

LITTOISTENJÄRVEN seuranta sinilevämyrkköjen suhteen

Date of analysis: 7.7.2022

Sample collection, immunoassay, data analysis and report by SULTANA AKTER

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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 with some modification based on concept of Akter et al., 2016

1. Prewash streptavidin coated strips (clear, KG 2007).
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)

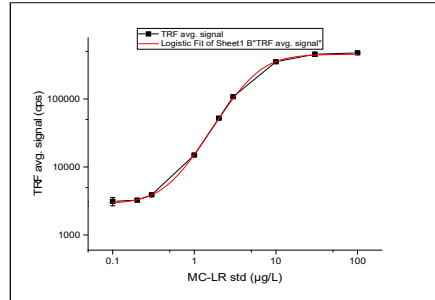
9.6.2022: working standard solution in reagent water: 100, 30, 10, 3, 2, 1, 0.3, 0.2 and 0.1 µg/L

Reagent mixture in assay buffer

1 µg/mL biotinylated anti-ADDA Antibody (stock 242 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-LR

(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	3102	2993	2958	2896	119	4.1	3254
0	2833	2841	2708				
0	2972	2849	2806				
0.1	3632	2845	2908	3128	437	14.0	
0.2	3408	3196	3175	3260	129	4.0	
0.3	3934	3887	3865	3895	35	0.9	
1	15049	14855	15141	15015	146	1.0	
2	52780	51613	52441	52278	600	1.1	
3	109144	103737	111405	108095	3940	3.6	
10	350933	351619	354166	352239	1703	0.5	
30	479228	418328	466658	454738	32152	7.1	
100	478195	482607	466456	475753	8348	1.8	

sample	TRF signal			(y)			*(x) From origin			DF	1x conc (µg/L)	Reported conc (µg/L)
	A	B	C	Avg	sig comments	std dev	cv%	conc µg/L				
21.6.2022												
1_A_Saarten taus	9244	8841	8974	9020		205	2.3	0.71	1	0.71	0.71	
2_B_Koilliselkä	5573	5640	5529	5581		56	1.0	0.48	1	0.48	0.48	
3_C_Luoteisselkä	10020	9746	9693	9820		176	1.8	0.76	1	0.76	0.76	
4_A'_Hiekkaranta	19887	20743	20804	20478		513	2.5	1.19	1	1.19	1.19	
5_D'_Ristikallion Uimaranta	25183	28214	27185	26861		1541	5.7	1.39	1	1.39	1.39	
blk+3SD (n=9)				3254				0.19				

Analytical DL (Detection limit) based on (blk+3SD) sig

3254

0.19 µg/L

set detection limit (based on used std signal) for reporting

3260

0.20 µg/L

Interpretation (07.07.2022 SA)

Collection of Raw water samples : 07.07.2022

Immunoassay analysis: 07.07.2022.

Before analysis, samples were 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 detects cyanobacterial peptide hepatotoxins (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 as follows:

07.07.2022 A_Saarten taus: 0.71 µg/L
 B_Koilliselkä: 0.48 µg/L
 C_Luoteisselkä: 0.76 µg/L
 A'_Hiekkaranta: 1.19 µg/L
 D'_Ristikallion Uimaranta: 1.39 µg/L

