Malachite Green ELISA Kit

Cat. No.:DEIA057
Pkg.Size:96T

Intended use

The Malachite Green ELISA Kit is a direct competitive enzyme immunoassay for the quantitative analysis of Malachite green in fish and shrimp samples. Malachite green is an organic compound that is used as a dyestuff and has emerged as a controversial agent in aquaculture. Malachite green is traditionally used as a dye for materials such as silk, leather, and paper. Although called malachite green, the compound is not related to the mineral malachite - the name just comes from the similarity of colour.
The unique features of the kit are:
1) Rapid extraction method for various samples with high recovery 90%±10%.
2) High sensitivity (0.05 ng/g or ppb) and low detection limit (0.5 ng/g or ppb for fish/shrimp, 0.3 ppb for water).
3) A quick ELISA assay (less than 2 hours regardless of number of samples).
4) High reproducibility.

Principle Of The Test

The method is based on a direct competitive ELISA assay. The anti-malachite green monoclonal antibody of interest has been coated in the plate wells. During the analysis, sample is added along with the HRP labeled Malachite green for the anti-Malachite green monoclonal antibody. If the target is pre-sent in the sample, it will compete for the antibody, thereby preventing the antibody from binding to the anti-malachite green attached to the well. The resulting color in-tensity, after addition of substrate, has an inverse relationship with the target concentration in the sample.

Reagents And Materials Provided

1. Microplate: 96 well polystyrene microplate (12 strips of 8 wells) coated with anti- malachite green monoclonal antibody;
2. Malachite Green Standards : 0, 0.05, 0.15, 0.45, 1.35, 4.05 ng/mL, 6 vials;
3. High concentration Malachite Green Standards: 100ppb, 1 vial;
4. HRP Labeled Malachite green: 7ml, 1 vial;
5. Wash Solution (10×) Concentrate: 50 ml, 1 vial;
6. TMB Solution A: 7ml, 1 vial; TMB Solution B: 7ml, 1 vial; TMB Stop Solution: 7ml, 1 vial;
7. Sample reconstitution solution(10×): 20ml, 1 vial; Cosolvent: 1vial;
8. Oxidant: 2 vials;
9. Microtiter plate sealers

Materials Required But Not Supplied

Reagents
Ethyl acetate
Alumina-N
Acetonitrile
n-Hexane
Methanol
Instruments

45-16 Ramsey Road Shirley, NY 11967, USA
Tel: 631-624-4882 ·Fax:631-614-7828
E-mail: info@creative-diagnostics.com
www.creative-diagnostics.com

Microtiter plate reader (450 nm)
Tissue Mixer
Rotary evaporator or Nitrogen Gas
Validated adjustable micropipettes, single and multi-channel
Shaker for microtiter plates (optional)
Electronic balance
Centrifuger

Storage

Unopened Kit: Store at 2 - 8°C. Do not use past kit expira-tion date.
Opened/Reconstituted Reagents: TMB Solution A; TMB Solution B; TMB Stop Solution; Wash Buffer; HRP-labeled Malachite green
The above mentioned reagents should be stored for up to 1 month at 2 - 8°C.
Microplate Wells: Return unused wells to the foil pouch con-taining the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 1 month at 2 - 8°C.

Reagent Preparation

1. Preparation of 1 x Oxidant Solution
Reconstitute Oxidant with 1 ml acetonitrile. Store at -20°C. Protect from light.
Mix 1 volume of Oxidant Solution with 89 volumes of acetonitrile before use. Prepare fresh before each assay.
2. Preparation of 1 x Sample reconstitution solution
Add 40 ml methanol and 50 ml distilled water to 10 ml sample reconstitution solution(10×).

Specimen Collection And Handling

1. Blend boneless fish/shrimp tissue in a blender/homogenizer until the sample has consistency of fine paste. Store the sample at -20ºC or in a freezer till analysis.
2. Take 1.0 g of homogenized fish/shrimp sample, add 1g Alumina-N, 0.3 ml acetonitrile and 6 ml of ethyl acetate. Vortex for 5 minutes at maximum speed.
3. Centrifuge the sample for 10 minutes at 4,000 x g at room temperature (20 – 25°C / 68 – 77°F). Transfer 3 ml of the supernatant to a 10-ml tube containing 100 uL of 1 x oxidant solution, add 20 ul cosolvent. Dry the sample by blowing nitrogen gas in a 50°C water bath.
4. Add 1 ml of n-hexane to dissolve the sample and then add 1 ml of 1 x sample reconstitution solution, vortex for 1 minute.
5. Leave the tube open and heat the sample at 85°C for 3 minutes (This step can be omitted if the lower aqueous layer is enough for the ELISA assay).
6. Centrifuge the sample for 10 minutes at 4,000 x g.
7. Discard the upper organic layer. Use 50 µl of the lower aqueous layer for the assay.

Assay Steps

1. Add 50µl of the standard solutions or samples (sample extracts) into the wells of the test strips according to the working scheme given. We recommend using duplicates or triplicates.
2. Add 50µl of HRP-labeled Malachite green to the individual
wells successively using a multi-channel pipette or a stepping pipette.
3. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop for about 30 seconds. Be careful not to spill contents.

