Cylindrospermopsin Plate Kit

Cat. # 20-0149-N

Instructional Brochure

Please read completely before use

INTENDED USE

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

ASSAY 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.

NOTE: Color is inversely proportional to cylindrospermopsin concentration.

Darker color = Lower concentration

Lighter color = Higher concentration

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REAGENTS AND MATERIALS PROVIDED

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 8ºC.

1	Plate containing 12 test strips of 8 wells each vacuum-sealed in an aluminized pouch with a desiccant.
5	Vials Cylindrospermopsin Calibrators (0, 0.05, 0.3, 0.75, 2 ppb)
1	Vial Cylindrospermopsin Positive Control (0.25 ppb)
1	Bottle Cylindrospermopsin HRP Enzyme Conjugate
1	Bottle Cylindrospermopsin Antibody
1	Bottle Substrate
1	Bottle Stop Solution (Caution! Contains 1N HCl. Handle with care.)

REAGENTS AND MATERIALS REQUIRED BUT NOT PROVIDED

Assay Procedure:

- Laboratory quality distilled or deionized water
- Pipette(s) with disposable tips capable of dispensing 50 and 100 µL
- Multi-channel pipette: 8 channels capable of dispensing 50 and 100 µL (recommended if running more than two strips at once.)
- Paper towels or equivalent absorbent material.
- Wash bottle
- Timer
- Orbital shaker (optional)
- Microwell plate or strip reader with 450 nm filter.

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%
7-Epi-Cylindrospermopsin	153%
7-Deoxy-Cylindrospermopsin	126%
Microcystin-LR	<1%
Nodularin	<1%

KIT HANDLING NOTES AND PRECAUTIONS

- Store all kit components at 4°C to 8°C (39°F to 46°F) when not in use.
- Running Calibrators, controls, and samples in duplicate will improve assay precision and accuracy.
- Precise transfer of samples and reagents by using an appropriate and calibrated pipette is critical to obtain proper assay results.
- If running more than two strips at once, the use of a multi-channel pipette is recommended when adding the Antibody, Substrate and Stop Solution.
- Each reagent is optimized for use in the Beacon Cylindrospermopsin Plate Kit. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other Beacon Cylindrospermopsin 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, 20 to 28ºC (62 to 82ºF) prior to use. Avoid prolonged (> 24 hours) storage at room temperature.
- Do not freeze kit components or expose them to temperatures greater than 37°C (99°F).
- 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.

SAMPLE PREPARATION

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

ASSAY PROCEDURE

(Note: Running calibrators and samples in duplicate will improve assay precision and accuracy.)

1. Allow reagents and sample extracts to reach room temperature prior to running the test.
2. Place the appropriate number of test wells into a micro well holder. Be sure to re-seal unused wells in the zip-lock bag with the desiccant to limit exposure to moisture.
3. Dispense **50 µL of Enzyme Conjugate** into each well.
4. Dispense **50 µL of Calibrators, Positive Control and Sample Extract(s)** into the appropriate wells. Be sure to use a clean pipette tip for each solution to avoid cross contamination.
5. Dispense **50 µL of Antibody** into each well. If running more than two strips at once, the use of a multi-channel pipette is recommended.
6. Gently shake the plate for 30 seconds using a back-and-forth motion and incubate the wells for **45 minutes** at room temperature.
7. Decant the contents of the wells into an appropriate waste container. Fill the wells to overflowing with laboratory quality distilled or deionized water and then decant. Repeat this wash step four times for a total of five washes. Following the last wash, tap the inverted wells onto absorbent paper to remove excess wash solution.
8. Dispense **100 µL of Substrate** to each well. If running more than two strips at once, the use of a multi-channel pipette is recommended.
9. Incubate for **45 minutes** at room temperature.
10. Dispense **100 µL of Stop Solution** into each well in the same order of addition as the Substrate. If running more than two strips at once, the use of a multi-channel pipette is recommended.
11. Measure and record the absorbance (Optical Density; OD) of the wells at 450 nm using a strip or plate reader. If the reader has dual wavelength capability, read at 450 nm minus 605 or 650 nm.

QUALITY CONTROL

The value of the 0.25 ppb positive control should fall within the range of 0.15 – 0.32 ppb.

RESULTS INTERPRETATION

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

Quantitative Interpretation: It is preferred for quantitative results to be determined using commercially available software for ELISA evaluation using a 4-parameter curve fit. Alternatively, a semi-log curve fit can be used if 4-parameter software is not available. A spreadsheet that will perform the curve fit and sample concentration calculation is available upon request. Please contact Beacon for further details. Samples with absorbances greater than the lowest calibrator or less than the highest calibrator must be reported as <0.05 ppb or >2 ppb, respectively.

Samples with absorbances lower than the highest calibrator contain a concentration of cylindrospermopsin too high for quantification. Further dilute the sample extract in laboratory quality distilled or deionized water and retest along with the calibrators and control. Samples should be diluted to fit into the standard curve. Results must then be multiplied by the dilution factor used.

EXAMPLE CALCULATIONS

Well Contents	OD	Average OD ± SD*	%RSD	%Bo**	Cylindrospermopsin Concentration (ppb)
0 ppb Calibrator	2.251 2.262	2.257 ± 0.007	0.32	100	N/A
0.05 ppb Calibrator	1.757 1.742	1.749 ± 0.011	0.60	78	N/A
0.3 ppb Calibrator	0.719 0.697	0.708 ± 0.016	2.21	31	N/A
0.75 ppb Calibrator	0.342 0.327	0.335 ± 0.010	3.13	15	N/A
2 ppb Calibrator	0.182 0.170	0.176 ± 0.008	4.75	8	N/A
0.25 ppb Positive Control	0.864 0.873	0.868 ± 0.006	0.67	38	0.23
Sample	1.320 1.360	1.340 ± 0.028	2.11	59	0.11

This data is for example purposes only.

* Standard Deviation

** $\%B_o$ = (Average OD / 0 ppb Average OD)*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 Material 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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Revision Log:

Name	Date	Changes Made
		Adjusted spelling, grammar, and formatting. Added page numbers, Beacon information and revision control/log. Removed product insert from materials provided list. Added dual wavelength. Reworded result interpretation and moved the last couple steps of the protocol to other sections. Redid sample calculations to be helpful.

Commented [KH1]: Add revision information