mix well.
3. **Solution 2 (Acetonitrile-Dichloromethane solution):** Prepare a mixture of **Acetonitrile** and **Dichloromethane** at a ratio of 1:4 (e.g.: 1 ml **Acetonitrile** + 4 ml **Dichloromethane**). Shake well.
4. **Solution 3 (Acetonitrile-Dichloromethane-0.1 M HCl solution):** Add 5 ml of **0.1 M HCl solution** to 100 ml of **Acetonitrile-Dichloromethane solution**. Shake well.
5. **Wash Buffer (1X):** If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals are completely dissolved. Dilute 10 ml of **Wash Buffer (20X)** into 190 ml deionized/distilled water to prepare 200 ml of **Wash Buffer (1X)**. Can be stored at 4℃ for one month.

6. **Standards Concentration:**

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

7. Sample Preparation:

Note: The prepared sample maybe stored for up to one day at 2-8°C.

- **Tissue (pork, duck, chicken, fish, shrimp, etc.)**
  1. Weigh 2 ± 0.05 g of the homogenized sample and put in 50 ml centrifugal tube.
  2. Add 8 ml of **Solution 2 (Acetonitrile-Dichloromethane solution)**, vortex for 5 min. Centrifuge at 4000 rpm for 10 mins at 15°C.
  3. Transfer 4 ml of supernatant into a new tube. Dry the sample at 56°C using nitrogen or rotary evaporator.
  4. Add 1 ml of **Redissolving Solution** and **1 ml of N-Hexane**, shake for 30 secs. Centrifuge at 4000 rpm for 5 mins at 15°C.
  5. Take 50 µl of sample for further analysis. (Dilution factor: 1)
- **Honey:**
  1. Weigh 2 ± 0.05 g of the homogenized sample and put in 50 ml centrifugal tube.
  2. Add 8 ml of **Solution 3 (Acetonitrile-Dichloromethane-0.1 M HCl solution)**, vortex for 3 min. Centrifuge at 4000 rpm for 10 mins at 15°C.
  3. Transfer 2 ml of supernatant into a new tube. Dry the sample at 56°C using nitrogen or rotary evaporator.
  4. Add 1 ml of **Redissolving Solution** and vortex for 1 min. Take 50 µl of sample for analysis. (Dilution factor: 2)

VIII. Assay Protocol:

Note: Bring all reagents and samples to room temperature 30 minutes prior to the assay.
It is recommended that all standards and samples be run at least in duplicate.
A standard curve must be run with each assay.

1. Prepare all reagents, samples and standards as instructed in **section VII**.
2. Calculate the number of wells to be used and return unused wells to the pouch containing dessicant. Seal the pouch and store at 4°C.
3. Add 50 µl of **Standard or Sample** per well. Then add 50 µl of **HRP-conjugate** to each well and 50 µl of **Antibody** to each well. Cover the microtiter plate with a new adhesive strip, mix well, and then incubate for 30 min at 25°C.
4. Aspirate each well and wash, repeating the process **4 times**. Wash by filling each well with 250 µl of **Wash Buffer (1X)** using a squirt bottle, multi-channel pipette, manifold dispenser, or autowasher, and let it stand for 30 seconds, complete removal of liquid at each step is essential to good performance.
5. Add 50 µl of **Substrate A** and 50 µl of **Substrate B** to each well, mix well. Incubate for 15 minutes at 25°C. Protect from light.
6. Add 50 µl of **Stop Solution** to each well, gently tap the plate to ensure thorough mixing.
7. Read result at 450 nm within 5 minutes.

IX. CALCULATION:

For calculation, **(the relative O.D.450) = (the O.D.450 of each well) – (the O.D.450 of Zero well**). The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The concentration of the samples can be interpolated from the standard curve. If the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

Note that the OD value of the sample has a negative correlation with fluoroquinolones in the sample.

X. RELATED PRODUCTS:

- Ampicillin sodium (Cat. No. 2484-5G, 25G, 100G)
- Carbenicillin disodium (Cat. No. 2485-1G, 5G, 10G)
- Colistin Sulfate (Cat. No. 9696-1G, 5G)
- Penicillin G sodium (Cat. No. 2503-100, 500)

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