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Malachite Green ELISA Kit

02/21

(Catalog # E4941-100, 96 assays, Store at 4°C)

I. Introduction:

Malachite Green is a triarylmethane dye that applications in numerous industries. It is used in the pigment industry wherein it is used as a dye to color silk, paper, wool, and leather. In the aquaculture industry, it is used as an ectoparasiticide, fungicide, and antiseptic. It is also widely used as a food coloring agent and food additive. However, the use of malachite green has been controversial due to its toxic effects. The dye is reported to cause mutagenesis, carcinogenesis, immunological, reproductive, and respiratory toxicity. BioVision's Malachite Green ELISA kit is used to quantitatively measure Malachite Green in muscle tissue samples. The kit is based on the Competitive ELISA principle. Samples and standards are added to the microwell plate that is pre-coated with an antigen and competes for binding to the anti-Malachite Green antibody. The HRP conjugate is added to each well and any unattached conjugates are washed off using Wash Buffer. The HRP enzymatic reaction is detected by the addition of substrate reagents. Finally, the reaction is terminated with an acidic stop solution. The color developed is inversely proportional to the concentration of Malachite Green in the samples.

II. Application:

This ELISA kit is used for *in vitro* quantitative determination of Malachite Green
Detection Limit: 0.1ppb for tissue (muscle) samples
Sensitivity: 0.025ppb
Cross reaction: Malachite Green 100%, Crystal violet 80%, Leucomalachite green (oxidized) 100%, Leucocrystal violet (oxidized) 80%

III. Sample Type:

Tissue (muscle)

IV. Kit Contents:

Components	E4941-100	Part No.
Micro ELISA Plate	8 X 12 Strips	E4941-100-1
Standard (S0 – S6)	1 ml X 7	E4941-100-2
HRP Conjugate	11 ml	E4941-100-3
Antibody working solution	5.5 ml	E4941-100-4
Substrate A	6 ml	E4941-100-5
Substrate B	6 ml	E4941-100-6
Stop Solution	6 ml	E4941-100-7
Wash Buffer (20X)	40 ml	E4941-100-8
Co-solvent	6 ml	E4941-100-9
Oxidant	3 ml	E4941-100-10
Reconstitution Buffer (10X)	20 ml	E4941-100-11
Plate Sealer	3	E4941-100-12

V. User Supplied Reagents and Equipment:

- Chemicals: deionized water, Acetonitrile, Ethyl acetate, Methanol
- Microplate reader, Nitrogen evaporator
- Clean eppendorf tubes and graduated cylinders for preparing standards or sample dilutions
- Absorbent paper

VI. Storage and Handling:

The entire kit may be stored at 4°C for up to 12 months from the date of shipment. Opened kit may be stable for 1 month at 4°C.

VII. Reagent and Sample Preparation:

Note: Bring all reagents to room temperature (20-25°C) 30 minutes before use.
Before using the kit, spin tubes and bring down all components to the bottom of tubes.

1. **Wash Buffer (1X):** Dilute 1 part of **Wash Buffer (20X)** with 19 parts of deionized water.

2. **Standards Preparation:** Add 3 ml **Reconstitution Buffer (1X)** in Standard S0 tube. Add 1.5 ml of **Reconstituted Buffer (1X)** in **Standards S1-S4** Standard tubes and add 2.88 ml of Reconstitution Buffer (1X) in **Standard S5** tube. Prepare Standard dilutions as follows:

   Standard S5: Add 120 µl of **Standard S6 (High Concentration – 10ppb)** and mix well with **Reconstitution Buffer (1X).** The concentration of S5 is **0.4ppb**.

   Standard S4: Add 1.5 ml of **Standard S5** into **Standard S4** tube to prepare a concentration of **0.2ppb**.

   Standard S3: Add 1.5 ml of **Standard S4** into **Standard S3** tube to prepare a concentration of **0.1ppb**.

Standard S2: Add 1.5 ml of **Standard S3** into **Standard S2** tube to prepare a concentration of **0.05ppb**.

Standard S1: Add 1.5 ml of **Standard S2** into **Standard S1** tube to prepare a concentration of **0.025ppb**.

Standard S0: This tube will contain only 3 ml **Reconstitution Buffer (1X)**.

