PRODUCT FLYER

ALEXIS® BIOCHEMICALS

Microcystins

& Other Cyanobacterial Toxins

highlight

Tomorrow's Reagents Manufactured Today®

International Version

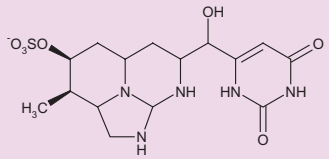
CONTENTS

NEW

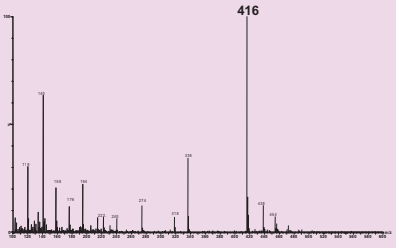
Product Highlight

Cylindrospermopsin

ALX-350-149-C025	25 µg
ALX-350-149-C100	100 µg



Isolated from *Cylindrospermopsis raciborskii*. Tricyclic alkaloid cytotoxin. Exhibits a completely different mechanism of toxicity than microcystins. Protein synthesis inhibitor.



MS Data Conditions: ESI + / 50 eV.

Toxins from Cyanobacteria

Cyanobacteria (blue-green algae) are a diverse group of photo-autotrophic organisms which are found in terrestrial and aquatic environments (Figure 1). They are an essential component of the food chain in many ecosystems, however, they can often form dense scums or blooms which have been shown to be hazardous to humans and animals. Routes of exposure may be via direct ingestion or inhalation during recreation, bathing or irrigation, whereas, indirect exposure is most likely to occur via ingestion of contaminated drinking water, vegetables or fish/shellfish. The apparent increase in the occurrence of blooms and associated toxic events has been associated with eutrophication and global warming.

The ecological function of cyanobacterial toxins remains under investigation. The toxic mechanisms to vertebrates are used to classify them into hepatotoxins (microcystins and nodularins), neurotoxins (anatoxin and saxitoxins), cytotoxins (cylindrospermopsin), dermatotoxins (lyngbyatoxin), and irritant toxins (lipopolysaccharide endotoxins). The microcystins are the most commonly encountered cyanotoxins and it is obvious that the detection of microcystins is a crucial factor of major public interest. Many regulatory authorities are now setting guidelines and accepted levels for drinking water/recreational water, etc. monitoring programs.

Additional concern on the occurrence and importance of cyanobacterial toxins is reflected by inclusion in the US Environmental Protection Agency (USEPA) drinking water contaminant list and by appearing in major reviews along with chemical warfare agents [1].

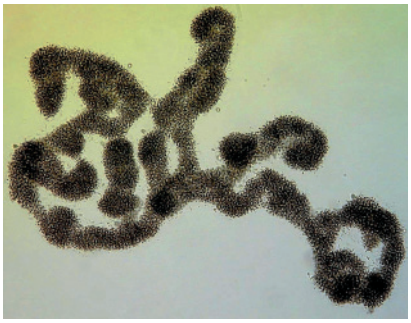


FIGURE 1: Cyanobacteria *Microcystis sp.*

Microcystins

Microcystins comprises a group of toxic, cyclic heptapeptides produced by several genera of cyanobacteria, most commonly, *Microcystis, Anabaena and Planktothrix*. They are characterized by a unique (2S,3S,8S,9S)-3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid (Adda) as shown in the general structure in Figure 2. Variation of amino acids at positions 2 and 4 (X and Z) provide the basis for microcystin nomenclature, for example, microcystin-LR has leucine (L) at position 2 and arginine (R) at position 4. Other variants are characterized by minor modifications such as methylation. The number of variants/congeners is over 70, creating a challenge for selection/development of robust methods for their detection.

ACKNOWLEDGEMENTS: We thank Linda Lawton and Christine Edwards (The Robert Gorden University, Aberdeen) for helpful discussions and data.

New Products

Hepatotox Set™ 1	Microcystins (Adda) ELISA Kit
See Page 5	See Page 8

Toxins from Cyanobacteria *continued*

Microcystins – Molecular Mechanism

Microcystins are potent inhibitors of the serine/threonine protein phosphatases type 1 (PP1) and 2A (PP2A) [2, 3], mediated through the Adda domain (Figure 3). PP1 and PP2A are two major protein phosphatases in eukaryotic cells which have been shown to be important in tumor suppression. PP2A is inhibited 1000-fold less potently, while six other phosphatases are unaffected. These results are strikingly similar to those obtained with the tumor promoter okadaic acid. The action of microcystin in inhibiting such enzymes might suggest that they act as tumor promoters [4]. All structural congeners of microcystin act as hepatotoxins [5, 6]. After accumulated in the liver they are involved in cytoskeletal disorganization, lipid peroxidation, loss of membrane integrity, DNA fragmentation, cell blebbing, apoptosis, cellular disruption, and necrosis.

Microcystins – Toxicology

Microcystins have been responsible for many acute poisonings, most famously the fatal intoxication of 50 dialysis patients in Brazil in 1996, whose water was contaminated by high concentrations of microcystins [7]. Epidemiological studies have shown that long term exposure to microcystins via drinking water supplies has been associated with primary liver cancer. Potential chronic toxicity from microcystins led the WHO to establish a guideline of 1 µg/l as a maximum concentration of microcystin-LR in drinking water [8]. In 2006, microcystin-LR, was classified as a carcinogen according to the International Agency for Research on Cancer (IARC) [9].

