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Olaquindox ELISA Kit

11/19

(Catalog # E4781-100; 96 assays, Storage at 4°C)

I. Introduction:

Olaquindox is a growth-promoting feed additive for food-producing animals. Its toxicities were reported to be closely related to the metabolism. Signs of toxicity in pigs are inappetence, lack of weight gain and cachexia, dehydration, cannibalism, anemia, muscle tremor, and weakness of the hind quarters and death. BioVision Olaquindox ELISA Kit is based on Competitive ELISA principle. The micro-plate provided in this kit has been pre-coated with Olaquindox. During the reaction, Olaquindox in the samples or standard competes with Olaquindox coated on the plate for binding to the anti-Olaquindox 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 Olaquindox. The concentration of Olaquindox in the samples can be calculated by comparing the OD of the samples to the standard curve.

II. Applications:

In vitro, quantitative determination of Olaquindox
Sensitivity: 0.5 ppb (ng/mL)
Detection Range: Tissue- 1.5 ppb, Feed- 150 ppb

Sample recovery rate: Tissue - 80%±15%, Feed- 85%±15%

Cross-reactivity: Olaquindox-100%, Carbadox- < 0.1%

III. Sample Type:

Tissue, Honey

IV. Kit Contents:

Components	E4781-100	Part Number
Micro ELISA Plate	96 wells	E4781-100-1
Standard	6 X 1 ml	E4781-100-2
HRP Conjugate	11 ml	E4781-100-3
Antibody Working Solution	5.5 ml	E4781-100-4
Substrate Reagent A	6 ml	E4781-100-5
Substrate Reagent B	6 ml	E4781-100-6
Stop Solution	6 ml	E4781-100-7
Wash Buffer (20X)	40 ml	E4781-100-8
Reconstitution Buffer (2X)	50 ml	E4781-100-9
Plate Sealer	3	E4781-100-10

V. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm
- Anhydrous acetonitrile, Methanol , N hexane
- 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 (2X):** Dilute 2X Reconstitution Buffer with deionized water. Mix 2x Reconstitution Buffer (V): Deionized water (V) =1:1). The Reconstitution buffer can be store at 4℃ for a month.
- **Sample Extract Solution:** Prepare sample extract solution by mixing Methanol with deionized water in the ratio Methanol (V) deionized water (V) 0.5: 9.5.
- **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 tissue (livestock):**
  Weigh 2 ± 0.05 g of homogenate sample without skin, bone and fat. Add 2 mL of deionized water and 8 mL of anhydrous acetonitrile and mix thoroughly. Incubate in water bath at 56 for 10 min, shake for 5 min, and centrifuge at 4000 r/min at room temperature for 10 min. Take 5 mL of supernatant to another tube, dry in 50 60 nitrogen evaporators/water bath. Redissolve the residual with 1 mL of Reconstitution Buffer, add 2 mL of N hexane and mix thoroughly Centrifuge at 4000 r/min at room temperature for 5 min, discard the upper organic phase, take 50 µl of lower liquid for analysis.
  Note: Sample dilution factor: 1, minimum detection limit: 1.5 ppb.

- **Pretreatment of feed:**
  Weigh 1 0.05 g homogenate feed, add 10 mL of Sample Extract Solution and mix thoroughly Incubate in water bath at 56 for 10 min, oscillate for 5 min, centrifuge at 4000 r/min at room temperature for 10 min. Take 50 µl of supernatant to another tube, add 450 µl of Reconstitution Buffer and mix thoroughly. Take 50 µl of supernatant from step (2) for analysis
  Note: Sample dilution factor: 100, minimum detection limit: 150 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.
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 **HRP Conjugate** to each well, incubate at 37°C for 30 min in dark.
5. Repeats wash 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 37°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:

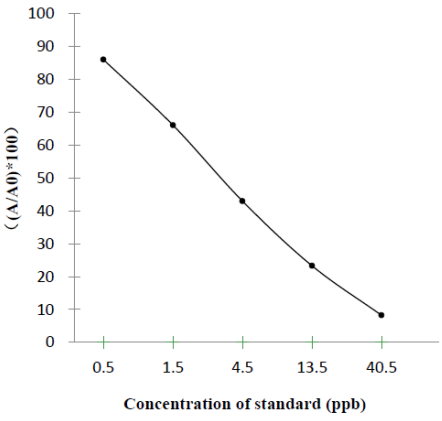
Create a standard curve by plotting the absorbance percentage of each standard on the y-axis against the log concentration on the x-axis to draw a semi logarithmic plot. Add average absorbance value of sample to standard curve to get corresponding concentration. If samples have been diluted, the concentration 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	2.0255	2.1055	2.0655
0.5	1.7990	1.7511	1.7751
1.5	1.3816	1.3456	1.3636
4.5	0.9028	0.8712	0.8870
13.5	0.4907	0.4713	0.4810
40.5	0.1621	0.1783	0.1702



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

XI. RELATED PRODUCTS:

- Florfenicol ELISA Kit (E4780)
- Norfloxacin ELISA Kit (E4776)
- Diazepam ELISA Kit (E4772)
- Cimaterol ELISA Kit (E4771)

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