Technical Manual

MG (Malachite Green) ELISA Kit

- Catalogue Code: FSES0011
- Competitive ELISA Kit
- Research Use Only

Contents

1. Key features and Sample Types

Sensitivity:

0.025 ppb (ng/mL)

Assay Procedure:

25°C, 30 min~30 min~15min

Detection Limit:

Muscle - 0.1 ppb

Cross Reactivity:

Malachite Green - 100%; Crystal violet - 80%;

Leucomalachite green (oxidized) - 100%; Leucocrystal violet (oxidized) - 80%

Sample Recovery rate:

Muscle - 85%±15%.

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 Malachite Green (MG) in samples, such as muscle, 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, MG in the samples or standard competes with coupled antigen on the solid phase supporter for sites of anti-MG 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 MG. The concentration of MG 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	High Concentrated Standard (10 ppb)	1 mL
3	Standards (empty bottles)	(0 ppb, 0.025 ppb, 0.05 ppb, 0.1 ppb, 0.2 ppb, 0.4 ppb)
4	HRP Conjugate	11 mL
5	Antibody Working Solution	5.5 mL
6	Substrate Reagent A	6 mL
7	Substrate Reagent B	6 mL
8	Stop Solution	6 mL
9	Cosolvent	6 mL
10	Oxidant	6 mL
11	10×Reconstitution Buffer	20 mL
12	20×Concentrated Wash Buffer	40 mL
13	Plate Sealer	3 pieces
14	Sealed Bag	1 piece
15	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

- **Instruments:** Microplate reader, Printer, Homogenizer, Nitrogen evaporators, Water bath, Vortex mixer, Centrifuge, Graduated pipette, Balance (sensitivity 0.01 g).
- **Micropipette:** Single channel (20-200 μL, 100-1000 μL), Multichannel (30-300 μL).
- **Reagents:** Acetonitrile, Ethyl acetate, Methanol.

5. Experimental Preparation

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

Open the micro-plate 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. Reagent preparation

Solution 1: Reconstitution Buffer

Dilute the **10× Reconstitution Buffer** with deionized water and **Methanol**. (10× Reconstitution Buffer (V): Deionized water (V): Methanol (V) =1:5:4), then mix fully.

Solution 2: Wash Buffer

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

3. Sample pre-treatment procedure

Do not use Oil-based Marker Pen during the marking process to avoid contaminating the sample.

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

Pre-treatment of low-fat muscle (carassius auratus, silver carp, shrimp) sample:

1. Remove the skin, bone and fat of fish, shrimp. Homogenize the sample, use Homogenizer.
2. Weigh 1 g of homogenate meat into a 50 mL centrifuge tube. Add 0.3 mL of **Acetonitrile** and 6 mL of **Ethyl acetate**, vortex for 5 min to ensure that the meat is not caked.
3. Centrifuge at 4000 r/min for 10 min at room temperature. Take 3 mL of the supernatant to another clean and dry glass tube, add 50 μL of **Oxidant,** vortex for 2 min, and add 50 μL of **Cosolvent** (Does not vortex).
4. Dry at 50°C with nitrogen evaporators or water bath. (There should be 1 drop of liquid in the bottom of tube after drying.)
5. Add 1 mL of **Reconstitution Buffer** (Solution 1) and mix fully. Centrifuge at 4000 r/min for 10 min at room temperature, remove the upper fat layer.
6. Take 50 μL of the lower liquid for analysis (Avoid inhaling the fat layer).
   Note: Sample dilution factor: 2, detection limit: 0.1 ppb

6. Assay Procedure

Bring 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°C.

Prepare the Standard Liquid. Standard Liquid of low concentration is unstable, prepare fresh solution before use.

Take 3 mL of **Reconstitution Buffe**r (Solution 1) into **0 ppb bottle**. Take 1.5 mL of **Reconstitution Buffe**r (Solution 1) into **0.025 ppb bottle**, **0.05 bottle**, **0.1 ppb bottle, 0.2 ppb bottle** respectively. Take 2.88 mL of **Reconstitution Buffe**r (Solution 1) into **0.4 ppb bottle**.

(1) **Standard Liquid 6**: Take 120 µL of **High Concentrated Standard (10 ppb)** into 0.4 ppb bottle, then mix fully. The concentration of Standard Liquid 6 is 0.4 ppb.
(2) **Standard Liquid 5**: Take 1.5 mL of Standard Liquid 6 into 0.2 ppb bottle, then mix fully. The concentration of Standard Liquid 5 is 0.2 ppb.
(3) **Standard Liquid 4**: Take 1.5 mL of Standard Liquid 5 into 0.1 ppb bottle, then mix fully. The concentration of Standard Liquid 4 is 0.1 ppb.
(4) **Standard Liquid 3**: Take 1.5 mL of Standard Liquid 4 into 0.05 ppb bottle, then mix fully. The concentration of Standard Liquid 3 is 0.05 ppb.
(5) **Standard Liquid 2**: Take 1.5 mL of Standard Liquid 3 into 0.025 ppb bottle, then mix fully. The concentration of Standard Liquid 2 is 0.025 ppb.
(6) **Standard Liquid 1**: Use Reconstitution Buffer directly. The concentration of Standard Liquid 1 is 0 ppb.

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, then add 50 µL of **Antibody Working Solution**, cover the plate with plate sealer, gently vortex for 5s to mix thoroughly. Incubate at 25°C for 30 min in the dark.
3. **Wash:** uncover the sealer carefully, remove the liquid in each well. Immediately add 300 µL of **Wash Buffer** (Solution 2) to each well and wash. Repeat 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. **Add HRP conjugate:** add 100 µL of **HRP Conjugate** to each well, incubate at 25°C for 30 min in the dark
5. **Wash:** Repeat step 3.
6. **Colour Development:** add 50 µL of **Substrate Reagent A** to each well, and then add 50 µL of **Substrate Reagent B**. Gently vortex for 5s to mix thoroughly. Incubate at 25°C for 15 min in the dark (The reaction time can be extended according to the actual colour change).
7. **Stop reaction:** Add 50 µL of **Stop Solution** to each well, vortex 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.

7. Data Analysis

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

   A: Average absorbance of standard or sample

   A_0: Average absorbance of 0ppb 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 Micro-plate should be covered by plate sealer. Avoid the kit to strong light.
5. **Each reagent is optimized for use in the FSES0011. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other FSES0011 with different lot numbers.**
6. Substrate Reagent should be abandoned if it turns blue colour. When OD value of standard (concentration: 0) < 0.5 unit (A450nm < 0.5), it indicates the reagent may 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.

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