Elabscience®

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MG(MalachiteGreen oxalate) ELISA Kit

Catalog No: E-FS-E013

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)
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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 Indirect-Competitive-ELISA as the method. It can be used to detect Malachite Green oxalate (MG) in samples. 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 MG antigen. During the reaction, MG in the samples or standard competes with MGantigen coated on the solid phase supporter for MGantibody. Then Horseradish Peroxidase (HRP) conjugate is added to each micro plate well, and TMB substrate 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.

Technical indicator

Sensitivity: 0.025ppb (ng/mL)

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

Detection limit: Fish/shrimp ---0.1ppb

Cross-reactivity: Malachite Green---100%, Crystal violet---80%, Recessive Malachite Green (oxidized) --- 100%, Recessive Crystal violet (oxidized) --- 80%

Sample recovery rate: Fish/shrimp and other aquatic products, Water sample---85%±15%.

Kits components

Item	Specifications
Micro ELISA Plate	96 wells
High Concentration Standard(10ppb)	1mL
Standard Liquid	1 mL each 0ppb, 0.025 ppb,0.05 ppb,0.1 ppb,0.2 ppb, 0.4 ppb.
HRP Conjugate(Red cap)	11mL
Antibody Working Solution (Blue cap)	5.5mL
Substrate Reagent A (Whitecap)	6mL
Substrate Reagent B (Black cap)	6mL
Stop Solution (Yellow cap)	6mL
Cosolvent (Yellow cap)	6mL
Oxygenant (Black cap)	6mL
20×Concentrated Wash Buffer (White cap)	40mL
10×Redissolved Buffer (Yellow cap)	20mL
Product Description	1 copy

Other supplies required

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

High-precision pipettor: Single-channel (20-200μL, 100-1000μL), Multi-channel (300μL).

Reagents: Acetonitrile (chromatographic pure), Ethyl acetate, Methanol.

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. **Notes for sample pretreatment:** experimental apparatus should be clean, and the pipette should be disposable to avoid cross-contamination during the experiment.

2. **Reagent preparation**

   Reagent 1: Re-dissolvesolution. Take 10 mL 10×Concentrated Re-dissolve solution, add50 mL deionized water and 40 mL methanol and mix fully.

   Reagent2: Wash buffer. Dilute 20×Concentrated Wash Bufferwith deionized water (20× Concentrated Wash Buffer (V): deionized water (V) =1:19).

3. **Sample pretreatmentprocedure**

Pretreatmentoffish/shrimpsample:

(1) Weigh1ghomogenatewithout bones intoa 50 mL EP tube.Add0.3mL acetonitrile and 6 mL ethyl acetate,oscillate fully (at least for 5 minutes).

(2) Centrifuge at 4000r/min for 10min at room temperature. Take 3 mL of the supernatant to a clean and dry glass tube, add 50 μLoxygenant and oscillate for 2 minutes.

(3) Add 50 μLcosolvent to each tube, dry with Nitrogen Evaporators in the condition of water bath at 50℃. (There should be 1 drop of liquid in the bottom of tube after drying. There will be residual yellow sticky liquid in the tube if the lipid content in sample is too high.)

(4) Add1 mL re-dissolve solutionand mix fully. Take 50μL for detection and analysis.

Note: Sample dilution factor: 2, minimum detection dose: 0.1ppb

Assay procedure

Bring all reagents and samples to room temperature before use.Centrifuge the sample again after thawing before the assay. **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℃.

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

Add 3 mL re-dissolve solution into 0 ppb standard solution, 1.5 mL re-dissolve solution for 0.025 ppb, 0.05 ppb, 0.1 ppb, and 0.2 ppb standard, and 2.88 mL re-dissolve solution for 0.4 ppb standard solution.

Standard solution 6: Take 120 μL of high concentration solution(10 ppb) into 2.88 mL re-dissolve solution, cover tightly and mix fully, then the concentration will be 0.04 ppb.

Standard solution 5: Take 1.5 mL of standard solution 6 into 1.5 mL re-dissolve solution, cover tightly and mix fully, then the concentration will be 0.2 ppb.

Standard solution 4: Take 1.5 mL of standard solution 5 into 1.5 mL re-dissolve solution, cover tightly and mix fully, then the concentration will be 0.1 ppb.

Standard solution 3: Take 1.5 mL of standard solution 4 into 1.5 mL re-dissolve solution, cover tightly and mix fully, then the concentration will be 0.05 ppb.

Standard solution 2: Take 1.5 mL of standard solution 3 into 1.5 mL re-dissolve solution, cover tightly and mix fully, then the concentration will be 0.025 ppb.

Standard solution 1: Use the re-dissolve solution directly, then the concentration will be 0 ppb.

1. **Number:** Number the sample and standard in order (multiple well), 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.Gently oscillate for 5s to mix thoroughly. Incubate for 30min at 25℃ in the dark.
3. **Wash:** Remove the liquid in each well. Immediately add 250μL of washing buffer to each well and wash. Repeat the 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. **Add HRP conjugate:** Add 100μL HRP conjugate to each well, incubate for 30min at 25℃in the dark.
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 for 15min at 25℃ in the dark(The reaction time can be 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 well at 450nm 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 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 valueof 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 of batch samples.

Notes

1. The overall OD values will be lower when reagents have not been brought to room temperature before use or theroom temperature is below25℃.
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 repeatability of this ELISA kit.
4. Micro ELISA plate should be covered with plate sealer.Prevent the reagents to strong light.
5. Do not useexpired kit and reagents of different batches.
6. TMB should be abandoned if it turns color.
7. When OD value of standard(concentration: 0)is below0.5 unit(A_{450nm}＜0.5), it indicates the reagent isdeteriorated.
8. Stop solution is caustic, avoid of contacting with the skin.

Storage and valid period

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

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