

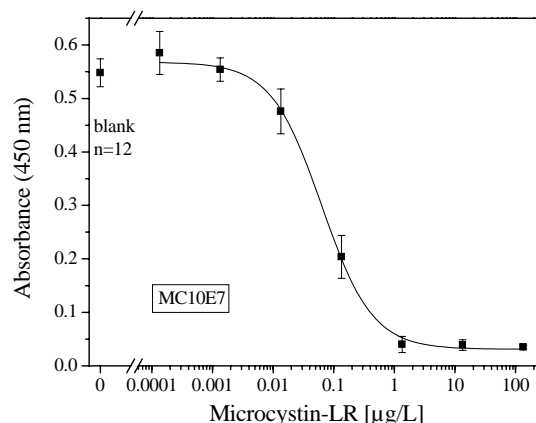
Monoclonal Antibody to Microcystin-LR (MC10E7) (Prod. No. ALX-804-320)

Recommended Immunoassay Procedure (direct competitive ELISA) for Measurement of Water Samples:

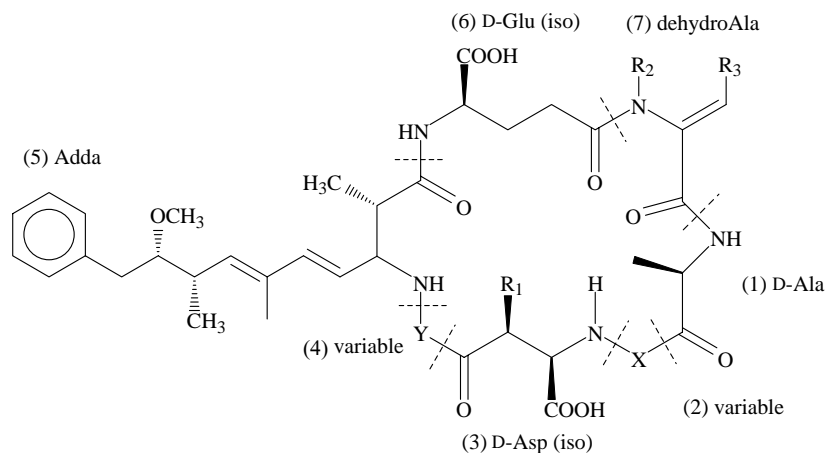
1. Coat microtiter plates with 250 µl/well of anti-mouse IgG (goat anti-mouse IgG, Fc specific) diluted 1:3'000 in coating buffer (40 mmol/l sodium carbonate, pH 9.6) and incubate overnight at room temperature.
2. Wash the plates three times with washing solution (7 mmol/l PBS, pH 7.6, containing 15 mmol/l of sodium chloride and 0.05% (v/v) of Tween 20).
3. Add 200 µl/well of MAb to Microcystin-LR (MC10E7) diluted 1:100'000* in PBS, 80 mmol/l sodium phosphate, pH 7.6) containing 8.5 g/l sodium chloride and incubate for one hour.
4. Wash the plates three times with washing solution (7 mmol/l PBS, pH 7.6, containing 15 mmol/l of sodium chloride and 0.05% (v/v) of Tween 20).
5. Add 20 µl/well of a buffer solution consisting of 1 mol/l TRIS, pH 7.4, containing 1% of BSA, 1% of EDTA sodium salt and 87.6 g/l of sodium chloride to suppress potential matrix interferences.
6. Add 200 µl/well of standard solutions, water samples or controls to the wells and pre-incubate for 30 minutes at room temperature.
7. Add 50 µl/well peroxidase tracer (microcystin-LR/horseradish peroxidase conjugate, 1 mg/ml, diluted about 1:10'000 in 0.1 mol/l TRIS, pH 7.4, containing 8.76 g/l of sodium chloride) to the wells and incubate for 15 min.
8. Wash the plates three times with washing solution (7 mmol/l PBS, pH 7.6, containing 15 mmol/l of sodium chloride and 0.05% (v/v) of Tween 20).
9. Add 200 µl/well of a freshly prepared substrate solution (hydrogen peroxide/TMB in citrate buffer, 0.2 mol/l citrate, 0.01% sorbic acid potassium salt, pH 3.8) to the wells and incubate until colour reaction occurs (~15 min.).
10. Stop the enzyme reaction by adding 100 µl of 5% (v/v) H₂SO₄.
11. Read optical density values of the wells at 450 nm, using 620 nm as reference wavelength.

*) Please note that concentration of the antibody and recommended dilution may vary between different lots.

Typical Standard Curve:



Chemical Structure of Microcystins and their Derivatives:



Microcystin Derivatives	R ₁ (3)	X (2)	Y (4)	R ₂ (7)	R ₃ (7)
Microcystin-LR	CH ₃	Leu	Arg	CH ₃	H
Microcystin-RR	CH ₃	Arg	Arg	CH ₃	H
Microcystin-YR	CH ₃	Tyr	Arg	CH ₃	H
Microcystin-LA	CH ₃	Leu	Ala	CH ₃	H
Microcystin-LW	CH ₃	Leu	Trp	CH ₃	H
Microcystin-LF	CH ₃	Leu	Phe	CH ₃	H
Microcystin-WR	CH ₃	Trp	Arg	CH ₃	H
Microcystin-LY	CH ₃	Leu	Tyr	CH ₃	H
3-Demethylmicrocystin-LR	H	Leu	Arg	CH ₃	H
3-Demethylmicrocystin-RR	H	Arg	Arg	CH ₃	H
3-Demethylmicrocystin-HTyrR	H	homoTyr	Arg	CH ₃	H
3-Demethyl-7-dehydrobutyrine-microcystin-RR	H	Arg	Arg	H	CH ₃

Cross-Reactivity Pattern:

Microcystin Derivatives	Cross-Reactivities [%] (molar)	Detection Limits [µg/L]	Affinity Constants [L/mol]
Microcystin-LR	100	0.008	7 x 10 ¹⁰
[Asp ³]-Microcystin-RR	134	0.006	1 x 10 ¹¹
Microcystin-RR	96	0.011	7 x 10 ¹⁰
Microcystin-YR	68	0.008	5 x 10 ¹⁰
Nodularin	7	0.095	4 x 10 ⁹
Microcystin-LY	0.07	29*	5 x 10 ^{7*}
Microcystin-LF	<10 ⁻⁴	>1'000	<10 ⁴
Microcystin-LW	<10 ⁻⁴	>1'000	<10 ⁴
Microcystin-LA	<10 ⁻⁴	>1'000	<10 ⁴
Adda	<10 ⁻⁴	>1'000	<10 ⁴
N-Acetyl-Adda	<10 ⁻⁴	>1'000	<10 ⁴

* estimated; not available

Literature Reference:

Highly sensitive immunoassay based on a monoclonal antibody specific for [4-arginine]microcystins: A. Zeck, et al.; Anal. Chim. Acta **441**, 1 (2001)