ADVANCES IN OCEANOGRAPHY AND LIMNOLOGY

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SUPPLEMENTARY MATERIAL

Non-competitive ELISA with broad specificity for microcystins and nodularins

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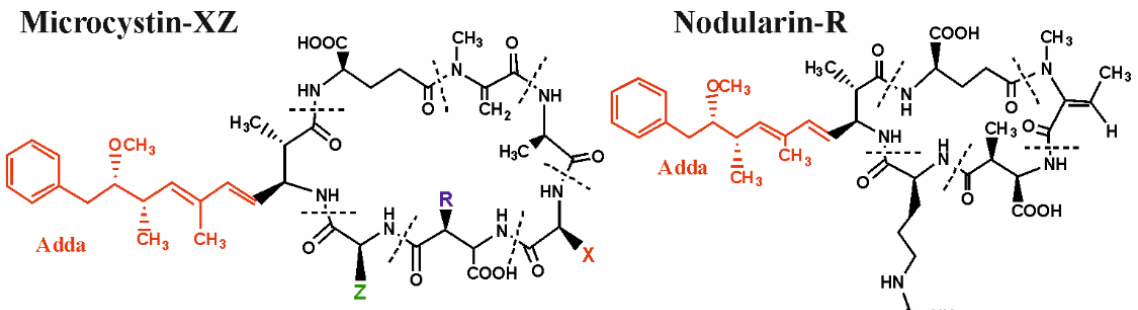

	Molecular weight	X	Z	R
MC-LR	995.2	Leu	Arg	CH_3
MC-dmLR	981.2	Leu	Arg	H
MC-RR	1038.2	Arg	Arg	CH_3
MC-dmRR	1024.2	Arg	Arg	H
MC-LA	910	Leu	Ala	CH_3
MC-LY	1002.2	Leu	Tyr	CH_3
MC-LF	986.2	Leu	Phe	CH_3
MC-LW	1025.2	Leu	Trp	CH_3
MC-YR	1045.2	Tyr	Arg	CH_3
MC-WR	1045.2	Trp	Arg	CH_3

Supplementary Fig. 1. Structure of microcystin-XZ (the 3-desmethylation site is indicated with R) and nodularin-R. The general structure of cyclic heptapeptide microcystin is cyclo(-D-Ala1-L-X2-D-erythro-β-methylAsp3(iso-linkage)-L-Z4-Adda5-D-Glu6(iso-linkage)-N-methyldehydro-Ala7) abbreviated as MC-XZ, where X and Z are variable amino acids. The general structure of pentapeptide nodularin is cyclo(-D-erythro-β-methylAsp1(iso-linkage)-L-Z2-Adda3-DGlu4(iso-linkage)-2-(methylamino)-2(Z)-dehydrobutyric acid5). The unique β-amino acid Adda, common in both microcystin and nodularin, stands for [(2S, 3S, 8S, 9S)-3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid].

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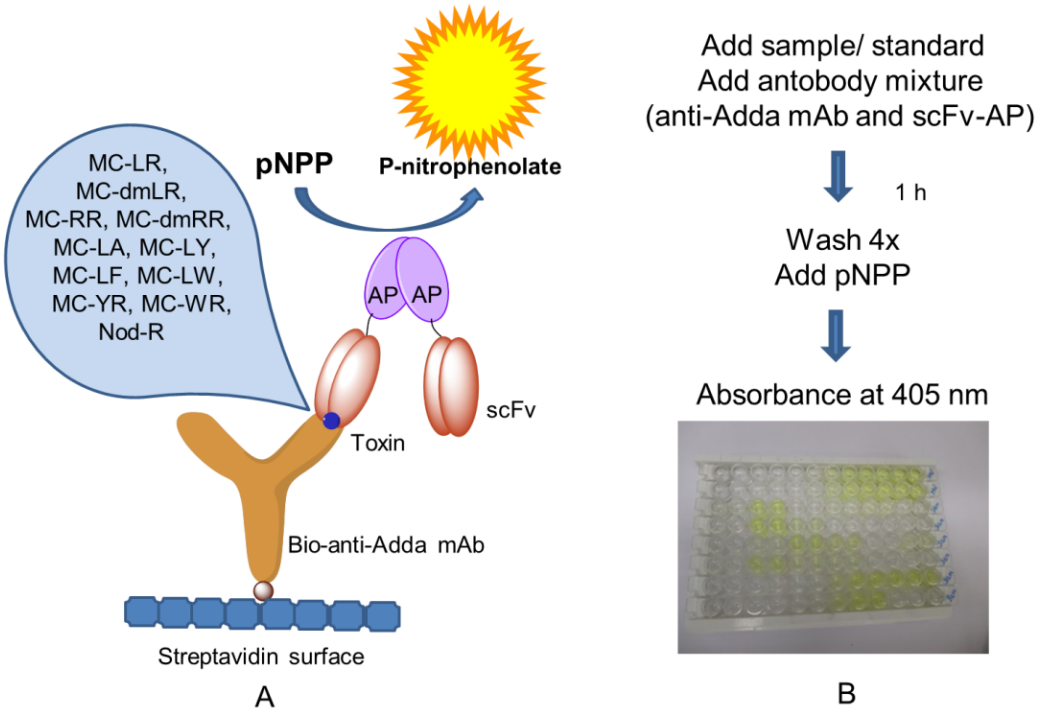

Supplementary Fig. 2. The non-competitive ELISA concept (A) and procedure (B). Alkaline phosphatase activity is detected (by measuring absorbance at 405 nm) after addition of pNPP substrate. Samples or standard, biotinylated anti-Adda mAb, anti-immunocomplex ScFv-AP are added in one step on streptavidin coated microtiter wells. In the presence of microcystin/nodularin in the sample, immunocomplex (anti-Adda mAb:MC/Nod) is formed which in turn is recognized by the anti-immunocomplex scFv-AP. The resulting sandwich (Adda mAb:microcystin/nodularin:scFv-AP) is detected by formation of yellow colored p-nitrophenolate from added para-Nitrophenylphosphate (pNPP) substrate (shown in B). In the absence of toxin, no immunocomplex is formed and scFv-AP is removed in wash step resulting in no signal generation (blank measurement).