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Enrofloxacin (ENR) ELISA Kit

rev 10/20

(Catalog # E4277-100, 100 assays, Store at 4°C)

I. Introduction:

Enrofloxacin (ENR) is a fluoroquinolone antibiotic. Enrofloxacin has demonstrated a significant post-antibiotic effect for both Gram-negative and Gram-positive bacteria and is active in both stationary and growth phases of bacterial replication. Enrofloxacin is currently approved by the FDA for the treatment of individual pets and domestic animals in the United States. It is prohibited for use in water to treat flocks of poultry, as this practice was noted to promote the evolution of fluoroquinolone-resistant strains of the bacterium Campylobacter, a human pathogen. BioVision's Enrofloxacin ELISA kit is a Competitive ELISA assay for the quantitative measurement of Enrofloxacin in animal tissues, honey, milk, egg, milk powder. The intensity of color is inversely proportional to the amount of Enrofloxacin captured from the samples.

II. Application:

This ELISA kit is used for *in vitro* quantitative determination of Enrofloxacin.
Detection Range: 0.1 – 8.1 ppb (ng/ml)
Sensitivity: < 0.1 ppb
Detection limit: 0.3 ppb for tissues, 0.4 ppb for honey, 3 bbp for milk and egg, 6 ppb for milk powder.

III. Sample Type:

Animal tissues, Honey, Milk, Egg, Milk Powder

IV. Kit Contents:

Components	E4277-100	Part No.	Cap Color
Micro ELISA Plate	8 X 12 strips	E4277-100-1	-
Standards (S1 – S6)	1.0 ml X 6	E4277-100-2-x	-
High standard (100 ng/ml)	1.0 ml	E4277-100-3	Black
Antibody working solution	5.5 ml	E4277-100-4	Blue
Enzyme Conjugate	5.5 ml	E4277-100-5	Red
Substrate A solution	6 ml	E4277-100-6	White
Substrate B solution	6 ml	E4277-100-7	Black
Stop Solution	6 ml	E4277-100-8	Yellow
Concentrated Wash Solution (20X)	40 ml	E4277-100-9	White
Redissolving Solution (5X)	50 ml	E4277-100-10	Yellow
Adhesive Membrane	1	E4277-100-11	-
Sealed bag	1	E4277-100-12	-

V. User Supplied Reagents and Equipment:

- Reagents: Acetonitrile, N-hexane, anhydrous acetonitrile, 0.15 M HCl, dichloromethane (CH_2Cl_2)
- Microplate reader capable of measuring absorbance at 450 nm
- Precision pipettes with disposable tips
- Distilled or deionized water
- Nitrogen-drying device
- 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.

VII. Reagents and Samples Preparation:

Note: Prepare reagents within 30 minutes before the experiment.
Before using the kit, spin tubes and bring down all components to the bottom of tubes.

1. **Standards:** ready to use.

Tube #	S1	S2	S3	S4	S5	S6
Concentration (ng/ml)	0	0.1	0.3	0.9	2.7	8.1

2. **Wash Buffer:** Dilute 40 ml of the concentrated washing buffer with the distilled or deionized water to 800 ml (or just to the required volume) for using.
3. **Sample Extract Solution:** mix 10 ml 0.15M HCl with 90 ml anhydrous acetonitrile.
4. **Redissolving Solution:** Dilute the concentrated redissolving solution (5X) with deionized water. Tt can be stored at 4°C up to a month.
5. **Sample Preparation**:

Note: Samples to be used within 5 days may be stored at 4°C, otherwise samples must be stored at -20°C (≤1 month) or -80°C (≤2 months) to avoid loss of bioactivity and contamination. Avoid multiple freeze-thaw cycles.

