Importance of Cylindrospermopsin Determination in Benthic Mats

Benthic mats are dense accumulations of algae that grow on the bottom of bodies of water and, under certain conditions, can become dislodged and rise to the surface. 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 cyanobacterial blooms are an emerging issue worldwide due to increased source water nutrient pollution caused by eutrophication. Cylindrospermopsin is a toxin produced by several different types of cyanobacteria (blue-green algae) and has been found in freshwater throughout the world. Certain strains of *Cylindrospermopsis raciborski* (found in Australia, Hungary, and the United States), *Umezakia natans* (found in Japan), and *Aphanizomenon ovalisporum* (found in Australia and Israel) have been found to produce Cylindrospermopsin. The production of Cylindrospermopsin seems to be strain specific rather than species specific.

Acute poisoning of humans and animals constitutes the most obvious problem from toxic cyanobacterial blooms and, in several cases, has led to death. Human and animal exposure to these toxins can occur through the ingestion of contaminated water, through drinking or during recreational activities in which water is swallowed, or food, such as fish. Dermal contact with Cylindrospermopsin may occur during showering or bathing, or during recreational activities such as swimming or boating. These toxins mediate their toxicity by inhibiting liver function and are potent inhibitors of protein synthesis and glutathione, leading to cell death.

To protect against adverse health effects, the U.S. Environmental Protection Agency (EPA) has established health advisories for Cylindrospermopsin in drinking water:

-For children pre-school age and younger (less than six years old), 0.7 µg/L (ppb)
-For school-age children and adults, 3.0 µg/L (ppb)

Performance Data

Test sensitivity: The limit of detection (LOD) with the ABRAXIS® Cylindrospermopsin Strip Test for benthic mats is 20 ppb (ng/g). At this level, the test line exhibits moderate intensity. At levels greater than 400 ppb (ng/g) the test line is not visible. When compared with samples of known Cylindrospermopsin concentration, it is possible to obtain a semi-quantitative result.
The limit of detection (LOD) with the ABRAXIS® Cylindrospermopsin ELISA test for benthic mats is 5 ppb (ng/g).

Selectivity: The ABRAXIS® Cylindrospermopsin test kits exhibit very good cross-reactivity with Cylindrospermopsin and Deoxy-Cylindrospermopsin.

Cell Lysing: A sample correlation between the ABRAXIS® QuikLyse™ reagents and the 3 cycle freeze/thaw method showed a good correlation.

Samples: A sample correlation between the ABRAXIS® Strip Test and ABRAXIS® ELISA methods showed a good correlation.

References

ITRC (Interstate Technology &Regulatory Council). 2022. Strategies for Preventing and Managing Harmful Benthic Cyanobacterial Blooms (HCB-2). Washington, D.C.: Interstate Technology & Regulatory Council, HCB Team. www.itrcweb.org.

ITRC (Interstate Technology &Regulatory Council). 2020. Strategies for Preventing and Managing Harmful Cyanobacterial Blooms (HCB-1). Washington, D.C.: Interstate Technology & Regulatory Council, HCB Team. www.itrcweb.org

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ABRAXIS® Benthic Mat Sample Extraction Kit for Cylindrospermopsin

Product No. 529917

1. General Description

The ABRAXIS® Benthic Mat Sample Extraction Kit for Cylindrospermopsin is for the collection of benthic mat samples and sample preparation/extraction of Cylindrospermopsin from benthic mat samples prior to testing with the ABRAXIS® Cylindrospermopsin Strip Test (PN 520029 or 520030). Extracts can also be used for confirmatory testing with the ABRAXIS® Cylindrospermopsin ELISA kit (PN 522011). Each extraction kit provides materials for 20 benthic mat samples.

2. Safety Instructions

Wear appropriate protective clothing (gloves, glasses, etc.) and avoid contact with skin and mucous membranes. The sample preparation procedure should be performed in a well-ventilated area. Avoid breathing aerosols. If contact with Cylindrospermopsin occurs, wash with copious amounts of water. Discard samples according to local, state and federal regulations.

3. Storage and Stability

The ABRAXIS® Benthic Mat Sample Extraction Kit for Cylindrospermopsin should be stored between 2-30°C. The benthic mat samples to be extracted and analyzed should be at room temperature before use.

