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Sarafloxacin ELISA Kit

10/19

(Catalog # E4777-100; 96 assays, Storage at 4°C)

I. Introduction:

Sarafloxacin is a veterinary fluoroquinolone antimicrobial agent that inhibits bacterial DNA gyrase. It has been used for the control of bacterial infections in poultry caused by either E. coli or Salmonella species. Sarafloxacin ELISA Kit is based on Competitive ELISA principle. The micro-plate provided in this kit has been pre-coated with Sarafloxacin. During the reaction, Sarafloxacin in the samples or standard competes with Sarafloxacin coated on the plate for binding to the anti-Sarafloxacin antibody. Then Horseradish Peroxidase (HRP) conjugate is added to each micro plate well, and TMB substrate is for color development. There is a negative correlation between the OD value of samples and the concentration of Sarafloxacin. The concentration of Sarafloxacin in the samples can be calculated by comparing the OD of the samples to the standard curve.

II. Applications:

In vitro, quantitative determination of Sarafloxacin
Sensitivity: 0.1 ppb (ng/mL)
Detection Range: Tissue (chicken, pork, fish, shrimp) - 0.3 ppb, Honey - 0.4 ppb, Milk - 3 ppb, Milk powder- 6 ppb, Egg - 3 ppb, Urine - 0.5 ppb

Sample recovery rate: Tissue, Honey, Milk, Milk powder, Eggs - 85%±15%.

Cross-reactivity: Sarafloxacin -100%

III. Sample Type:

Tissue, Urine, Feed

IV. Kit Contents:

Components	E4777-100	Part Number
Micro ELISA Plate	96 wells	E4777-100-1
Standard	6 X 1 ml	E4777-100-2
HRP Conjugate	5.5 ml	E4777-100-3
Antibody Working Solution	5.5 ml	E4777-100-4
Substrate Reagent A	6 ml	E4777-100-5
Substrate Reagent B	6 ml	E4777-100-6
Stop Solution	6 ml	E4777-100-7
Wash Buffer (20X)	40 ml	E4777-100-8
Reconstitution Buffer (5X)	50 ml	E4777-100-9
Plate Sealer	3	E4777-100-10

V. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm
- anhydrous acetonitrile、n-hexane、concentrated HCl, methylene dichloride
- Clean Eppendorf tubes for preparing standards or sample dilutions

VI. Storage and Handling:

Store at 4ºC**.**

VII. Reagent and Sample Preparation:

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

- **Wash Buffer (20X):** Dilute 20X Concentrated Wash Buffer to 1X with deionized water.
- **Reconstitution Buffer (5X):** Dilute 5X Reconstitution Buffer with deionized water. Mix 5x Reconstitution Buffer (V): Deionized water (V) =1:4). The Reconstitution buffer can be store at 4℃ for a month.
- **0.15 M HCl:** Dissolve 5 ml of concentrated hydrochloric acid (HCl) to 400 ml.
- **Sample Extract:** Measure 10 ml of 0.15 M HCl to 90 ml of anhydrous acetonitrile, mix well.
- **Standard:**

Standard	S1	S2	S3	S4	S5	S6
Concentration (ppb)	0	0.1	0.3	0.9	2.7	8.1

VIII. Sample Preparation:

Sample pretreatment:

- **Pretreatment of tissue (chick, pig, fish, shrimp):**
  Weigh 2 ± 0.05 g of crushed homogenate tissue sample; add 8 mL of sample extract. Mix well for 5 min, centrifuge at a speed of over 4000 r/min for 10 min at room temperature. Remove 2 ml of the clear upper organic phase to a clean and dry glass tube, dry at 50- 60°C with nitrogen evaporators or water bath. Add 1 ml of N hexane and shake for 2 min. Add 1 ml of Reconstitution Buffer Solution and shake for 30 sec to mix well. Centrifuge for 5 min at 4000 r/min at room temperature. Remove the N hexane upper layer; take 50 µl of the lower water layer solution for analysis.
  Note: Sample dilution factor: 2, minimum detection limit: 0.3 ppb.

