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

Catalog No: E-FS-E150

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 muscle sample. 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,15 min

Detection limit: Muscle---0.2 ppb

Cross-reactivity: MQCA ---100%,

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

Sample recovery rate: 90%±30%

Kits components

Item	Specifications
ELISA Microtiter plate	96 wells
Standard Liquid	1.5 mL each (ppb=ng/mL=ng/g) (0ppb,0.2ppb,0.5ppb,1ppb,2ppb,5ppb)
Concentrated Antibody Working Solution	0.8 mL
Concentrated HRP Conjugate	0.8 mL
HRP Conjugate Dilution	8 mL
4 M H_2SO_4	50 mL
Sample Diluent	50 mL
Substrate Reagent A	7 mL
Substrate Reagent B	7 mL
Stop Solution	7 mL
20×Concentrated Wash Buffer	25 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, 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-E150. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other E-FS-E150 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

   Dilute the **4 M H_2SO_4** with deionized water. (4 M H_2SO_4 (V): Deionized water (V) = 1:1)

   Solution 2: Wash Buffer

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

   Solution 3: Antibody Working Solution

   Dilute **Concentrated Antibody Working Solution** with **Concentrated HRP Conjugaten** and **HRP Conjugate Dilution**. (Concentrated Antibody Working Solution (V): Concentrated HRP Conjugate (V): HRP Conjugate Dilution (V) =1:1:10) (Prepare and use immediately, do not store for long term)

3. Sample pretreatment procedure

3.1 Pretreatment of muscle (chicken, duck, pork) sample:

(1) Weigh 2±0.05 g of homogenate sample without fat to 50mL centrifuge tube, add 1 mL of **Deionized water** and 8 mL of **Ethyl acetate**, oscillate for 1min.

(2) Add 1 mL of **2 M H_2SO_4 Solution** (Solution 1), oscillate for 1min, Centrifuge at 4000 r/min at room temperature for 5 min.

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

(4) Dissolve the dried material with 2 mL of **N-hexane**, add 0.5mL of **Sample Diluent**, gently invert it up and down 10 times. Do not shake it vigorously, centrifuge at 4000 r/min at room temperature for 5min.

(5) Remove the upper N-hexane and the impurities in the middle layer; take 40 µL of lower liquid for analysis.

Note: Sample dilution factor: 0.5, Minimum detection limit: 0.2 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 40 μL of **Standard or Sample** per well, then add 60 μL of **Antibody Working Solution (**Solution 3**)** to each well, cover the plate with plate sealer, oscillate for 10s 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 260 μL of **Wash Buffer** (Solution 2) to each well and wash. Repeat wash procedure for 4 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. **Color Development:** add 100 μL of **Substrate Reagent A** and **Substrate Reagent B** mixture. (Substrate Reagent A and Substrate Reagent B are mixed 1:1 according to volume, must be fully mixed, the mixture is used within 5 minutes, avoid the use of metal container, avoid stirring reagents.) Gently oscillate for 5 s to mix thoroughly. Incubate 25℃ for 15-20 min at in shading light.
5. **Stop reaction:** add 50 μL of **Stop Solution** to each well, oscillate gently to mix thoroughly.
6. **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-E150) **Standard Curve**