

CALCULATE RESULTS

1. After you read all of the wells, average the OD of each set of calibrators, controls and samples, and calculate the %Bo as follows:

$$\%B^{\circ} = \frac{\text{(average OD of calibrator, control or sample} \times 100)}{\text{average OD of negative control}}$$

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 ± SD*	%RSD	%Bo**	MCYN conc. (ppb)
Negative Control	1.478 1.552	1.515 ± 0.052	3.4	100	N/A
0.1 ppb Calibrator	1.255 1.194	1.225 ± 0.043	3.5	80.8	N/A
0.3 ppb Calibrator	0.941 0.932	0.937 ± 0.006	0.68	61.8	N/A
0.8 ppb Calibrator	0.626 0.602	0.614 ± 0.017	2.7	40.5	N/A
2.0 ppb Calibrator	0.389 0.386	0.302 ± 0.008	0.54	25.6	N/A
Sample	0.634 0.610	0.622 ± 0.017	2.7	31.5	0.909

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

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 **Beacon**
Analytical Systems Inc.



Microcystin Plate Kit

Cat.# 20-0068

Instructional Booklet

READ COMPLETELY BEFORE USE.

INTENDED USE

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

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USE PRINCIPLES

The Beacon Microcystin Plate 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 well, followed by antibody solution. The conjugate competes with any Microcystins in the sample for the same antibody binding sites. The test well is coated with anti-rabbit IgG to capture the rabbit anti-microcystin added.
- Wash away any unbound molecules, after you incubate this mixture for 30 minutes.
- Add clear substrate solution to each well. 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 well, and each well 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 PLATE KIT

- 1 plate containing 12 strips of 8 wells coated with sheep anti-rabbit antibodies
- 1 vial of Negative Control (0.0 ppb Microcystin-LR)
- 1 vial each of 0.1, 0.3, 0.8 and 2.0 ppb Microcystin-LR Calibrator
- 1 vial 1.0 ppb Microcystin control.
- 1 vial of Microcystin-HRP Enzyme Conjugate
- 1 vial Rabbit anti-microcystin antibody solution
- 1 vial of Substrate
- 1 vial of Stop Solution
- 1 vial 100X Wash Solution

You also need these items:

- Microtiter plate reader
- Tape or Parafilm®
- Watch or timer
- Clean running water or a wash bottle containing tap or deionized water.
- Orbital shaker (optional)

PERFORMANCE CHARACTERISTICS

SPECIFICITY

The Beacon Microcystin Plate 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. All concentrations are in parts per billion (ppb).

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 strips 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 2.0 ppb should be diluted prior to analysis.

ASSAYPROCEDURE

1. Bring all kit reagents and samples to be run to room temperature.
2. Remove the required number of antibody coated strips from the re-sealable foil bag. Be sure to re-seal the bag with the desiccant to limit exposure of the strips to moisture.
3. Prepare 1X wash solution by diluting the 100X concentrate, i.e. 5 mL of the 100X plus 495mL deionized water in 500 ml wash bottle.
4. Add **50 µL of Enzyme Conjugate** to each well.
5. Pipet **50 µL of calibrators, control and samples** into the appropriate wells. Be sure to use a clean pipet tip for each solution to avoid cross contamination.
6. Add **50 µL of Antibody Solution** to each well.
7. Swirl the plate rapidly to mix the contents and cover the wells with tape or Parafilm. Alternately, the plate may be incubated on a rotater for continuous mixing during incubation.
8. Incubate for **30 minutes**.
9. After incubation, remove the covering and vigorously shake the contents of the wells into a sink. Flood the wells completely with 1X wash solution, then shake to empty. Repeat this wash step four times for a total of five washes. Invert the plate on absorbent paper and tap out as much water as possible.
10. Add **100 µL of Substrate** to each well.
11. Cover the wells and incubate for **30 minutes**.
12. Add **100 µL of Stop Solution** to each well in the same order of addition as the Substrate.
WARNING: Stop Solution is 1N hydrochloric acid. Handle carefully.
13. Read the plate on a microtiter plate reader at 450nm. If the plate reader has dual wavelength capability, read at 450nm minus 605 or 650nm.
14. If the microtiter plate reader has data reduction capabilities, use either a semi-log linear or 4-parameter curve fit. If manual data reduction is required, proceed with next section.