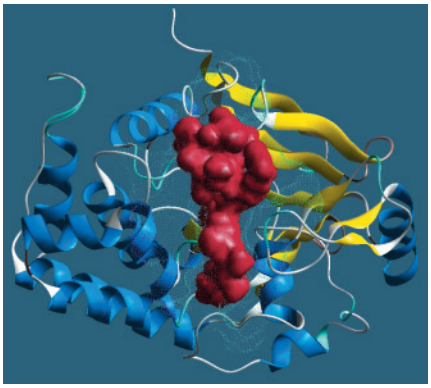


FIGURE 3: 3D-structure of microcystin-LR/PP1A-crystalline complex. *Courtesy of Prof. Marcel Jaspars, Marine Natural Products Laboratory, Department of Chemistry, University of Aberdeen.*

Nodularin & Variants Thereof

Nodularin, produced by brackish and freshwater species of *Nodularia* (most commonly *N. spumigena*), is a cyclic pentapeptide, similar to microcystin-LR, also possessing a characteristic Adda amino acid [10, 11], but with increased water solubility. Nodularin is a potent inhibitor of the serine/threonine protein phosphatases type 1 (PP1) and 2A (PP2A) [12, 13]. Several variants of nodularin have been characterized [14]. Whilst toxicity and mode of action of nodularin is similar to that of microcystins, a major difference is that the binding to protein phosphatases is irreversible. Nodularin is a great complimentary tool to microcystins for studying cellular processes.

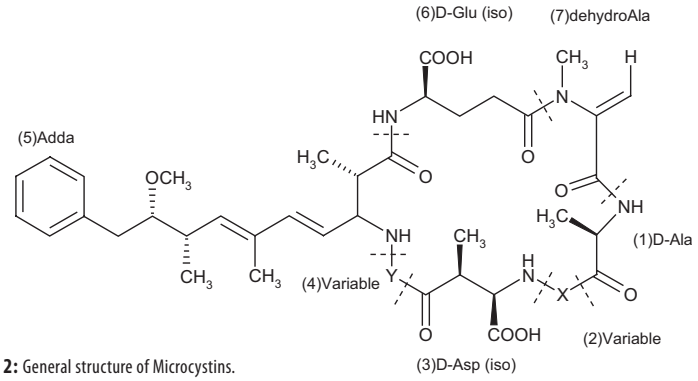


FIGURE 2: General structure of Microcystins.

Overview on Selected Microcystin Derivatives

Prod. No.	Name	Monoisotopic Mass	MW	LD_{50} (Mouse Intraperitoneal)	Isolated from	X (2)	Y (4)
ALX-350-096	Microcystin-LA	909	910.1	50	*M. aeruginosa*	Leu	Ala
ALX-350-081	Microcystin-LF	985	986.2	toxic	*M. aeruginosa*	Leu	Phe
ALX-350-012	Microcystin-LR	994	995.2	50	*M. aeruginosa*	Leu	Arg
ALX-350-080	Microcystin-LW	1024	1025.2	not determined	*M. aeruginosa*	Leu	Trp
ALX-350-148	Microcystin-LY	1001	1002.2	90	*M. aeruginosa*	Leu	Tyr
ALX-350-043	Microcystin-RR	1037	1038.2	600	*M. aeruginosa*	Arg	Arg
ALX-350-044	Microcystin-YR	1044	1045.2	70	*M. aeruginosa*	Tyr	Arg
	Microcystin-WR	1067	1068.2	150-200	*M. species*	Trp	Arg

Cylindrospermopsin

Cylindrospermopsin is a cyanobacterial cytotoxin comprising a tricyclic guanidine moiety combined with a hydroxymethyl uracil. It is produced by species of several genera, *Cylindrospermopsis raciborskii, Umezakia natans* and *Aphanizomenon ovalisporum*, in temperate and tropical regions and exhibits a completely different mechanism of toxicity than microcystins [15–17]. Cylindrospermopsin inhibits plant protein synthesis [18].

LIT: **[1]** Water analysis: emerging contaminants and current issues: S.D. Richardson & T.A. Ternes; Anal. Chem. **77,** 3807 (2005) ▪ **[2]** Characterization of microcystin-LR, a potent inhibitor of type 1 and type 2A protein phosphatases: R.E. Honkanen, et al.; J. Biol. Chem. **265,** 19401 (1990) ▪ **[3]** Cyanobacterial microcystin-LR is a potent and specific inhibitor of protein phosphatases 1 and 2A from both mammals and higher plants: C. MacKintosh, et al.; FEBS Lett. **264,** 187 (1990) **[4]** ▪ Liver tumor promotion by the cyanobacterial cyclic peptide toxin microcystin-LR: R. Nishiwaki-Matsushima, et al.; J. Cancer Res. Clin. Oncol. **118,** 420 (1992) ▪ **[5]** Comparison of in vivo and in vitro toxic effects of microcystin-LR in fasted rats: G.A. Miura, et al.; Toxicon **27,** 1229 (1989) ▪ **[6]** Inhibition of protein phosphatases by microcystins and nodularin associated with hepatotoxicity: S. Yoshizawa, et al.; J. Cancer Res. Clin. Oncol. **116,** 609 (1990) ▪ **[7]** Fatal microcystin intoxication in haemodialysis unit in Caruaru, Brazil: S. Pouria, et al.; Lancet **352,** 21 (1998) ▪ **[8]** World Health Organization. 1998. Guidelines for drinking water quality, 2nd ed. Addendum to vol. 2. Health criteria and other supporting information. World Health Organization, Geneva: 95-110. ▪ **[9]** Carcinogenicity of nitrate, nitrite, and cyanobacterial peptide toxins: Y. Grosse, et al.; Lancet Oncol. **7,** 628 (2006) ▪ **[10]** Nodularin, microcystin and the configuration of Adda: K.L. Rinehart, et al.; JACS **110,** 8557 (1988) ▪ **[11]** Toxicity and partial structure of a hepatotoxic peptide produced by the cyanobacterium Nodularia spumigena Mertens emend. L575 from New Zealand: W.W. Carmichael, et al.; Appl. Environ. Microbiol. **54,** 2257 (1988) ▪ **[12]** Nodularin, a potent inhibitor of protein phosphatases 1 and 2A, is a new environmental carcinogen in male F344 rat liver: T. Ohta, et al.; Cancer Res. **54,** 6402 (1994) ▪ **[13]** Cyanobacterial nodularin is a potent inhibitor of type 1 and type 2A protein phosphatases: R.E. Honkanen, et al.; Mol. Pharmacol. **40,** 577 (1991) ▪ **[14]** Characterization of nodularin variants in Nodularia spumigena from the Baltic Sea using liquid chromatography/mass spectrometry/mass spectrometry: H. Mazur-Marzec, et al.; Rapid Commun. Mass Spectrom. **20,** 2023 (2006) ▪ **[15]** Severe hepatotoxicity caused by the tropical cyanobacterium (blue-green alga) Cylindrospermopsis raciborskii (Woloszynska) Seenaya and Subba Raju isolated from a domestic water supply reservoir: P.R. Hawkins, et al.; Appl. Environ. Microbiol. **50,** 1292 (1985) ▪ **[16]** Cylindrospermopsin, a potent hepatotoxin from the blue-green alga Cylindrospermopsis raciborskii: I. Ohtani, et al.; JACS **114,** 7941 (1992) ▪ **[17]** The Palm Island mystery disease 20 years on: a review of research on the cyanotoxin cylindrospermopsin: D.J. Griffiths & M.L. Saker; Environ. Toxicol. **18,** 78 (2003) ▪ **[18]** Inhibition of plant protein synthesis by the cyanobacterial hepatotoxin, cylindrospermopsin: J.S. Metcalf, et al.; FEMS Microbiol. Lett. **235,** 125 (2004)

