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（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

OQX(Olaquindox) ELISA Kit

Catalog No: E-FS-E007

96T

This manual must be read attentively and completely before using this product.

If you have any problems, please contact our Technical Service Center for help.

Phone: 240-252-7368(USA)240-252-7376(USA)
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number(on the outside of the box) of the kit for more efficient service.

Test principle and application

This kit uses Indirect-Competitive-ELISA as the method. It can detect OQX in samples, such as tissue, fodder, etc. This kit is composed of Micro ELISA Plate, HRP conjugate, antibody, standard and other supplementary reagents. The micro-plate provided in this kit has been pre-coated with OQX. During the reaction, OQX in the samples or standard competes with OQX on the solid phase supporter for sites of OQX 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 OQX. You can calculate the concentration of OQX in the samples by comparing the OD of the samples to the standard curve.

Technical indicator

Sensitivity: 0.5ppb(ng/mL)
Reaction mode: 37℃, 30min～30min～15min
Detection limit: Tissue---1.5ppb
Fodder---150ppb
Cross-reactivity: Olaquindox---100%,
Carbadox---＜0.1%
Sample recovery rate: Tissue ---80%±15%
Fodder---85%±15%

Kits components

Item	Specifications
Micro ELISA Plate	96 wells
Standard Liquid(black cap)	1mL each (0ppb,0.5ppb,1.5ppb,4.5ppb,13.5ppb,40.5ppb)
High ConcentratedStandard(100 ppb)	1mL
HRP Conjugation(red cap)	11mL
Antibody Working Solution(blue cap)	5.5mL
Substrate Reagent A(white cap)	6mL
Substrate Reagent B(black cap)	6mL
Stop Solution(yellow cap)	6mL
20×Concentrated Wash Buffer(white cap)	40mL
2×RedissolveSolution(yellow cap)	50mL
Product Description	1 copy

Other supplies required

Instruments: Micro-plate reader, Printer, Homogenizer, Nitrogen Evaporators, Oscillators, Centrifuge, Graduated pipette, Balance(sensibility 0.01g).

High-precision transferpettor: single channel(20-200μL, 100-1000μL), Multichannel(300μL).

Reagents: Anhydrous acetonitrile, methyl alcohol.

Experimental preparation

Bring all reagents and samples to room temperature before use.

Open the micro-plate reader in advance, preheat the instrument, and set the testing parameters.

1. **Sample pretreat ment Notice:** experimental apparatus should be clean, and the pipette should be disposable to avoid cross-contamination during the experiment.

2. Solution preparation

Solution 1: Sample extraction buffer

Diluterequisite CH_3OH with deionized water,and mix thoroughly. CH_3OH(V):deionized water(V)=0.5:9.5

Solution 2: Redissolve Solution

Double dilute the 2× Reconstitution fluid with deionized water (Redissolve Solution (V):deionized water (V) =1:1). Redissolve Solutioncan be stored 1 month at 4℃

Solution 3: Washing Buffer

Dilute 20×Concentrated Wash Buffer with deionized water (20×Concentrated Wash Buffer (V): deionized water (V) = 1:19).

3. Sample pretreatment procedure

3.1 Pretreatment ofTissue(pork liver, pork,etc.):

(1) Weigh2±0.05g homogenatesample without fat, add 2mL deionized water and 8mL Anhydrous acetonitrile, and mixthoroughly.Incubate inwater bath at 56℃ for 10min, oscillate for 5min, centrifuge at 4000r/min at room temperature for 10min

(2) Take 5mL of upper liquid to another tube, blow-dry in 50-60℃nitrogen or air .

(3) Redissolvethe residual with 1 mLredissolve Solution, add2mL n-hexane, mix thoroughly. Centrifuge at 4000r/min at room temperature for 5 min.

(4) Discard the upperorganic phase, take 50uLlower liquid foranalyze.

Note: Sample dilution factor: 1, minimum detection dose: 1.5ppb

3.2 Pretreatment ofFodder

(1) Weigh1±0.05g choppedfodder, add 10mL of sample extraction bufferand mix thoroughly.Incubate inwater bath at 56℃ for 10min, oscillate for 5min, centrifuge at 4000r/min at room temperature for 10min

(2) Take 50μLupper liquid to another tube, add 450μLredissolvesolution,and mix thoroughly.

(3) Take 50μL upper liquid from step(2) foranalyze.

Note: Sample dilution factor: 100, minimum detection dose: 150ppb

Assay procedure

Centrifuge the sample again after thawing before the assay. Bring all reagents to room temperature for 30 min before use.**All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming.**Take out the Micro plate and frame of needed amount, and keep the remained Micro plate into the valve bag and stored at 2-8℃.

1. **Number:** number the sample and standard in order(multiplewell), and keep a record of standard wells and sample wells.
2. **Add sample:** add 50μL of Standard, or Sample per well, then add 50μL of antibody working solution, cover the plate with sealer we provided, oscillate for 5s gently to mix thoroughly, incubate for 30 min at 37℃ in the dark.
3. **Wash:** uncover the sealer carefully, remove the liquid in each well. Immediately add 250μL of washing buffer to each well and wash. Repeat wash procedure for 5 times, 30s intervals/time. Invert the plate and pat it against thick clean absorbent paper (If bubbles exist in the wells, clean tips can be used to prick them).
4. **HRP conjugate:**add 100μL HRP conjugate to each well, shading light incubation for 30min at 37℃.
5. **Wash:** repeat step 3.
6. **Color Development:** add 50μL of substrate solution A to each well, and then add 50μL of substrate solution B. Gently oscillate for 5s to mix thoroughly. Incubate shading light for 15min at 37℃(The reaction time can be or extended according to the actual color change).
7. **Stop reaction:** add 50μL of stop solution to each well, oscillate gently to mix thoroughly.
8. **OD Measurement:** determine the optical density(OD value) of each wellat 450 nm with a micro-plate reader (the 450/630 nm double wavelength is recommended). This step should be finished in 10min after stop reaction.

Result analysis

1. **Absorbance (%)=A/A_0×100%**

   A: Average absorbance of standard or sample

   A_0: Average absorbance of 0 ppb Standard

2. **Drawing and calculation of standard curve**

   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 sampleto 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.

   For this kit, it is more convenient to use professional analysis software for accurate and fast analysis on a large number of samples.

Notes

1. Overall OD value will be lower when reagents is not brought to room temperature before use or room temperature is below25℃.
2. During the washing procedure, if the wells turn dry, it will lead to bad linear standard curve and poor repeatability, move on to the next step immediately after wash.
3. Mix thoroughly and wash the plate completely. The consistency of wash procedure can strongly affect the reproducibility of this ELISA kit.
4. Micro ELISA plate should be covered by plate sealer.Avoid the reagents to strong light.
5. Do not use expired kit and reagents of different batches of kits.
6. TMB should be abandoned if it turns color. When OD value of standard(concentration: 0)is below0.5 unit(A_{450nm}＜0.5), it indicates reagent is deteriorated.
7. Stop solution is caustic, avoid contact withskinandeyes.

Storage and valid period

Storage: Store at 2-8℃. Avoid freeze / thaw cycles.

Valid Period: 1 year, production date is on the packing box.