4. Warnings and Precautions

-This procedure is intended for use with benthic mat samples. Other matrices should be thoroughly validated before use.
-Due to the high variability of compounds that may be found in benthic mat samples, test interferences caused by matrix effects cannot be completely excluded. Please see the corresponding test kit user's guide for additional information on test limitations and interferences.
-The Cylindrospermopsin Strip Test provides only a preliminary qualitative test result. An alternate, more quantitative analytical method, such as ELISA or instrumental analysis, should be used to obtain a confirmed quantitative analytical result. See step 9 in section D (Extraction Procedure), below.
-The cellular makeup of benthic mats can vary widely throughout the mat, which can therefore cause the toxin content of the mat to also vary widely throughout the mat. Because of this, whenever possible, the collected sample should be a composite, made up of pieces sampled from throughout the mat to provide the most accurate assessment of toxin concentration contained within the entire benthic mat. See section C, Sample Collection, for instructions on collecting a representative mat sample and ITRC HCB-2 and HCB-1 (https://hcb-2.itrcweb.org and https://hcb-1.itrcweb.org) for additional information.
-Distilled or deionized water **must** be used for sample preparation. Do **not** use tap water for sample preparation, as chlorine and other water treatment chemicals present in tap water will degrade Cylindrospermopsin, causing inaccurate, biased low sample results.
-Re-usable equipment (scissors, tweezers, etc.) used for collection or extraction of benthic mat samples must be thoroughly cleaned between samples to avoid cross-contamination which may cause inaccurate sample results. Clean equipment after each use with a 10% bleach solution (1 part bleach in 9 parts water) and then rinse with clean water.
*Note: Tap water may be used for the preparation of the 10% bleach solution and for the rinsing of equipment after cleaning with the 10% bleach solution, but do **not** use tap water for sample preparation/extraction, as chlorine and other water treatment chemicals present in tap water will degrade Cylindrospermopsin, causing inaccurate, biased low sample results.*
-Samples should be allowed to reach room temperature before testing.

A. Materials Provided

1. 40 mL amber glass Sample Collection Vials with caps and labels (20)
2. Disposable sample extraction tubes with disposable pestles (20)
3. Sample extraction tube holder (1)
4. Foam vial holder (1)
5. Disposable graduated pipettes (20, bags marked with blue sticker to indicate appropriate step for use)
6. Disposable transfer pipettes (20, bag marked with yellow sticker to indicate appropriate step for use)
7. Dilution vials (20)
8. 4 mL glass Final Sample Extract Vials with caps and labels (20)
9. User's guide

B. Additional Materials (not provided with the extraction kit)

1. Portable scale with two decimal place display (i.e., 0.00 g), PN 709049 or equivalent
2. Disposable gloves
3. Protective glasses
4. Scissors
5. Tweezers
6. 10% bleach solution
7. Tap water (for rinsing equipment, see section 4, Warnings and Precautions, above)
8. Distilled or deionized water
9. Paper towels
10. Disposable spatulas (optional), PN 705043 or equivalent
11. Permanent marker
12. Timer, PN 709055 or equivalent
13. ABRAXIS® Cylindrospermopsin Strip Test, PN 520029 and 520030 or ABRAXIS® Cylindrospermopsin ELISA kit PN 522011, for confirmatory analysis

C. Sample Collection

1. Using clean scissors, remove pieces of mat material from throughout the benthic mat and place into a single clean, appropriately labeled amber 40 mL Sample Collection vial.
2. Clean scissors with 10% bleach solution and rinse with water. Remove and appropriately dispose of gloves.
3. If the sample is to be tested immediately, proceed to section D, Extraction Procedure. If the sample is to be stored for later extraction/testing, place tightly closed vial in a cooler with ice packs. Store samples refrigerated.

D. Extraction Procedure

1. Unfold the cardboard vial holder from the sample extraction kit and place on the portable scale. Place one sample extraction tube into the vial holder and press the "tare" button to bring displayed weight to "0.00 g".
2. Remove the benthic mat sample from the amber vial and place on paper towels. Blot to remove excess moisture.
3. Using tweezers or a disposable spatula, remove small pieces of the mat material from throughout the sample and place in the sample extraction tube. Transfer a total of 0.25 g of benthic mat to the tube.
   Note: Tweezers must be cleaned with 10% bleach solution and rinsed with water after each use and gloves must be cleaned or changed if they come into contact with benthic mat sample or water that is removed during blotting of the sample to prevent cross-contamination of extraction/testing materials or samples which can cause inaccurate test results.
4. Using a clean disposable graduated pipette from bag 1 (bag with blue sticker), add 0.5 mL of **distilled or deionized water** to the tube, being careful to avoid touching the sample or tube with the pipette to prevent cross-contamination.
5. Using a clean disposable pestle, thoroughly grind the sample and water solution for 4 minutes, being careful to avoid causing the sample or water to overflow or spill from the tube. When thoroughly ground, the sample will have a thin, mud-like appearance, with no large mat pieces visible in the solution.
   Note: If sample or water overflows from the tube during extraction, wipe the tube with a paper towel saturated with 10% bleach solution and clean or change gloves to prevent contact with Cylindrospermopsin or cross-contamination of extraction/testing materials that can produce inaccurate results.
6. Using the same disposable graduated pipette used in step 4, transfer 9.5 mL of **distilled or deionized water** to a clean, appropriately labeled Dilution Vial.
7. Using the same disposable graduated pipette used in step 6, transfer all of the sample extract from step 5 to the Dilution Vial. Discard pipette after use. Cap vial tightly and shake for 30 seconds to thoroughly mix. Allow to settle for 10 minutes.
8. Using a clean transfer pipette from bag 2 (yellow sticker), transfer the top of the upper liquid portion of the extract from the Dilution Vial into a clean, appropriately labeled amber Final Extract vial.
9. Analyze the diluted benthic mat sample extract (from step 8, above) as directed in section E, Test Strip Sample Analysis Procedure. This extract may also be used for confirmatory testing of positive results using the ABRAXIS® Cylindrospermopsin ELISA kit (PN 522011) if desired. Sample extracts require an additional 1:2.5 dilution of the final extracts (0.4 mL of the sample from the final extract vial into 0.6 mL of Cylindrospermopsin sample diluent) prior to analysis. The diluted sample should be analyzed as described in the Assay Procedure section of the plate kit user's guide. Final results for samples are determined by multiplying the ELISA results by a factor of 100.