- **Animal tissue (chicken, pork, fish, shrimp)**: Weigh 2 g Homogeneous sample into 50 ml centrifuge tube. Add 8 ml Sample Extract Solution and mix with vortex for 5 min. Centrifuge at 4000 rpm at room temperature (25°C) for 10 min. Take 2 ml clear organic phase of upper into the clean, dry, 10 ml, glass tube, and dry at 50 - 60 °C water bath with nitrogen. Add 1 ml N-hexane, mix with vortex for 2 min.

for 5 min. Remove the upper N-hexane, take 50 µl Lower water phase to be analyses. (Dilution factor: 2)

- **Honey**: Weigh 1 g homogeneous sample into 50 ml centrifuge tube, add 6 ml sample extract solution, oscillate 5 min for complete dissolve. Add 3 ml redissolving solution, then add 11 ml dichloromethane (CH_2Cl_2), oscillate 5 min, centrifuge at 4000 rpm at room temperature for 5 min. Remove out the upper phase, take 8 ml organic phase to dry container, and dry with nitrogen at 50 - 60°C water bath. Dissolve the dry residue with 1 ml redissolving solution, then add 1ml N-hexane, mix 30 sec, centrifuge at 3000 rpm at RT for 5 min. Remove the upper phase, take 50 µl lower phase for analyses. (Dilution factor: 2)
- **Milk**: Take 25 µl milk sample and 475 µl redissolving solution, mix and oscillate 1 min to make it dissolves fully. Use 50 µl solution for analyses. (Dilution factor: 20)
- **Milk Powder**: Weigh 0.5 g homogeneous sample into 10 ml centrifuge tube, add 5 ml deionized water, oscillate and make it dissolves fully. Mix 100 µl sample with 400 µl redissolving solution and oscillate 1 min. Use 50 µl solution for analyses. (Dilution factor: 50)
- **Eggs**: Weigh 1 g homogeneous sample into 10 ml centrifuge tube, add 5 ml deionized water, oscillate and make it dissolves fully. Mix 100 µl sample with 400 µl redissolving solution and oscillate for 1 min. Use 50µl solution for analyses. (Dilution factor: 30)

VIII. Assay Protocol:

Note: Bring all reagents and samples to room temperature 30 minutes prior to the assay. Shake the reagent bottles if there is any crystal. 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 **diluted standards** or **samples** into marked well. Add 50 µl **Enzyme Conjugate** into each well, then add 50 µl **antibody working solution** into each well.
3. Oscillate the plate for 5 sec, cover the well and incubate in dark for 45 min at RT (25°C).
4. Discard solution, wash plate 5 times with **1X Wash Solution**. Wash by filling each well with Wash Buffer (250 µl) using a multi-channel pipette or autowasher. Let it soak for 1 min, and then remove all residual wash-liquid from the wells. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Clap the plate on absorbent filter papers or other absorbent materials.
5. Pipette 50 µl **Substrate A solution**, then pipette 50 µl **Substrate B solution** to each well, oscillate gently for 5 sec, avoid the light preservation for 15 min at RT.
6. Add 50 µl **Stop Solution** to each well and oscillate gently to stop the reaction.
7. Read result at 450 nm within 10 minutes.

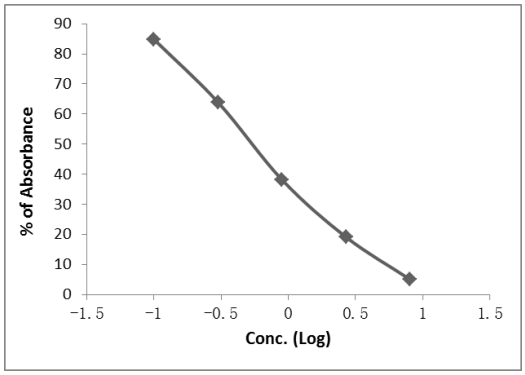
IX. Calculation:

Percentage of absorbance value (%) = A/A_0 X 100%

A: the average (double wells) OD value of the sample or the standard solution; A_0: the average OD value of the 0 ppb standard solution.

To draw the standard curve and calculate, take absorbance percentage of standards as Y-axis, the corresponding log of standards concentration (ppb) as X-axis. Draw the standard semilog curves with X-axis and Y-axis. Take absorbance percentage of samples substitute into standard curve, then can get the corresponding concentration from standard curve; last, Multiplied by the corresponding dilution times is the actual concentration of Sal of samples.

Figure: Typical Standard Curve: These standard curves are for demonstration only. A standard curve must be run with each assay.



X. Related Products:

- Sulfonamides residue ELISA Kit (Cat. No. K4207-100)
- Salbutamol (SALB) ELISA Kit (Cat. No. K4209-100)
- Kanamycin ELISA Kit (Cat. No. K4210-100)
- Streptomycin ELISA Kit (Cat. No. K4272-100)
- Fluoroquinolones ELISA Kit (Cat. No. K4205-100)