Importance of Cylindrospermopsin Determination

Most of the world's population relies on surface freshwaters as its primary source for drinking water. The drinking water industry is constantly challenged with surface water contaminants that must be removed to protect human health. Toxic cyanobacteria (blue-green algae) blooms are an emerging issue in the U.S. and the world because of increased source water nutrient pollution caused by eutrophication. Cylindrospermopsin is a naturally produced toxin of several cyanobacterial strains and has been found in fresh water throughout the world. Certain strains of *Cylindrospermopsis raciborskii* (Australia, Hungary, United States), *Umezakia natans* (Japan), *Aphanizomenon ovalisporum* (Australia, Israel) have been found to produce cylindrospermopsin. The production of cylindrospermopsin seems to be strain specific and not species specific.

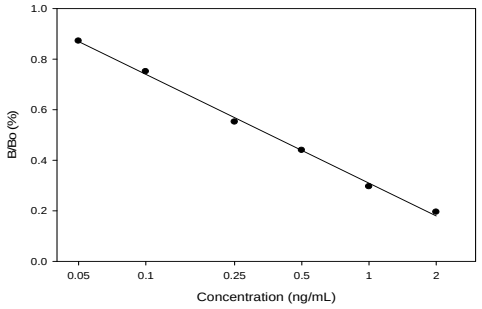
Acute poisoning of humans and animals constitutes the most obvious problem from toxic cyanobacterial blooms, and in several cases has lead to death. These toxins mediate their toxicity by inhibiting liver function and are potent inhibitors of protein synthesis and glutathione, leading to cell death. Human exposure to cylindrospermopsin may occur by ingestion of toxin contaminated water during recreational activities or by ingestion of food (fish) or water contaminated with the toxin. Dermal contact with cylindrospermopsin may occur during showering or bathing, or during recreational activities such as wading, swimming, boating, or water skiing. To protect consumers from adverse health effects caused by algal toxins, the WHO has proposed limits for some toxins (i.e microcystin-LR) in drinking water and in recreational waters. A tolerable daily intake (TDI) of cylindrospermopsin along with the guideline values for human exposure have been calculated based on acute toxicity studies in mice. The TDI is 0.02 g/Kg body weight/day. It was estimated that GVs for adult, children, and infants are 0.48, 0.16, and 0.11 g/L, respectively, based on a drinking water consumption of 2L for a 60-Kg adult, 1 L for a 10-Kg child, and 0.75 L for a 5-Kg infant.

Performance Data

Test sensitivity: The detection limit for this assay is 0.040 ppb (ug/L)

Test reproducibility: Coefficients of variation (CVs) for standards: <10%, for samples: <15%.

Selectivity: The assay exhibits very good cross-reactivity with cylindrospermopsin and not with other non-related algal toxins tested.



Samples: A sample correlation between the ELISA and HPLC methods showed a good correlation.

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R022111

Cylindrospermopsin ELISA (Microtiter Plate)

Enzyme-Linked Immunosorbent Assay for the Determination of Cylindrospermopsin Water Samples

Product No. 522011

1. General Description

The Abraxis Cylindrospermopsin ELISA is an immunoassay for the quantitative and sensitive detection of Cylindrospermopsin in water samples. A pre-sample concentration is not required. If necessary, positive samples can be confirmed by HPLC, or other conventional methods.

2. Safety Instructions

The substrate solution contains tetramethylbenzidine (TMB) and the stop solution contains diluted sulfuric acid. Avoid contact of the TMB and stopping solution with skin and mucous membranes. If these reagents come in contact with the skin, wash with water.

3. Storage and Stability

The Cylindrospermopsin ELISA should be stored in the refrigerator (4–8°C). Solutions should be allowed to reach room temperature (20-25°C) before use. Reagents may be used until the expiration date on the box. Consult state, local and federal regulations for proper disposal of all reagents.

4. Test Principle

The test is a direct competitive ELISA that allows the detection of Cylindrospermopsin. It is based on the recognition of Cylindrospermopsin by specific antibodies. Cylindrospermopsin, when present in a sample, and a Cylindrospermopsin-HRP analogue compete for the binding sites of rabbit anti-cylindrospermopsin antibodies in solution. The cylindrospermopsin antibodies are then bound by a second antibody (sheep anti-rabbit) immobilized in the plate. After a washing step and addition of the substrate solution, a color signal is generated. The intensity of the blue color is inversely proportional to the concentration of the Cylindrospermopsin present in the sample. The color reaction is stopped after a specified time and the color is evaluated using an ELISA reader. The concentrations of the samples are determined by interpolation using the standard curve constructed with each run.

