

# LITTOISTENJÄRVEN seuranta sinilevämyrkykjen suhteen

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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 KG1739).
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)

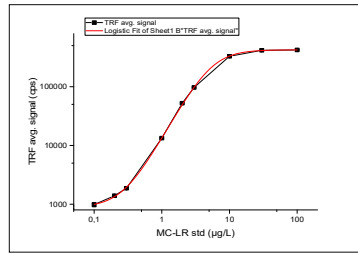
30.9.2019SA: Further working standard solution in reagent water: 100, 30, 10, 3, 2, 1, 0.3, 0.2 and 0.1 µg/L

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)		
	A	B	C	avg sig	std dev	cv%
MC-LR (µg/L) std	0	0	0			
	0	886	928	891		
	0	916	871	870		
	0	890	882	860	888	22
						2.5
	0.1	1062	904	988	985	79
	0.2	1441	1406	1337	1395	53
	0.3	1815	1930	1858	1868	58
	1	13586	13163	13194	13314	236
	2	52217	51090	53761	52356	1341
	3	96511	96043	98835	97130	1495
	10	327939	331979	329122	329680	2077
	30	415678	419225	414585	416496	2426
	100	422284	420723	423808	422272	1543

sample	TRF signal			(y)	sig	comments	std dev	cv%	*(x) From origin		DF	1x conc (µg/L)	reported conc (µg/L)
	A	B	C						conc µg/L	DF			
A_Saarten taus	1	A	1024	1005	996	1008	close to blk+3SD	14	1.4	0.11	1	0.11	<0.2
B_Koilliselkä	2	B	1100	1116	1008	1075	close to blk+3SD	58	5.4	0.13	1	0.13	<0.2
C_Luoteisselkä	3	C	1118	1069	991	1059	close to blk+3SD	64	6.0	0.13	1	0.13	<0.2
A'_Hiekkaranta	4	A'	1002	1086	1160	1083	close to blk+3SD	79	7.3	0.13	1	0.13	<0.2
B'_Pirtan Laituri(1), near Littoistenjärvi	5	B'	1287	1096	1045	1143	close to blk+3SD	128	11.2	0.15	1	0.15	<0.2
C'_Bussilaituri(2) Rauhaniemi, bus stop	6	C'	1127	1097	1064	1096	close to blk+3SD	32	2.9	0.14	1	0.14	<0.2
D'_Ristikallion Uimaranta	7	D'	1096	1056	1206	1119	close to blk+3SD	78	6.9	0.14	1	0.14	<0.2
E'_Kuoviluo	8	E'	955	908	912	925	below blk+3SD	26	2.8	--	1	#VALUE!	<0.2
F'_Rantapolun laituri(3)	9	F'	850	895	934	893	below blk+3SD	42	4.7	--	1	#VALUE!	<0.2

DL based on(blk+3SD) sig	954	--	µg/L
DL based on true standard above (blk+3SD) signal	1395	0.2	µg/L

## Interpretation (20.8.2020 SA)

Raw water samples were analyzed fresh on 20.8.2020.

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, the detected cyanobacterial peptide hepatotoxin concentrations (µg/L) (free and cell bound) were shown below from the following samples:

- 20.8.2020 A\_Saarten taus: <0.2 µg/L
- 20.8.2020 B\_Koilliselkä: <0.2 µg/L
- 20.8.2020 C\_Luoteisselkä: <0.2 µg/L

- 20.8.2020 A'\_Hiekkaranta: <0.2 µg/L
- 20.8.2020 B'\_Pirtanlaituri, near Littoistenjärvi: <0.2 µg/L
- 20.8.2020 C'\_Bussilaituri, Rauhaniemi, bus stop 6378: <0.2 µg/L

- 20.8.2020 D'\_Ristikallion Uimaranta: <0.2 µg/L
- 20.8.2020 E'\_Kuoviluo: <0.2 µg/L
- 20.8.2020 F'\_Rantapolun laituri: <0.2 µg/L

