

LITTOISTENJÄRVEN seuranta sinilevämyrkyjen suhteen

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

Date: 4.6.2020

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.

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)

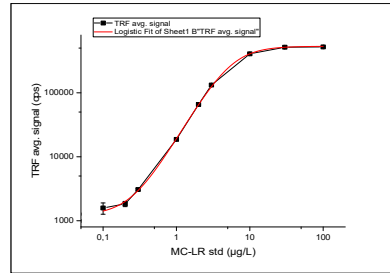
30.9.2019SA: Further working standard solution in reagent water: 100, 30, 10, 3, 2, 1, 0.3, 0.2 and 0.1 µg/L

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 138 µg/ml).



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	1126	1034	1045				
0	1071	1062	1092				
0	1058	1646	1038	1130	196	17.3	1717
0.1	1304	1928	1513	1582	318	20.1	
0.2	1770	2029	1704	1834	172	9.4	
0.3	2997	3196	3015	3069	110	3.6	
1	18359	18546	19198	18701	440	2.4	
2	66017	66733	64210	65653	1300	2.0	
3	128934	130754	137306	132331	4403	3.3	
10	407993	405542	405768	406434	1355	0.3	
30	518845	517227	513758	516610	2599	0.5	
100	523713	533713	507627	521684	13161	2.5	

	TRF signal				(y)	sig comments	std dev	cv%	*(x) From (origin)	
	A	B	C	Avg					*conc µg/L	Toxin conc (µg/L)
2.1.2020 Littoisten Uimaranta (A')	1308	1249	1305	1287	sig below DL	33	2.6	--	<DL	
23.2.2020 Littoisten Uimaranta (A')	1516	2026	1504	1682	sig below DL	298	17.7	0.15	<DL	
28.3.2020 Littoisten Uimaranta (A')	1266	1258	1184	1236	sig below DL	45	3.7	--	<DL	
28.3.2020 Laituri 1, near Littoistenjärventie 109 (B')	1440	1260	1366	1355	sig below DL	90	6.7	--	<DL	
28.3.2020 Laituri 2, Rauhaniemi, bus stop 6378 (C')	1302	1246	1480	1343	sig below DL	122	9.1	--	<DL	
28.3.2020 Ristikallion Uimaranta(D')	1238	1797	1228	1421	sig below DL	326	22.9	0.10	<DL	
25.4.2020 Littoisten Uimaranta (A')	1165	1252	1182	1200	sig below DL	46	3.8	--	<DL	
25.4.2020 Laituri 1, near Muurikinkatu, bus stop 6373 (B')	1088	1144	1266	1166	sig below DL	91	7.8	--	<DL	
25.4.2020 Laituri 2, Rauhaniemi, bus stop 6378 (C')	1282	1290	1548	1373	sig below DL	151	11.0	--	<DL	
25.4.2020 Ristikallion Uimaranta (D')	1266	1664	1214	1381	sig below DL	246	17.8	--	<DL	
25.4.2020 Kuoviluoto (E')	1179	1120	1145	1148	sig below DL	30	2.6	--	<DL	
14.5.2020 location A	1221	1222	1360	1268	sig below DL	80	6.3	--	<DL	
14.5.2020 location B	1295	1294	1612	1400	sig below DL	183	13.1	--	<DL	
14.5.2020 location C	1634	1339	1385	1453	sig below DL	159	10.9	0.11	<DL	
14.5.2020 Littoisten Uimaranta(A')	1689	2011	1134	1605	sig below DL	442	27.5	0.14	<DL	
14.5.2020 Laituri 1, near Littoistenjärventie 109 (B')	1261	1153	1253	1222	sig below DL	60	4.9	--	<DL	
14.5.2020 Laituri 2, Rauhaniemi, bus stop 6378 (C')	1096	1185		1141	sig below DL	63	5.5	--	<DL	
14.5.2020 Ristikallion Uimaranta (D')	1614	1325	1319	1419	sig below DL	169	11.9	0.10	<DL	
14.5.2020 Kuoviluoto (E')	1323	1336	1282	1314	sig below DL	28	2.1	--	<DL	
14.5.2020 Laituri 3, Ranta polku (F')	1279	1239	1295	1271	sig below DL	29	2.3	--	<DL	
calculated DL (blk+3SD)				1717					0.16	
DL based on true standard				1834					0.20	

Interpretation

Samples were stored at -20°C until analysis, which was performed on 4.6.2020.

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 (free and cell bound) were below detection limit (below 0.2 µg/L) from the following samples

- 2.1.2020 Littoisten Uimaranta (A')
- 23.2.2020 Littoisten Uimaranta (A')

- 28.3.2020 Littoisten Uimaranta (A')
- 28.3.2020 Littoisten Uimaranta (A')
- 28.3.2020 Laituri 1, near Littoistenjärventie 109 (B')
- 28.3.2020 Laituri 2, Rauhaniemi, bus stop 6378 (C')
- 28.3.2020 Ristikallion Uimaranta (D')

- 25.4.2020 Littoisten Uimaranta (A')
- 25.4.2020 Laituri 1, near Muurikinkatu, bus stop 6373 (B')
- 25.4.2020 Laituri 2, Rauhaniemi, bus stop 6378 (C')
- 25.4.2020 Ristikallion Uimaranta (D')
- 25.4.2020 Kuoviluoto (E')

- 14.5.2020 Location A
- 14.5.2020 Location B
- 14.5.2020 Location C
- 14.5.2020 Littoisten Uimaranta (A')
- 14.5.2020 Laituri 1, near Littoistenjärventie 109 (B')
- 14.5.2020 Laituri 2, Rauhaniemi, bus stop 6378 (C')
- 14.5.2020 Ristikallion Uimaranta (D')
- 14.5.2020 Kuoviluoto (E')
- 14.5.2020 Laituri 3, Ranta polku (F')