5. Limitations of the Cylindrospermopsin ELISA, Possible Test Interference

Numerous organic and inorganic compounds commonly found in water samples have been tested and found not to interfere with this test. However, due to the high variability of compounds that might be found in water samples, test interferences caused by matrix effects can't be completely excluded. The presence of the following substances up to 10,000 ppm were found to have no significant effect on the Cylindrospermopsin Assay results: aluminum oxide, calcium chloride, calcium sulfate, manganese sulfate, magnesium sulfate, magnesium chlorides, sodium chloride, phosphate, sodium thiosulfate. Sodium nitrate, zinc sulfate up to 1,000 ppm. Humic acid, ferric sulfate up to 100 ppm. Copper chloride up to 10 ppm. Lugol's solution up to 0.01%. Salt water up to 50%.

Mistakes in handling the test can also cause errors. Possible sources for such errors can be:
Inadequate storage conditions of the test kit, wrong pipetting sequence or inaccurate volumes of the reagents, too long or too short incubation times during the immune and/or substrate reaction, and extreme temperatures during the test performance (lower than 10°C or higher than 30°C). The assay procedure should be performed away from direct sun light.

As with any analytical technique (GC, HPLC, etc…..) positive results requiring some action should be confirmed by an alternative method.

A. Materials Provided

1. Microtiter plate coated with a second antibody (sheep anti rabbit).
2. Standards (7) and Control (1): 0, 0.05, 0.10, 0.25, 0.50, 1.0, 2.0 ng/mL. Control at 0.75 ng/mL
3. Antibody solution (Rabbit anti-Cylindrospermopsin), 6 mL
4. Cylindrospermopsin-HRP, 6 mL
5. Diluent/zero, 25 mL. Use to dilute samples with concentration above 2 ppb.
6. Wash Solution 5X Concentrate, 100 mL
7. Color Solution (TMB), 12 mL
8. Stop Solution, 2 X 6 mL

B. Test Preparation

Micro-pipetting equipment and pipette tips for pipetting the standards and the samples are necessary. We recommend using a multi-channel pipette or a stepping pipette for adding the antibody, enzyme conjugate, substrate solution, and the stop solution in order to equalize the incubations periods of the standard solutions and the samples on the entire microtiter plate. Please only use the reagents and standards from one package lot in one test, as they have been adjusted in combination. Read and understand the instructions and precautions given in this insert before proceeding.

1. Adjust the microtiter plate and the reagents to room temperature before use.
2. Remove the number of microtiter plate strips required from the foil bag. The remaining strips are stored in the foil bag and zip-locked closed. Store the remaining kit in the refrigerator (4-8°C).
3. The standard, control, antibody solution, enzyme conjugate, substrate and stop solutions are ready to use and do not require any further dilutions.
4. The wash solution is a 5X concentrated solution and needs to be diluted with deionized water. In a 1L container dilute the 5X solution 1:5 (i.e. 100 mL of the 5X wash solution plus 400 mL of deionized water). The diluted solution is used to wash the microtiter wells.
5. The stop solution has to be handled with care as it contains diluted H_2SO_4.

C. Assay Procedure

1. Add 50 µL of the standard solutions, control or samples into the wells of the test strips according to the working scheme given. We recommend using duplicates or triplicates.
2. Add 50 µL of the enzyme conjugate solution to the individual wells successively using a multi- channel pipette or a stepping pipette.
3. Add 50 µL of antibody solution to the individual wells successively using a multi- channel pipette or a stepping pipette. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a rapid circular motion on the benchtop for about 30 seconds. Be careful not to spill contents. Incubate the strips for forty five (45) minutes at room temperature.
4. After incubation, remove the covering and vigorously shake the contents of the wells into a sink. Wash the strips four times using the 1X washing buffer solution. Please use at least a volume of 250 µL of washing buffer for each well and each washing step. Remaining buffer in the wells should be removed by patting the plate dry on a stack of paper towels.
5. Add 100 µL of substrate/color solution to the wells using a multi- channel pipette or a stepping pipette. The strips are incubated for 30-45 minutes at room temperature. Protect the strips from sunlight.
6. Add 100 µL of stop solution to the wells in the same sequence as for the substrate/color solution using a multi- channel pipette or a stepping pipette.
7. Read the absorbance at 450 nm using a microplate ELISA photometer within 15 minutes after stopping the reaction.

