Instructions for Use
Version: 1.0.1

Revision date: March 2022

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Malachite Green (MG) ELISA Kit

Catalog No: abx364825

Size: 96T

Detection limit: 0.1 ppb

Sensitivity: 0.025 ppb

Cross reactivity: Malachite Green - 100%; Crystal violet – 80%; Leucomalachite green (oxidised) – 100%; Leucocrystal violet (oxidised) – 80%

Sample recovery rate: Muscle – 85% ± 15%.

Storage: Store at 2-8°C for 6 months.

Application: The qualitative detection of MG in Pig serum, plasma and other biological fluids.

Principle of the Assay: This kit is based on competitive enzyme-linked immuno-sorbent assay technology. An antigen is pre-coated onto a 96-well plate. Standards, test samples, and a HRP-conjugated MG are added to the wells n and incubated. A competitive inhibition reaction takes place between the HRP-labelled MG and the unlabelled-MG, with the pre-coated antigen. Unbound conjugates are removed using wash buffer. TMB substrate is used to quantify the HRP enzymatic reaction. After TMB substrate is added, only wells that contain sufficient MG will produce a blue coloured product, which changes to yellow after adding the acidic stop solution. The intensity of the colour yellow is inversely proportional to the MG amount bound on the plate. The OD is measured spectrophotometrically at 450 nm in a microplate reader, from which the concentration of MG can be calculated.

Kit Components

- Pre-coated 96-Well Microplate: 12 x 8
- Wash Buffer (20X): 40 ml
- Standard (10 ppb): 1 ml
- Detection Reagent A: 11 ml
- Detection Reagent B: 5.5 ml
- Substrate A: 6 ml
- Substrate B: 6 ml
- Stop Solution: 6 ml
- Cosolvent: 6 ml
- Oxidant: 6 ml
- Reconstitution Buffer (10X): 20 ml
- Plate Sealer: 3
- Hermetic Bag: 1

Materials Required But Not Provided

- 37°C incubator
- Multi and single channel pipettes and sterile pipette tips
- Squirt bottle or automated microplate washer
- 1.5 ml tubes
- Distilled water
- Absorbent filter papers
- 100 ml and 1 liter graduated cylinders
- Microplate reader (wavelength: 450 nm)
- ELISA Shaker
- Acetonitrile
- Ethyl Acetate
- Methanol
- Water Bath OR Nitrogen Evaporator

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Protocol

A. Sample Preparation

Analyse immediately or store samples at 2-8°C (within 24 hrs). For long term storage, aliquot and store at -20°C or -80°C. Avoid multiple freeze-thaw cycles.

- **Muscle:** Remove any skin, bone and fat from the sample, then homogenise. Weigh 1g of homogenate into a 50 ml centrifuge tube. Add 0.3 ml of Acetonitrile and 6 ml of Ethyl acetate, then vortex for 5 minutes. Centrifuge the sample at 4000 x g for 10 minutes at room temperature. Take 3 ml of the supernatant into another centrifuge tube and add 50 µl of Oxidant. Vortex for 2 minutes, then add 50 µl of Cosolvent. Dry at 50°C with either a water bath or nitrogen evaporator until one drop of liquid remains. Add 1 ml of Reconstitution Buffer and mix fully. Centrifuge at 4000 x g for 10 minutes at room temperature. Remove the upper layer of fat. Use 50 µl of the lower liquid for analysis, avoiding the fat layer. Dilute the sample 2-fold.

Notes:

- **Analyse samples at a 2-fold (1/2) dilution.**
- Store frozen samples undiluted. Once ready to analyse, thaw samples and dilute.
- Fresh samples, or recently obtained samples, are recommended to prevent protein degradation and denaturation that may lead to erroneous results.
- NaN_3 cannot be used as a test sample preservative, since it inhibits HRP.
- If possible, prepare solid samples using sonication and/or homogenization, as lysis buffers may (on occasion) interfere with the kit's performance.
- If a sample is not indicated in the manuals applications, a preliminary experiment to determine the suitability of the kit will be required.

