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 Microcystin 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 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.
- Samples with absorbances lower than the highest calibrator contain a concentration of Microcystin too high for quantification. Further dilute the sample extract in laboratory quality distilled or deionized water to fit into the standard curve and retest along with the calibrators and controls. Results must then be multiplied by the dilution factor used.
- Samples with Microcystin absorbances greater than the lowest calibrator or less than the highest calibrator must be reported as < 0.3 ppb or > 5 ppb, respectively.

Technical Assistance

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

Beacon Analytical Systems, Inc.
82 Industrial Park Rd. Saco, ME 04046
Tel. 207-571-4302
info@beaconkits.com | www.beaconkits.com

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.

General Limited Warranty

Beacon Analytical Systems Inc.®

Microcystin BX 40 Tube Kit Product Brochure

Catalog No. 20-0311

Rev. A

Intended Use

The Beacon Microcystin BX 40 Tube Kit is an immunoassay for the detection of dissolved and particulate Microcystins in both marine and freshwater samples. This product is intended for research use only.

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 Bags each containing 20 test tubes that are vacuum sealed in an aluminized pouch with a desiccant.
5 Vials of Microcystin Calibrators (0, 0.3, 0.8, 2, and 5 ppb).
1 Vial of Microcystin Control (1 ppb).
1 Bottle of Microcystin HRP Enzyme Conjugate.
1 Bottle of Microcystin Antibody.
1 Bottle of 100X Wash Concentrate (dilute prior to use).
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).
- 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 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, 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 BX 40 Tube Kit. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other Beacon Microcystin BX 40 Tube 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 Microcystin BX 40 Tube Kit can detect several Microcystin congeners. The percent cross reactivity of Microcystin congeners relative to Microcystin-LR (used in the calibrators and controls) is shown in the table below.

Compound	% Cross-Reactivity
Microcystin-LR	100
Microcystin-RR	104
Microcystin-YR	56
Microcystin-LA	38
Microcystin-LF	31
Microcystin-LW	29
Nodularin	62

Up to 100 ppm Humic Acid was tested and was found to not interfere with the detection and quantification of Microcystin in this assay.

Sample Collection

- Water samples should be collected in glass or PETG storage containers.
- Visible particulates should be removed by filtration with a GF/F glass fiber filter.
- Water and/or filtered material containing particulates can be lysed using 3 freeze-thaw cycles to release toxins or by following the extraction procedure outlined below.

Sample Preparation

Water: (Dilution Factor: 1)

1. Load one GF/F glass filter into a Swinnex filter unit using an "O" ring. Tighten by hand.
2. Remove the plunger from the barrel. Screw the barrel into the Swinnex filter unit.
3. Pour the sample into the barrel to the 10 mL mark.
4. Insert the plunger into the barrel and slowly push the sample through the filter. Collect the filtrate in a clean glass container. (Dissolved fraction can be sampled here)
   Note: To filter additional volume, unscrew the barrel from the Swinnex filter unit and repeat steps 2 – 4.
5. Remove the filter from the Swinnex filter unit and place it in the bottom of the label side of a NASCO filter bag.
6. Roll the filter in the bag for 1 minute using a seam roller.
7. Add the contents of the filtrate collected in step 4 to the bag and mix thoroughly.
8. Transfer the contents of the bag to a clean glass vial for use in the assay.

Assay Procedure

When running the test kit for the first time, run the Calibrators, Controls, and Sample Extract(s). In subsequent assays, only the 0 ppb Calibrator, 1 ppb Positive Control and Sample Extract(s) need to be run. It is recommended that a new calibration curve be generated each day or more frequently if the positive control value falls outside of the expected range.

1. Allow kit components and the sample extract(s) to reach room temperature prior to running the test.
2. Prepare 1X Wash Solution by diluting 5 mL of 100X Wash Concentrate in 495 mL of laboratory quality distilled or deionized water. Gently swirl to mix. Transfer to a wash bottle for use in the assay.
3. Place the appropriate number of test tubes into a tube rack. Label the tubes one inch from the top with the calibrator concentration or sample identification. Be sure to re-seal unused tubes in the zip-lock bag with the desiccant to limit exposure to moisture.
4. Dispense **500 µL of Enzyme Conjugate** into each tube.
5. 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.
6. Dispense **500 µL of Antibody** into each tube.
7. Gently shake the tubes for 30 seconds using a back-and-forth motion and incubate for **20 minutes** at room temperature.
8. 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.
9. Dispense **500 µL of Substrate** into each tube.
10. Gently shake the tubes for 30 seconds using a back-and-forth motion and incubate for **20 minutes** at room temperature.
11. Dispense **500 µL of Stop Solution** into each tube in the same order of addition as the Substrate.
12. Gently shake the tubes for 30 seconds using a back-and-forth motion.
13. 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.