Technical Manual

QCA (Quinoxaline-2-carboxylic acid) ELISA Kit)

- Catalogue Code: FSES0058
- Competitive ELISA Kit
- Research Use Only

Contents

1. Key features and Sample Types

Sensitivity:

0.1 ppb (ng/mL)

Assay Procedure:

25°C, 30 min~15 min

Detection Limit:

Muscle - 1 ppb

Cross Reactivity:

Quinoline-2-Carboxylic Acid (QCA) - 100%; Desoxycarbadox (DCBX) - < 0.1%,

3-Methylquinoline-2-Carboxylic Acid (MQCA) - 78%

Sample Recovery rate:

90%±30%.

Storage:

2-8°C for 6 months.

Expiry:

See Kit Label

2. Storage

Store the kit at 2~8°C. Do not freeze any test kit components.
Return any unused microwells to their original foil bag and reseal them together with the desiccant provided and further store at 2 – 8 °C.

3. Test Principle

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

4. Kit Contents

Each kit contains reagents for 96 assays including:

No.	Component	96-WellKit
1	ELISA Microtiter Plate	96 wells
2	Standards	1 mL each (0 ppb, 0.1 ppb, 0.3 ppb, 0.9 ppb, 2.7 ppb, 8.1 ppb)
3	HRP Conjugate Diluent	7 mL
4	11×Concentrated HRP Conjugate	1 mL
5	Substrate Reagent A	6 mL
6	Substrate Reagent B	6 mL
7	Stop Solution	6 mL
8	20×Concentrated Wash Buffer	25 mL
9	5×Concentrated Sample Solution	25 mL
10	Plate Sealer	3 pieces
11	Sealed Bag	1 piece
12	Manual	1 copy

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

Additional materials required:

Other materials required but not supplied

- **Instrument:** Microplate reader, Homogenizer, Vortex mixer, Centrifuge, Nitrogen evaporators, Water bath, Balance (sensitivity 0.01 g).
- **Micropipette:** Single channel (20-200 μL, 100-1000 μL), Multichannel (30-300 μL).
- **Reagents:** H_2SO_4, Ethyl acetate, N-hexane.

5. Experimental Preparation

Bring all reagents and samples to room temperature before use.

Open the microplate reader in advance, preheat the instrument, and set the testing parameters.

1. Sample pre-treatment Notice:

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

2. Solution preparation

Please prepare solution according to the number of samples. Don't use up all components in the kit at once!

Solution 1: 2 M H_2SO_4 Solution

Dilute 10.65 mL of **H_2SO_4** slowly to 100 mL with deionized water. Mix fully.

Solution 2: Wash Buffer

Dilute **20×Concentrated Wash Buffer** with deionized water.

(20×Concentrated Wash Buffer (V): Deionized water (V) = 1:19).

Solution 3: Sample Solution.

Dilute **5×Concentrated Sample Solution** with deionized water.

(5×Concentrated Sample Solution (V): Deionized water (V) = 1:4).

Solution 4: HRP Conjugate

Dilute **11×Concentrated HRP Conjugate** with **HRP Conjugate Diluent**.

(11× Concentrated HRP Conjugate (V): HRP Conjugate Diluent (V) = 1:10).

Note: Please use immediately, it cannot be stored.

3. Sample pre-treatment procedure

Targets may be distributed unevenly, resulting in no detection. To avoid this, ensure to take sufficient samples when sampling.

3.1 Pre-treatment of meat (livestock) sample:

1. Weigh 2±0.05 g of homogenate sample into a 50 mL centrifuge tube.
2. Add 1 mL of deionized water and 8 mL of **Ethyl acetate** to vortex for 3 min. Add 1 mL of **2 M H_2SO_4 Solution** (Solution 1), and vortex for 1 min.
3. Centrifuge for 5 min at 4000 r/min.
4. Take 2 mL of the supernatant organic phase into another clean centrifuge tube, dry at 50-60°C with nitrogen evaporators or water bath. Add 0.5 mL of **N-hexane** to dissolve residues, then add 0.5 mL of **Sample Solution** (Solution 3), mix fully.
5. Centrifuge for 5 min at 4000 r/min.
6. Remove the supernatant N-hexane and intermediate residues completely.
7. Take 50 µL of lower layer liquid for detection.

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

6. Assay Procedure

Bring all reagents and samples to room temperature (25°C) 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°C.

1. **Number:** number the sample and standard in order (multiple well), and keep a record of standard wells and sample wells. **Standard and Samples must be tested in duplicate.**
2. **Add Sample:** add 50 µL of **Standard or Sample** per well, add 50 µL of **HRP Conjugate** (Solution 4) into each well. Gently vortex for 10s to mix thoroughly and cover the plate with plate sealer. Incubate at 25°C for 30 min away from direct sunlight.
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 the wash procedure for 5 times, 30s intervals/time. Invert the plate and pat it against absorbent paper (If bubbles exist in the wells, clean tips can be used to prick them).
4. **Colour Development:** add 50 µL of **Substrate Reagent A** to each well, and then add 50 µL of **Substrate Reagent B**. Gently vortex for 10s to mix thoroughly. Incubate at 25°C for 15 min away form direct sunlight (The reaction time can be extended according to the actual colour change).
5. **Stop Reaction:** add 50 µL of **Stop Solution** to each well, vortex gently for 10s 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.

7. Data Analysis

1. **Absorbance (%) = $A/A_0 \times 100\%$**

   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 of batch samples.

8. 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°C.
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. ELISA Microtiter plate should be covered by plate sealer. Avoid the kit to strong light.
5. **Each reagent is optimized for use in the FSES0058. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other FSES0058 with different lot numbers.**
6. Substrate Reagent should be abandoned if it turns blue colour. When OD value of standard (concentration: 0) < 0.8 unit (A450nm < 0.8), it indicates the reagents are 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.

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