Beacon Analytical Systems Inc.

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.

Instructional Brochure

READ COMPLETELY BEFORE USE.

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 the microcystin concentration.
Darker color = Lower concentration
Lighter color = Higher concentration

MATERIALS PROVIDED

2 Bags Antibody Coated Tubes – each containing 20 tubes

1 Vial Negative Control - containing 5 mL of 0.0 ppb Microcystin-LR

4 Vials Microcystin Calibrators – containing 5 mL with concentrations of 0.3, 0.8, 2.0, and 5.0 ppb Microcystin-LR

1 Vial Positive Control – containing 5 mL of 1.0 ppb Microcystin-LR

1 Bottle Microcystin-HRP Enzyme Conjugate – containing 24 mL

1 Bottle Microcystin Antibody Solution - containing 24 mL

1 Bottle Substrate – containing 25 mL

1 Bottle Stop Solution – containing 25 mL (Caution! 1N HCI. Handle with care.)

1 Bottle 100X Wash Solution – containing 25 mL (Must be diluted before use. See Assay Procedure Step 3.)

MATERIALS REQUIRED BUT NOT PROVIDED

- Laboratory grade water or deionized water
- Pipette capable of delivering 500 µL
- Wash bottle
- Paper towels or equivalent absorbent material
- Watch or timer
- Photometer capable of reading optical densities of 12 mm tubes at 450 nm

SPECIFICITY

The Beacon Microcystin Plate Kit can detect several microcystin congeners. The % cross reactivity (% CR) of microcystin, other microcystin congeners relative to microcystin-LR is shown in the table below.

Congeners	% 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.
- Each reagent is optimized for use in the Beacon Microcystin Tube Kit. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other Beacon Microcystin Tube Kit with different lot numbers.
- Dilution or adulteration of reagents or samples not called for in the procedure may result in inaccurate results.
- Do not use reagents after expiration date.
- Reagents should be brought to room temperature, 20 to 28ºC (62 to 82ºF) prior to use. Avoid prolonged (> 24 hours) storage at room temperature.
- The Stop Solution is 1N hydrochloric acid. Avoid contact with skin and mucous membranes. Immediately clean up any spills and wash area with copious amounts of water. If contact should occur, immediately flush with copious amounts of water.
- Transfer of samples and reagents by pipette requires constant monitoring of technique. Pipetting errors are the major source of error in immunoassay methodology.
- Use approved methodologies to confirm and positive results.

ASSAY PROCEDURE

1. Bring all kit reagents and samples to room temperature.
2. Remove the required number of antibody coated tubes from the re-sealable foil bag. Place the tubes in a rack and label each with a sample or calibrator level. Be sure to re-seal the bag with the desiccant to reduce moisture exposure.
3. Prepare 1X wash solution by diluting the 100X concentrate (i.e. 5 mL of the 100X plus 495 mL of deionized water in a 500 mL wash bottle).
4. Dispense **500 µL of the Enzyme Conjugate** into each tube.
5. Add **500 µL of the Calibrators, Control and Samples** into the appropriate tubes. Be sure to use a clean pipette tip for each solution to avoid cross contamination.
6. Dispense **500 µL of the Antibody Solution** into each tube.
7. Swirl the tubes rapidly to mix the contents.
8. Incubate the tubes for **20 minutes** at room temperature.
9. Decant the contents of the tubes into an appropriate waste container. Flood the tubes completely with 1X wash solution, then decant. Repeat this wash step three times for a total of four washes. Invert the rack onto absorbent paper and tap out as much water as possible.
10. Add **500 µL of the Substrate** to each tube.
11. Swirl the tubes rapidly to mix the contents.
12. Incubate the tubes for **20 minutes** at room temperature.
13. Add **500 µL of the Stop Solution** to each tube in the same order of addition as the Substrate.
    WARNING: Stop Solution is 1N hydrochloric acid. Handle with care.
14. Read the tubes with a spectrometer or tube reader at 450 nm within 20 minutes of stopping reaction. If the reader has dual wavelength capability, read at 450 nm minus 605 or 650 nm.

CALCULATE RESULTS

1. It is preferred for quantitative results to be determined using commercially available software for ELISA evaluation such as a 4-parameter curve fit. Alternatively, a semi-log curve fit can be used if 4-parameter software is not available.
2. Semi-quantitative results can be derived by simple comparison of the sample absorbances to the absorbance of the calibrator wells: Samples containing less color than a calibrator will have a concentration of microcystin-LR greater than the concentration of the calibrator. Samples containing more color than a calibrator well have a concentration less than the concentration of the calibrator.
3. Samples with absorbances greater than the lowest calibrator or less than the highest calibrator must be reported as < 0.3 ppb or > 5 ppb, respectively.
4. If the absorbance of a sample is lower than the highest calibrator (5.0 ppb), the concentration of microcystin is too high and is out of range of the standard curve. Dilute the sample in laboratory grade water and rerun. Samples should be diluted to fit into the standard curve (0.3 ppb to 5.0 ppb). Results must then be multiplied by the dilution factor used.

QUALITY CONTROL

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

1.0 ppb Microcystin control	0.80 – 1.30 ppb

SAMPLE CALCULATIONS

Well Contents	OD	Average OD ± SD*	%RSD	%Bo**
Negative Control	1.067 1.072	1.070 ± 0.004	0.33	100
0.3 ppb Calibrator	0.785 .777	0.781 ± 0.006	0.72	73.9
0.8 ppb Calibrator	0.586 0.579	0.583 ± 0.005	0.85	54.9
2.0 ppb Calibrator	0.399 0.396	0.398 ± 0.002	0.53	37.5
5.0 ppb Calibrator	0.271 0.272	0.272 ± 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 or contact us at info@beaconkits.com.

SAFETY

To receive complete safety information on this product, contact Beacon Analytical Systems, Inc. and request 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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