

CALCULATE RESULTS

1. After you read all of the tube, average the OD of each set of calibrators, controls and samples, and calculate the %Bo as follows:
$$\%Bo = \frac{\text{(average OD of calibrator, control or sample} \times 100)}{\text{average OD of negative control}}$$
2. Graph the %Bo of each calibrator on the Y (linear) axis against its microcystin concentration on the X (log) axis using semi-log graph paper. Draw the best-fit line through the calibrator points.
3. Determine the Microcystin concentration of each sample by finding its %Bo value and the corresponding concentration level on the graph.
4. Calculation of sample concentration is only valid if the %Bo of the sample falls within the range of the %Bo's set by the calibrators. If the sample falls outside of that range, the results must be reported as less than the lowest calibrator value or greater than the highest calibrator value.

Quality Control

1. The value of the 1.0 ppb control should fall within the following range:

1.0 ppb Microcystin control 0.80 – 1.30ppb

SAMPLE CALCULATIONS

| Well Contents | OD | Average OD \pm SD* | %RSD | %Bo** |
|--------------------|----------------|----------------------|------|-------|
| Negative Control | 1.067 | 1.070 \pm 0.004 | 0.33 | 100 |
| 0.3 ppb Calibrator | 0.785 .777 | 0.781 \pm 0.006 | 0.72 | 73.9 |
| 0.8 ppb Calibrator | 0.586 0.579 | 0.583 \pm 0.005 | 0.85 | 54.9 |
| 2.0 ppb Calibrator | 0.399 0.396 | 0.398 \pm 0.002 | 0.53 | 37.5 |
| 5.0 ppb Calibrator | 0.271 0.272 | 0.272 \pm 0.001 | 0.26 | 25.6 |

Actual values may vary; this data is for example purposes only.

* Standard deviation

** %Bo equals average sample absorbance divided by average negative control absorbance times 100%.

TECHNICAL ASSISTANCE

For questions regarding this kit or for additional information about Beacon products, call (207) 571-4302.

SAFETY

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

General Limited Warranty

Beacon Analytical Systems, Inc. ("Beacon") warrants the products manufactured by it against defects in materials and workmanship when used in accordance with the applicable instructions for a period not to extend beyond a product's printed expiration date. BEACON MAKES NO OTHER WARRANTY, EXPRESSED OR IMPLIED. THERE IS NO WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE. The warranty provided herein and the data, specifications and descriptions of Beacon products appearing in published catalogues and product literature may not be altered except by express written agreement signed by an officer of Beacon. Representations, oral or written, which are inconsistent with this warranty or such publications are not authorized and, if given, should not be relied upon.

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Microcystin Tube Kit Cat.# 20-0098



www.epa.gov/etv

Microcystin tube kit was verified by the U.S. EPA ETV (Environmental Technology Verification) program.

<http://www.epa.gov/nrmrl/std/etv/vt-ams.html#itkm>

Instructional Booklet

READ COMPLETELY BEFORE USE.

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INTENDED USE

The Beacon Microcystin Tube Kit is an immunological laboratory test for the quantitation of Microcystins in water.

USE PRINCIPLES

The Beacon Microcystin Tube Kit uses a polyclonal antibody that binds both Microcystins and a Microcystin-enzyme conjugate. Microcystins in the sample compete with the Microcystin-enzyme conjugate for a limited number of antibody binding sites. In the assay procedure you will:

- Add Microcystin-enzyme conjugate and a sample containing Microcystins to a test tube, followed by antibody solution. The conjugate competes with any Microcystins in the sample for the same antibody binding sites. The test tube is coated with anti-rabbit IgG to capture the rabbit anti-microcystin added.
- Wash away any unbound molecules, after you incubate this mixture for 20 minutes.
- Add clear substrate solution to each tube. In the presence of bound Microcystin-enzyme conjugate, the substrate is converted to a blue compound. One enzyme molecule can convert many substrate molecules.

Since the same number of antibody binding sites are available in every tube, and each tube receives the same number of Microcystin-enzyme conjugate molecules, a sample containing a low concentration of Microcystins allows the antibody to bind many Microcystin-enzyme conjugate molecules. The result is a dark blue solution. Conversely, a high concentration of Microcystins allows fewer Microcystin-enzyme conjugate molecules to be bound by the antibodies, resulting in a lighter blue solution.