D. Evaluation

The evaluation of the ELISA can be performed using commercial ELISA evaluation programs (4-parameters, Logit/Log or alternatively point to point). For a manual evaluation, calculate the mean absorbance value for each of the standards. Calculate the $\%B/B_0$ for each standard by dividing the mean absorbance value for each standard by the Zero Standard (Standard 0) mean absorbance. Construct a standard curve by plotting the $\%B/B_0$ for each standard on a vertical linear (y) axis versus the corresponding Cylindrospermopsin concentration on horizontal logarithmic (x) axis on graph paper. $\%B/B_0$ for controls and samples will then yield levels in ppb of Cylindrospermopsin by interpolation using the standard curve.

The concentrations of the samples are determined using the constructed standard curve (do not use a previously stored curve). Samples showing a lower concentration than 0.05 ppb of Cylindrospermopsin are considered to be negative. Samples showing a higher concentration than standard 6 (2.0 ppb) must be diluted to obtain more accurate results. The concentration of the positive control should be in the range given on the bottle (±20%).

E. Additional Materials (not delivered with the test kit)

1. Micro-pipettes with disposable plastic tips (50-250 µL)
2. Multi-channel pipette (50-250 µL) or stepper pipette with plastic tips (50-250 µL)
3. Reagent reservoir for multichannel pipettes
4. Microtiter plate washer (optional)
5. Microtiter plate reader (wave length 450 nm)
6. Shaker for microtiter plates (optional)

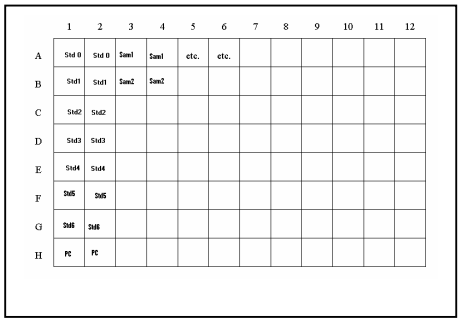
F. Working Scheme

The microtiter plate consists of 12 strips of 8, which can be used individually. The standards must be run with each test. Never use the values of standards which have been determined in a test performed previously.

Sd0-Sd6: Standards

PC (Positive Control): 0.75 ppb

Sam1, Sam2, Sam3, etc.: Samples



Recovery

Four (4) groundwater samples, were spiked with various levels of Cylindrospermopsin and then assayed using the Abraxis Cylindrospermopsin Assay. The following results were obtained:

Amount of Cylindrospermopsin Added (ppb)	Mean (ppb)	S.D. (ppb)	Recovery (%)
0.1	0.101	0.019	101
0.25	0.269	0.026	108
0.50	0.514	0.038	103

Precision

The following results were obtained:

Control	1	2	3
Replicates	3	3	3
Days	3	3	3
n	9	9	9
Mean (ppb)	0.198	0.501	1.01
% CV (within assay)	6.2	4.3	5.2
% CV (between assay)	8.3	5.3	4.9

Sensitivity

The Abraxis Cylindrospermopsin ELISA has an estimated minimum detectable concentration, based on 90% B/Bo of 0.040 ppb.

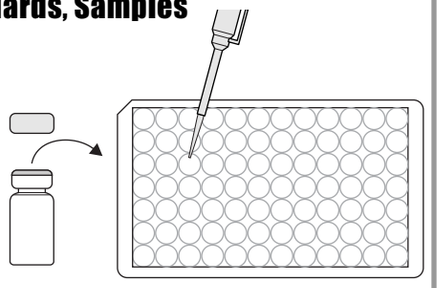
G. References

(1) Cylindrospermopsin, Review of Toxicological Literature. Prepared by Integrated Laboratory Systems for Scott Masten, National Institute of Health Sciences, RTP, NC. Contract Number N01-ES-65402, December 2000.

