

## LITTOISTENJÄRVEN seuranta sinilevämyrkköjen suhteen

Menetelmä: Immunomääritys (Akter et al 2016 / Turun yliopisto)

PVM: 19.7.2019

1. Prewash streptavidin coated strips (yellow, normal, Lot KG1574).
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 at least 5 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)

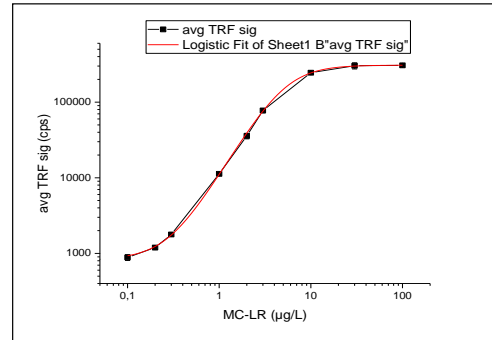
Further working standard solution in reagent water: 100, 30, 10, 3, 2, 1, 0.3, 0.2 and 0.1 µg/L (stored at 4C for short term, -20 C for long term)

### Reagent mixture in assay buffer

1 µg/mL biotinylated anti-ADDA Antibody; +

1 µg/mL anti-immunocomplex scFv-AP +

0.5 µg/mL N1-Eu-anti AP Ab.



standard curve of microcystin-LR

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(x)	TRF signal (counts per second)			(y)			
MC-LR (µg/L) std	A	B	C	avg sig	std	cv%	blk+3SD (n=9)
0	1006	911	960				
0	926	910	844				
0	1048	780	841	914	84	9.2	1167
0.1	940	795	920	885	79	8.9	
0.2	1266	1201	1118	1195	74	6.2	
0.3	1898	1710	1731	1780	103	5.8	
1	10822	11571	11376	11256	389	3.5	
2	33290	34794	39099	35728	3015	8.4	
3	82044	78513	71778	77445	5216	6.7	
10	253561	230045	251591	245066	13046	5.3	
30	330617	273982	303806	302802	28331	9.4	
100	312713	314112	291293	306039	12790	4.2	

samp	Date	Dilution	TRF signal			Avg	sig comments	std dev	cv%	*(x) From standard curve using logistic fit (origin)			
			A	B	C					conc µg/L	DF	1x conc (µg/L)	1x Avg conc (µg/L)
samp1	11.7.2019_1	(1/10) (1x)	1760 98220	1842 105500	1802 108069	1801 103930	reliable more reliable	41 5109	2.3 4.9	0.3 3.7	10 1	3.1 3.7	3.4
samp2	11.7.2019_2	(1/10) (1x)	1828 69808	1628 67979	1555 76636	1670 71474	reliable more reliable	141 4563	8.5 6.4	0.3 2.9	10 1	2.9 2.9	2.9
samp3	11.7.2019_3	(1/10) (1x)	2361 104557	2336 107295	2106 108235	2268 106696	reliable more reliable	141 1911	6.2 1.8	0.4 3.8	10 1	3.7 3.8	3.8
samp4	11.7.2019_4	(1/10) (1x)	2639 116308	2428 127622	2542 132449	2536 125460	reliable reliable	106 8285	4.2 6.6	0.4 4.3	10 1	4.1 4.3	4.2
samp5	11.7.2019_5	(1/10) (1x)	1814 92646	1895 99289	1916 97800	1875 96578	reliable more reliable	54 3486	2.9 3.6	0.3 3.5	10 1	3.2 3.5	3.4
samp6	11.7.2019_6	(1/10) (1x)	2791 131658	2705 134989	2641 136536	2712 134394	reliable reliable	75 2493	2.8 1.9	0.4 4.6	10 1	4.3 4.6	4.4
samp7	18.7.2019_7	(1/10) (1x)	1095 16202	1070 17396		1083 16799	not reliable reliable	18 844	1.6 5.0	0.2 1.3	10 1	1.6 1.3	1.3

### Interpretation

Samples were stored at -20°C until analysis, which was performed on 19.7.2019.

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

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.

Sample 1: 11.7.2019 22:07-22:09 at Pirjeläntie 8, 20m from peer, temperature at surface 19c degrees, WGS84-coordinates N60.44754, E22.39226, water depth 1.2m at the point of sampling.

Sample 2: 11.7.2019 22:13-22:15 at Ristikallion uimaranta, above underwater pipeline (sign a shore), 20c degrees, N60.44729, E22.39686, depth < 1m.

Sample 3: 11.7.2019 22:28-22:30 at Kuoviluoto peer, 19c degrees, N60.45304, E22.39780, depth 1.2m.

Sample 4: 11.7.2019 22:34-22:36 at Hamppekallio peer, 19c degrees, N60.45376, E22.40251, depth 1.2m.

Sample 5: 11.7.2019 22:56-22:58 at Littoisten hiekkaranta a.k.a. Lido di Litsa, 20c degrees N60.45800, E22.37683, depth < 1m.

Sample 6: 11.7.2019 22:03-22:05 at Pirtanranta peer (where the cyanometer is), 20c degrees N60.45582 E22.37748, depth 1.3m.

Sample7:18.7.2019, 15:00, Heikkaranta,

### Result:

In Littoistenjärvi water, the detected cyanobacterial peptide hepatotoxin concentrations (free and cell bound) were as follows:

Sample 1 (11.7.2019 at Pirjeläntie 8): **3,4 µg/L**

Sample 2 (11.7.2019 at Ristikallion uimaranta): **2,9 µg/L**

Sample 3 (11.7.2019 at Kuoviluoto peer): **3,8 µg/L**

Sample 4 (11.7.2019 at Hamppekallio): **4,2µg/L**

Sample 5 (11.7.2019 at Littoisten hiekkaranta): **3,4µg/L**

Sample 6 (11.7.2019 at Pirtanranta peer): **4,4 µg/L**