**Importance of Anatoxin-a 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. These mats can produce toxins, including Anatoxin-a, which is an alkaloid neurotoxin produced by some species of cyanobacteria (blue-green algae). It is one of the most toxic of the cyanobacterial toxins. In humans and other animals, the skeletal neuromuscular junction constitutes a primary target for Anatoxin-a (Anatoxin-a can also cross the blood-brain barrier). The neuromuscular junction is specialized for the rapid transmission of neuronal information from the pre-synaptic nerve terminal to the post-synaptic muscle fiber. This transmission is mediated by the synchronous release of the neurotransmitter acetylcholine (ACh), which activates nicotinic acetylcholine receptors (nAChRs) in the muscle endplate, triggering a series of events that lead to muscle contraction. Most ACh molecules are hydrolyzed by acetylcholinesterases, which are highly concentrated at the neuromuscular junction. Anatoxin-a functions as an agonist of nAChRs, like ACh, but is about 20 times more potent. Unlike ACh, it is not degraded by acetylcholinesterases and produces sustained depolarization of the muscle endplate, causing overstimulation of the muscles, leading to muscle fatigue and ultimately paralysis. Symptoms begin within 5 minutes of ingestion of Anatoxin-a and progress rapidly, resulting in cyanosis, convulsions, cardiac arrhythmia, and respiratory paralysis, which ultimately results in death due to suffocation.

Humans and other animals may be exposed to Anatoxin-a through contact with or ingestion of contaminated water or benthic mats. Due to the potential for serious harm and even death, many countries are expanding monitoring programs to include Anatoxin-a and are establishing regulations regarding the amount of Anatoxin-a in drinking and recreational waters. New Zealand is among those taking regulatory action, establishing a 6.0 µg/L provisional maximum acceptable value (MAV) for Anatoxin-a, and the U.S. Environmental Protection Agency (EPA) will be announcing drinking and recreational water health advisories.

**Performance Data**

Test sensitivity: The limit of detection (LOD) with the ABRAXIS® Anatoxin-a Strip Test for benthic mats is 40 ppb (ng/g). At this level, the test line exhibits moderate intensity. At levels greater than 250 ppb (ng/g), the test line is not visible. When compared with samples of known Anatoxin-a concentration, it is possible to obtain a semi-quantitative result.
The limit of detection (LOD) with the ABRAXIS® Anatoxin-a ELISA test for benthic mats is 15 ppb (ng/g).

Selectivity: The ABRAXIS® Anatoxin-a test kits exhibit very good cross-reactivity with (+) Anatoxin-a and Homoanatoxin-a.

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

*The monoclonal antibody and conjugate (Patent Application P201531661) included in the ABRAXIS® Anatoxin-a test kits has been licensed from the Spanish National Research Council (CSIC) and the University of Valencia (UVEG).

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# ABRAXIS® Benthic Mat Sample Extraction Kit for Anatoxin-a

**Product No. 529918**

**1. General Description**

The ABRAXIS® Benthic Mat Sample Extraction Kit for Anatoxin-a is for the collection of benthic mat samples and sample preparation/extraction of Anatoxin-a from benthic mat samples prior to testing with the ABRAXIS® Anatoxin-a Strip Test (PN 520042 or 520043). Extracts produced can also be used for confirmatory testing with the ABRAXIS® Anatoxin-a ELISA kit (PN 520060). Each extraction kit provides materials for 20 benthic mat samples.

**2. Safety Instructions**

Anatoxin-a is one of the most toxic of the cyanobacterial toxins. 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 Anatoxin-a 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 Anatoxin-a should be stored between 2-30°C. The extraction solution and 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 test limitations and interferences.
-The ABRAXIS® Benthic Mat Extraction Solution contains the reagents necessary for Anatoxin-a sample preservation, therefore benthic mat sample extracts do **not** require the use of the Preservation Vials included in the Anatoxin-a Strip Test Kit. Do not use Anatoxin-a Strip Test Kit Sample Preservation Vials with benthic mat samples as this **will produce inaccurate results**.
-The Anatoxin-a 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 12 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 in the entire benthic mat. See section C, Sample Collection, for instructions on collecting a representative 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 (step 8 in section D, Extraction Procedure). Do **not** use tap water for sample preparation, as chlorine and other water treatment chemicals present in tap water will degrade Anatoxin-a, causing inaccurate, biased low 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 Anatoxin-a, causing inaccurate, biased low sample results.*
-Reagents and samples should be allowed to reach room temperature before testing.
-Prior to use, ensure that the product has not expired by verifying that the date of use is prior to the expiration date on the label.
-Samples should be protected from exposure to natural and artificial light, as light exposure will degrade Anatoxin-a.