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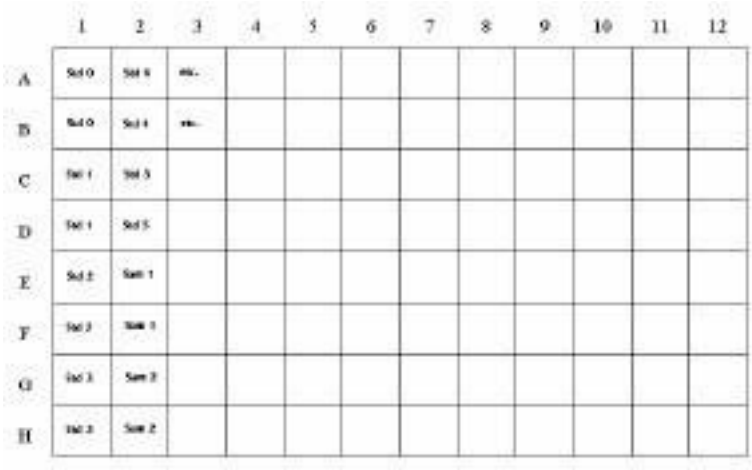
4. Incubate the strips for 45 minutes at room temperature.
5. After incubation, remove the covering and vigorously shake
the contents of these wells into a sink. Wash the strips three times using the 1× washing buffer solution. Use at least a volume of 250 µl of washing buffer for each well and each washing step. Remaining buffer in the wells should be removed by patting the plate dry on a stack of paper towels.
6. Dispense 50 µl of TMB Solution A and 50 µl TMB Solution B into each well. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop for about 30 seconds. Incubate the strips for 15-20 minutes at room temperature. Protect the strips from direct sunlight.
7. Add 50 µl of stop solution to the wells in the same sequence as for the substrate solution.
8. Read the absorbance at 450 nm and 630 nm using a mi-croplate ELISA photometer within 5 minutes after the addition of the stopping solution.

Working Scheme

The microtiter plate consists of 12 strips of 8 wells, which can be used individually for the test. The standards must be run with each test. Never use the values of standards which have been determined in a test performed previously.
Std 0-Std 5: Standards
0, 0.05, 0.15, 0.45, 1.35, 4.05 ng/mL
Sam1, Sam2, etc.: Samples



Performance Data

A standard curve can be constructed by plotting the mean relative absorbance (%) obtained from each reference stan-dard against its concentration in ng/mL on a logarithmic curve.
Relative absorbance (%) = absorbance standard (or sample) x 100/ absorbance zero standard.
Use the mean relative absorbance values for each sample to determine the corresponding concentration of the tested drug in ng/mL from the standard curve.
The ELISA sensitivity is 0.05 ng/mL, and the range of the standard curve is 0.05-4.05 ng/mL.

Evaluation

The evaluation of the ELISA can be performed using commercial ELISA evaluation programs (4-Parameter (preferred) or Logit/Log. For manual evaluation, calculate the mean absorbance value for each of the standards. Calculate the %B/ B0 for

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each standard by dividing the mean absorbance value for each standard by the Zero Standard (Standard 0) mean absorbance. Construct a standard curve by plotting the %B/ B0 for each standard on the vertical linear (y) axis versus the corresponding Enrofloxacin concentration on the horizontal logarithmic (x) axis on graph paper. %B/B0 for samples will then yield levels in ppb of a malachite green by interpolation using the standard curve. Samples showing lower concentra-tions of malachite green compared to Standard 1 (0.05 ng/mL) are considered as negative. Samples showing a higher con-centration than Standard 5 (4.05 ng/mL) must be diluted further to obtain accurate results.

Sensitivity

Sample TYPE	Detection Limit(ng/g or ppb)
Fish, shrimp	0.5
Water	0.3

Specificity

Malachite green……...100%
crystal violet……92%

Indications of instability or deterioration of the reagents

1. Values of the Positive or Negative controls, which are out of the indicated Quality control range, are indicator of possible deterioration of the reagents and/or operator or equipment errors. In such case, the results should be considered as invalid and the samples must be retested. In case of constant erroneous results classified as due to deterioration or instability of the reagents, immediately substitute the reagents with new ones, or contact our technical support for further assistance.
2. If after mixing of the TMB Solution A and B into the wells, the color of the mixture turns blue within few minutes, do not continue carrying out the testing and replace the reagents with fresh ones.

Precautions

1. The kit should be equilibrated to room temperature (20-23°C) before opening any vials and starting the assay. It is highly recommended that the solutions be used as soon as possible after rehydration.
2. Do not mix or substitute reagents with those from other lots or sources.
3. To a void cross-contamination, change pipette tips between
additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
5. Crystals could appear in the 10× wash solution due to high salt concentration in the stock solutions. Crystals are readily dissolved at room temperature or at 37°C before dilution of the buffer solutions.
6. Keep TMB Substrate protected from light.
7. The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

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