

Fluoroquinolones ELISA Kit

rev 05/18

(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 food containing low Fluoroquinolones drugs residues, thus resulting in bad effect on the treatment of human disease. This kit is a detection product developed based on competitive ELISA technology, with operation time as short as 50 min and a sensitivity of 0.3 ppb, and linear range from 0.3 ppb to 24.3 ppb.

II. Application:

This ELISA kit is used for *in vitro* quantitative determination of Fluoroquinolones including Ofloxacin, Ciprofloxacin, Enrofloxacin.

Detection Range: 0.3 – 24.3 ppb (ng/ml)

Sensitivity: 0.3 ppb

Detection limitation: 3 ppb for milk and tissue, 2 ppb for eggs, 0.3 ppb for honey

III. Sample Type:

Milk, tissues, eggs, 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
TMB substrate	12 ml	K4205-100-5
Stop Solution	10 ml	K4205-100-6
Wash Buffer (10X)	30 ml	K4205-100-7
Sample Diluent 1	40 ml	K4205-100-8
Sample Diluent 2	15 ml	K4205-100-9
Extraction Solution (50X)	15 ml	K4205-100-10
Extractant	6 g	K4205-100-11
Plate sealers	4	K4205-100-12

V. User Supplied Reagents and Equipment:

- Concentrated HCl, Acetonitrile
- 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. **Extraction Solution 1 (for tissue use only):** Dilute 5 ml of **Extraction Solution (50X)** into 245 ml ddH₂O, Keep it at 4°C for one month.
2. **Extraction Solution 2 (for honey use only):** Transfer 1 mL of **Concentrated HCl** into 119 mL ddH₂O, mix well and add 480 ml of **Acetonitrile**, mix thoroughly.
3. **Sample Diluent A (for honey and milk use only):** Dilute 10 ml of Sample Diluent 2 into 40 ml ddH₂O, then add 1.5 g of Extractant. Shake well.
4. **Sample Diluent B (for egg use only):** Dilute 10 ml of Sample Diluent 2 into 40 ml ddH₂O, then add 2.5 g of Extractant. 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 (10X) into 90 mL deionized water to prepare 100 mL of Wash Buffer (1X). Can be stored at 4°C for one month.

FOR RESEARCH USE ONLY! Not to be used on humans.

6. Standards Concentration:

Standards	S0	S1	S2	S3	S4	S5
Concentration (ppb)	0	0.3	0.9	2.7	8.1	24.3

7. Sample Preparation:

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

• Milk and fresh milk

1. Bring the milk sample to room temperature.
2. Mix 100 µl of sample with 900 µl of **Sample Diluent A** in a new centrifugal tube.
3. Take 50 µl of sample for further analysis.

• Tissue(pork, duck, chicken, fish, shrimp)

1. Weigh 1 g of the homogenized sample and add 5 mL of **Extraction Solution 1**, vortex properly for 5 min.
2. Centrifuge at 4000 rpm for 5 min. Transfer 100 µl of supernatant into a new tube.
3. Add 100 µl of **Sample Diluent 1**, shake well and take 50 µl of sample for further analysis. (Dilution factor:10)

• Honey:

1. Weigh 1 g of the homogenized sample and add 4 mL of **Extraction Solution B**, vortex properly for 5 min.
2. Centrifuge at 4000 rpm for 10 min. Transfer 2 ml of supernatant into a new tube.
3. Dry the sample by blowing nitrogen gas at 60°C.
4. Add 1 ml of Sample Diluent A and vortex for 1min. Take 50 µl of sample for analysis. (Dilution factor:2)

• Eggs:

1. Weigh 1 g of the homogenized sample and add 4 ml of ddH₂O, vortex properly for 5 min.
2. Take 500 µl of sample into 500 µl Sample Diluent B. Shake well. Take 50 µl of sample for analysis. (Dilution factor:10)

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. 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 and mix well, then incubate for 30 min at 25°C.
3. Aspirate each well and wash, repeating the process 4 times. Wash by filling each well with 250 µl of **Wash Buffer** 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.
4. Add 100 µl of **TMB Substrate** to each well, mix well. Incubate for 15 minutes at 25°C. Protect from light.
5. Add 50 µl of **Stop Solution** to each well, gently tap the plate to ensure thorough mixing.
6. Read result at 450 nm within 10 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)