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Tylosin ELISA Kit

rev 04/21

(Catalog # E4779-100; 96 assays, Storage at 4°C)

I. Introduction:

Tylosin is an antibiotic and a bacteriostatic feed additive used in veterinary medicine. It has a broad spectrum of activity against Gram-positive organisms and a limited range of Gram-negative organisms. It is found naturally as a fermentation product of Streptomyces fradiae. It is a macrolide antibiotic. Tylosin is used in veterinary medicine to treat bacterial infections in a wide range of species and has a high margin of safety. It has also been used as a growth promotant in some species, and as a treatment for colitis in companion animals. Tylosin ELISA Kit is based on Competitive ELISA principle. The micro-plate provided in this kit has been pre-coated with Tylosin. During the reaction, Tylosin in the samples or standard competes with Tylosin coated on the plate for binding to the anti-Tylosin 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 Tylosin. The concentration of Tylosin in the samples can be calculated by comparing the OD of the samples to the standard curve.

II. Applications:

In vitro, quantitative determination of Tylosin
Sensitivity: 0.5 ppb (ng/mL)

Detection Range: Tissue (muscle) - 1 ppb, Honey- 0.5 ppb

Sample recovery rate: Tissue - 85%±10%, Honey- 85%±15%

Cross-reactivity: Tylosin - 100%, Erythromycin - 1%, other macrolides ＜1%

III. Sample Type:

Tissue, Honey

IV. Kit Contents:

Components	E4779-100	Part Number
Micro ELISA Plate	96 wells	E4779-100-1
Standard	6 X 1 ml	E4779-100-2
HRP Conjugate	11 ml	E4779-100-3
Antibody Working Solution	5.5 ml	E4779-100-4
Substrate Reagent A	6 ml	E4779-100-5
Substrate Reagent B	6 ml	E4779-100-6
Stop Solution	6 ml	E4779-100-7
Wash Buffer (20X)	40 ml	E4779-100-8
Reconstitution Buffer (2X)	50 ml	E4779-100-9
Plate Sealer	3	E4779-100-10

V. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm
- Sodium Hydroxide, Methanol, concentrated Hydrochloric Acid, Acetonitrile, Trichloromethane
- 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 (1X):** Dilute **Wash Buffer (20X)** to 1X with deionized water.
- **Reconstitution Buffer (1X):** Dilute **Reconstitution Buffer (2X)** with deionized water. Mix **Reconstitution Buffer (2X)** (V): Deionized water (V) =1:1). The diluted Reconstitution buffer can be store at 4℃ for a month.
- **0.1 M NaOH:** Dissolve 0.4 g of solid **NaOH** with 100 mL of deionized water
- **Extraction Solution:** Mix 84 mL of **Acetonitrile** and 16 mL of **HCl (0.1M)** then add 18 ml of **Methanol** and mix well.
- **0.1M HCl:** Dissolve 0.86 ml of **Concentrated HCl** to 100 ml with deionized water
- **Standard:**

Standard	S1	S2	S3	S4	S5	S6
Concentration (ppb)	0	0.5	1.5	4.5	13.5	40.5

VIII. Sample Preparation:

Sample pretreatment:

- **Pretreatment of muscle tissue (livestock):**
  Weigh 2 ± 0.05 g of homogeneous sample into 50 ml centrifuge tube add 8 ml of **Extraction Solution**, shake well for 5 min, centrifuge at 4000 r/min for 10 min at room temperature. Take 2 ml of the supernatant, add 1 ml of **0.1 M NaOH Solution** and mix well, then add 3 ml **Trichloromethane** and mix well for 5 min, centrifuge at 4000 r/min for 10 min at room temperature. Discard the upper phase, take the lower organic phase to another centrifuge tube, dry at 56°C with nitrogen evaporators or water bath. Dissolve the residue with 1 ml of **Reconstitution Buffer**, mix well. Take 50 µl for detection and analysis.
  Note: Sample dilution factor: 2, minimum detection limit: 1ppb.

- **Pretreatment of honey sample:**
  Weigh 1 ± 0.05 g of honey into a 50 ml tube, add 2 ml of deionized water, mix well for 2 min to dissolve fully. Add 10 ml **Trichloromethane** and shake well for 5 min, centrifuge at 4000 r/min for 10 min at room temperature; Discard the upper phase, take the lower organic phase to another centrifuge tube, dry at 56 °C with nitrogen evaporators or water bath. Dissolve the residue with 1 ml of **Reconstitution Buffer**, mix well. Take 50 µl for detection and analysis.
  Note: Sample dilution factor: 1, 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 **Antibody Working Solution**. Cover the plate with the sealer provided in the kit. Gently mix and incubate for 30 min. at 25°C in dark.
3. Aspirate the solution from each well add 300 µl of **Wash Buffer (1X)** 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 100 µl of **HRP Conjugate** to each well, incubate for 30 min. at 25°C in dark.
5. Perform Washing as mentioned in **step 3**.
6. 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.
7. 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.
8. 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:

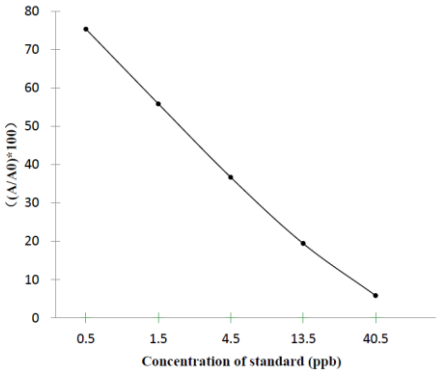
Create a standard curve by plotting the absorbance percentage of each standard on the y-axis against the log concentration on the x-axis to draw a semi logarithmic plot. Add average absorbance value of sample to standard curve to get corresponding concentration. If samples have been diluted, the concentration 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

Concentration of standard (ppb)	OD-1	OD-2	Average OD
0	2.0312	1.9665	1.9989
0.5	1.5047	1.5062	1.5055
1.5	1.1218	1.1108	1.1163
4.5	0.7243	0.7423	0.7333
13.5	0.3922	0.3852	0.3887
40.5	0.1238	0.1118	0.1178



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

XI. RELATED PRODUCTS:

- Sarafloxacin ELISA Kit (E4777)
- Norfloxacin ELISA Kit (E4776)
- Diazepam ELISA Kit (E4772)

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