

8. References:

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- 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. Chemo-Biological Interactions* 2016 (246) 45-51; doi:10.1016/j.cbi.2015.12.016

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ABRAXIS® Uri-Standards Set – ACE Microcystins

Urine Standard Curve

Product Number 520101

1. General Description

The **ABRAXIS® Uri-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 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. Urine 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 urine 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 Uri-Standards Set – ACE Microcystins consists of a matrix-matched Microcystin-LR standard curve re-suspended in a synthetic urine 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 urine 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 Uri-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 kit)

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 2,000 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 Evaluation of Downstream Assay)

C. Instructions

1. Add 1 mL of room temperature ACE Uri-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 urine samples.

Note on Urine Sample Collection and Preparation

Microcystins and nodularins peptides bind quickly and readily to most plastics. Correct urine sample collection and handling is paramount for accurate concentration determination. Urine 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 urine 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 2,000 x g for 5 minutes to pellet any precipitates. Transfer the urine supernatant to a new Protein LoBind® tube and discard the pellet, if present.

3. If using the Microcystins/Nodularins PP2A Kit (GSD PN 520032) or ABRAXIS® Microcystins-ADDA ELISA Kit (GSD PN 520011) for downstream analysis, substitute extracted Uri-Standards and Control in place of the standard curve included in the assay kit. **Customers planning to run ABRAXIS® Microcystins-ADDA ELISA Kit 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 Uri-Standard Curve and Control from this matrix-matched standard set.**

7. Evaluation of Urine 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 Uri-Standards
 Control: ACE Microcystins Uri-Control
 Samp 1, Samp 2, etc: Urine 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										

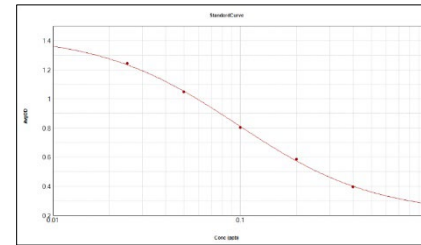
B. Evaluation of Downstream Assay

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 urine samples are interpolated and calculated against the extracted Uri-Standard Curve run with each test. Samples prepared using the ABRAXIS® ACE Kit - Microcystins showing a lower concentration of microcystins than Uri-Standard 1 should be reported as containing < 0.025 ppb of microcystins. Samples showing a higher concentration than Uri-Standard 5 should be reported as containing > 0.40 ppb of microcystins, or may be diluted in a Protein LoBind® tube or glass vial with the ACE Uri-Standard Sample Diluent/Zero Standard and re-tested to obtain results that measure within the standard curve. Any dilution must be factored into the final calculation. Determinations closer to the middle of the standard curve give the most accurate results. The concentration of the Uri-Standard Control provided must be 0.075 ± 0.0185 ppb.

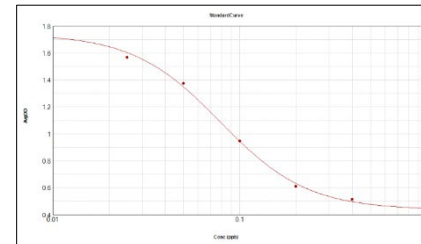
Semi-quantitative results can be derived by simple comparison of the sample absorbances to the absorbances of the standards. Samples with lower absorbances than a standard will have concentrations of microcystins greater than that standard. Samples which have higher absorbances than a standard will have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

C. Uri-Standard Curve in Microcystins-ADDA ELISA Assay



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

D. Uri-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.

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).