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Quinolone ELISA Kit

rev 04/21

(Catalog # E4530-100; 100 assays, Store kit at -20 °C)

I. Introduction:

Quinolones are one of the most commonly prescribed classes of antibiotics used to treat various bacterial infections in humans and domestic animals. Quinolones that contain additional fluorine atom in the core structure are called fluoroquinones. Both of these antibiotics can kill bacteria by inhibiting DNA gyrase in gram-negative bacteria or topoisomerase IV in gram-positive bacteria. However, excessive quinolone or fluoroquinolone in foods or drinks can pose a serious threat to humans as it may cause central nervous system toxicity, blood disorders, tendonitis, brain, liver and gastrointestinal dysfunction. As a consequence, it is important to monitor their levels in food products such as in meat and milk. The traditional techniques/instruments (HPLC or GC-MS) for detecting quinolone are complex, expensive, laborious, and time-consuming. Immunoassay techniques, such as ELISAs, are commonly preferred as a simple, reliable and rapid method. **BioVision's Quinolone ELISA Kit** is a competitive-based ELISA that can be used to detect both quinolone and fluoroquinolone in tissues, urine and serum. This detection kit offers ready-to-use reagents, and can quantify quinolone (0.12 – 10 ng/ml) within 90 minutes.

II. Applications:

- *In vitro,* quantitative determination of quinolone
- **Detection Range:** 0.12 - 10 ppb (ng/ml)
- **Sensitivity:** 0.12 ppb
- **Detection limit:** 1 ppb (serum), 2 ppb (milk and urine)
- **Cross Reactivity:** Nalidixic Acid - 100%, Oxolinic Acid - 100%, Pefloxacin - 100%, Levofloxacin - 50%, Norfloxacin - 37%, Enrofloxacin- 15% and Ciprofloxacin - 15%

III. Sample Types:

Serum, urine and milk

IV. Kit Contents:

Components	E4350-100	Cap Code	Part Number
ELISA Microplate	8 X 12 Strips	--	E4530-100-1
Quinolone Standard	2 vials	Yellow	E4530-100-2
HRP conjugate	10 µl	Blue	E4530-100-3
Antibody	7 ml	Red/NM	E4530-100-4
TMB substrate	10 ml	Amber/NM	E4530-100-5
Stop Solution	10 ml	Blue/NM	E4530-100-6
Sample Diluent	20 ml	NM	E4530-100-7
Wash Buffer	50 ml	NM	E4530-100-8
Serum Solution	2 ml	Purple	E4530-100-9
Extraction Solution	2 ml	Brown	E4530-100-10
Standard Buffer	20 ml	WM	E4530-100-11
Conjugate Buffer	7 ml	Green/NM	E4530-100-12
Plate Sealers	4	--	E4530-100-13

V. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 and 650 nm
- Precision pipettes with disposable tips
- Clean eppendorf tubes for preparing standards and sample dilutions

VI. Storage and Handling:

The entire kit may be stored at -20 °C for up to 12 months from the date of shipment. Opened kit may be stable for 1 month at -20 °C.

VII. Reagent and Standard Preparation:

Bring all reagents to room temperature (RT) before use. Before using the kit, spin tubes and bring down all components to the bottom of tubes.

- **Antibody, TMB Substrate, Stop Solution, Sample Diluent, Serum Solution, Extraction Solution, Standard Buffer and Conjugate Buffer:** Ready to be used. Bring bottle to RT before use. After each use, store them at 4 °C.
- **HRP Conjugate:** Spin briefly before opening the tube. Pipet 6 µl of HRP Conjugate Stock into Conjugate Buffer (7.5 ml) bottle to prepare the conjugate working solution. Vortex the bottle for a minute. The conjugate working solution is stable at 4 °C for 2 months.
- **Wash Buffer (10X):** Bring bottle to RT. If crystals are present, warm up to RT and mix gently until the crystals are completely dissolved. Prepare 100 ml of 1X Wash Buffer by diluting 10 ml of Wash Buffer (10X) with 90 ml deionized water. The 1X solution can be stored at 4 °C for one month.
- **Quinolone Standard:** Add 1.5 ml of Standard Buffer into one vial of Quinolone Standard to prepare 10 ng/ml (S5). Perform 3-fold serial dilutions from S5 (e.g. 300 µl in 600 µl of Standard buffer) to prepare S4 to S1 standards sequentially. S0 contains Standard Buffer only. **Note:** Prepared Standards are stable at -20 °C for 2 weeks.

