

LITTOISTENJÄRVEN seuranta sinilevämyrkköjen suhteen

Date of analysis: 15.10.2021

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 Spooft, 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, low, KG1904).
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)

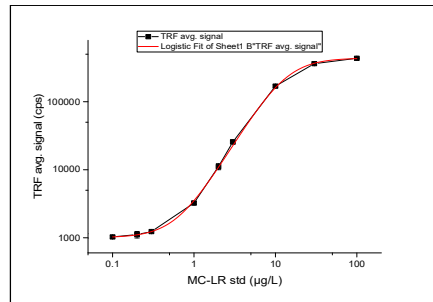
30.9.2019SA: Further working standard solution in reagent water: 100, 30, 10, 3, 2, 1, 0.3, 0.2 and 0.1 µg/L

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).



standard curve of microcystin-

[x] MC-LR (µg/L) std	TRF signal (counts per second)			[y]			blk+3SD (9 blank)
	A	B	C	avg sig	std dev	cv%	
0	1124	1032	1064	1065	82	7.7	1312
0	1111	990	1007				
0	1102	944	1214				
0.1	1084	1004	1000	1029	47	4.6	
0.2	1250	1010	1090	1117	122	10.9	
0.3	1226	1234	1240	1233	7	0.6	
1	3242	3047	3453	3247	203	6.3	
2	11184	10006	12262	11151	1128	10.1	
3	23787	25778	27503	25689	1860	7.2	
10	160897	171996	175657	169517	7686	4.5	
30	366140	364601	359034	363258	3738	1.0	
100	436761	438959	430309	435343	4496	1.0	

sample	TRF signal			[y]	sig comments	std dev	cv%	*(x) From origin		DF	1x conc (µg/L)	Reported conc (µg/L)
	A	B	C					conc µg/L	DF			
19.8.2021	A_Saarten taus	1346	1240	1228	1271	below blk+3SD	65	5.1	0.31	1	0.31	below DL (0.35)
	B_Koilliselkä	1314	1254	1148	1239	below blk+3SD	84	6.8	0.29	1	0.29	below DL (0.35)
	C_Luoteisselkä	1514	1378	1448	1447	low	68	4.7	0.40	1	0.40	0.40
	A'_Hiekkaranta	2284	1766	1756	1935	low	302	15.6	0.57	1	0.57	0.57
	D'_Ristikallion Uimaranta	1411	1468	1332	1404	close to DL	68	4.9	0.38	1	0.38	0.38
9.9.2021	A_Saarten taus	1232	1204	1156	1197	below blk+3SD	38	3.2	0.26	1	0.26	below DL (0.35)
	B_Koilliselkä	1229	1202	1260	1230	below blk+3SD	29	2.4	0.28	1	0.28	below DL (0.35)
	C_Luoteisselkä	1211	1136	1266	1204	below blk+3SD	65	5.4	0.26	1	0.26	below DL (0.35)
	A'_Hiekkaranta	1201	1158	1184	1181	below blk+3SD	22	1.8	0.24	1	0.24	below DL (0.35)
	D'_Ristikallion Uimaranta	1310	1262	1332	1301	below blk+3SD	36	2.8	0.33	1	0.33	below DL (0.35)
23.9.2021	A_Saarten taus	1250	1198	1198	1215	below blk+3SD	30	2.5	0.27	1	0.27	below DL (0.35)
	B_Koilliselkä	1235	1113	1150	1166	below blk+3SD	63	5.4	0.23	1	0.23	below DL (0.35)
	C_Luoteisselkä	1252	1156	1194	1201	below blk+3SD	48	4.0	0.26	1	0.26	below DL (0.35)
	A'_Hiekkaranta	1186	1254	1164	1201	below blk+3SD	47	3.9	0.26	1	0.26	below DL (0.35)
	D'_Ristikallion Uimaranta	1962	1508	1933	1801	low	254	14.1	0.53	1	0.53	0.53
13.10.2021	A_Saarten taus	1306	1198	1284	1263	below blk+3SD	57	4.5	0.31	1	0.31	below DL (0.35)
	B_Koilliselkä	1242	1206	1226	1225	below blk+3SD	18	1.5	0.28	1	0.28	below DL (0.35)
	C_Luoteisselkä	1254	1274	1290	1273	below blk+3SD	18	1.4	0.31	1	0.31	below DL (0.35)
	A'_Hiekkaranta	1284	1328	1315	1309	below blk+3SD	23	1.7	0.33	1	0.33	below DL (0.35)
	D'_Ristikallion Uimaranta	1336	1356	1402	1365	low	34	2.5	0.36	1	0.36	0.36
blk+3SD (n=9)				1312					0.34			
DL (Detection limit) based on (blk+3SD) sig				1312					0.34 µg/L			

Interpretation (15.10.2021 SA)

Raw water samples (collected on 19.8.2021, 9.9.2021, 23.09.2021, 13.10.2021) were stored at -20 C until analysis on 15.10.2021.

Before analysis, samples were thawed and 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 below 0,3 µg/L from the following samples:

- 19.8.2021
 - A_Saarten taus: below DL
 - B_Koilliselkä: below DL
 - C_Luoteisselkä: 0,40 µg/L
 - A'_Hiekkaranta: 0,57 µg/L
 - D'_Ristikallion Uimaranta: 0,38 µg/L
- 9.9.2021
 - A_Saarten taus: below DL
 - B_Koilliselkä: below DL
 - C_Luoteisselkä: below DL
 - A'_Hiekkaranta: below DL
 - D'_Ristikallion Uimaranta: below DL
- 23.9.2021
 - A_Saarten taus: below DL
 - B_Koilliselkä: below DL
 - C_Luoteisselkä: below DL
 - A'_Hiekkaranta: below DL
 - D'_Ristikallion Uimaranta: 0,53 µg/L
- 13.10.2021
 - A_Saarten taus: below DL
 - B_Koilliselkä: below DL
 - C_Luoteisselkä: below DL
 - A'_Hiekkaranta: below DL
 - D'_Ristikallion Uimaranta: 0,36 µg/L

