

## LITTOISTENJÄRVEN seuranta sinilevämyrkykjen suhteen

Date of analysis: 22.10.2020

Sample collection, immunoassay, data analysis and report by SULTANA AKTER

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Assay method reference:

Sultana Akter, Markus Vehniäinen, Lisa Spoo, Sonja Nybom, Jussi Meriluoto, and Urpo Lamminmäki. Broad-spectrum noncompetitive immunocomplex immunoassay for cyanobacterial peptide hepatotoxins (microcystins and nodularins), Analytical Chemistry, 2016, 88, 10080–10087. (PMID:27657987)

Assay method: Immunoassay based on Akter et al., 2016 with slight modification

1. Prewash streptavidin coated strips (yellow, normal, Lot KG1891).
2. Add blank (reagent water), MC-LR standard or sample, 50 µL/well as Triplicate.
3. Add Reagent Mixture, 50 µL/well
4. Incubate with slow shaking for 1 hour at RT.
5. Wash 4 x.
6. Add Enhancement solution 200 µL per well. Use the Plate Dispenser.
7. Incubate with slow shaking for 10 min at RT.
8. Measure the Time resolved fluorescence (TRF) signal with Plate fluorometer.
9. Resolve standard curve with Origin 2016 and logistic fit.

microcystin-LR (MC-LR) standard

MC-LR (Enzo Life sciences, ALX350-431)

Prepared original stock of 1000 µg/L in reagent water+5% Methanol. Stored at (-20C)

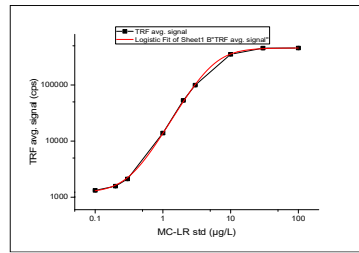
30.9.2019SA: Further working standard solution in reagent water: 100, 30, 10, 3, 2, 1, 0.3, 0.2 and 0.1 µg/L

Reagent mixture in assay buffer

1 µg/mL biotinylated anti-ADDA Antibody (stock 256 µg/ml); +

1 µg/mL anti-immunocomplex scFv-AP (stock 440 µg/ml) +

0.5 µg/mL N1-Eu-anti AP pAb (stock 200 µg/ml, 16.1.2020).



standard curve of microcystin-LR

(x)	TRF signal (counts per second)			(y)		
MC-LR (µg/L) std				avg sig	std dev	cv%
0	1502	998	1086	1118	130	11.6
0	1050	958	998			
0	1054	1076	1012			
0	1224	1196	1030			
0	1146	1134	1110			
0	1302	1140	1104			
0.1	1308	1342	2136	1325	24	1.8
0.2	1510	1561	1654	1575	73	4.6
0.3	2145	2025	2183	2118	82	3.9
1	13582	13413	14738	13911	721	5.2
2	53749	51920	53721	53130	1048	2.0
3	99573	97873	100840	99429	1489	1.5
10	346077	338271	375142	353163	19430	5.5
30	473409	438685	451134	454409	17592	3.9
100	449968	473804	444971	456248	15408	3.4

sample	TRF signal				(y)	*(x) From origin			DF	1x conc (µg/L)	reported conc (µg/L)
	A	B	C	Avg	sig comments	std dev	cv%	conc µg/L			
A_Saarten taus	1248	1176	1244	1223	below blk+3SD	40	3.3	--	1	#VALUE!	<0.2
B_Koilliselkä	1318	1228	1166	1237	below blk+3SD	76	6.2	--	1	#VALUE!	<0.2
C_Luoteiselkä	1276	1170	1236	1227	below blk+3SD	54	4.4	--	1	#VALUE!	<0.2
A'_Hiekkaranta	1230	1254	1398	1294	below blk+3SD	91	7.0	--	1	#VALUE!	<0.2
B'_Pirtan Laituri(1), near Littoistenjärvi	1234	1282	1437	1318	below blk+3SD	106	8.1	0.11	1	0.11	<0.2
C'_Bussilaituri(2) Rauhaniemi, bus stop	1827	1241	1242	1437	below blk+3SD	338	23.5	0.15	1	0.15	<0.2
D'_Ristikallion Uimaranta	1305	1341	1312	1319	below blk+3SD	19	1.4	0.11	1	0.11	<0.2
E'_Kuoviluoto	1476	1208	1230	1305	below blk+3SD	149	11.4	--	1	#VALUE!	<0.2
F'_Rantapolun laituri(3)	1256	1216	1190	1221	below blk+3SD	33	2.7	--	1	#VALUE!	<0.2

DL based on (blk+3SD) sig	1506	0.17	µg/L
DL based on true standard above (blk+3SD) signal	1575	0.2	µg/L

### Interpretation (22.10.2020 SA)

Raw water samples were analyzed fresh on 22.10.2020.

Before analysis, samples were heated at 80 °C for 10 min to release cell bound toxins if any.

Hence, the results represent the total peptide hepatotoxin amount (already released toxin in water and the cell bound toxin).

The immunoassay (Akter et al., 2016) detects cyanobacterial peptide hepatotoxins (eg microcystins).

For quantification, microcystin-LR was used as standard.

### Result:

In Littoistenjärvi water sample of 22.10.2020, the detected cyanobacterial peptide hepatotoxin concentrations (µg/L) (free and cell bound) were shown below from the following samples:

A\_Saarten taus: <0.2 µg/L

B\_Koilliselkä: <0.2 µg/L

C\_Luoteiselkä: <0.2 µg/L

A'\_Hiekkaranta: <0.2 µg/L

B'\_Pirtanlaituri, near Littoistenjärventie 109: <0.2 µg/L

C'\_Bussilaituri, Rauhaniemi, bus stop 6378 : <0.2 µg/L

D'\_Ristikallion Uimaranta: <0.2 µg/L

E'\_Kuoviluoto: <0.2 µg/L

F'\_Rantapolun laituri: <0.2 µg/L