Selected Review Articles

Cyanobacteria secondary metabolites - the cyanotoxins: W.W. Carmichael; J. Appl. Bacteriol. **72,** 455 (1992) ▪ The toxins of cyanobacteria: W.W. Carmichael; Sci. Am. **270,** 78 (1994) ▪ The cyanotoxins: W.W. Carmichael; Adv. Bot. Res. **27,** 211 (1997) ▪ The toxicology of microcystins: R.M. Dawson; Toxicon **36,** 953 (1998) ▪ The microcystins and nodularins: cyclic polypeptide inhibitors of PP1 and PP2A: B.M. Gulledgea, et al.; Curr. Med. Chem. **9,** 1991 (2002) ▪ Role of oxidative stress and mitochondrial changes in cyanobacteria-induced apoptosis and hepatotoxicity: W.X. Ding & C. Nam Ong; FEMS Microbiol. Lett. **220,** 1 (2003) ▪ Guidance values for microcystins in water and cyanobacterial supplement products (blue-green algal supplements): a reasonable or misguided approach?: D. Dietrich & S. Hoeger; Toxicol. Appl. Pharmacol. **203,** 273 (2005) ▪ Detection of the cyanobacterial hepatotoxins microcystins: J. McElhiney & L.A. Lawton; Toxicol. Appl. Pharmacol. **203,** 219 (2005) ▪ Cyanobacterial toxins - occurrence, biosynthesis and impact on human affairs: E. Dittmann & C. Wiegand; Mol. Nutr. Food Res. **50,** 7 (2006) ▪ Methods for determining microcystins (peptide hepatotoxins) and microcystin-producing cyanobacteria: L.N. Sangolkar, et al.; Water Res. **40,** 3485 (2006) ▪ Cyanobacterial (blue-green algal) toxins in water supplies: Cylindrospermopsins: I.R. Falconer & A.R. Humpage; Environ. Toxicol. **21,** 299 (2006)) ▪ Algal toxins as guidance to identify phosphoproteins with key roles in apoptotic cell death: T. Solstad & K.E. Fladmark; Curr. Pharm. Biotechnol. **7,** 209 (2006) Toxins of cyanobacteria: M.E. van Apeldoorn, et al.; Mol. Nutr. Food Res. **51,** 7 (2007)

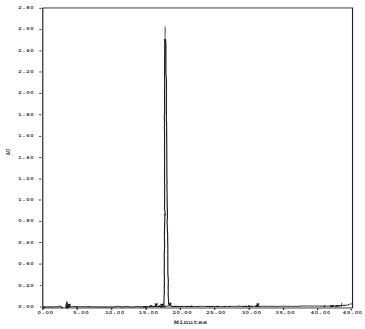
The Widest Panel of Microcystins!

Microcystin-LR – The Standard

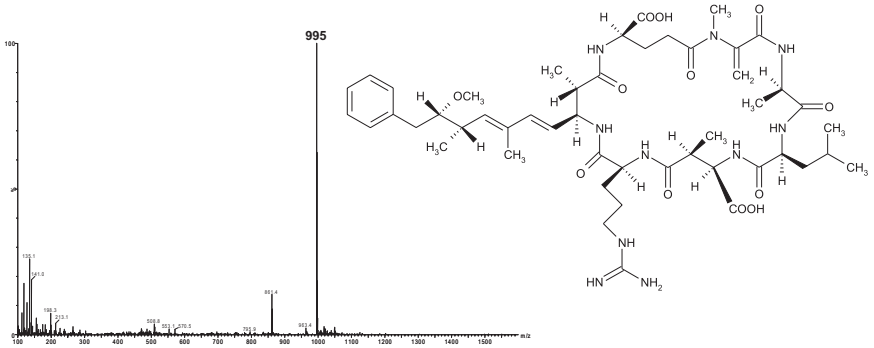
ALX-350-012-C050	50 µg
ALX-350-012-C100	100 µg
ALX-350-012-C500	500 µg
ALX-350-012-M001	1 mg

Isolated from *Microcystis aeruginosa*. For details see Page 2.

MS Data Conditions: ESI + / 70 eV.