- **Pretreatment of honey sample:**
  Weigh 1 ± 0.05 g of honey into a 50 ml tube, add 6 mL of Sample Extract and shake for 5 min to ensure it is thoroughly dissolved. Add 3 ml of Reconstitution Buffer and 11 ml of methylene dichloride shake well for 5 min. Then centrifuge at 4000 r/min for 5 min at room temperature. Remove the supernatant and transfer 8 ml of the upper layer organic solution to a dry container. Dry at 50-60 °C with nitrogen evaporators or water bath. Dissolve the dry residue with 1 ml of Reconstitution Buffer. Add 1 mL of N hexane and oscillate for 30 s. Centrifuge for 5 min at 3 000 r/min at room temperature. Remove the N hexane upper layer take 50 µl of the lower layer solution for analysis.
  Note: Sample dilution factor: 2, minimum detection limit: 0.4 ppb.

- **Pretreatment of milk sample:**
  Dilute the milk with Reconstitution Buffer for 20 times (eg add 25 µl of milk into 475 µl of Reconstitution Buffer), shake for 1 min to dissolve it well. Take 50 µl for detection and analysis
  Note: Sample dilution factor: 20, minimum detection limit: 3 ppb

- **Pretreatment of milk powder sample:**
  Weigh 0.5 ± 0.02 g of homogenate sample into a 10 ml tube, add 5 mL of deionized water and oscillate to dissolve well. Mix 100 µl of sample solution with 400 µl of Reconstitution Buffer. Dissolve for 1 min. Take 50 µ L for detection and analysis
  Note: Sample dilution factor: 50 , minimum detection limit: 6 ppb

- **Pretreatment of eggs sample:**
  Weigh 1 ± 0.02 of homogenate egg into a 10 ml tube, add 5 ml of deionized water and shake to dissolve it well. Mix 100 µl of sample solution with 400 µl of Reconstitution Buffer. Shake well for 1 min. Take 50 µl for detection and analysis
  Note: Sample dilution factor: 30 minimum detection limit: 3 ppb

- **Pretreatment of urine sample:**
  Add 4 m of Reconstitution Buffer into 1 mL of clear urine sample, mix for 30 s. Take 50 µl for detection and analysis.
  Note: Sample dilution factor: 5, minimum detection limit: 0.5 ppb

IX. 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. Add 50 µl of each **standard or samples** into appropriate wells.
2. Add 50 µl of **HRP Conjugate** to each well. Add 50 µl of Antibody **Working Solution**. Cover the plate with the sealer provided in the kit. Gently mix and incubate for 45 min. at 25°C.
3. Aspirate the solution from each well add 300 µl of **1x wash buffer** to each well. Leave it for 30 sec, aspirate the solution from each well and pat it dry against clean absorbent paper. Repeat this wash step 5 times.
4. Add 50 µl of **Substrate Reagent A** to each well and then add 50 µl of **Substrate Reagent B.** Cover with a plate sealer. Incubate for about 15 min at 25°C. Protect the plate from light.

   Note: the reaction time can be shortened or extended according to the actual color change, but not more than 30 min.
5. Add 50 µl of **Stop Solution** to each well. Note: adding the stop solution should be done in the same order as the substrate solution.
6. Read the absorbance in micro plate reader set to 450 nm reference wavelength 630 nm. This step should be performed within 10 min after stop reaction.

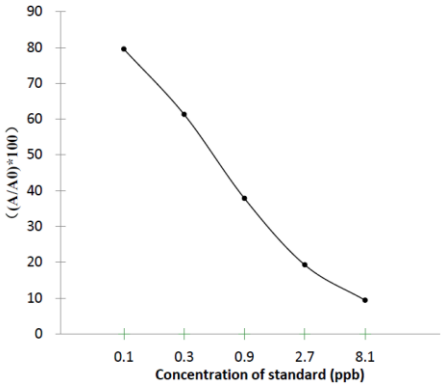
X. Calculation:

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.

Absorbance (%)=A/A_0 ×100%

A: Average absorbance of standard or sample

A_0 : Average absorbance of 0 ppb Standard



Typical standard curve and data is provided below for reference only. A standard curve must be run with each assay

Concentration of standard (ppb)	OD-1	OD-2	Average OD
0	2.2456	2.2085	2.2123
0.1	1.7589	1.7654	1.7589
0.3	1.3452	1.3385	1.3549
0.9	0.8341	0.8309	0.8362
2.7	0.4278	0.4254	0.4255
8.1	0.2078	0.2085	0.2070

XI. RELATED PRODUCTS:

- Vancomycin ELISA Kit (E4605)
- Gentamicin (serum/urine) ELISA Kit (K4315)
- Diazepam ELISA Kit (E4772)
- Enrofloxacin (ENR) ELISA Kit (E4277)

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