3. **Sample Preparation:**
Note: The prepared sample maybe stored for up to one day at 2-8°C.

Sample pre-treatment: The following method must be used for pre-treatment of any kind of sample:
Note: Only the disposable tips can be used for the experiments and the tips must be changed when used for absorbing different reagents.

Solution preparation before sample pre-treatment:

1) Reconstitution Buffer (1X): Dilute 1 part of **Reconstitution Buffer (10X)** with 5 parts of deionized water and 4 parts of methanol (1:5:4). Mix well.

Sample Preparation and pre-treatment (for low-fat muscle samples):

Detection limit: 0.1ppb

- The pretreatment can be performed on muscle samples of *Carassius auratus*, silver carp, and shrimp.
- De-skin, de-bone, and remove fat from fish and shrimp and then homogenize the sample.
- Take 1 gram of the homogenized sample in a 50 ml centrifuge tube, add 0.3 ml **Acetonitrile** and 6 ml **Ethyl acetate**, mix thoroughly for 5 mins, check if the meat has not caked.
- Centrifuge at 4000 r/min for 10 mins at room temperature (RT). Take 3 ml of the supernatant in a new dry glass tube, add 50 µl **Oxidant**, mix for 2 mins, then add 50 µl **Co-solvent**, do not oscillate.
- Dry the sample at 50°C with nitrogen evaporator or water bath. (After drying, there should be 1 drop of liquid at the bottom of the tube).
- Add 1 ml **Reconstitution buffer (1X)** and mix thoroughly. Centrifuge at 4000 r/min for 10 mins at RT. Discard the upper fat layer.
- Take 50 µl lower liquid for analysis.
- **NOTE: To prevent contamination of the sample, avoid using an oil-based marker pen. Also, do not inhale the upper fat layer during sample preparation.**
- **Fold of dilution of the sample: 2**

VIII. Assay Protocol:

Note: Bring all reagents and samples to room temperature 30 minutes prior to the assay.
It is recommended that all standards and samples be run at least in duplicate.
A standard curve must be run with each assay.

1. Add 50 µl of the **sample or standards** to separate duplicate wells, and then add 50 µl of the **Antibody working solution** into each well. Mix gently by shaking the plate manually, seal the microplate with the plate sealer, and incubate at 25 ^{0}C for 30 mins in dark.
2. Remove the plate sealer carefully, aspirate liquid out of microwells, and add 300 µl of **Wash Buffer (1X)** to each well. Wash for 30 secs, and then discard the buffer. Repeat the washing step five times. After the final wash step, tap to dry (if there are the bubbles after tapping, remove them with the clean tips).
3. Add 100 µl of the **HRP conjugate** in each well, cover the plate with plate sealer, manually shake the plate gently and incubate at 25 ^{0}C for 30 mins in dark.
4. Discard the solution and wash the plate as mentioned in **step 2**.
5. Add 50 µl of the **Substrate A** and then 50 µl of the **Substrate B** into each well. Mix gently by shaking the plate manually, and incubate at 25 ^{0}C for 15 mins at dark.
6. Add 50 µl of the **Stop Solution** into each well. Mix gently by shaking the plate manually. Set the wavelength of the microplate reader at 450 nm to determine the OD value (Recommend reading the OD value at the wavelength 450 nm within 5 mins).

IX. Calculation:

- **Quantitative determination**

The mean values of the absorbance values obtained for the standards and the samples are divided by the absorbance value of the first standard (zero standard) and multiplied by 100%. The zero standard is thus made equal to 100% and the absorbance values are quoted in percentages.

Absorbance Value (%) = B/B_0 X 100%

B: The average absorbance value of the sample or standard

B_0: The average absorbance value of the 0 ppb standard

To draw a standard curve: Take the absorbency value of standards as y-axis, logarithmic of the concentration of the Malachite green standards solution (ppb) as x-axis. The Malachite green concentration of each sample (ppb), which can be read from the calibration curve, is multiplied by the corresponding dilution factor of each sample followed, and the actual concentration of sample is obtained.

X. Related Products:

Sulfonamides residue ELISA Kit (K4207)
Doxycycline ELISA Kit (E4613)
Furazolidone ELISA Kit (K4231)
Fluoroquinolones ELISA Kit (K4205)
Enrofloxacin (ENR) ELISA Kit (E4277)