**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. ABRAXIS® Anatoxin-a/Saxitoxin Benthic Mat Sample Extraction Solution (12 mL)
6. 1 mL disposable graduated pipettes (20, bag marked with red sticker to indicate appropriate step for use)
7. 3 mL disposable graduated pipettes (20, bags marked with blue stickers to indicate appropriate step for use)
8. Disposable transfer pipettes (20, bag marked with yellow sticker to indicate appropriate step for use)
9. Dilution Vial A (small glass vial containing 2 mL of extraction solution, 20 vials)
10. Dilution Vial B (large glass vial, 20)
11. 4 mL amber glass Final Sample Extract Vials with caps and labels (20)
12. 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® Anatoxin-a Strip Test, PN 520042 or 520043, or ABRAXIS® Anatoxin-a ELISA PN 520060, for confirmatory analysis

**C. Sample Collection**

1. Using clean scissors, remove pieces of mat material from throughout the benthic mat and place in 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 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 red sticker), add 0.5 mL of Anatoxin-a/Saxitoxin Benthic Mat Extraction Solution to the tube.
5. Using a clean disposable pestle, thoroughly grind the sample and extraction solution for 4 minutes, being careful to avoid causing the sample or extraction solution 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 extraction solution 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 Anatoxin-a, which can cause harm, or cross-contamination of extraction/testing materials that can produce inaccurate results.*
6. Using the same disposable pipette used in step 4, transfer all of the sample extract from step 5 to Dilution Vial A (small dilution vial). Discard pipette after use. Cap vial tightly and shake for 30 seconds to thoroughly mix.
7. Allow to settle for 10 minutes.
8. Using a clean disposable graduated pipette from bag 2 (bag with blue sticker), add 9 mL of **distilled or deionized water** to Dilution Vial B (large dilution vial).
9. Using the same disposable graduated pipette used in step 8, transfer 1 mL of the top of the upper liquid portion of the sample extract from Vial A (small vial) into Vial B (large vial). Discard pipette after use. Cap tightly and shake for 30 seconds to thoroughly mix.
10. Allow sample to settle for 10 minutes.
11. Using a clean transfer pipette from bag 3 (yellow sticker), transfer the top of the upper liquid portion of the extract from Vial B (large vial) into a clean, appropriately labeled amber Final Extract vial.
12. Analyze the diluted benthic mat sample extract from the Final Extract vial (from step 11 above) as described in section E, Test Strip Sample Analysis Procedure. This extract may also be used for confirmatory testing of positive results using the ABRAXIS® Anatoxin-a ELISA kit (PN 520060) if desired. No additional preparation/dilution of the final extract is required prior to ELISA analysis. Final sample results are determined by multiplying the ELISA results by a factor of 100.
    *Note: The Benthic Mat Extraction Solution contains the reagents necessary for Anatoxin-a sample preservation, therefore benthic mat sample extracts from step 11 are ready for testing and do not require the use of the Preservation Vials included in the Anatoxin-a Strip Test kit. (Preservation vials included in the Anatoxin-a Strip Test kit are for use with water samples only.)*

**E. Test Strip Sample Analysis Procedure** (Analysis using ABRAXIS® Anatoxin-a Strip Test, PN 520042 or 520043, not included with Benthic Mat Sample Extraction Kit)

1. Using a new disposable exact volume transfer pipette for each sample, transfer 200 µL of the benthic mat sample extract (from section D, above) to the appropriately labeled conical test vial (see pipette package for usage instructions).
2. 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).
3. Incubate the conical test vial at room temperature for 10 minutes.
4. Insert the test strip (arrows down) into the conical vial.
5. Allow the test to develop for 10 minutes.
6. Remove the test strip. Lay the strip flat and allow to continue developing for 5 minutes.
7. Read the results visually, as explained below in section F, Interpretation of Results.

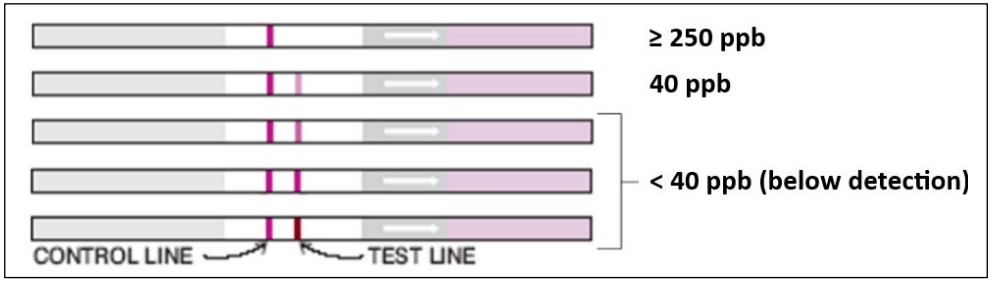
**F. Interpretation of Benthic Mat Sample Results**

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. 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 (< 40 ppb). Test strips with a test line which is lighter than the control line indicates a result which is ≥ 40 ppb. Test strips with no test line visible (only the control line is visible) indicates a result which is ≥ 250 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 | ≥ 250 ppb |
| Control line present | Test line present | Between 0 and <250 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 Anatoxin-a 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 which must be multiplied by 100 to obtain final sample concentrations. For example, the AbraScan reader result of ~0.4 ppb x 100 = ~40 ppb of Anatoxin-a in a benthic mat sample.

**G. Additional Analysis**

If desired, positive samples can be confirmed by ELISA, HPLC, or other conventional methods. These services are available from commercial analytical laboratories.