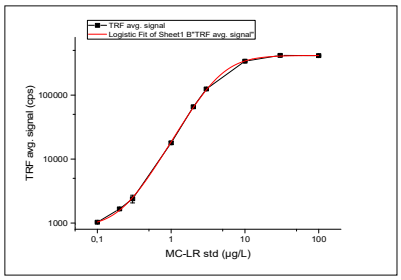
LITTOISTENJÄRVEN seuranta sinilevämyrkkyjen suhteen

Assay method: Immunoassay (Akter et al 2016 / Department of Biotechnology, University of Turku)

Date: **13.6.2020**

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.



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)

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).

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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=15)
0	908	978	880				
0	946	938	894				
0	984	924	854				
0	878	810	806				
0	852	950	880	899	55	6.1	1064
0.1	1018	1018	1051	1029	19	1.9	
0.2	1542	1456	1500	1499	43	2.9	
0.3	2207	1944	2110	2087	133	6.4	
1	16619	16868	18264	17250	887	5.1	
2	61444	61463	64049	62319	1499	2.4	
3	117202	117009	126617	120276	5492	4.6	
10	349594	363451	369723	360923	10300	2.9	
30	427628	491832	462920	460793	32155	7.0	
100	432340	454602	475831	454258	21748	4.8	

		TRF signal			(y)				*(x) From origin			Avg 1x conc (µg/L)
	Dilution	A	B	C	Avg	sig comments	std dev	cv%	conc µg/L	DF	1x conc (µg/L)	
11.6.2020 1_A	1x	79455	93075	98995	**90508**	reliable	10020	11.1	2.47	1	**2.47**	**2.1**
11.6.2020 1_A (1/5x)	1/5x	3073	3169	3059	**3100**	reliable	60	1.9	0.35	5	**1.75**	
11.6.2020 2_B	1x	46938	49703	48820	**48487**	reliable	1412	2.9	1.70	1	**1.70**	1.4
11.6.2020 2_B(1/5x)	1/5x	1678	1780	1826	**1761**	low range	76	4.3	0.22	5	1.11	
11.6.2020 3_C	1x	72173	74787	74552	**73837**	reliable	1446	2.0	2.18	1	2.18	1.9
11.6.2020 3_C(1/5x)	1/5x	2505	2610	2571	**2562**	reliable	53	2.1	0.31	5	1.53	
11.6.2020 4_A'	1x	17321	20780	18549	**18883**	reliable	1754	9.3	1.01	1	1.01	0.9
11.6.2020 4_A'(1/5x)	1/5x	1165	1226	1406	**1266**	low range	125	9.9	0.15	5	0.75	
11.6.2020 5_B'	1x	23507	24978	29028	**25838**	reliable	2859	11.1	1.20	1	1.20	1.0
11.6.2020 5_B'(1/5x)	1/5x	1422	1418	1247	**1362**	low range	100	7.3	0.17	5	0.83	
11.6.2020 6_C'	1x	31587	31432	28117	**30379**	reliable	1960	6.5	1.31	1	1.31	1.1
11.6.2020 6_C'(1/5x)	1/5x	1376	1291	1436	**1368**	low range	73	5.3	0.17	5	0.84	
11.6.2020 7_D'	1x	112837	122018	129119	**121325**	reliable	8163	6.7	3.01	1	3.01	**2.6**
11.6.2020 7_D'(1/5x)	1/5x	4099	4515	4745	**4453**	reliable	327	7.4	0.44	5	2.22	
11.6.2020 8_E'	1x	146346	162995	160821	**156721**	reliable	9050	5.8	3.64	1	3.64	3.1
11.6.2020 8_E'(1/5x)	1/5x	5521	6012	6096	**5876**	reliable	311	5.3	0.52	5	2.62	
11.6.2020 9_F'	1x	48452	53079	54391	**51974**	reliable	3120	6.0	1.77	1	1.77	1.5
11.6.2020 9_F'(1/5x)	1/5x	1944	1908	2056	**1969**	low range	77	3.9	0.25	5	1.23	

calculated DL (blk+3Sd)	1064	0.11 µg/L
DL based on true standard	1499	0.20 µg/L

Interpretation

Samples were stored at -20˚C until analysis, which was performed on 13.6.2020.
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:

11.6.2020 Location A: 2,1 µg/L
11.6.2020 Location B: 1,4 µg/L
11.6.2020 Location C : 1,9 µg/L

11.6.2020 Littoisten Uimaranta (A'): 0,9 µg/L
11.6.2020 Laituri 1, near Littoistenjärventie 109 (B'): 1,0 µg/L
11.6.2020 Laituri 2 Rauhaniemi, bus stop 6378 (C'): 1,1 µg/L

11.6.2020 Ristikallion Uimaranta (D'): 2,6 µg/L
11.6.2020 Kuoviluoto (E'): 3,1 µg/L
11.6.2020 Laituri 3, Ranta polku (F'): 1,5 µg/L