7. Importance of Microcystins/Nodularins Determination

Toxic cyanobacterial blooms are an emerging issue worldwide due to increased source water nutrient pollution caused by eutrophication. Microcystins and nodularins are cyclic toxin peptides. Microcystins (of which there are many structural variants, or congeners) have been found in fresh water throughout the world. To date, approximately 270 variants of microcystin have been isolated. The most common variant is Microcystin-LR. Other common variants include YR, RR, and LW. These toxins are produced by many types of cyanobacteria (blue-green algae), including *Microcystis, Anabaena, Oscillatoria, Nostoc,* and *Hapalosiphon.* Nodularins are produced by the genus *Nodularia* and are found in marine and brackish water. To protect consumers from adverse health effects caused by these toxins, the World Health Organization (WHO) has proposed a provisional upper limit for Microcystin-LR of 1.0 ppb (µg/L) in drinking water, and the U.S. EPA of 1.6 ppb. 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 occurs most frequently through ingestion of water through drinking or during recreational activities in which water is swallowed. These toxins mediate their toxicity by inhibiting liver function and are potent inhibitors of the serine/threonine protein phosphatases, and therefore may act as tumor promoters. Determining concentration in biological samples including urine and blood can help public health laboratories survey uptake in human and animal populations.

8. References:

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U.S. Environmental Protection Agency; Office of Water. *Cyanobacteria and Cyanotoxins: Information for Drinking Water Systems.* 2019; EPA-810F11001

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The ABRAXIS® Affinity Capture and Extraction (ACE) Kit – Microcystins is intended for research and *in vitro* use only. This product was not tested or certified for diagnostic use.

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ABRAXIS® Affinity Capture and Extraction (ACE) Kit - Microcystins

Immuno-Magnetic Separation Bead Kit for Purification of Microcystins and Nodularins from Biological Matrices

Product Number 520100 (20 purifications)

1. General Description

The **ABRAXIS® ACE Kit - Microcystins** is an Immuno-Magnetic Separation (IMS) bead-based extraction kit for the selective capture of microcystins and nodularins toxins (ADDA region) from urine and blood serum in advance of a compatible downstream detection assay. Anti-ADDA monoclonal antibody-coupled superparamagnetic beads efficiently bind to the ADDA region of microcystins and nodularins toxins in the sample, resulting in a high sensitivity capture with minimal carryover of other matrix components which might otherwise cause downstream assay interference. It is possible to use this kit for other matrices; however it must be validated prior to use. The kit is intended for manual or automated extractions using a magnetic separator or automated magnetic separation instrument.

2. Safety Instructions

Use appropriate protective equipment (including but not limited to gloves, lab coats, and safety glasses) when collecting biological samples and using the kit. Urine and blood serum samples may contain biohazards, and must be disposed of properly. The kit contains acetonitrile and formic acid, which can be be irritating or toxic to the eyes, skin, and respiratory system. Preparation of the Elution Buffer with these chemicals must be in a biological/chemical safety cabinet or fume hood. Refer to Safety Data Sheet for further information.

3. Storage and Stability

Upon delivery of the kit, store at 2-8°C. **Do not freeze the magnetic beads solution**. Do not use after the printed expiration date.

4. Kit Principle

The **ABRAXIS® ACE Kit - Microcystins** uses a simple, efficient, biotinylated antibody/streptavidin magnetic bead-based procedure for microcystins and nodularins toxin extraction, as illustrated below in **Figure 1**:

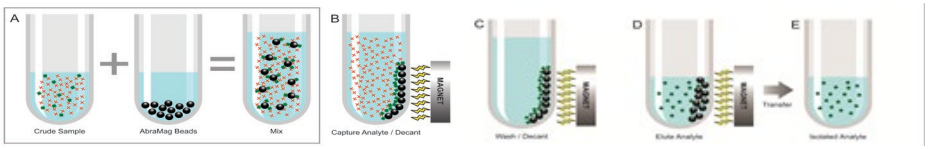

Figure 1. Schematic of the **ABRAXIS® ACE Kit - Microcystins** process.

A. **Binding:** The biological sample is added to the biotinylated, monoclonal, anti-ADDA antibody/streptavidin magnetic bead complex, which captures microcystins and nodularins toxins from the sample.