HPLC Spectrum



LIT: Structural studies on cyanoginosins-LR, -YR, -YA, and -YM, peptide toxins from Microcystis aeruginosa: D.P. Botes et al.; JCS Perkin Trans. **I,** 2747 (1985) ▪ Nodularin, microcystin, and the configuration of Adda: K.L. Rinehart, et al.; JACS **110,** 8557 (1988) ▪ Cyanobacterial microcystin-LR is a potent and specific inhibitor of protein phosphatases 1 and 2A from both mammals and higher plants: C. MacKintosh, et al.; FEBS Lett. **264,** 187 (1990) ▪ Characterization of microcystin-LR, a potent inhibitor of type 1 and type 2A protein phosphatases: R.E. Honkanen, et al.; J. Biol. Chem. **265,** 19401 (1990) ▪ Protein phosphatase 2A is a specific protamine-kinase-inactivating phosphatase: G.D. Amick, et al.; Biochem. J. **287,** 1019 (1992) ▪ Liver tumor promotion by the cyanobacterial cyclic peptide toxin microcystin-LR: R. Nishiwaki-Matsushima, et al.; J. Cancer Res. Clin. Oncol. **118,** 420 (1992) ▪ Two significant aspects of microcystin-LR: specific binding and liver specificity: R. Nishiwaki, et al.; Cancer Lett. **83,** 283 (1994) ▪ Negative regulation of ERK and Elk by protein kinase B modulates c-Fos transcription: I. Galetic, et al.; J. Biol. Chem. **278,** 4416 (2003)

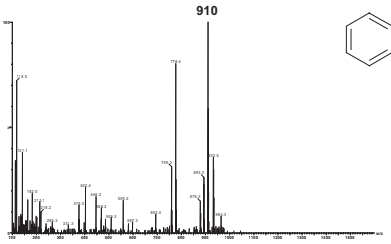
Microcystin-LA

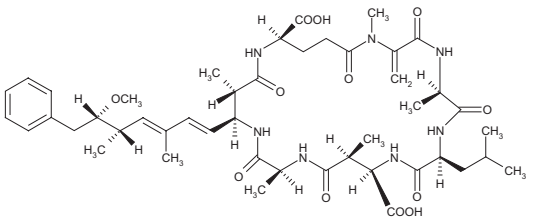
ALX-350-096-C025	25 µg
ALX-350-096-C100	100 µg

Isolated from *Microcystis aeruginosa*. Analog of microcystin-LR (Prod. No. ALX-350-012) with methyl substituted in place of Ala.

Inhibits protein phosphatase 2A (PP2A) and protein phosphatase 3 (PP3) more potently than protein phosphatase 1 (PP1).

MS Data Conditions: ESI + / 70 eV.





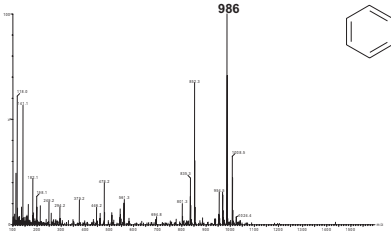
LIT: The structure of cyanoginosin-LA, a cyclic heptapeptide toxin from the cyanobacterium Microcystis aeruginosa: D.P. Botes et al.; J. Chem. Soc. **1,** 2311 (1984) ▪ Microcystin composition of an axenic clonal strain of Microcystis viridis and Microcystis viridis - containing waterblooms in Japanese freshwaters: K. Kaya & M.M. Watanabe; J. App. Phycol. **2007,** 173 (1990)

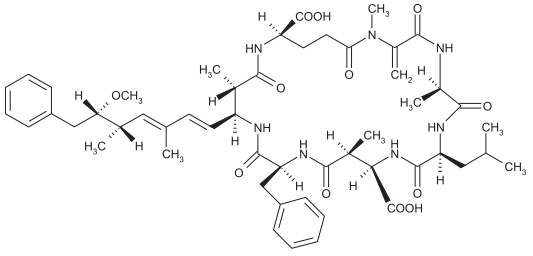
Microcystin-LF

ALX-350-081-C025	25 µg
ALX-350-081-C100	100 µg

Isolated from *Microcystis aeruginosa*. Analog of microcystin-LR (Prod. No. ALX-350-012) with Phe substituted in place of Arg. Hydrophobic and believed to be more cell permeable than other microcystins.

MS Data Conditions: ESI + / 70 eV.





LIT: Extraction and high-performance liquid chromatographic method for the determination of microcystins in raw and treated waters: L.A. Lawton, et al.; Analyst **119,** 1525 (1994) ▪ First report of microcystins from a Brazilian isolate of the cyanobacterium Microcystis aeruginosa: S.M.F.O. Azevedo, et al.; J. Appl. Phycology **6,** 261 (1994) ▪ Isolation and characterization of microcystins from laboratory cultures and environmental samples of Microcystis aeruginosa and from an associated animal toxicosis: L.A. Lawton, et al.; Nat. Toxins **3,** 50 (1995)

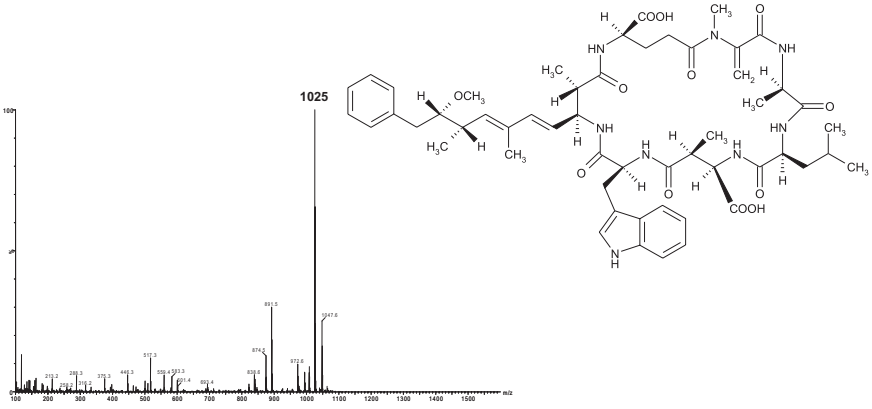
The Widest Panel of Microcystins!

