8. References:

Cunningham, B.R; Wharton, R.E; Lee, C; Mojica, M.A; Krajewski, L.C; Gordon, S.C; Schaefer, A.M; Johnson, R.C; Hamelin, E.I. *Measurement of Microcystin Activity in Human Plasma Using Immunocapture and Protein Phosphatase Inhibition Assay*. Toxins 2022 (14) 813; doi.org/10.3390/toxins14110813

Heussner, A.H; Altaner, S; Kamp, L; Rubio, F; Dietrich, D. Pitfalls in Microcystins Extraction and Recovery in Human Blood Serum. Chemico-Biological Interactions 2014 (223) 87-94; doi.org/10.1016/ j.cbi. 2014.08.010

Kamp, L; Church, J; 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*. Chemico-Biological Interactions 2016 (246) 45-51; doi:10.1016/j.cbi.2015.12.016

The ABRAXIS® Seri-Standards Set – ACE 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® Seri-Standards Set – ACE Microcystins

Blood Serum Standard Curve

Product Number 520102

1. General Description

The **ABRAXIS® Seri-Standards Set - ACE Microcystins** is a standard set designed for use with the **ABRAXIS® Affinity Capture and Extraction (ACE) Kit - Microcystins**, a bead-based extraction kit for the capture and extraction of microcystins and nodularins toxins (ADDA region) from biological matrices in advance of analysis using an appropriate detection assay or technique.

2. Safety Instructions

Use appropriate protective equipment (including but not limited to gloves, lab coats, and safety glasses) when collecting and working with biological samples. Blood serum samples may contain biohazards, and must be collected, transported, stored, and disposed of properly. The standard set contains no biological material and uses a synthetic blood serum substitute. Refer to Safety Data Sheet for further information.

3. Storage and Stability

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

4. Test Principle

This Seri-Standards Set – ACE Microcystins consists of a matrix-matched Microcystin-LR standard curve re-suspended in a synthetic blood serum substitute. When extracted alongside unknown samples, the standards allow concentrations of unknown samples to be interpolated and calculated.

5. Limitations and Precautions

Due to the high variability of compounds that may be found in individual blood serum samples, test interferences caused by matrix effects cannot be completely excluded. Mistakes in handling the samples and/or standards may cause errors. Possible sources for such errors include inadequate storage conditions and incorrect pipetting in preparation of standards. It should be noted that different microcystin congeners may also degrade at different rates.

6. Working Instructions

A. **Materials Provided**

1. ACE Seri-Standard Sample Diluent/Zero Standard, 45 mL
2. Protein LoBind® Tubes, quantity of 30 in re-sealable bag
3. ACE Microcystins Standard 0, 0.000 ppb
4. ACE Microcystins Standard 1, 0.025 ppb
5. ACE Microcystins Standard 2, 0.050 ppb
6. ACE Microcystins Standard 3, 0.100 ppb
7. ACE Microcystins Standard 4, 0.200 ppb
8. ACE Microcystins Standard 5, 0.400 ppb
9. ACE Microcystins Control, 0.075 ± 0.0185 ppb

Standard and Control vials supplied lyophilized, 1 mL/vial after reconstitution
Note: Vials are vacuum-sealed, open carefully to not disturb dried standard

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

1. ABRAXIS® Affinity Capture and Extraction (ACE) Kit – Microcystins (GSD PN 520100)
2. Materials for appropriate detection assay/technique, e.g. Microcystins/Nodularins PP2A Kit (GSD PN 520032), ABRAXIS® Microcystins-ADDA ELISA Kit (GSD PN 520011), or LC-MS/MS
3. Centrifuge or microcentrifuge capable of 10,000 x *g,* GSD PN 709068 or equivalent
4. Vortex, GSD PN 709045 or equivalent
5. 2-8°C refrigerator
6. -20°C freezer
7. Glass vials *(optional, see Note on Blood Serum Sample Collection and Preparation)*

C. **Instructions**

1. Add 1 mL of room temperature ACE Seri-Standard Sample Diluent/Zero Standard to each ACE Microcystins Standard vial and Control vial. Vortex ~ 5 seconds to mix well. Visually confirm that all material has been re-suspended. Re-hydrated standards must be used within 8 hours of preparation.
2. Follow instructions in the **ABRAXIS® ACE Kit - Microcystins** User Guide to extract each matrix-matched standard curve point and control alongside unknown blood serum samples.

   Note on Blood Serum Sample Collection and Preparation
   Microcystins and nodularins peptides bind quickly and readily to most plastics. Correct blood serum sample collection and handling is paramount for accurate concentration determination. After separation of blood serum from whole blood, serum samples must be frozen immediately and stored frozen (-20°C) until just prior to extraction – including shipping frozen on dry ice, if required. Avoid freeze/thaw of samples by aliquoting prior to freezing if necessary. Different microcystin congeners may degrade at different rates during collection and freeze/thaw.