B. **Isolation:** A magnet is used to collect the beads, with toxins attached.

C. **Washing:** Remaining sample matrix is removed in a wash step.

D. **Elution:** Microcystins and nodularins toxins are then eluted and transferred to a new tube.

E. **Downstream Applications:** Microcystins and nodularins toxins extracted from the sample may then be used in a corresponding downstream detection assay.

5. Limitations and Precautions

Due to the high variability of compounds that may be found in unique biological samples, specifically urine and blood serum, test interferences caused by matrix effects cannot be completely excluded. Mistakes in handling the samples and/or kit may cause errors. Possible sources for such errors include: inadequate storage conditions of the samples and/or test kit, incorrect pipetting sequence and/or inaccurate volumes of the reagents, too long or too short incubation times during the immunocapture and elution steps, and extreme temperatures during the test performance (lower than 10°C or higher than 30°C). The kit procedure should be performed away from direct sunlight.

6. Working Instructions

A. **Materials Provided**

1. AbraMag® ACE anti-Microcystins Magnetic Beads, 0.6 mL
2. ACE Bead Wash Buffer, 2 mL
3. ACE Sample Wash Buffer (DI-water with preservative), 15 mL
4. ACE Acetonitrile Elution Buffer, 2 x 1 mL
5. ACE Formic Acid, 1 mL
6. ACE Neutralization Buffer, 1 mL
7. Protein LoBind® Tubes, quantity of 42 in re-sealable bag

B. **Additional Materials and Equipment Required** (not included with the kit)

1. Matrix-matched standard curve set for biological sample of interest – Urine (Uri-Standards Set - Microcystins, GSD PN 520101) or Blood Serum (Seri-Standards Set - Microcystins, GSD PN 520102)
2. Materials for appropriate detection assay/technique, e.g. Microcystins/Nodularins PP2A kit (GSD PN 520032), ABRAXIS® Microcystins-ADDA ELISA microtiter plate kit (GSD PN 520011), or LC-MS/MS
3. Microcentrifuge tube rack
4. Magnetic microcentrifuge tube separator, such as Multi-6 (GSD PN 472260), Multi-32 (GSD PN 472287) or equivalent
5. Micro-pipettes with disposable plastic tips (GSD PN 704072, 704044, 704054) or equivalent
6. 4°C refrigerator
7. -20°C freezer
8. Biological/chemical safety cabinet or fume hood
9. Vortex, GSD PN 709045 or equivalent
10. Microcentrifuge capable of 2,000 x g, GSD PN 709068 or equivalent
11. Microcentrifuge vial mixer capable of 1400 rpm (*for detection with Microcystins/Nodularins PP2A kit or LC-MS)* or Heated microcentrifuge vial mixer (thermomixer) capable of 80°C (*for detection with ABRAXIS® Microcystins-ADDA ELISA microtiter plate kit*), Eppendorf® 5350 or equivalent
    Note: If vial mixer is not available, contact GSD for alternate vortex-only protocol

C. **Sample Collection and Preparation**

See appropriate GSD Standard Set User Guide for biological sample collection and preparation requirements for respective sample types.

D. **Kit Reagent Preparation**

1. Allow kit reagents to come to room temperature before use.
2. If the downstream detection method selected is the PP2A kit or LC-MS, the ACE Acetonitrile Elution Buffer will be used. This buffer must be prepared with fresh Formic Acid immediately before use as follows:

    In a fume hood or biological/chemical safety cabinet, add 5 µL Formic Acid to one vial of Elution Buffer, and mark the bottle label to indicate it has been added. Two vials of Elution Buffer are included for convenience. Once Formic Acid is added, the elution buffer must be used within one hour.

E. **Kit Procedure**

Note: See the appropriate GSD Standard Set instructions for preparing biological samples and simultaneously extracting the matrix-matched standard curve (required). It is essential to use the included Protein LoBind tubes for all steps. Do not substitute!