NOTE: Color is inversely proportional to Microcystin concentration.

Darker color = Lower concentration

Lighter color = Higher concentration

MATERIALS PROVIDED IN THE BEACON MICROCYSTIN TUBE KIT

- 40 antibody coated tubes
- 1 vial of Negative Control (0.0 ppb Microcystin-LR)
- 1 vial each of 0.3 ppb, 0.8, 2.0 and 5.0 ppb Microcystin-LR Calibrator
- 1 vial 1.0 ppb Microcystin control
- 1 vial of Microcystin-HRP Enzyme Conjugate
- 1 vial of Microcystin Antibody Solution
- 1 vial of Substrate
- 1 vial of Stop Solution
- 1 vial of 100X Wash Solution

You also need these items:

- Photometer capable of reading optical density of 12 mm tubes at 450nm.
- Tape or Parafilm®
- Pipette capable of delivering 500ul.
- Watch or timer
- Laboratory grade water or deionized water.

PERFORMANCE CHARACTERISTICS

SPECIFICITY

The Beacon Microcystin Tube Kit does not differentiate between Microcystin-LR (used as kit calibrators) and other microcystin variants, but detects their presence at varying degrees. The following table shows the relative values for the percent cross-reactivity (%CR) versus Microcystin-LR.

| Variant | %CR |
|----------------|-----|
| Microcystin-LR | 100 |
| Microcystin-RR | 73 |
| Microcystin-YR | 58 |
| Microcystin-LA | 2 |
| Microcystin-LF | 3 |
| Microcystin-LW | 4 |
| Nodularin | 126 |

PRECAUTIONS

- Store all kit components at 4°C to 8°C (39°F to 46°F) when not in use.
- Do not freeze kit components or expose them to temperatures greater than 37°C (99°F).
- Allow all reagents and samples to reach ambient temperature before you begin the test.
- Do not use kit components after the expiration date.
- Do not mix reagents or test tubes from kits with different lot numbers.
- Transfer of samples and reagents by pipette requires constant monitoring of technique. Pipetting errors are the major source of error in immunoassay methodology.
- The assay is not specific for microcystin and will react with related structures. See table in Performance Characteristics for specific information.
- Samples found to have or expected to have concentrations of microcystin greater than 5.0 ppb should be diluted prior to analysis.

SAMPLE PREPARATION

If required, samples containing live algae can be lysed before analysis to release the toxins in the cells. A simple freeze/ thaw cycle will accomplish this. Be sure the sample temperature is ambient before running in the assay.

ASSAY PROCEDURE

1. Bring all kit reagents and samples to be run to room temperature.
 2. Prepare 1X wash solution by diluting the 100X wash concentrate with DI water. 1 mL concentrate per 99 mL DI water.
 3. Remove the required number of anti-Rabbit IgG coated tubes from the re-sealable foil bag. Place tubes in rack and label with samples or calibrator level. Be sure to re-seal the bag with the desiccant to limit exposure of the tubes to moisture.
 4. Add **500 µL of Enzyme Conjugate** to each tube.
 5. Pipet **500 µL of calibrators, control or samples** into the appropriate tubes. Be sure to use a clean pipet tip for each solution to avoid cross contamination.
 6. Add **500 µL of Antibody solution** to each tube.
 7. Swirl the tubes rapidly to mix the contents.
 8. Incubate for **20 minutes**.
 9. After incubation, remove the covering and vigorously shake the contents of the tubes into a sink. Flood the tubes completely with wash solution, then shake to empty. Repeat this wash step four times for a total of five washes. Invert the rack on absorbent paper and tap out as much water as possible.
 10. Add **500 µL of Substrate** to each tube.
 11. Cover the tubes and incubate for **20 minutes**.
 12. Add **500 µL of Stop Solution** to each tube in the same order of addition as the Substrate.
WARNING: Stop Solution is 1N hydrochloric acid. Handle carefully.
 13. Read the tubes with a spectrometer or tube reader at 450nm within 20 minutes of stopping reaction. If the reader has dual wavelength capability, read at 450nm minus 605 or 650nm.
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