Cylindrospermopsin Plate, Detailed ELISA Procedure

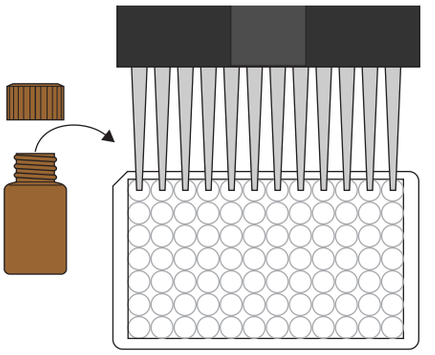
1. Addition of Standards, Samples

Add 50 ul of the standard solutions, control or samples into the wells of the test strips according to the working scheme given. We recommend using duplicates or triplicates.



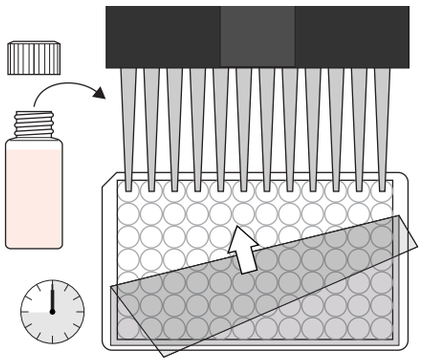
2. Addition of Enyzme Conjugate

Add 50 uL of the enzyme conjugate to the individual wells successively using a multi- channel pipette or a stepping pipette.



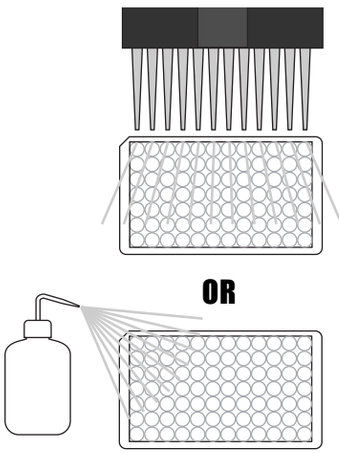
3. Addition of Antibody Solution

Add 50 ul of the Cylindrospermopsin anitbody solution to the individual wells successively using a multi-channel pipette. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a rapid circular motion on the benchtop. Be careful not to spill contents. Incubate the strips for 45 min at room temperature.



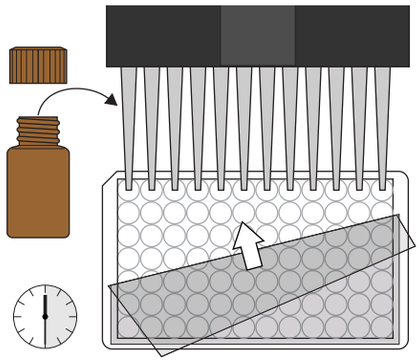
4. Washing of Plates

After incubation, remove the covering and vigorously shake the contents of the wells into a sink. Wash the strips four times with a multi-channel pipette or wash bottle using the diluted 1X washing buffer solution. Please use at least a volume of 250 uL of washing buffer for each well and each washing step. Remaining buffer in the wells should be removed by patting the plate dry on a stack of paper towels.



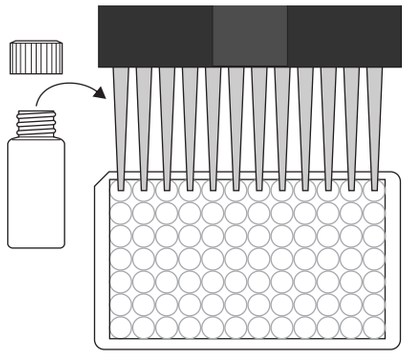
5. Addition of Substrate/Color Solution

Add 100 uL of substrate/color solution to the individual wells successively using a multi-channel pipette or a stepping pipette. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a rapid circular motion on the benchtop. Be careful not to spill contents. Incubate the strips for 30-45 min at room temperature.



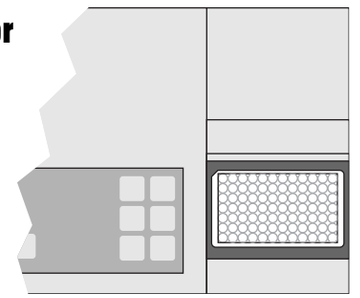
6. Addition of Stopping Solution

Add 100 uL of stop solution to the wells in the same sequence as for the substrate solution using a multi- channel pipette or a stepping pipette.



7. Measurement of Color

Read the absorbance at 450 nm using a microplate ELISA reader. Calculate results.



