Cylindrospermopsin and Microcystins in Benthic Mats
Sample Preparation for ABRAXIS® ELISA Microtiter Plate Kits

1. Intended Use

Sample preparation for the detection of Cylindrospermopsin and Microcystins in benthic mats using the ABRAXIS® Cylindrospermopsin, ABRAXIS® Microcystins-ADDA, and ABRAXIS® Microcystins-DM ELISA microtiter plate kits.

2. Sensitivity

5 ppb of Cylindrospermopsin in matrix
15 ppb of Microcystins in matrix

3. Materials and Reagents Required

Bead ruptor (Omni Bead Ruptor 24 or equivalent)
Disposable bead ruptor sample tubes with caps (Omni 7 mL or equivalent)
Stainless steel beads (Omni 2.4 mm or equivalent)
Analytical balance
Micropipettes with disposable plastic tips
Fume hood
Centrifuge (9000 rpm capability)
Disposable gloves
Protective glasses
Scissors
Tweezers
Disposable spatulas (optional), PN 705043 or equivalent
40 mL amber glass vials with Teflon-lined caps, PN 701034 or equivalent
20 mL glass vials with Teflon-lined caps, PN 701029 or equivalent
10% bleach solution
Distilled or deionized water (for sample preparation, see section 4, Notes and Precautions, below)
Tap water (for use in cleaning equipment only, see section 4, Notes and Precautions, below)
Paper towels
Permanent marker
4 mL glass vials with Teflon-lined caps, PN 701031 or 701032 or equivalent
Vortex mixer, PN 709045 or equivalent
Timer, PN 709055 or equivalent
Cooler with ice packs (see section 5, Sample Collection, below)
ABRAXIS® Cylindrospermopsin ELISA Kit (PN 522011)
ABRAXIS® Microcystins-ADDA (PN 520011) or ABRAXIS® Microcystins-DM (PN522015) ELISA kit

4. Notes and Precautions

This procedure is intended for use with benthic mat samples. Other matrices should be thoroughly validated before use with this procedure.

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.

Wear appropriate protective clothing (gloves, glasses, etc.) and avoid contact with skin and mucous membranes. The sample preparation procedure should be performed in a fume hood. Avoid breathing aerosols. If contact with Cylindrospermopsin or Microcystins occurs, wash with copious amounts of water.

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 sample that is collected should be a composite, made up of pieces sampled from throughout the mat in order to provide the most accurate assessment of toxin concentration contained within the entire benthic mat. See section 5, Sample Collection, for instructions on collecting a representative mat sample and ITRC HCB-2 and HCB-1 (https://hcb-2.itrcweb.org and https://hbc-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 and Microcystins, causing inaccurate, biased low sample results.

Re-useable equipment (scissors, tweezers, etc.) used for the 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 water and dry thoroughly using clean paper towels.
*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 and Microcystins, causing inaccurate, biased low sample results.*

Microcystins have been found to adsorb onto many types of plastic, which may result in adsorptive loss of Microcystins with prolonged contact, producing inaccurate (falsely low) results. To minimize potential loss of analyte, samples should be transferred to glass vials immediately after bead ruptor processing (do not store processed sample in bead ruptor tubes).

5. Sample Collection

5.1 Using clean scissors, remove pieces of mat material from throughout the benthic mat and place into a single clean, appropriately labelled amber 40 mL glass vial.

5.2 Clean scissors with 10% bleach solution and rinse with water, then dry thoroughly using clean paper towels. Remove and appropriately dispose of gloves.

5.3 If the sample is to be tested immediately, proceed to section 6, 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.

6. Extraction Procedure

6.1 Remove the benthic mat sample from amber vial and place on paper towels. Blot to remove excess moisture.

6.2 Using tweezers or a disposable spatula, remove small pieces of the mat material from throughout the sample and place into bead ruptor tube containing stainless steel beads. Transfer a total of 0.5 g of benthic mat to the bead ruptor tube.
Note: Tweezers must be cleaned with 10% bleach solution and rinsed with water after each use to prevent cross-contamination of samples which can cause inaccurate test results.

6.3 Add 2 mL of **distilled or deionized water** to the tube.

6.4 Process with bead ruptor (4 cycles at 6 m/s for 25 seconds, 0.05 dwell pause between cycles).
Note: As Microcystins have been found to adsorb onto may types of plastic, after processing, immediately proceed to step 6.5 below. Do not store processed samples in bead ruptor tubes, as this may result in adsorptive loss of Microcystins which will produce inaccurate (biased low) results.

6.5 Add 3 mL of **distilled or deionized water** to an appropriately labelled clean 20 mL glass vial. Add the processed benthic mat extract from step 6.4 to the vial. Vortex thoroughly.

6.6 Centrifuge for 5 minutes at 9000 rpm.

6.7 Add 1.8 mL of **distilled or deionized water** to a clean, appropriately labelled amber 4 mL vial. Add 0.2 mL of the upper liquid layer of the concentrated sample extract from step 6.6. Vortex thoroughly.

6.8 Analyze benthic mat sample extract (from step 6.7 above) as described in the Assay Procedure section of the ABRAXIS® Cylindrospermopsin, ABRAXIS® Microcystins-ADDA, or ABRAXIS® Microcystin-DM ELISA plate kit user's guides.

7. Evaluation of Results

7.1 Cylindrospermopsin

The Cylindrospermopsin concentration in samples is determined by multiplying the ELISA result by a factor of 100. Sample extracts showing concentrations lower than standard 1 (0.05 ppb) should be reported as < 5 ppb of Cylindrospermopsin. Samples showing a higher concentration than Standard 6 (2 ng/mL) should be reported as containing > 200 ppb of Cylindrospermopsin. If a quantitative result is necessary, samples which exceed the calibration range of the assay must be diluted further using the sample diluent provided in the ELISA kit and re-analyzed to obtain accurate quantitative results.

7.2 Microcystins

The Microcystins concentration in samples is determined by multiplying the ELISA result by a factor of 100. Sample extracts showing concentrations lower than standard 1 (0.150 ppb) should be reported as < 15 ppb of Microcystins. Samples showing a higher concentration than Standard 5 (5 ng/mL) should be reported as containing > 500 ppb of Microcystins. If a quantitative result is necessary, samples which exceed the calibration range of the assay must be diluted further using the sample diluent provided in the ELISA kit and re-analyzed to obtain accurate quantitative results.

8. 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.

9. Assistance

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