LITTOISTENJÄRVEN seuranta sinilevämyrkkyjen suhteen

Date of analysis: **23.7.2021**

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

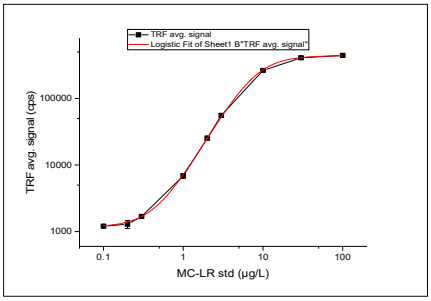
Sultana Akter (sultana.akter@utu.fi), Researcher, Department of Life Technologies (Biotechnology), Faculty of Technology, University of Turku

Assay method reference:

Sultana Akter, Markus Vehniäinen, Lisa Spoof, 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).
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-

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).

(x)	TRF signal (counts per second)			(y)			
MC-LR (µg/L) std	A	B	C	avg sig	std dev	cv%	blk+3SD (9 blank)
0	1276	1302	1236	**1206**	66	5.5	1405
0	1186	1219	1195				
0	1182	1070	1186				
0.1	1274	1182	1160	**1205**	60	5.0	
0.2	1110	1298	1463	**1290**	177	13.7	
0.3	1728	1584	1756	**1689**	92	5.5	
1	6296	6925	7354	**6858**	532	7.8	
2	23237	26517	26190	**25315**	1807	7.1	
3	52738	55263	58873	**55625**	3083	5.5	
10	263132	255153	272436	**263574**	8650	3.3	
30	403714	399452	422139	**408435**	12058	3.0	
100	442205	458063	431440	**443903**	13392	3.0	

	TRF signal			(y)				*(x) From origin			
sample	A	B	C	Avg	sig comments	std dev	cv%	conc µg/L	DF	1x conc (µg/L)	**reported conc (µg/L)**
1_A_Saarten taus (1x)	1641	1650	1656	**1649**	low	8	0.5	0.29	1	0.29	0.29
2_B_Koilliselkä(1x)	1600	1596	1472	**1556**	low	73	4.7	0.26	1	0.26	0.26
3_C_Luoteisselkä(1x)	1649	1938	1710	**1766**	low	152	8.6	0.32	1	0.32	0.32
4_A'_Hiekkaranta(1x)	1887	1948	2385	**2073**	low	272	13.1	0.39	1	0.39	0.39
5_D'_Ristikallion Uimaranta(1x)	1860	1479	1650	**1663**	low	191	11.5	0.29	1	0.29	0.29

DL based on (blk+3SD) sig	1405	0.20 µg/L
DL based on true standard above (blk+3SD) signal	**1689**	0.30 µg/L

Interpretation (23.7.2021 SA)

Raw water samples (collected on 22-23.7.2021) were stored at +4 C until analysis on 23.7.2021.

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 (Akter et al., 2016) detects cyanobacterial peptide hepatotoxins (eg 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 shown below from the following samples:

22.7.2021, A_Saarten taus : 0,29 µg/L
22.7.2021, B_Koilliselkä : 0,26 µg/L
22.7.2021, C_Luoteisselkä : 0,32 µg/L
23.7.2021, A'_Hiekkaranta : 0,39 µg/L
23.7.2021, D'_Ristikallion Uimaranta : 0,29 µg/L