E. Test Strip Sample Analysis Procedure (Analysis using ABRAXIS® Cylindrospermopsin Strip Test, PN 520029 and 520030, not included with Benthic Mat Sample Extraction Kit)

1. Using a new graduated disposable pipette for each sample, draw the benthic mat sample extract to the 1 mL line (graduation mark slightly below bulb) and add 1 mL of sample to the lysis vial.
2. Cap the vial and shake for 2 minutes, then allow the sample in the vial to incubate at room temperature for 8 minutes.
3. Using the forceps provided, add 1 reagent paper to the lysis vial.
4. Cap the vial and shake for 2 minutes, then allow the sample in the vial to incubate at room temperature for 8 minutes.
5. Label conical test vials for each sample to be tested.
6. Using a new disposable exact volume transfer pipette for each sample, transfer 200 µL of the sample from the lysis vial to the appropriately labeled conical test vial (see pipette package for usage instructions).
7. Close the conical test vial and shake for 30 seconds. Examine the vial to ensure all dried reagents are completely dissolved (dried reagents will dissolve, turning the sample purple).
8. Incubate the conical test vial at room temperature for 10 minutes.
9. Insert test strip (arrows down) into the conical vial.
10. Allow the test to develop for 10 minutes.
11. Remove the test strip. Lay the strip flat and allow to continue developing for 5 minutes.
12. Read the results visually, as explained below in Section F, Interpretation of Results.

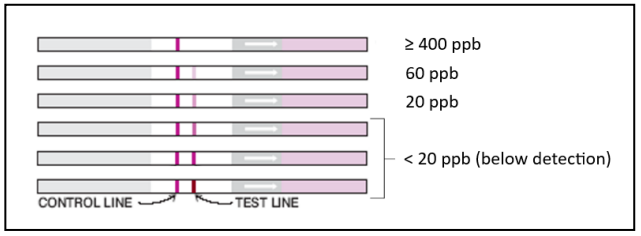
F. Interpretation of Results

Sample concentrations are determined by comparison of the intensity of the test line to the intensity of the control line on the same test strip. Although control line intensity may vary, a visible control line must be present for results to be considered valid. Test strips with a test line which is darker than or of equal intensity to the control line indicates a result which is below the limit of detection of the test (< 20 ppb). Test strips with a test line which is lighter than the control line indicates a result which is ≥ 20 ppb. Test strips with no test line visible (only the control line is visible) indicates a result which is ≥ 400 ppb. Results should be determined within 5-10 minutes after completion of the strip test procedure. Determination made using strips which have dried for more or less than the required time may be inaccurate, as line intensities may vary with drying time.

Control Line	***Test Line***	***Interpretation***
No control line present	No test line present	Invalid result
Control line present	No test line present	≥400 ppb
Control line present	Test line present	Between 0 and <400 ppb

The appearance of test strips may also be compared to the illustration below to determine approximate sample concentration ranges. Please note that the illustration is intended for the demonstration of test line to control line intensity only. Results should not be determined by comparing the intensity of test lines from test strips to the test line intensity of the illustration, as the overall intensity of test strips may vary slightly with different lots of reagents. To obtain semi-quantitative results, solutions of known Cylindrospermopsin concentration (control solutions) must be tested concurrently with samples. Sample test line intensities can then be compared with control solution test line intensities, yielding approximate sample concentrations. Do not use strips run previously to determine semi-quantitative sample concentrations, as test line intensities may vary once strips are completely dry.

Visual Interpretation:



Alternately, test strips can also be interpreted using the AbraScan test strip reader (PN 475025), which provides objective determination of line intensities for consistent interpretation of results as well as a digital photographic record of all test strips. Please note that benthic mat sample results interpreted using the AbraScan test strip reader are raw results that must be multiplied by 40 to obtain final sample concentrations. For example, the AbraScan test strip reader result of ~0.5 ppb x 40 = ~20 ppb of Cylindrospermopsin in a benthic mat sample.