   Immediately prior to testing and use of the ABRAXIS® ACE Kit - Microcystins, thaw serum sample(s) and transfer 1.4 mL to a Protein LoBind® tube. Use only Protein LoBind® tubes included with this standard set, do not substitute! In a centrifuge or microcentrifuge, spin at 10,000 x *g* for 5 minutes to separate any precipitates. Transfer 0.5 mL of the serum supernatant (clear liquid portion) to a new Protein LoBind® tube or glass vial and discard any precipitate or flocculant remainder. Add 1.0 mL ACE Seri-Standard Sample Diluent/Zero Standard and vortex well, resulting in a 1:3 dilution. *Magnetic beads must be able to move through serum easily and be visible against the magnet.* If diluted serum is still too thick to easily pipet, additional ACE Seri-Standard Sample Diluent/Zero Standard may be added. Account for the dilution factor in the final concentration by multiplying the calculated concentration by 3 (or final dilution factor, if more Seri-Standard has been added).
3. If using the Microcystins/Nodularins PP2A kit (GSD PN 520032) or ABRAXIS® Microcystins-ADDA ELISA microtiter plate kit (GSD PN 520011) for downstream analysis, substitute extracted Seri-Standards and Control in place of the standard curve included in the assay kit. Customers planning to run ABRAXIS® Microcystins-ADDA ELISA automated on the CAAS Cube should contact GSD Horsham Technical Support at support.abraxis@us.goldstandarddiagnostics.com for the appropriate Technical Bulletin.
   DO NOT USE the standards/control that come with the assay kit! REPLACE standards/control with the extracted Seri-Standard Curve and Control from this matrix-matched standard set.

7. Evaluation of Blood Serum Samples Using Microcystins/Nodularins PP2A Kit (GSD PN 520032) or ABRAXIS® Microcystins-ADDA ELISA Kit (GSD PN 520011)

A. **Working Scheme**

Std 0 – Std 5: ACE Microcystins Seri-Standards
Control: ACE Microcystins Seri-Control
Samp 1, Samp 2, etc: Blood Serum Samples

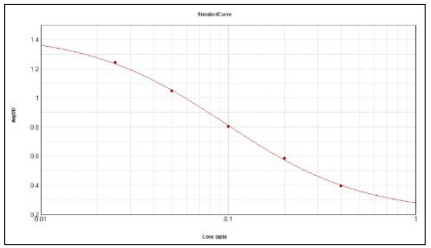
	1	2	3	4	5	6	7	8	9	10	11	12
A	Std 0	Std 4	Samp 2									
B	Std 0	Std 4	Samp 2									
C	Std 1	Std 5	etc.									
D	Std 1	Std 5	etc.									
E	Std 2	Control										
F	Std 2	Control										
G	Std 3	Samp 1										
H	Std 3	Samp 1										

B. **Evaluation of Downstream Assay**
Using a plate reader, read the microtiter plate at the wavelength specified in the downstream assay User Guide. The evaluation of the absorbance readings can be performed using commercial ELISA evaluation programs using 4-Parameter Logistic function to calculate the standard curve. Results can also be determined using a spreadsheet macro available from Gold Standards Diagnostics upon request.

The concentrations of unknown blood serum samples are interpolated and calculated against the extracted Seri-Standard Curve run with each test. The 1:3 dilution (or other dilution factor) from step C.2 must be factored into the final calculation by multiplying the concentration by 3 (or by other dilution factor). Samples prepared using the ABRAXIS® ACE Kit – Microcystins showing a lower concentration of microcystins than Seri-Standard 1 (0.025 ppb) should be reported as containing < 0.075 ppb of microcystins (with dilution factor of 3). Samples showing a higher concentration than Seri-Standard 5 (0.40 ppb) should be reported as containing > 1.2 ppb of microcystins (with dilution factor of 3), or may be diluted in a Protein LoBind® tube or glass vial with the ACE Seri-Standard Sample Diluent/Zero Standard to obtain results that measure within the standard curve. Determinations closer to the middle of the standard curve give the most accurate results. The concentration of the Seri-Standard Control provided should be 0.075 ± 0.0185 ppb.

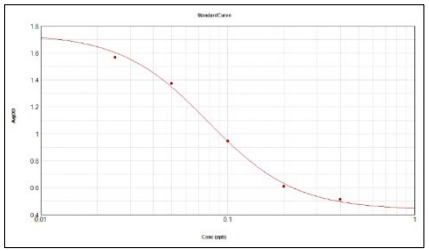
Semi-quantitative results can be derived by simple comparison of the sample absorbances to the absorbances of the standards. Samples with lower absorbances than a standard will have concentrations of microcystins greater than that standard. Samples which have higher absorbances than a standard will have concentrations of microcystins less than that standard.

C. **Seri-Standard Curve in Microcystins-ADDA ELISA Assay**



For demonstration purposes only. Not for use in sample interpretation.

D. **Seri-Standard Curve in Microcystins/Nodularins PP2A Assay**



For demonstration purposes only. Not for use in sample interpretation.

Test Sensitivity: The Limit of Detection (LOD) for this standard set, based on MC-LR, is 0.025 ppb.
When diluted 1:3, the LOD for the sample is 0.075 ppb.

Test Reproducibility: Coefficients of variation (CVs) for standards: < 10%; for samples: < 15%.

Selectivity: The assay exhibits cross-reactivity with all cyanobacterial cyclic peptide toxin congeners tested to date (This includes MC-HiLR, MC-LA, MC-LR, MC-LW, MC-YR).