Beacon Analytical Systems Inc.®

Cylindrospermopsin Plate Kit

Cat. # 20-0149-N
Product Insert

PLEASE READ COMPLETELY BEFORE USE

INTENDED USE

The Cylindrospermopsin Plate Kit is a competitive ELISA for the quantitative analysis of cylindrospermopsin in water.

USE PRINCIPLES

The Beacon Cylindrospermopsin Plate Kit is a competitive enzyme-labeled immunoassay. Cylindrospermopsin-HRP enzyme conjugate is pipetted into the test wells followed by calibrators or samples. Cylindrospermopsin Antibody Solution is added into the test wells to initiate the reaction. During the 45 minute incubation period, cylindrospermopsin from the sample and cylindrospermopsin-HRP conjugate compete for binding to the cylindrospermopsin antibody. Following this incubation, the wells are washed to remove any unbound cylindrospermopsin or cylindrospermopsin-HRP conjugate. After washing, a colorless substrate is added to the wells and any bound cylindrospermopsin-HRP conjugate will convert the substrate to a blue color. Following a 45 minute incubation, the reaction is stopped 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 cylindrospermopsin concentration of the sample is derived. The color intensity is inversely proportional to the amount of cylindrospermopsin present in the sample.

MATERIALS PROVIDED IN THE BEACON CYLINDROSPERMOPSIN PLATE KIT

- **Plate** – (1) containing 12 strips of 8 wells each, vacuum-packed in aluminized pouch with indicating desiccant.
- **Cylindrospermopsin Calibrators** – (5) vials containing 2 mL each, labeled as 0, 0.05, 0.3, 0.75 and 2.0 ppb Cylindrospermopsin.
- **Cylindrospermopsin Positive Control** – (1) vial containing 2 mL of 0.25 ppb Cylindrospermopsin control
- **Cylindrospermopsin HRP Enzyme Conjugate** – (1) bottle containing 7 mL
- **Cylindrospermopsin Antibody Solution** – (1) bottle containing 7 mL
- **Substrate** – (1) bottle containing 14 mL
- **Stop Solution** – (1) bottle containing 14 mL (Caution! Contains 1N HCl. Handle with care.)
- **Sample Diluent -** (1) bottle containing 25 mL
- **Wash Solution –** (1) bottle containing 25 mL (Must be diluted before use. See assay procedure.)
- **Product Insert** containing instructions for use.

MATERIALS REQUIRED BUT NOT PROVIDED IN THE BEACON CYLINDROSPERMOPSIN PLATE KIT

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

SPECIFICITY: Cylindrospermopsin residues can be detected by this assay. Common cyanotoxins which can be found in water samples were tested in the assay and their reactivity is listed in the table below.

Compound	% Reactivity
Cylindrospermopsin	100%
Microcystin-LR	<1%
Nodularin	<1%

KIT HANDLING NOTES and PRECAUTIONS

- Running calibrators and samples in duplicate will improve assay precision and accuracy.
- Reagents should be brought to room temperature, 20 to 28°C (62 to 82°F prior to use). Avoid storing kits for extended periods (>24 hr.) at room temperature.
- The kit in its original packaging can be used until the end of the month indicated on the box label when stored at 2 to 8°C. The kit expires one year from the date of manufacturing.
- Store all kit components at 2°C to 8°C when not in use. Do not use kit components after the expiration date.
- Do not freeze kit components or expose them to temperatures greater than 37°C (99°F).
- Cylindrospermopsin is a toxic substance and should be handled safely.
- Do not mix reagents or test strips from kits with different lot numbers or components from any other manufactured kit.
- The intended user of this kit is a trained laboratory technician. Familiarity with ELISA is recommended. Please contact Beacon for technical support if you have any questions about the use of this kit.
- The use of a multichannel pipette to dispense the Enzyme Conjugate, Antibody Solution, Substrate, and Stop Solution is recommended when running 2 strips or more.
- Dilution or adulteration of reagents or samples not called for in the procedure may result in inaccurate results.
- 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.

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. Prepare the 1X wash solution by diluting the (100X) Wash solution (i.e. 5 mL of the wash solution plus 495mL of deionized water in a 500 ml wash bottle).
3. Remove the required number of test wells from the re-sealable foil bag. Re-seal the remaining strips in the bag containing the desiccant to limit moisture exposure.
4. Dispense **50 µL of the HRP Enzyme Conjugate** into each well.
5. Add **50 µL of the Calibrators, Positive Control or 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. Shake the plate gently to mix contents.
7. Incubate the wells for **45 minutes** at room temperature.
8. After this incubation, decant the contents of the wells into an appropriate waste container. Flood the wells completely with laboratory grade water, then decant. Repeat this wash step three times for a total of four washes. Invert the plate on absorbent paper and tap out as much of the water wash solution as possible.
9. Add **100 µL of Substrate** to each well. Shake the plate gently to mix contents.
10. Incubate the wells at room temperature for **45 minutes**.
11. Add **100 µL of Stop Solution** to each well. Shake the plate gently to mix contents.

12. Measure and record the absorbance (Optical Density; OD) of each well using a microtiter plate reader at 450nm.
13. If the absorbance of a sample is lower than the highest calibrator (2.0 ppb), the concentration of cylindrospermopsin 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 (0.05 ppb to 2.0 ppb). Results must then be multiplied by the dilution factor used.
14. The value of the 0.25 ppb control should fall within the following range:

0.25 ppb Cylindrospermopsin Positive Control: 0.15 – 0.32 ppb

CALCULATE RESULTS

1. Semi-quantitative results can be derived by simple comparison of the sample absorbance to the absorbance of the calibrator wells. Samples with lower absorbance (less color) than a calibrator well, have a concentration of cylindrospermopsin greater than the concentration of the calibrator. Samples with higher absorbance (more color) than a calibrator well, have a concentration less than the concentration of the calibrator.

2. Quantitative interpretation requires graphing the absorbance (OD) from the calibrator wells (Y axis) versus the calibrator concentration (X axis). This can be done using a plate reader with software which uses either a 4-Parameter or Semi-log curve fit. If your plate reader software does not provide these curve fits, a spreadsheet that will perform the curve fit and sample concentration calculation is available upon request.

SAMPLE CALCULATIONS

Well Contents	Average OD ± SD*	RSD%	**Bo%
0 ppb Calibrator	2.15 ± 0.04	1.67	100
0.05 ppb Calibrator	1.88 ± 0.02	1.23	87
0.3 ppb Calibrator	1.02 ± 0.02	2.92	47
0.75 ppb Calibrator	0.51 ± 0.02	3.07	24
2.0 ppb Calibrator	0.23 ± 0.005	2.2	11
Positive Control 0.25 ppb	1.09 ± 0.03	2.4	51

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

* Standard deviation

**Bo% equals the average sample absorbance divided by the average 0 ppb Calibrator absorbance multiplied by 100.

TECHNICAL ASSISTANCE

For questions regarding this kit or for additional information about Beacon products, contact Beacon at (207) 571-4302 or info@beaconkits.com or your local representative.

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.

In the event of a breach of the foregoing warranty, Beacon's sole obligation shall be to repair or replace, at its option, any product or part thereof that proves defective in materials or workmanship within the warranty period, provided the customer notifies Beacon promptly of any such defect. The exclusive remedy provided herein shall not be deemed to have failed of its essential purpose so long as Beacon is willing and able to repair or replace any nonconforming Beacon product or part. Beacon shall not be liable for consequential, incidental, special or any other indirect damages resulting from economic loss or property damage sustained by a customer from the use of its products. However, in some states the purchaser may have rights under state law in addition to those provided by this warranty.

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