Microcystin-LW

ALX-350-080-C025	25 µg
ALX-350-080-C100	100 µg

Isolated from *Microcystis aeruginosa*. Analog of microcystin-LR (Prod. No. ALX-350-012) with Trp substituted in place of Arg. Microcystin-LW has a characteristically different absorption spectrum compared to other microcystins, making it a useful reference compound for HPLC analysis. The Trp confers an absorption maximum at 222nm, whereas most microcystins have a characteristic maximum at 239nm. Hydrophobic and believed to be more cell permeable than other microcystins. May prove useful in biochemical studies in intact cells.

MS Data Conditions: ESI + / 70 eV.



LIT: Mass spectral analyses of microcystins from toxic cyanobacteria using on-line chromatographic and electrophoretic separations: K.P. Bateman, et al.; J. Chromatog. A, **712,** 253 (1995) ▪ Extraction and high-performance liquid chromatographic method for the determination of microcystins in raw and treated waters: L.A. Lawton, et al.; Analyst **119,** 1525 (1994) ▪ Isolation and characterization of microcystins from laboratory cultures and environmental samples of Microcystis aeruginosa and from an associated animal toxicosis: L.A. Lawton, et al.; Nat. Toxins **3,** 50 (1995)

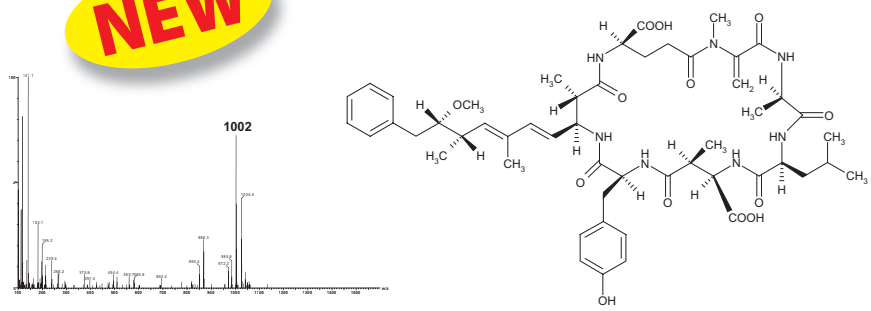
Microcystin-LY

NEW

ALX-350-148-C025	25 µg
ALX-350-148-C100	100 µg

Isolated from *Microcystis aeruginosa*. Analog of microcystin-LR (Prod. No. ALX-350-012) with Tyr substituted in place of Arg.

MS Data Conditions: ESI + / 70 eV.



LIT: The effects of single L-amino acid substitutions on the lethal potencies of the microcystins: R.D. Stoner, et al.; Toxicon **27,** 825 (1989) ▪ Identification and characterization of microcystin-LY from Microcystis aeruginosa (strain 298): S. Rudolph-Bohner, et al.; Biol. Chem. Hoppe Seyler **374,** 635 (1993)

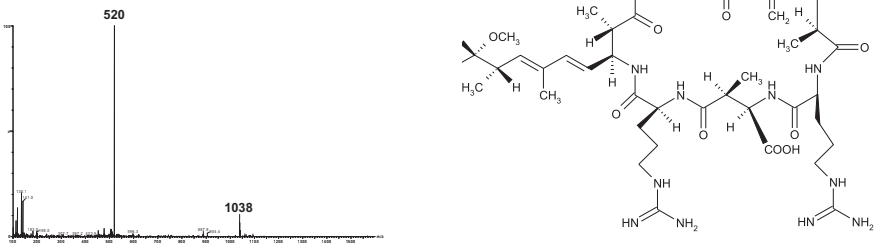
Microcystin-RR

ALX-350-043-C050	50 µg
ALX-350-043-C100	100 µg
ALX-350-043-C250	250 µg
ALX-350-043-C500	500 µg
ALX-350-043-M001	1 mg

Isolated from *Microcystis aeruginosa*. Arg-Arg analog of microcystin-LR (Prod. No. ALX-350-012). Hepatotoxic, although found to be up to 10-fold less toxic than microcystin-LR on i.p. injection in mice.

Potent inhibitor of protein phosphatase 2A (PP2A).

MS Data Conditions: ESI + / 70 eV.



LIT: The structure of a cyclic peptide toxin, cyanogenosin-RR from Microcystis aeruginosa: P. Painuly, et al.; Tetrahedron Lett., 29, 11 (1988) ▪ Toxicity and toxins of natural blooms and isolated strains of Microcystis spp. (Cyanobacteria) and improved procedure for purification of cultures: M. Shirai, et al.; Appl. Environ. Microbiol. **57,** 1241 (1991) ▪ Inhibition of protein phosphatases activates glucose-6-phosphatase in isolated rat hepatocytes: S. Claeyssens, et al.; FEBS Lett. **315,** 7 (1993) ▪ Extraction and high-performance liquid chromatographic method for the determination of microcystins in raw and treated waters: L.A. Lawton, et al.; Analyst **119,** 1525 (1994) ▪ Determination of some physicochemical parameters of microcystins (cyanobacterial toxins) and trace level analysis in environmental samples using liquid chromatography: C. Rivasseau, et al.; J. Chromatogr. A **799,** 155 (1998)

The Reliable Source! *continued*

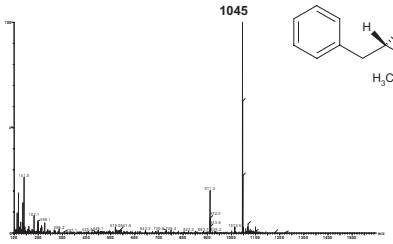
Microcystin-YR

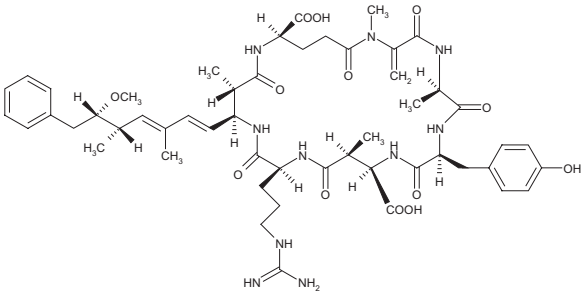
ALX-350-044-C025	25 µg
ALX-350-044-C100	100 µg

