Beacon Analytical Systems Inc.

Microcystin BX Plate Kit

Cat. # 20-0300

Product Insert

READ COMPLETELY BEFORE USE.

INTENDED USE

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

USE PRINCIPLES

The Beacon Microcystin BX 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 calibrator or 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 colorless 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 to 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 BX concentration.

Darker color = Lower concentration

Lighter color = Higher concentration

MATERIALS PROVIDED

- **Plate** – (1) containing 12 strips of 8 wells coated with sheep anti-rabbit antibodies
- **Negative Control** – (1) vial containing 2 mL of 0.0 ppb (µg/L) Microcystin-LR
- **Microcystin BX Calibrators** – (4) vials containing 2mL with a concentration of 0.1, 0.3, 0.8 and 2.0 ppb of Microcystin-LR
- **Positive Control** – (1) vial containing 2 mL of 1.0 ppb Microcystin-LR control
- **Microcystin BX HRP Enzyme Conjugate** – (1) bottle containing 8 mL
- **Anti-Microcystin BX Antibody Solution** – (1) bottle containing 8 mL
- **Substrate** – (1) bottle containing 14 mL
- **Stop Solution** – (1) bottle containing 14 mL
- **100X Wash Solution** – (1) bottle containing 25 mL (Must be diluted before use. See Assay Procedure Step 3.)

MATERIALS REQUIRED BUT NOT PROVIDED

- Microtiter plate reader or strip reader with 450 nm filter
- Clean running water or a wash bottle containing tap 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
- Orbital shaker (optional)

SPECIFICITY

The Beacon Microcystin BX Plate Kit can detect several Microcystin congeners. The % cross reactivity of Microcystin congeners relative to Microcystin-LR is shown in the table below.

Compound	% CR
Microcystin-LR	**100**
Microcystin-RR	**86**
Microcystin-LA	**41**
Microcystin-LF	**34**
Microcystin-LW	**29**
Microcystin-LY	**30**
Microcystin-YR	**53**
Nodularins	**58**

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.

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. Bring all kit reagents and samples 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 of deionized water in a 500 ml wash bottle).
4. Dispense **50 µL of the Enzyme Conjugate** into each well.
5. Add **50 µL of the calibrators, control and samples** into the appropriate wells. Be sure to use a clean pipette tip for each solution to avoid cross contamination.
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.
8. Incubate the wells for **30 minutes** at room temperature.
9. After this incubation, decant the contents of the wells into an appropriate waste container. Flood the wells completely with 1X wash solution, then decant. Repeat this wash step four times for a total of five washes. Invert the plate on absorbent paper and tap out as much of the wash solution as possible.
10. Add **100 µL of Substrate** to each well.
11. Shake the plate gently for 30 seconds using a back and forth motion.
12. Incubate the wells at room temperature for **30 minutes.**
13. 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.
14. Measure and record the absorbance on a microtiter plate reader at 450nm. If the plate reader has dual wavelength capability, read at 450nm minus 605 or 650nm.
15. For a microtiter plate reader that has data reduction capabilities, use a 4-parameter curve fit. Alternatively, a semi-log fit can also be used if a 4-parameter curve fit is not available. If manual data reduction is required, proceed with the next section.
16. If the absorbance of a sample is lower than the highest calibrator (2.0 ppb), the concentration of Microcystin BX 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 2.0 ppb). Results must then be multiplied by the dilution factor used.

CALCULATE RESULTS

1. Semi-quantitative results can be derived by simple comparison of the sample absorbances to the absorbance of the calibrator wells: Sample 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 will have a concentration less than the concentration of the calibrator.
2. 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. Samples with absorbances greater than the lowest calibrator or less than the highest calibrator must be reported as < 0.1 ppb or > 2 ppb, respectively. Beacon can supply a spreadsheet template which can be used for data reduction. Please contact Beacon for further details.

Quality Control

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	%B_0**	MCYN conc. (ppb)
Negative Control	1.478 1.552	1.515 ± 0.052	3.5	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	2.8	40.5	N/A
2.0 ppb Calibrator	0.389 0.386	0.388 ± 0.002	0.55	25.6	N/A
Sample	0.769 0.771	0.770 ± 0.001	0.18	50.8	0.495

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 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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