

Intended Use

The Beacon Microcystin 40 Tube Kit is an immunoassay for detecting Microcystin in water samples. Verified by the U.S. EPA ETV program, it is intended for research use only - not for clinical or diagnostic purposes - under U.S. regulations. The kit is suitable for environmental and drinking water monitoring, and users should follow local regulatory requirements when using it for compliance purposes.

Principles

Microcystin HRP Enzyme Conjugate is pipetted into the test tubes followed by the Calibrators, Controls, and the Sample Extract(s). A soluble polyclonal Microcystin antibody solution is then added to the test tubes to initiate the reaction. During an incubation, Microcystin and Microcystin HRP Enzyme Conjugate compete for binding to the soluble Microcystin antibody which is in turn immobilized on the test tubes. Following the incubation, the tubes are washed to remove any unbound Microcystin and Microcystin HRP Enzyme Conjugate. After washing, a colorless substrate is added to the tubes 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 tube is measured. The color of the unknown sample is compared to the color of the calibrators and the Microcystin concentration of the sample is derived.

Reagents and Materials Provided

2 Unit	Bags each containing 20 test tubes that are vacuum sealed in an aluminized pouch with a desiccant.
5 X 5 mL	Vials of Microcystin Calibrators (0, 0.3, 0.8, 2, and 5 ppb).
1 X 5 mL	Vial of Microcystin Control (1 ppb).
1 X 24 mL	Bottle of Microcystin HRP Enzyme Conjugate.
1 X 24 mL	Bottle of Microcystin Antibody.
1 X 25 mL	Bottle of 100X Wash Concentrate (dilute prior to use).
1 X 25 mL	Bottle of Substrate.
1 X 25 mL	Bottle of Stop Solution.

Reagents and Materials Required but Not Provided

- Pipette(s) with disposable tips capable of dispensing the required volume(s).
- Repeater pipette(s) with disposable tips capable of dispensing the required volume(s) (recommended if running more than five tubes at once).
- Laboratory quality distilled or deionized water.
- Reagents and materials for sample preparation.
- Materials for 1X wash solution preparation.
- Personal protective equipment.
- Paper towels or equivalent absorbent material.
- Permanent Marker.
- Tube rack.
- Timer.
- Photometer capable of reading absorbance at 450 nm in 12 mm x 75 mm tubes.

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 2°C to 8°C (36°F to 46°F) when not in use.
- Reagents should be brought to room temperature, 20°C to 28°C (68°F 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, Controls, 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 five tubes at once, the use of a repeater pipette is recommended when adding the Antibody, 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 Microcystin 40 Tube Kit. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other Beacon Microcystin 40 Tube Kits with different lot numbers.
- Do not reuse test tubes.
- 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 Microcystin 40 Tube Kit does not differentiate between Microcystin-LR (used in the calibrators and controls) and other Microcystin variants but detects their presence at varying degrees. The following table shows the relative values for the percent cross reactivity versus Microcystin-LR.

Compound	% Cross-Reactivity
Microcystin-LR	100
Microcystin-RR	73
Microcystin-YR	58
Microcystin-LY	6
Microcystin-LA	2
Microcystin-LF	3
Microcystin-LW	4
Nodularin	126

Sample Collection, Storage, and Preparation

Drinking Water Samples:

1. Collect a representative sample (approximately 100 mL) in a clean amber glass or Polyethylene Terephthalate Glycol (PETG) bottle with a tight fitting lid.
2. If the sample is expected to contain chlorine or other oxidizers, immediately add sodium thiosulfate (ex. 4 ppm chlorine needs 0.01g/L) in order to neutralize the compound and use in the assay.

Note: Test the sample as soon as possible. If immediate testing is not possible, store refrigerated. In the event that testing cannot commence for more than 5 days, store frozen.

Recreational Water Samples:

1. Collect a representative sample (approximately 100 mL) in a clean amber glass or Polyethylene Terephthalate Glycol (PETG) bottle with a tight fitting lid.

Note: If immediate processing is not possible, store refrigerated. In the event that testing cannot commence for more than 5 days, store frozen.

2. Aliquot the sample into at least two 1.5 mL microcentrifuge tubes.
3. Complete three freeze/thaw cycles to disrupt the algal cells.
4. Centrifuge at 2,000 X g for 3 minutes.
5. Carefully transfer the supernatants to a clean amber glass or PETG container taking care not to disturb the pellets. Mix thoroughly and use in the assay.

1X Wash Solution Preparation

1. Measure 495 mL of laboratory quality distilled or deionized water and transfer to a clean container with a tight-fitting lid.
2. Measure 5 mL of the 100X Wash Concentrate and add to the container.
3. Gently swirl to mix.
4. Transfer the 1X Wash Solution to a wash bottle to use in the assay.

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 tubes into a tube rack. Label the tubes one inch from the top with the calibrator concentration, control concentration/identification, or sample identification. Be sure to re-seal unused tubes in the zip-lock bag with the desiccant to limit exposure to moisture.
3. Dispense **500 µL of Enzyme Conjugate** into each tube.
4. Dispense **500 µL of Calibrators, Controls and Sample Extract(s)** into the appropriate tube. Be sure to use a clean pipette tip for each solution to avoid cross contamination.
5. Dispense **500 µL of Antibody** into each tube.
6. Gently shake the tubes for 30 seconds using a back-and-forth motion and incubate for **20 minutes** at room temperature.
7. Decant the contents of the tubes into an appropriate waste container. Fill the tubes to overflowing with 1X Wash Solution and then decant. Repeat this wash step three times for a total of four washes. Following the last wash, tap the inverted tubes onto absorbent paper to remove excess wash solution.
8. Dispense **500 µL of Substrate** into each tube.
9. Gently shake the tubes for 30 seconds using a back-and-forth motion and incubate for **20 minutes** at room temperature.
10. Dispense **500 µL of Stop Solution** into each tube in the same order of addition as the Substrate.
11. Gently shake the tubes for 30 seconds using a back-and-forth motion.
12. Carefully wipe the optical surface with a soft, lint-free wipe. Measure and record the absorbance (Optical Density; OD) of each tube at 450 nm using a tube reader within 10 minutes of stopping the assay. Be sure to blank the reader with laboratory quality distilled or deionized water prior to measuring.
13. Dispose of used test tubes in an appropriate waste container.

Quality Control (QC) Criteria

- The correlation coefficient (R^2) of the calibration curve, analyzed using a 4-parameter logistic regression, must be ≥ 0.99 .
- The average absorbance of the zero calibrator replicates must be ≥ 1.0 .
- The average absorbance of calibrator and control replicates must have a coefficient of variation (%CV) $< 15\%$.
- The average absorbance of sample replicates must have a coefficient of variation (%CV) $< 20\%$.
- The concentration of the control must fall between 0.8 and 1.3 ppb.

Result 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.

To ensure the validity of the results, please adhere to the following:

- Ensure QC criteria are met.
- The concentration of Microcystin 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.
- In the event that the average absorbance of the sample is lower than the highest calibrator, further dilute the sample extract in laboratory quality distilled or deionized water to fit into the standard curve and retest alongside the calibrators and controls. Sample results must be multiplied by the total dilution factor used.

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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