Isolated from *Microcystis aeruginosa.* Analog of microcystin-LR (Prod. No. ALX-350-012) with Tyr substituted in place of Leu. As for all microcystins the conjugated double bonds in the ADDA moiety cause a characteristic absorption maximum at 238nm. The Tyr residue in position 2 of microcystin-YR confers an absorption maximum at 232nm. Useful as a reference compound in environmental analysis. The hydroxyl group of the Tyr residue may prove useful for linking microcystin-YR via conjugation to other chemicals.

Potent inhibitor of eukaryotic protein phosphates 1 and 2A.

MS Data Conditions: ESI + / 70 eV.





LIT: Structural studies on cyanoginosins-LR, -YR, -YA, and -YM, peptide toxins from Microcystis aeruginosa: D.P. Botes et al.; J. Chem. Soc., Perkin Transactions, I, 2747 (1985) ▪ Cyanobacteria secondary metabolites - the cyanotoxins: W.W. Carmichael; J. Appl. Bacteriol. **72,** 445 (1992) ▪ Characterization of natural toxins with inhibitory activity against serine/threonine protein phosphatases: R.E. Honkanen, et al.; Toxicon **32,** 339 (1994) ▪ Extraction and high-performance liquid chromatographic method for the determination of microcystins in raw and treated waters: L.A. Lawton, et al.; Analyst **119,** 1525 (1994) ▪ Isolation and characterization of microcystins from laboratory cultures and environmental samples of Microcystis aeruginosa and from an associated animal toxicosis: L.A. Lawton, et al.; Nat. Toxins **3,** 50 (1995) ▪ The cyanotoxins: W.W. Carmichael; Adv. Bot. Res. **27,** 211 (1997) ▪ The toxicology of microcystins: R.M. Dawson; Toxicon **36,** 953 (1998) ▪ Molecular mechanisms underlying inhibition of protein phosphatases by marine toxins: J.F. Dawson and C.F. Holmes; Front. Biosci. **4,** D646 (1999) ▪ Isolation and detection of microcystins and nodularins, cyanobacterial peptide hepatotoxins: J. Meriluoto, et al.; Methods Mol. Biol. **145,** 65 (2000) ▪ Toxicology and evaluation of microcystins: P.K. Lam, et al.; Ther. Drug. Monit. **22,** 69 (2000)

Nodularin

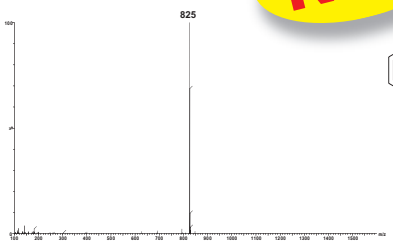
NEW

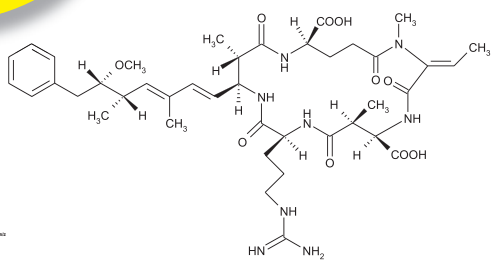
ALX-350-061-C050	50 µg
ALX-350-061-C100	100 µg
ALX-350-061-C250	250 µg
ALX-350-061-M001	1 µg

Isolated from *Nodularia spumigena.*

Inhibitor of protein phosphatase 1 (PP1) (IC_{50}=1.8nM), protein phosphatase 2A (PP2A) (IC_{50}=0.026nM) and to a lesser extent protein phosphatase 2B (PP2B) (IC_{50}=8.7µm). Similar to microcystin-LR (Prod. No. ALX-350-012) but with increased water solubility.

MS Data Conditions: ESI + / 70 eV.





LIT: Nodularin, microcystin and the configuration of Adda: K.L. Rinehart, et al.; JACS **110,** 8557 (1988) ▪ Toxicity and partial structure of a hepatotoxic peptide produced by the cyanobacterium Nodularia spumigena Mertens emend. L575 from New Zealand: W.W. Carmichael, et al.; Appl. Environ. Microbiol. **54,** 2257 (1988) ▪ Inhibition of protein phosphatases by microcystins and nodularin associated with hepatotoxicity: S. Yoshizawa, et al.; J. Cancer Res. Clin. Oncol. **116,** 609 (1990) ▪ Rapid purification of the peptide toxins microcystin-LR and nodularin: C. Martin, et al.; FEMS Microbiol. Lett. **56,** 1 (1990) ▪ *In vitro* and *in vivo* effects of protein phosphatase inhibitors, microcystins and nodularin, on mouse skin and fibroblasts: R. Matsushima, et al.; BBRC **171,** 867 (1990) ▪ Internal surface reversed-phase high-performance liquid chromatographic separation of the cyanobacterial peptide toxins microcystin-LA, -LR, -YR, -RR and nodularin: J.A. Meriluoto, et al.; J. Chromatogr. **509,** 390 (1990) ▪ Cyanobacterial nodularin is a potent inhibitor of type 1 and type 2A protein phosphatases: R.E. Honkanen, et al.; Mol. Pharmacol. **40,** 577 (1991) ▪ Degradation of the cyanobacterial hepatotoxin, nodularin, under light and dark conditions: H. Twist and G.A. Codd; FEMS Microbiol. Lett. **151,** 83 (1997) ▪ Isolation and detection of microcystins and nodularins, cyanobacterial peptide hepatotoxins: J. Meriluoto, et al.; Methods Mol. Biol. **145,** 65 (2000) ▪ Influence of microcystin-YR and nodularin on the activity of some proteolytic enzymes in mouse liver: A. Lankoff and A. Kolataj; Toxicon **39,** 419 (2001) ▪ Nodularin-Har: a new nodularin from Nodularia: K. Saito, et al; J. Nat. Prod. **64,** 139 (2001) ▪ Detection of nodularin in flounders and cod from the Baltic Sea: V. Sipia, et al.; Environ. Toxicol. **16,** 121 (2001)