Standards	S0	S1	S2	S3	S4	S5
Concentrations (ppb)	0	0.12	0.37	1.11	3.33	10

VIII. Sample Preparation:

- **Serum**

1. Add 30 µl of Serum Solution into 270 µl of serum in an Eppendorf tube and vortex well.
2. Incubate the sample at 37 °C for 45 min.
3. After 45 min, incubate the sample at 85-90 °C for 10 min.
4. Dilute the sample 10 fold using the Sample Diluent. For example, mix 20 µl of serum with 180 µl of Sample Diluent.
5. Use 50 µl per well for the assay.

Note: Dilution factor: 10

- **Urine**

1. Centrifuge 0.5 ml of urine at 10,000 x g for 5 min and collect the supernatant.
2. Dilute the supernatant 10 fold using the Sample Diluent. (For example, mix 20 µl of urine with 180 µl of Sample Diluent.)
3. Use 50 µl per well for the assay.

Note: Dilution factor: 10

- **Milk**

1. Add 20 µl of Extraction Solution to 1 ml of milk and vortex well.
2. Centrifuge the sample at 10,000 x g for 20 min at 4 °C and collect the clear supernatant.
3. Dilute the supernatant 10 fold with Sample Diluent. For example, mix 20 µl of the supernatant with 180 µl of Sample Diluent.
4. Use 50 µl per well for the assay.

Note: Dilution factor: 10

IX. Quinolone ELISA Assay Protocol:

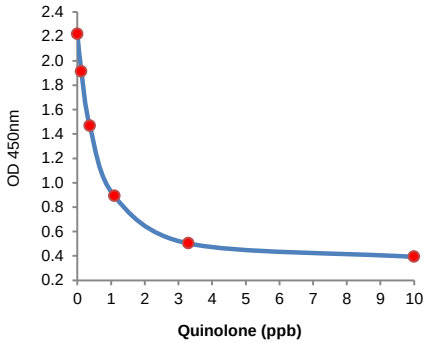
Notes: It is recommended that all standards and samples should be run at least in duplicate. Standard curves must be run each time an assay is performed.

1. Prepare all reagents, Standards and samples as sections VII and VIII respectively.
2. Add 50 µl of Standards or Samples per well. Then add 50 µl of HRP-conjugate and 50 µl of Antibody to the above wells.
3. Cover the microtiter plate with plate sealer and mix well. Incubate the plate at room temperature (25°C) for 60 min.
4. Aspirate all reagents and wash each well 4 times. Add 250 µl of 1X Wash Buffer and incubate for 30 seconds. Remove 1X Wash buffer completely before the next wash. **Note:** This is essential for accurate results. Repeat this step 3 more times.
5. Add 100 µl of TMB Substrate to each well. Tap or shake the plate to ensure complete mixing.
6. Check the OD at 650 nm for the well containing no Quinolone (S0). When its reading is between 0.95 and 1.05 (usually between 10-30 min after adding the TMB Substrate), add 50 µl of Stop Solution and gently tap the plate to ensure thorough mixing.
7. Measure the OD at 450 nm for the Standards and samples within 10 min.

X. Calculation:

The Standard Curve is done by plotting the OD at 450 nm vs. Quinolone concentrations. The concentration of Quinolone in each sample (ng/ml), which can be read from the calibration curve, is multiplied by the corresponding dilution factor.

A.



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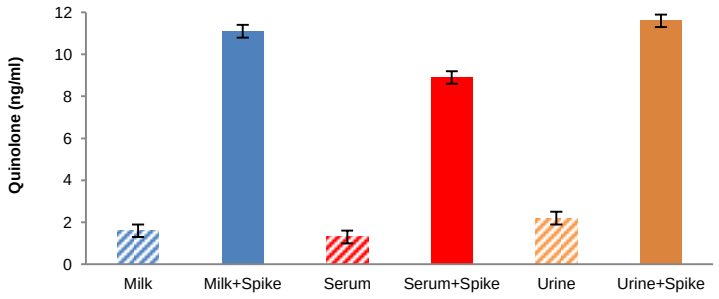

Figures. **A.** Quinolone Standard Curve for Quinolone ELSIA Kit (*This standard curve is for demonstration only. A Standard Curve must be run with each assay*). **B.** Spike recovery experiment: Human serum, urine and milk samples were assayed without and with spike of 10 ng/ml quinolone. Recovery rate: 80-100%.

XI. Related Products:

Gentamicin (serum/urine) ELISA Kit (Cat. No. K4315-100)
Ampicillin ELISA Kit (Cat. No. E4350-100)
Enrofloxacin (ENR) ELISA Kit (Cat. No. E4277-100)
Folic Acid ELISA Kit (Cat. No. 4523-100)
Kanamycin ELISA Kit (Cat. No. K4210-100)

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