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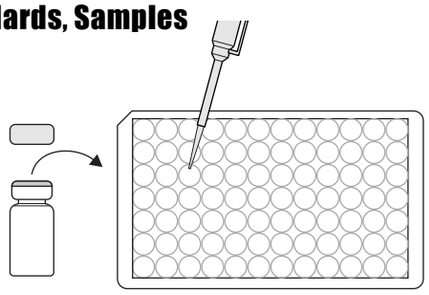
Cylindrospermopsin Plate, Concise ELISA Procedure

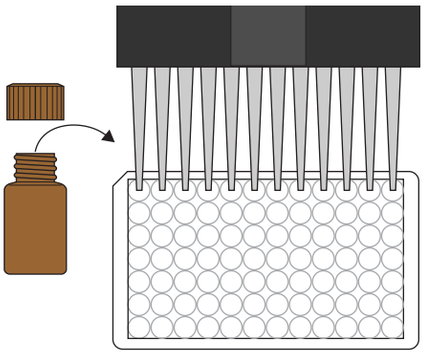
1. Addition of Standards, Samples

Add 50 uL of standard solutions, control or samples.



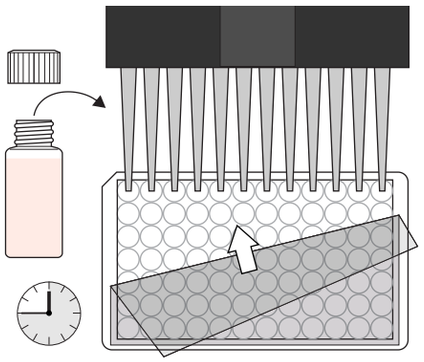
2. Addition of Enyzme Conjugate

Add 50 uL of enzyme conjugate.



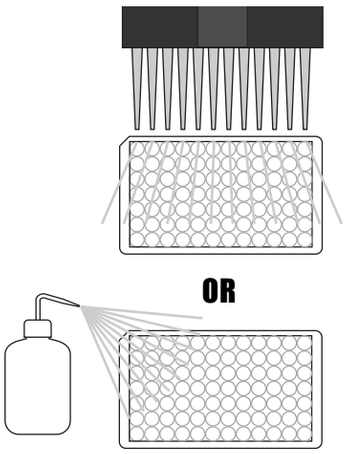
3. Addition of Antibody Solution

Add 50 uL of the antibody solution. Cover and mix for 30 seconds by rotating on benchtop. Incubate for 45 minutes at room temperature.



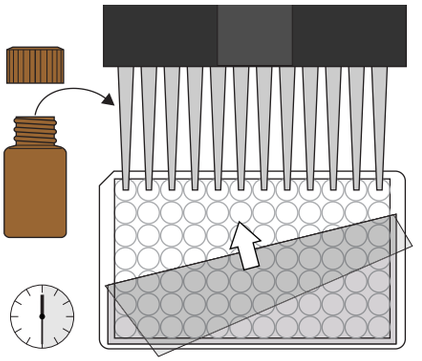
4. Washing of Plates

Wash the plates four times with 250 uL of diluted 1X washing buffer.



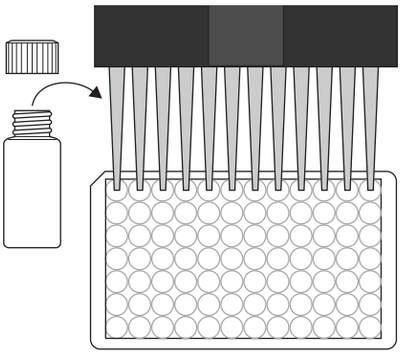
5. Addition of Substrate/Color Solution

Add 100 uL of substrate/color solution. Incubate 30-45 minutes at room temperature and away from direct sunlight.



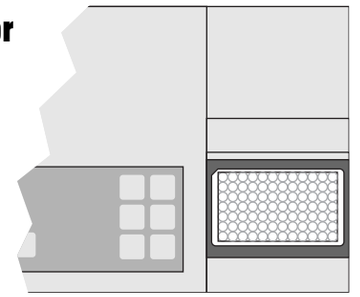
6. Addition of Stopping Solution

Add 100 uL of stop solution.



7. Measurement of Color

Measure color at 450 nm. Calculate results.



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