Intended Use

The Beacon Malachite Green Plate Kit is an immunoassay for the detection of Malachite Green in aquatic samples. This product is intended for research use only.

Principles

Calibrators and the Sample Extract(s) are pipetted into the test wells followed by Malachite Green HRP Enzyme Conjugate. During an incubation, Malachite Green in the calibrator/sample and Malachite Green HRP Enzyme Conjugate compete for binding to the polyclonal Malachite Green antibody immobilized on the test wells surface. Following the incubation, the wells are washed to remove any unbound Malachite Green and Malachite Green HRP Enzyme Conjugate. After washing, a colorless substrate is added to the wells and any bound enzyme conjugate will convert the substrate to a blue color. Following an incubation, the reaction is stopped with the addition of Stop Solution and the amount of color in each well is measured. The color of the unknown sample is compared to the color of the calibrators and the Malachite Green concentration of the sample is derived.

Reagents and Materials Provided

1 Plate containing 12 test strips of 8 wells each that are vacuum sealed in an aluminized pouch with a desiccant.
1 Vial of Malachite Green Calibrator Stock (100 ppb). Dilute prior to use.
1 Bottle of 30X Malachite Green HRP Enzyme Conjugate. Dilute prior to use.
1 Bottle of Dilution Buffer 1.
1 Bottle of Dilution Buffer 2.
1 Bottle of 4X Reagent A (dilute prior to use).
1 Bottle of Reagent B.
1 Bottle of Substrate.
1 Bottle of Stop Solution.

Reagents and Materials Required but Not Provided

- Pipette(s) with disposable tips capable of dispensing the required volume(s).
- Positive displacement pipette(s) with disposable tips capable of dispensing the required volume(s).
- Multichannel pipette(s) (8 channels) with disposable tips capable of dispensing the required volume(s) (optional).
- Laboratory quality distilled or deionized water.
- Reagents and materials for sample preparation.
- Reagents and materials for 1X Reagent A preparation.
- Vials for 1X enzyme conjugate and calibrator preparation.
- Personal protective equipment.
- Paper towels or equivalent absorbent material.
- Wash bottle (optional).
- Timer.
- Microtiter plate or strip reader capable of reading at 450 nm.

Kit Handling Notes and Precautions

- Read the product brochure in its entirety prior to use.
- The kit, in its original packaging, can be used until the end of the month indicated on the box label.
- Do not use reagents after expiration date.
- Store all kit components at 4°C to 8°C (39°F to 46°F) when not in use.
- Reagents should be brought to room temperature, 20°C to 28°C (62°C to 82°F), prior to use. Avoid prolonged (> 24 hours) storage at room temperature.
- Do not freeze kit components or expose them to temperatures greater than 37°C (99°F).
- Running Calibrators and Samples in duplicate will improve assay precision and accuracy.
- Precise transfer of samples and reagents by using a calibrated pipette that is capable of dispensing the required volume is critical to obtain proper assay results.
- If running more than two strips at once, the use of a multi-channel pipette is recommended when adding the Substrate and Stop Solution.
- All procedural steps should be completed without interruption. Ensure all reagents, materials and equipment are ready at the appropriate time.
- Each reagent is optimized for use in the Beacon Malachite Green Plate Kit. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other Beacon Malachite Green Plate Kits with different lot numbers.
- Dilution or adulteration of reagents or samples not called for in the procedure may result in inaccurate results.
- Damage to or obstruction of the optical surface may cause unsatisfactory results.

Specificity

The Beacon Malachite Green Plate Kit cannot differentiate between Malachite Green metabolites and structurally related compounds but detects their presence to differing degrees.

Compound	% Cross-Reactivity
Malachite Green	100
Leucomalachite	< 1
Crystal Violet	120
Leucocrystal Violet	< 1

1X Reagent A Preparation

Note: Prepare immediately before use.

1. Dilute the 4X Reagent A 1:4 in acetonitrile.
2. Thoroughly mix and use in the assay.

Sample Preparation

Fish and Shrimp: (Dilution Factor: 10)

1. Homogenize the sample using a stomacher, mixer or ultra turrax.
2. Weigh 5 g of sample into a conical tube. Let the sample dry completely before proceeding.
3. Measure 10 mL of acetonitrile and add to the tube. Mix for 10 minutes on a shaker.
4. Centrifuge for 5 minutes at approximately 3,000 x g.
5. Measure 3 g alumina, neutral (100-200 mesh) and add to the sample. Vortex or shake to mix.
6. Centrifuge for 5 minutes at approximately 3000 x g.
7. Transfer 2 mL of the supernatant to a clean vial and dry with a gentle nitrogen steam.
8. Redissolve the dried extract with 0.5 mL of 1X Reagent A. Allow to sit for 30 minutes before proceeding.
9. Measure 0.1 g of Reagent B and add to the vial. Mix for 3 seconds and let sit for 5 minutes before proceeding.
10. Dilute the top layer 1:20 in Dilution Buffer 2. Thoroughly mix and use in the assay.

