

## LITTOISTENJÄRVEN seuranta sinilevämyrkköjen suhteen

Date of analys 21.7.2022

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

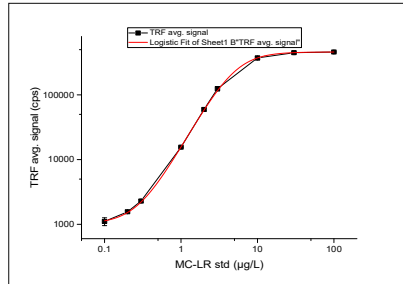
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Assay method reference:

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 with some modification based on concept of Akter et al., 2016

1. Prewash streptavidin coated strips (yellow,41-07 TV, KG 2109).
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.



standard curve of microcystin-LR

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)

9.6.2022: 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 242 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).

(x)	TRF signal (counts per second)			(y)				
MC-LR (µg/L)	A	B	C	avg sig	std dev	cv%		blk+3SD (9 blank)
0	984	1030	950	991	45	4.6		1127
0	956	973	1088					
0	1005	945	988					
0.1	1285	1022	1018	1108	153	13.8		
0.2	1640	1521	1528	1563	67	4.3		
0.3	2356	2137	2375	2289	132	5.8		
1	15522	15325	15849	15565	265	1.7		
2	55993	61679	60763	59478	3053	5.1		
3	128099	113922	132353	124791	9650	7.7		
10	400601	359734	355820	372052	24802	6.7		
30	433580	450084	461098	448254	13850	3.1		
100	468211	446995	463481	459562	11138	2.4		

sample	TRF signal			(y)	sig	comments	std dev	cv%	*(x) From origin		1x conc (µg/L)	Reported conc (µg/L)
	A	B	C	Avg					conc µg/L	DF		
1_A_Saarten taus	1400	1218	960	1193	very low	221	18.5	0.12	1	0.12	below 0.2 µg/L	
2_B_Koilliselkä	1038	1314	993	1115	below DL	174	15.6	--	1	#VALUE!	below 0.2 µg/L (below analytical DL)	
3_C_Luoteisselkä	1186	888	1056	1043	below DL	149	14.3	--	1	#VALUE!	below 0.2 µg/L (below analytical DL)	
4_A'_Hiekkaranta	1116	1318	1322	1252	very low	118	9.4	0.14	1	0.14	below 0.2 µg/L	
5_D'_Ristikallion Uimaranta	1198	1118	1078	1131	below DL	61	5.4	--	1	#VALUE!	below 0.2 µg/L (below analytical DL)	
blk+3SD (n=9)				1127								

Analytical DL (Detection limit) based on (blk+3SD) sig	1127	~0.11 µg/L
set Detection Limit (based on used std signal) for reporting	1563	0.20 µg/L

### Interpretation (21.07.2022 SA)

Collection of Raw water samples : 21.07.2022

Immunoassay analysis: 21.07.2022.

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

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

The immunoassay detects cyanobacterial peptide hepatotoxins ( microcystins and/or nodularin).

For quantification, microcystin-LR was used as standard.

#### Result:

In Littoistenjärvi water, the detected cyanobacterial peptide hepatotoxin ( free and cell bound microcystin) concentrations (µg/L) are as follows:

- 21.07.2022
- A\_Saarten taus: below 0.2 µg/L
  - B\_Koilliselkä: below 0.2 µg/L (below analytical DL)
  - C\_Luoteisselkä: below 0.2 µg/L (below analytical DL)
  - A'\_Hiekkaranta: below 0.2 µg/L
  - D'\_Ristikallion Uimaranta: below 0.2 µg/L (below analytical DL)

