Microcystin Plate Kit
Cat.# 20-0068-N

Product Insert

READ COMPLETELY BEFORE USE.

INTENDED USE

The Beacon Microcystin Plate Kit is a competitive ELISA for the quantitative analysis of Microcystins in water.

USE PRINCIPLES

The Beacon Microcystin plate kit is a competitive enzyme-labeled immunoassay. Microcystin HRP enzyme conjugate is pipetted into the test wells followed by calibrators or sample extract. A Microcystin antibody solution is then added into the test wells to initiate the reaction. During a 30 minute incubation period, microcystin from the sample and Microcystin HRP conjugate compete for binding to the Microcystin antibody. Following this incubation, the wells are washed to remove any unbound microcystin and Microcystin HRP conjugate. After washing, a colorless substrate is added to the wells and any bound enzyme conjugate will convert the substrate to a blue color. Following another 30 minute incubation, the reaction is stopped with the addition of stop solution and the amount of color in each well 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. The color intensity is inversely proportional to the amount of microcystin present.

MATERIALS PROVIDED IN THE BEACON MICROCYSTIN PLATE KIT

- **Plate** – (1) containing 12 test strips of 8 wells coated each, vacuum-packed in aluminized pouch with indicating desiccant
- **Microcystin Calibrators** – (6) vials containing 2 mL with a concentration of 0.0 ppb (µg/L), 0.1, 0.3, 0.8, 2.0 and 5.0 ppb of Microcystin-LR
- **Positive Control** – (1) vial containing 2 mL of 1.0 ppb Microcystin-LR control
- **Microcystin HRP Enzyme Conjugate** – (1) bottle containing 8 mL
- **Microcystin Antibody Solution** – (1) bottle containing 8 mL
- **Substrate** – (1) bottle containing 14 mL
- **Stop Solution** – (1) bottle containing 14 mL (Caution! Contains 1 N HCl. Handle with care.)
- **100X Wash Solution** – (1) bottle containing 25 mL (Must be diluted before use. See Assay Procedure Step 2.)

MATERIALS REQUIRED BUT NOT PROVIDED IN THE BEACON MICROCYSTIN PLATE KIT

- Microtiter plate reader or strip reader with 450 nm filter
- Laboratory grade or deionized water
- Pipette with disposable tips capable of dispensing 50 µL
- Multi-channel pipette; 8 channel capable of dispensing 50 and 100 µL
- Paper towels or equivalent absorbent material
- Timer
- Wash bottle
- Vortex mixer

SPECIFICITY

The Beacon Microcystin Plate Kit can detect several microcystin congeners. The percent cross reactivity (based on IC_{50}) of microcystin congeners relative to Microcystin-LR is shown in the table below.

Congeners	% Cross Reactivity
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 Plate Kit. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other Beacon Microcystin Plate Kits 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 (RT), 20 to 28ºC (68 to 82ºF) prior to use. Avoid prolonged (> 24 hours) storage at room temperature.
- Microcystin is a toxin and should be treated with care.
- The Stop Solution is 1N hydrochloric acid, which is corrosive and an irritant. 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.
- Precise transfer of samples and reagents by using an appropriate and calibrated pipette is critical to obtain proper assay results. Please pipette carefully.
- If running more than two strips at once, the use of a multichannel pipette is required.

SAMPLE PREPARATION

Water samples should be free of particles and adjusted to a neutral pH. If necessary centrifuge or filter samples prior to running in the assay.

ASSAY PROCEDURE

1. Allow reagents and sample extracts to reach room temperature prior to running the test.
2. Prepare the 1X wash solution by diluting the 100X concentrate (i.e. 5 mL of the 100X plus 495mL of deionized water in a 500 ml wash bottle).
3. Place the appropriate number of test wells into a micro well holder. Be sure to re-seal any unused wells in the zip-lock bag with desiccant.
4. Dispense **50 µL of the HRP Enzyme Conjugate** into each well.
5. Using a pipette with disposable tips, add **50 µL of the Calibrators, Positive Control, or sample extract** into the appropriate test wells. Be sure to use a clean pipette tip for each.
6. Dispense **50 µL of the Antibody Solution** into each well.
7. Shake the plate gently for 30 seconds using a back and forth motion. Then incubate the wells for **30 minutes** at RT.
8. Decant the contents of the wells into an appropriate waste container. Fill the wells to overflowing with 1X wash solution and then decant. Repeat 4X for a total of five washes.
9. Following the last wash, tap the inverted wells onto absorbent paper to remove the last of the wash solution.
10. Dispense **100 µL of the Substrate** into each well. Shake the plate gently for 30 seconds using a back and forth motion.
11. Incubate the wells for **30 minutes** at RT.
12. Dispense **100 µL of the Stop Solution** into each well.

13. Measure and record the absorbance (Optical Density; OD) of the wells at 450 nm. If the plate reader has dual wavelength capability, read at 450 nm minus 605 or 650 nm.
14. If the absorbance of a sample is lower than the highest calibrator (5.0 ppb), the concentration of Microcystin is too high and 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 (0.1 ppb to 5.0 ppb). Results must then be multiplied by the dilution factor used.
15. The value of the 1.0 ppb control should fall within the following range:

1.0 ppb Microcystin Positive Control: 0.80 – 1.30ppb

CALCULATE RESULTS

1. 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 well 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.
2. It is preferred that quantitative results from the ELISA are determined using commercially available software using a 4-Parameter curve fit. Alternatively, a semi-log curve fit can be used if 4-Parameter software is not available. Samples with absorbances greater than the lowest calibrator or less than the highest calibrator must be reported as < 0.1 ppb or > 5 ppb, respectively. Beacon can supply a spreadsheet template which can be used for data reduction. Please contact Beacon for further details.

SAMPLE CALCULATIONS

Well Contents	OD	Average OD ± SD*	%RSD	$\%B_0$**	MCYN conc. (ppb)
Negative Control	1.78 1.75	1.77 ± 0.025	1.40	100	0
0.1 ppb Calibrator	1.36 1.44	1.40 ± 0.054	3.83	79	0.10
0.3 ppb Calibrator	1.01 1.01	1.01 ± 0.001	0.11	57	0.32
0.8 ppb Calibrator	0.76 0.73	0.74 ± 0.017	2.30	42	0.75
2.0 ppb Calibrator	0.54 0.55	0.55 ± 0.006	1.14	31	1.97
5.0 ppb Calibrator	0.45 0.45	0.45 ± 0.003	0.66	25	5.57
Sample	0.67 0.66	0.67 ± 0.007	1.12	38	1.03

Actual values may vary; this data is for example purposes only.
* standard deviation
** $\%B_0$ equals average sample absorbance divided by average Negative Control absorbance multiplied by 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 Safety Data Sheets. Stop Solution is 1N hydrochloric acid. Handle with care.

General Limited Warranty

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BEACON ANALYTICAL SYSTEMS, INC.
82 Industrial Park Road
Saco, ME 04072
Tel. (207) 571-4302
Fax (207) 602-6502
www.beaconkits.com