1X Enzyme Conjugate Preparation

Note: Prepare fresh before each assay.

1. Dilute the 30X Malachite Green Enzyme Conjugate 1:30 in Dilution Buffer 1.
2. Thoroughly mix and use in the assay.

Calibrator Preparation

Note: Prepare fresh before each assay.

Calibrator Concentration	Diluent Volume	Stock Volume	Stock Description
0.405 ppb	4 mL	0.0162 mL	100 ppb Stock
0.135 ppb	1 mL	0.5 mL	0.405 ppb Calibrator
0.045 ppb	1 mL	0.5 mL	0.135 ppb Calibrator
0.015 ppb	1 mL	0.5 mL	0.045 ppb Calibrator
0.005 ppb	1 mL	0.5 mL	0.015 ppb Calibrator
0 ppb	1 mL	N/A	N/A

- Dilute stocks in Dilution Buffer 2.
- The use of a positive displacement pipette is recommended when pipetting the stock solutions.
- Tightly cap the vials to prevent evaporation.
- Thoroughly mix calibrators in-between dilution steps and prior to use.

Assay Procedure

1. Allow kit components and the sample extract(s) to reach room temperature prior to running the test.
2. Place the appropriate number of test wells into a holder. Be sure to re-seal unused test wells in the zip-lock bag with the desiccant to limit exposure to moisture.
3. Dispense **100 µL of Calibrators and Sample Extract(s)** into the appropriate well. Be sure to use a clean pipette tip for each solution to avoid cross contamination.
4. Dispense **100 µL of 1X Enzyme Conjugate** into each well.
5. Gently shake the wells for 30 seconds using a back-and-forth motion and incubate for **30 minutes** at room temperature.
6. Decant the contents of the wells into an appropriate waste container. Fill the wells to overflowing with laboratory quality distilled or deionized water and then decant. Repeat this wash step four times for a total of five washes. Following the last wash, tap the inverted wells onto absorbent paper to remove excess wash solution.
7. Dispense **100 µL of Substrate** into each well.
8. Incubate for **30 minutes** at room temperature.
9. Dispense **100 µL of Stop Solution** into each well in the same order of addition as the Substrate.
10. Gently shake the wells for 30 seconds using a back-and-forth motion.
11. Carefully wipe the optical surface with a soft, lint-free wipe. Measure and record the absorbance (Optical Density; OD) of each well at 450 nm using a plate or strip reader within 10 minutes of stopping the assay. If the reader has dual wavelength capability, read at 450 nm minus 605 or 650 nm.

Result Interpretation

Semi-Quantitative Interpretation: Semi-quantitative results can be derived by simple comparison of the sample absorbances to the absorbance of the calibrators:

- Samples with a lower absorbance (less color) than a calibrator have a concentration of Malachite Green greater than the concentration of the calibrator.
- Samples with a higher absorbance (more color) than a calibrator have a concentration less than the concentration of the calibrator.

Quantitative Interpretation: It is preferred for quantitative results to be determined using commercially available software for ELISA evaluation using a 4-parameter curve fit. Alternatively, a semi-log curve fit can be used if 4-parameter software is not available. A spreadsheet that will perform the curve fit and sample concentration calculations is available upon request. Please contact Beacon for further details.

- The concentration of Malachite Green in a sample is determined by comparing the average sample absorbance to the standard curve. This value must then be multiplied by the dilution factor used.
- Samples with absorbances lower than the highest calibrator contain a concentration of Malachite Green too high for quantification. Further dilute the sample extract in Dilution Buffer 2 to fit into the standard curve and retest along with the calibrators. Results must then be multiplied by the dilution factor used.
- Samples with Malachite Green absorbances greater than the lowest calibrator or less than the highest calibrator must be reported as < 0.005 ppb or > 0.405 ppb, respectively.

Technical Assistance
For questions regarding this kit or for additional information about Beacon products, contact us.

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Safety
Stop Solution is 1N hydrochloric acid. Handle with care. To receive complete safety information on this product, contact Beacon Analytical Systems, Inc., and request Safety Data Sheets.

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