HPLC Profile

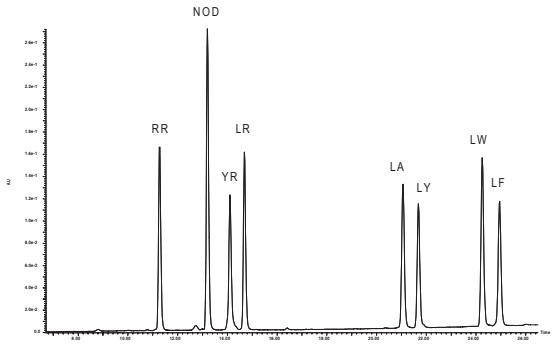


Figure 4: Microcystins/nodularin were separated by HPLC on a Waters Sunfire™ C18 column (2.1 mm ID x 150 mm long; 5 µm particle size) maintained at 40 °C. Mobile phase was Milli-Q water (A) and acetonitrile (B) both containing 0.05% TFA. Components were eluted using a linear gradient from 15% to 65% B over 25 minutes at a flow of 0.3 ml/min.

Hepatotox Set™ 1

ALX-850-325-KI01	1 Set

Set of major microcystins (MC-LA (25µg); MC-LF (25µg); MC-LR (50µg); MC-LW (25µg); MC-LY (25µg); MC-PR (50µg); MC-YR (25µg) and Nodularin (50µg)).

NEW

Detection of Microcystins – Antibodies

MAb to Microcystins (Adda specific) (AD4G2)

ALX-804-585-C100 **100 µg**

CLONE: AD4G2. **ISOTYPE:** Mouse IgG1. **IMMUNOGEN:** N-Acetyl-Adda-KLH and N-Acetyl-Adda-BSA. **SPECIFICITY:** Recognizes all microcystins. **APPLICATION:** ELISA.

With a direct competitive enzyme-linked immunosorbent assay (ELISA) using antibody AD4G2, IC_{50} values for microcystin-LR of 0.06 µg/l have been obtained. The provisional guideline value proposed by the WHO is 1µg/l for drinking water. The detection limit for microcystin-LR is 0.07µg/l. All microcystin variants show similar IC_{50} values and detection limits. No microcystins are known, that are not recognized by this antibody. Microcystin-LR spiked water samples in the concentration range between 0.1 and 1µg/l were measured and a mean recovery of 113 ± 23% was found. The antibody is well suited for the sensitive analysis of all microcystins in drinking as well as surface water. Due to the very even cross-reactivity pattern of the antibody, a calibration with one microcystin (preferably MC-LR); (Prod. No. ALX-350-012) is sufficient for the quantitative determination of a sum concentration of all microcystins contained in a sample.

LIT: Generic microcystin immunoassay based on monoclonal antibodies against Adda: A. Zeck, et al.; Analyst **126**, 2002 (2001) ▪ Multidimensional biochemical detection of microcystins in liquid chromatography: A. Zeck, et al.; Anal. Chem. **73,** 5509 (2001) ▪ Highly sensitive immunoassay based on a monoclonal antibody specific for [4-arginine]microcystins : A. Zeck, et al.; Anal. Chim. Acta **441,** 1 (2001) ▪ Development of a direct competitive microcystin immunoassay of broad specificity: M.G. Weller, et al.; Anal. Sci. **17,** 1445 (2005)

For research purposes only. Due to patent restricitons cannot be used for commercial ELISA development.

Microcystin Derivative	Prod. No.	ALX-804-320 (MC10E7)		ALX-804-585 (AD4G2)	
		Cross-reactivity [%] (molar)	Detection Limit [µg/l]	Cross-reactivity [%] (molar)	Detection L imit [µg/l]
Microcystin-LR	ALX-350-012	100	0.006	100	0.07
[Asp^3]-Microcystin-RR		134	0.006	109	0.03
Microcystin-RR	ALX-350-043	96	0.011	70	0.07
Microcystin-YR	ALX-350-044	68	0.008	129	0.04
Nodularin	ALX-350-061	7	0.095	163	0.03
Microcystin-LY	ALX-350-148	0.07	29*	103	0.06
Microcystin-LF	ALX-350-081	$<10^{-4}$	>1000	69	0.14
Microcystin-LW	ALX-350-080	$<10^{-4}$	>1000	84	0.09
Microcystin-LA	ALX-350-096	$<10^{-4}$	>1000	66	0.06
Adda		$<10^{-4}$	>1000	27	0.09
N-Acetyl-Adda		$<10^{-4}$	>1000	25	0.14
N-Acetyl-Adda-methylamide		n.d.	n.d.	99	0.02

*estimated

MAb to Microcystin-LR (MC10E7)

ALX-804-320-C200 **200 µg**

CLONE: MC10E7. **ISOTYPE:** Mouse IgG1. **IMMUNOGEN:** Microcystin-LR linked via N-methyl-dehydroalanine to cationized ovalbumin. **SPECIFICITY:** Recognizes all 4-Arg microcystins. **APPLICATION:** ELISA.