1. Prepare **Anti-Microcystins Magnetic Beads** by gently shaking or rotating the vial until beads are fully re-suspended, then measure 25 µL of bead solution per sample/standard into an included Protein LoBind tube. To run all 20 tests (13 samples and 7 standard/control points), the vial should contain 500 µL (25 µL x 20) of bead solution. To account for any pipetting loss, it is recommended to calculate for 1 extra sample.
2. Place the tube with the bead solution on the magnetic separator until the supernatant has cleared, ~ 1 minute. Leaving the tube on the separator, use a pipette to remove and discard the supernatant, without disturbing the beads that have collected at the magnet.
3. Re-suspend the resulting pellet with 75 µL of **Bead Wash Buffer** per sample/standard, or 1.5 mL (75 µL x 20) for all 20 tests. Pipette up and down or vortex until homogenized.
4. Place the tube on the magnetic separator until the supernatant has cleared, ~ 1 minute. Leaving the tube on the separator, remove and discard the supernatant using a pipette, without disturbing the beads that have collected at the magnet.
5. Re-suspend the pellet with 25 µL of **Bead Wash Buffer** per sample/standard, or 500 µL (25 µL x 20) for all 20 tests**.** Pipette up and down or vortex until homogenized. For 20 tests, distribute the 500 µL pool into 25 µL beads per sample in 20 Protein LoBind tubes.
6. Add 1 mL of the first biological sample or standard into the first tube containing the re-suspended beads. Proceed with remaining samples/standards, add to a new tube of re-suspended beads. Vortex gently.
7. Place the tubes containing beads and sample/standard in a mixer or thermomixer and mix for 5 minutes at 1400 rpm at room temperature.
8. Pulse spin the tubes in a microcentrifuge for ~1 second to pull down any liquid in the cap. Place the tubes on the magnetic separator until the supernatant has cleared, 2-3 minutes. Leaving the tubes on the separator, use a pipette to remove and discard the supernatant, without disturbing the beads that have collected at the magnet. When opening cap lids, use caution so as not to create aspirates. Use a different pipette tip with each standard/sample.
9. Add 500 µL of **Sample Wash Buffer** and vortex gently to mix.
10. Pulse spin the tubes in a microcentrifuge for ~1 second to pull down any liquid in the cap. Place the tubes on the magnetic separator until supernatant has cleared, 2-3 minutes. Leaving the tubes on the separator, use a pipette to remove and discard the supernatant, without disturbing the beads that have collected at the magnet. If necessary, use a smaller size pipette tip to remove all remaining liquid. Use a different pipette tip with each standard/sample.
11. Add eluting buffer depending on downstream assay. Vortex gently to mix.
    - *Microcystin PP2A, LC-MS*: Add 43 µL of **Elution Buffer** to each tube, prepared as directed in step D.2
    - *Microcystin ADDA ELISA*: Add 110 µL of **Sample Wash Buffer** (DI-water) to each tube
12. Place the tubes in a mixer or thermomixer and mix for 5 minutes at 1400 rpm:
    - *Microcystin PP2A, LC-MS (Elution Buffer)*: Mix at room temperature
    - *Microcystin ADDA ELISA (Sample Wash Buffer)*: Mix at **80°C**
13. Pulse spin the tubes in a microcentrifuge for ~1 second to pull down any liquid in the tube cap or sides. Place the tubes on the magnetic separator until the supernatant has cleared, 2-3 minutes. Transfer each standard/sample elution supernatant to a new Protein LoBind tube. Discard bead tube.
14. Post-elution modification:
    - *Microcystin PP2A (Elution Buffer)*: Add 7 µL of **Neutralization Buffer** to each tube and vortex gently to mix. Add 60 µL of **Sample Wash Buffer** (DI-water) and vortex gently to mix. Final volume is 110 µL.
    - *Microcystin ADDA ELISA (Sample Wash Buffer)*: None. Final volume is 110 µL.
    - *LC-MS (Elution Buffer)*: None. Final volume is 43 µL.
15. The standards and sample(s) are ready for the downstream detection assay (or store at 2-8°C; recommend to use within 24 hours). When using the Microcystins/Nodularins PP2A kit or ABRAXIS® Microcystins-ADDA ELISA microtiter plate kit, **DO NOT USE the standards/control that come with the kit! REPLACE standards/control with the extracted matrix-matched standard curve and control.**