

ABRAXIS® Affinity Capture and Extraction (ACE) – Microcystins Kit Urine and Blood Serum Sample Collection Recommendations

1. Intended Use

To provide customers with best practice recommendations regarding collection, storage, and shipping of urine and blood serum biological samples to minimize degradation and loss of microcystins prior to being processed with the ABRAXIS® Affinity Capture and Extraction (ACE) – Microcystins Kit (PN 520100) and either the ABRAXIS® Uri-Standards Set – Microcystins (PN 520101) or ABRAXIS® Seri-Standards Set – Microcystins (PN 520102).

2. Safety Instructions

Urine and blood serum samples may contain biohazards. Use appropriate protective equipment (including but not limited to gloves, lab coats, and safety glasses) when working with pre- and post-extracted samples. Collect, ship, store, and discard pre- and post-extracted samples according to local, state, and federal regulations.

3. Limitations, Possible Interferences, and Precautions

Microcystins in biological samples may be degraded or lost due to storage in certain plastics where the microcystins adsorb to the container walls. Likewise, freeze/thaw and changes in temperature may cause loss or degradation. Follow the recommendations in this guideline for best practices to minimize these types of losses. Comply with all local, state, and federal regulations and privacy protocols regarding individually identifiable biological samples.

4. Collection Container Recommendations

Microcystins and Nodularins peptides are known to adsorb to certain types of plastic used in collection containers, thereby removing the toxins from the sample matrix, leading to lower recoveries or even false negatives. Different congeners may have different rates of adsorption loss. The best storage container for preserving microcystins in urine or blood serum is glass, however this may be impractical. Gold Standard Diagnostics and other groups have published data on microcystins adsorption to various plastic types, and indicated that PET/PETG (Polyethylene terephthalate/glycol) is an acceptable alternative to glass^{1,2}. Other types of plastic should not be used, or if they must be used, proceed <u>immediately</u> to freezing the sample (Section 5) to minimize toxin loss via plastic adsorption onto the collection container sides. For blood serum, proceed immediately to freezing after separation of the serum from whole blood. Collection containers should be sterile^{3,4}.

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5. Storage Recommendations

After collection of the urine or blood serum sample, freeze the sample and container at or below 0°F (-18°C), which is the standard freezer temperature. According to Center for Disease Control (CDC) publications, if a deep freezer (-80°C) is available, this should be used^{3,4}. Avoid freeze/thaw of samples by aliquoting prior to freezing, if necessary, as repeated freeze/thaw cycles will degrade any microcystin or nodularins that may be present in the biological sample.

6. Shipping Recommendations

If the biological sample is to be shipped, keep frozen until shipping, and ship on dry ice in an insulated container such as Styrofoam to maintain the sample in a frozen state until arrival.

7. Preparation for ACE Kit Protocol

Keep the biological sample frozen until ready to begin the ACE kit protocol, at which point thaw the sample or aliquot at room temperature. Following the detailed instructions in the Standard Sets User Guides section 6.C.2, the sample will be immediately transferred into the Eppendorf Protein LoBind[®] Tubes that are provided. These tubes have been successfully evaluated for minimal microcystins adsorption loss by Gold Standard Diagnostics and other groups^{3,4,5}. Continue to follow the Standards Sets and ACE Kit User Guides for the remaining procedure.

8. References

1. Kamp L, Church JL, Carpino J, Faltin-Mara E, Rubio F. The Effects of Water Sample Treatment, Preparation, and Storage Prior to Cyanotoxin Analysis for Cylindrospermopsin, Microcystin and Saxitoxin. *Chem Biol Interact* 2016, 246:45-51. <u>https://doi.org/10.1016/j.cbi.2015.12.016</u>

2. Seo C, Lee JW, Jung W-K, Lee Y-M, Lee S, Lee SG. Examination of Microcystin Adsorption by the Type of Plastic Materials Used During the Procedure of Microcystin Analysis. *Toxins* 2022, 14, 625. <u>https://doi.org/10.3390/toxins14090625</u>

3. Wharton RE, Cunningham BR, Schaefer AM, Guldberg SM, Hamelin EI, Johnson RC. Measurement of Microcystin and Nodularin Activity in Human Urine by Immunocapture-Protein Phosphatase 2A Assay. *Toxins* 2019, 11, 729. <u>https://doi.org/10.3390/toxins11120729</u>

4. Cunningham BR, Wharton RE, Lee C, Mojica MA, Krajewski LC, Gordon SC, Schaefer AM, Johnson RC, Hamelin EI. Measurement of Microcysint Activity in Human Plasma Using Immunocapture and Protein Phosphatase Inhibition Assay. *Toxins* 2022, 14, 813. <u>https://doi.org/10.3390/toxins14110813</u>

5. Heussner AH, Altaner S, Kamp L, Rubio F, Dietrich D. Pitfalls in Microcystin Extraction and Recovery from Human Blood Serum. *Chem Biol Interact* 2014, 223, 87-94. <u>https://doi.org/10.1016/j.cbi.2014.08.010</u>

9. For ordering or technical assistance contact:

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Date this Technical Bulletin is effective: 02/07/2025

Version: 01