

LITTOISTENJÄRVEN seuranta sinilevämyrkköjen suhteen

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

Date of analysis: 24.6.2020

1. Prewash streptavidin coated strips (yellow, normal, Lot KGI739).
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)

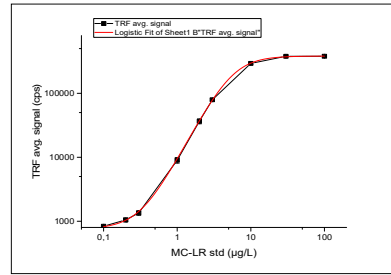
Prepared original stock of 1000 µg/L in reagent water+5%Methanol. Stored at (-20C)

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).

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standard curve of microcystin-LR

(x)	TRF signal (counts per second)			(y)			blk+3SD (n=9)
	A	B	C	avg sig	std	cv%	
MC-LR (µg/L) std							
0	695	666	714				
0	771	710	708				
0	714	650	686	702	34	4.9	805
0.1	846	820	838	835	13	1.6	
0.2	992	1025	1132	1050	73	7.0	
0.3	1234	1446	1366	1349	107	7.9	
1	8406	8572	10010	8996	882	9.8	
2	33970	35598	40087	36552	3168	8.7	
3	79769	76426	81537	79244	2596	3.3	
10	300901	288321	291140	293454	6602	2.2	
30	380742	387275	369330	379116	9082	2.4	
100	385164	380675	373779	379873	5735	1.5	

sample of 24.6.2020	TRF signal			(y)			*(x) From origin		DF	1x conc (µg/L)	Avg 1x conc (µg/L)	
	A	B	C	Avg	sig comments	std dev	cv%	conc µg/L				
location A	1_A	179393	195472	189637	188167	high	8140	4.3	5.56	1	5.56	5.2
	1_A (1/10x)	2435	2688	2845	2656	reliable	207	7.8	0.49	10	4.89	
location B	2_B	195281	202453	199270	199001	high	3594	1.8	5.86	1	5.86	5.3
	2_B (1/10x)	2679	2532	2635	2615	reliable	75	2.9	0.48	10	4.84	
location C	3_C	184041	193517	208970	195509	high	12583	6.4	5.76	1	5.76	5.4
	3_C (1/10x)	2868	2635	2843	2782	reliable	128	4.6	0.50	10	5.04	
Littoisten Uimaranta (A')	4_A'	133080	145407	141923	140137	high	6355	4.5	4.38	1	4.38	4.0
	4_A' (1/10x)	1725	1692	1790	1736	low range,	50	2.9	0.36	10	3.59	
Laituri 1, near Littoistenjärvi	5_B'	165931	174494	166564	168996	high	4772	2.8	5.07	1	5.07	4.9
	5_B' (1/10x)	2497	2579	2490	2522	low range	49	2.0	0.47	10	4.72	
Laituri 2 Rauhaniemi, bus s 6	6_C'	185664	178300	173364	179109	high	6190	3.5	5.32	1	5.32	5.2
	6_C' (1/10x)	2864	2694	2745	2768	reliable	87	3.2	0.50	10	5.02	
Ristikallion Uimaranta (D')	7_D'	196987	206416	218235	207213	high	10646	5.1	6.10	1	6.10	5.4
	7_D' (1/10x)	2581	2566	2531	2559	reliable	26	1.0	0.48	10	4.77	
Kuoviluoto (E')	8_E'	117466	118816	118217	118166	high	676	0.6	3.89	1	3.89	3.7
	8_E' (1/10x)	1924	1557	1508	1663	low, cv%	227	13.7	0.35	10	3.46	
Laituri 3, Ranta polku (F')	9_F'	179465	181490	180541	180499	high	1013	0.6	5.36	1	5.36	4.9
	9_F' (1/10x)	2088	2370	2368	2275	low range	162	7.1	0.44	10	4.40	

(blk+3Sd) sig 805
DL based on true standard signal 835 0.10 µg/L

Interpretation

Raw fresh water samples were analyzed fresh on 24.6.2020.

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:

- 24.6.2020 Location A: 5,2 µg/L
- 24.6.2020 Location B: 5,3 µg/L
- 24.6.2020 Location C: 5,4 µg/L

- 24.6.2020 Littoisten Uimaranta (A'): 4,0 µg/L
- 24.6.2020 Laituri 1, near Littoistenjärventie 109 (B'): 4,9 µg/L
- 24.6.2020 Laituri 2 Rauhaniemi, bus stop 6378 (C'): 5,2 µg/L

- 24.6.2020 Ristikallion Uimaranta (D'): 5,4 µg/L
- 24.6.2020 Kuoviluoto (E'): 3,7 µg/L
- 24.6.2020 Laituri 3, Ranta polku (F'): 4,9 µg/L

