LITTOISTENJÄRVEN seuranta sinilevämyrkkyjen suhteen

Menetelmä: Immunomääritys (Akter et al 2016 / Turun yliopisto)

PVM: **15.8.2019**

1. Prewash streptavidin coated strips (yellow, normal, Lot KG1574).
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 at least 5 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)

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

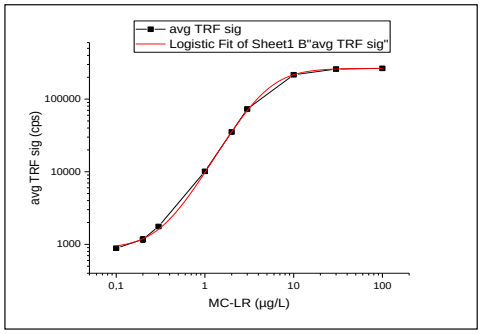
(stored at 4C for short term, -20 C for long term)

Reagent mixture in assay buffer

1 µg/mL biotinylated anti-ADDA Antibody; +

1 µg/mL anti-immunocomplex scFv-AP +

0.5 µg/mL N1-Eu-anti AP pAb.



standard curve of microcystin-LR

Tekijä: Sultana Akter (sultana.akter@utu.fi)

(x)	TRF signal (counts per second)			(y)			
MC-LR (µg/L) std	A	B	C	avg sig	std	cv%	blk+3SD (n=9)
0	**872**	**909**	**930**				
0	**914**	**864**	**886**				
0	**874**	**910**	**802**	885	38	4.3	999
0.1	**866**	**916**	**870**	884	28	3.1	
0.2	**1280**	**1170**	**1064**	1171	108	9.2	
0.3	**1786**	**1714**	**1779**	1760	40	2.3	
1	**10199**	**9647**	**10504**	10117	434	4.3	
2	**35200**	**35206**	**35297**	35234	54	0.2	
3	**73734**	**72210**	**72935**	72960	762	1.0	
10	**213098**	**217166**	**218035**	216100	2636	1.2	
30	**252353**	**264901**	**260336**	259197	6351	2.5	
100	**263641**	**265503**	**265481**	264875	1069	0.4	

*(x) From standard curve using logistic fit (origin)

			TRF signal			(y)						
		Dilution	A	B	C	Avg	sig comments	std dev	cv%	conc µg/L	DF	1x conc (µg/L)
samp1	09.08.2019_1	(1x)	1272	1221	1080	**1191**	sig at lower range	99	8.4	0.20	1	0.20
samp2	14.08.2019_2	(1x)	1092	1250	1050	**1131**	sig at lower range	105	9.3	0.18	1	0.18
					detection limit	999				0.13		

Interpretation

Samples were stored at -20˚C until analysis, which was performed on 15.08.2019.

Before analysis, samples were heated at 80 ˚C for 10 min to release any possible cell bound toxins.
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.

Sample 1: 09.08.2019 Littoistenjärvi hiekkaranta, knee dept water, ~15-20 cm below surface. Turbid water, weather : ~19˚C.
Sample 2: 14.08.2019 Littoistenjärvi hiekkaranta, knee dept water,~15-20 cm below surface. Clear water. weather: ~15 ˚C

Result:

In Littoistenjärvi water, the detected cyanobacterial peptide hepatotoxin concentrations (free and cell bound) weres as follows:
Sample 1 (09.08.2019, Littoistenjärvi hiekkaranta): ~ **0,2** µg/L
Sample 2 (14.08.2019, Littoistenjärvi hiekkaranta): below **0,2** µg/L