B. Reagent Preparation

Wash Buffer: Dilute the concentrated Wash buffer 20-fold (1/20) with distilled water. If crystals have formed in the concentrated Wash Buffer, warm to room temperature and mix gently until the crystals have completely dissolved.

Reconstitution Buffer: Dilute the reconstitution buffer with deionised water and methanol to a ratio of 1:5:4. Mix fully.

Standard: Add 3 ml of Reconstitution buffer into a tube labelled 0 ppb. Add 1.5 ml of Reconstitution buffer into 5 tubes labelled 0.025 ppb, 0.05 ppb, 0.1 ppb and 0.2ppb respectively. Add 2.88 ml Reconstitution buffer into a tube labelled 0.4 ppb and add 120 µl of the Standard, then mix fully. Take 1.5 ml of the 0.4 ppb standard and add to the 0.2 ppb standard tube. Mix fully. Then take 1.5 ml of the 0.2 ppb standard and add to the 0.1 ppb standard tube and mix fully. Repeat for all standard concentrations, except 0 ppb, which remains solely Reconstitution buffer.

C. Assay Protocol

Equilibrate the kit components and samples to room temperature prior to use. It is recommended to measure in duplicate.

1. Set 2 of each standard concentration and test sample wells on the pre-coated plate respectively, and record their positions. *Add the solution to the bottom of each well without touching the side walls. Pipette the standards and samples up and down to mix before adding to the wells. Avoid foaming or bubbles.*
2. Aliquot 50 µl of each standard into their respective wells.
3. Aliquot 50 µl of appropriately diluted sample into the test sample wells. Gently tap the plate to mix, or use a microplate shaker.
4. Cover the plate with a plate sealer and incubate for 30 mins at 25°C in the dark.
5. Remove the cover and discard the liquid. Wash the plate 5 times with 1X Wash Buffer. *Fill each well completely with Wash buffer (300 µl) using a multi-channel Pipette or autowasher (1-2 mins soaking period is recommended). Complete removal of*

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liquid at each step is essential for good performance. After the final wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean absorbent paper towels.

6. Aliquot 100 µl of Detection Reagent to each well (except the blank well). Cover the plate with a plate sealer and incubate for 30 mins at 25°C in the dark.
7. Remove the cover, discard the liquid, and repeat the wash process as described above, 5 times.
8. Aliquot 50 µl of TMB Substrate A and 50 µl of TMB Substrate B into each well. Cover the plate with the plate sealer and gently tap the plate to mix thoroughly. Incubate at 25°C for 15 mins. The incubation time is for reference only, the optimal time should be determined by end user. Avoid exposure to light.
9. Aliquot 50 µl of Stop Solution into each well. It is important that the Stop Solution is mixed quickly and uniformly throughout the microplate to inactivate the enzyme completely.
10. Ensure that there are no fingerprints or water on the bottom of the plate, and that the fluid in the wells is free of bubbles. Measure the OD at 450 nm immediately.

Data Analysis:

$$Absorbance\ (\%) = \frac{Average\ Absorbance\ of\ Sample}{Average\ Absorbance\ 0\ ppb\ Standard} x\ 100$$

The standard curve can be plotted as the absorbance percentage of each reference standard solution (X), against the respective concentration of each standard solution (Y). The concentration of the samples can be interpolated from the standard curve. If the samples measured were diluted, multiply the concentrations from interpolation by the dilution factor, to obtain the concentration before dilution.

Precautions:

- Before using the kit, centrifuge the tubes to bring down the contents trapped in the lid.
- Do not leave the wells uncovered for extended periods between incubations. The addition of reagents for each step should not exceed 10 mins.
- Ensure that the plate is properly sealed or covered during the incubation steps, and that the time and temperature are controlled.
- Do not reuse pipette tips and tubes.
- Do not use expired components, or components from a different kit.
- The TMB substrate should be used under sterile conditions, and light exposure should be minimised. Unused substrate should be colorless, or a very light yellow in appearance. Do not discard any residual solution back into the vial.
- Please note that this kit is optimised for detection of native samples, rather than recombinant proteins or synthetic chemicals. We are unable to guarantee detection of recombinant proteins, as they may have different sequences or tertiary structures to the native protein.

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