MQCA (3-methyl quinoxaline-2-carboxylic acid) ELISA Kit

Catalog No: E-FS-E008

96T/96T*3

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.

Toll-free: 1-888-852-8623 Tel: 1-832-243-6086 Fax: 1-832-243-6017

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

This kit uses Competitive-ELISA as the method for the quantitative detection. It can detect 3-methyl quinoxaline-2-carboxylic acid (MQCA) in samples, such as tissue, liver, etc. This kit is composed of ELISA Microtiter plate, HRP conjugate, antibody working solution, standard and other supplementary reagents. The microtiter plate in this kit has been pre-coated with coupled antigen. During the reaction, MQCA in the samples or standard competes with coupled antigen on the solid phase supporter for sites of anti-MQCA antibody. Then Horseradish Peroxidase (HRP) conjugate is added to each microtiter plate well, and substrate reagent is added for color development. There is a negative correlation between the OD value of samples and the concentration of MQCA. The concentration of MQCA in the samples can be calculated by comparing the OD of the samples to the standard curve.

Technical indicator

Reaction mode: 25℃, 30 min~30 min~15 min

Detection limit: Tissue ---1 ppb; Liver---2 ppb

Cross-reactivity: MQCA ---100%,

N-(2-hydroxyethyl)-3-methylquinoxaline-2-carboxamide ---＜1.0%

QCA, Olaquindox, Quinacetone, Desoxyquinoceton, Mequindox ---＜1.0%,

Sample recovery rate: 85%±25%

Kits components

Item	Specifications
ELISA Microtiter plate	96 wells
Standard Liquid	1 mL each (ppb=ng/mL=ng/g) (0ppb,0.3ppb,0.9ppb,2.7ppb,8.1ppb,24.3ppb)
HRP Conjugate	11 mL
Antibody Working Solution	6 mL
Substrate Reagent A	6 mL
Substrate Reagent B	6 mL
Stop Solution	6 mL
20×Concentrated Wash Buffer	40 mL
2×Reconstitution Buffer	50 mL
Plate Sealer	3 pieces
Sealed Bag	1 piece
Manual	1 copy

Note: All reagent bottle caps must be tightened to prevent evaporation and microbial pollution.

Other materials required but not supplied

Instruments: Microplate reader, Printer, Homogenizer, Nitrogen Evaporators, Water bath, Oscillators, Centrifuge, Graduated pipette, Balance (sensibility 0.01 g).

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

Reagents: Ethyl acetate, Concentrated H_2SO_4, N-hexane.

Notes

1. The overall OD value will be lower when reagents have not been brought to room temperature before use or room temperature is below 25℃.
2. If the wells turn dry during the washing procedure, it will lead to bad linear standard curve and poor repeatability. Operate 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. FOR RESEARCH USE ONLY. ELISA Microtiter plate should be covered by plate sealer. Avoid the kit to strong light.
5. **Each reagent is optimized for use in the E-FS-E008. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other E-FS-E008 with different lot numbers.**
6. Substrate Reagent should be abandoned if it turns blue color. When OD value of standard (concentration: 0)＜0.5 unit (A450nm＜0.5), it indicates the reagent be deteriorated.
7. Stop solution is caustic, avoid contact with skin and eyes.
8. As the OD values of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique, washing technique or temperature effects), the operator should establish a standard curve for each test.
9. Even the same operator might get different results in two separate experiments. In order to get reproducible results, the operation of every step in the assay should be controlled.
10. If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.
11. The kit is used for rapid screening of actual samples. If the test result is positive, the instrument method such as HPLC, LC/MS, etc. can be used for quantitative confirmation.

Storage and valid period

Store at 2~8℃ for 1 year. Avoid freeze.

Please store the opened plate at 2~8℃, the shelf life of the opened kit is up to 1 month.

Expiry date: expiration date is on the packing box.

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 pretreatment Notice:**

   Experimental apparatus should be clean, and the pipette should be disposable to avoid cross-contamination during the experiment.

2. **Solution preparation**

   Solution 1: 2 M H_2SO_4 Solution

   Take 15 mL **Concentrated H_2SO_4,** slowly add to 120 mL deionized water, mix well, store at room temperature

   Solution 2: Reconstitution Buffer

   Dilute the **2×Reconstitution Buffer** with deionized water. It can be stored at 4℃ for one month. (2×Reconstitution Buffer (V): Deionized water (V) = 1:1)

   Solution 3: Wash 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 of tissue (livestock, fish, shrimp) sample:

(1) Weigh 1±0.05 g of homogenate sample without fat, add 8 mL of **Ethyl acetate**, add 1 mL of **2 M H_2SO_4 Solution (**Solution 1), oscillate for 30s, Centrifuge at 4000 r/min at room temperature for 5 min.

(2) Take 4 mL of upper liquid (Ethyl acetate layer) to a clean glass tube, dry at 50-60℃ nitrogen evaporators or water bath.

(3) Dissolve the dried material with 1 mL of n-hexane, oscillate 30s and add 1mL of **Reconstitution Buffer (**Solution 2), mix fully, centrifuge at 4000 r/min at room temperature for 5min.

(4) Remove the upper organic phase, take 50 μL of lower liquid for analysis.

Note: Sample dilution factor: 2, minimum detection limit: 1 ppb

3.2 Pretreatment of liver sample:

(1) Weigh 1±0.05 g of homogenate sample without fat, add 8 mL of **Ethyl acetate**, then add 1mL of **2 M H_2SO_4 Solution (**Solution 1), oscillate for 30s, centrifuge at 4000 r/min at room temperature for 5min.

(2) Take 2 mL of upper liquid (Ethyl acetate layer) to a clean glass tube, dry at 50-60℃ nitrogen evaporators or water bath.

(3) Dissolve the dried material with 2 mL of **N-hexane**, oscillate 30s and add 1 mL of **Reconstitution Buffer (**Solution 2), mix fully, centrifuge at 4000 r/min at room temperature for 5 min.

(4) Remove the upper organic phase, take 50 μL lower liquid for analysis.

Note: Sample dilution factor: 4, minimum detection limit: 2 ppb

Assay procedure

Restore all reagents and samples to room temperature before use. All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming. The unused ELISA Microtiter plate should be sealed as soon as possible and stored at 2~8℃.

1. **Number:** number the sample and standard in order (multiple well), and keep a record of standard wells and sample wells. **Standard and Samples need test in duplicate.**
2. **Add sample:** add 50 μL of **Standard or Sample** per well, then add 50 μL of **Antibody Working Solution** to each well, cover the plate with plate sealer, oscillate for 5s gently to mix thoroughly. Incubate at 25℃ for 30min in shading light.
3. **Wash:** uncover the sealer carefully, remove the liquid in each well. Immediately add 300 μL of **Wash Buffer** (Solution 3) to each well and wash. Repeat wash procedure for 5 times, 30sintervals/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 100 μL of **HRP Conjugate** to each well, incubation at 25℃ for 30 min in shading light.
5. **Wash:** repeat step 3.
6. **Color Development:** add 50 μL of **Substrate Reagent A** to each well, and then add 50 μL of **Substrate Reagent B**. Gently oscillate for 5s to mix thoroughly. Incubate at 25℃ for 15min in shading light (The reaction time can be or extended according to the actual color change).
7. **Stop reaction:** add 50 μL of **Stop Solution** to each well, oscillate gently to mix thoroughly.
8. **OD Measurement:** determine the optical density (OD value) of each well at 450 nm (reference wavelength 630 nm) with a microplate reader. This step should be finished in 10 min 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 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.**

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

Olaquindox Metabolites (E-FS-E008) **Standard Curve**