With a direct competitive enzyme-linked immunosorbent assay (ELISA) using antibody MC10E7, IC_{50} values for microcystin-LR of 0.06 µg/l have been obtained. The provisional guideline value proposed by the WHO is 1 µg/l for drinking water. The detection limit for microcystin-LR is 0.006 µg/l. All microcystin variants containing an arginine at position 4 show similar IC_{50} values and detection limits, whereas other microcystins, such as microcystin-LA are not recognized. The affinity constant for MC10E7 was determined to be at least $7x10^{10}$ l/mol.

The antibody was tested for its robustness against interferences (humic acids, pH, salt content, surfactants or organic solvents) and was found to be very stable. Microcystin-LR spiked water samples in the concentration range between 0.01 and 0.1µg/l were measured and a mean recovery of 99.9±16.4% was found. The antibody is well suited for the sensitive analysis of microcystins in drinking as well as surface water.

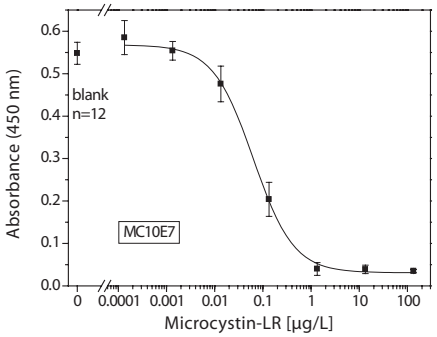


FIGURE: Typical Standard Curve.

LIT: Highly sensitive immunoassay based on a monoclonal antibody specific for [4-arginine]microcystins: A. Zeck, et al.; Anal. Chim. Acta **441,** 1 (2001) ▪ Development of a direct competitive microcystin immunoassay of broad specificity: M.G. Weller, et al.; Anal. Sci. **17,** 1445 (2001)

Microcystins (Adda specific) ELISA Kit

ALX-850-319-KI01 **1 Kit**

Enzyme-linked immunosorbent assay for the congener-independent determination of microcystins and nodularins in water samples.

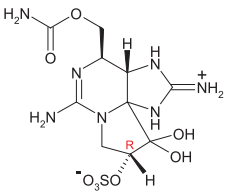
See Backcover for more information.

Other Cyanobacterial Toxins & Related Compounds

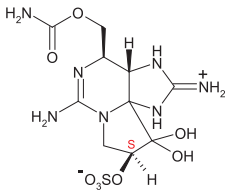
Gonyautoxin 2/3 Epimers

ALX-350-307-C010	10 µg

Isolated from *Alexandrium tamarense*. Epimeric mixture of gonyautoxin 2 (GTX II, C-11α-hydroxysaxitoxinsulfate) and gonyautoxin 3 (GTX III, C-11β-hydroxysaxitoxinsulfate). Equally potent and selective Na^+ channel blockers. Neurotoxin.



Gonyautoxin 2 (GTX II)



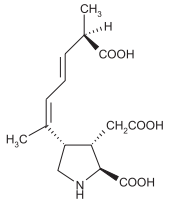
Gonyautoxin 3 (GTX III)

LIT: Letter: Structures of gonyautoxin II and III from the East Coast toxic dinoflagellate Gonyaulax tamarensis: Y. Shimizu, et al.; JACS **98,** 5414 (1976) ▪ Gonyautoxin associated with RNA-containing fraction in the toxic scallop digestive gland: M. Kodama, et al.; J. Biochem. **92,** 105 (1982) ▪ Structure and function of voltage-gated sodium channels: E. Marban, et al.; J. Physiol. **508 (Pt 3),** 647 (1998) ▪ Toxicokinetics and toxicodynamics of gonyautoxins after an oral toxin dose in cats: D. Andrinolo, et al.; Toxicon **40,** 699 (2002) ▪ The gonyautoxin 2/3 epimers reduces anal tone when injected in the anal sphincter of healthy adults: R. Garrido, et al.; Biol. Res. **37,** 395 (2004) ▪ Gonyautoxin: new treatment for healing acute and chronic anal fissures: R. Garrido, et al.; Dis. Colon Rectum **48,** 335 (2005)

Domoic acid

ALX-550-152-M001	1 mg

Isolated from *Nitzschia pungens f. multiseries*. Glutamate/kainate excitatory amino acid agonist with highest affinity for the kainate receptor of all known kainic acid analogs.



LIT: Structure-activity relations of excitatory amino acids on frog and rat spinal neurones: T.J. Biscoe, et al.; Br. J. Pharmacol. **58,** 373 (1976) ▪ A kainic acid receptor from frog brain purified using domoic acid affinity chromatography: D.R. Hampson & R.J. Wenthold; J. Biol. Chem. **263,** 2500 (1988) ▪ Identification of domoic acid, a neuroexcitatory amino acid, in toxic mussels from eastern Prince Edward Island: P. Wright, et al.; Can. J. Chem. **67,** 481 (1989) ▪ Domoic acid, the alleged „mussel toxin," might produce its neurotoxic effect through kainate receptor activation: an electrophysiological study in the dorsal hippocampus: G. Debonnel, et al.; Can. J. Physiol. Pharmacol. **67,** 29 (1989) ▪ Domoic acid: a dementia-inducing excitotoxic food poison with kainic acid receptor specificity: G.R. Stewart, et al.; Exp. Neurol. **110,** 127 (1990) ▪ Pharmacology of systemically administered domoic acid in mice: R.A. Tasker, et al.; Can. J. Physiol. Pharmacol. **69,** 378 (1991) ▪ Transfer constants for blood-brain barrier permeation of the neuroexcitatory shellfish toxin, domoic acid: E. Preston & I. Hynie; Can. J. Neurol. Sci. **18,** 39 (1991) ▪ Interaction of domoic acid and several derivatives with kainic acid and AMPA binding sites in rat brain: D.R. Hampson, et al.; Eur. J. Pharmacol. **218,** 1 (1992) ▪ Parenteral domoic acid impairs spatial learning in mice: B.F. Petrie, et al.; Pharmacol. Biochem. Behav. **41,** 211 (1992) ▪ **For a comprehensive bibliography please visit our website.**

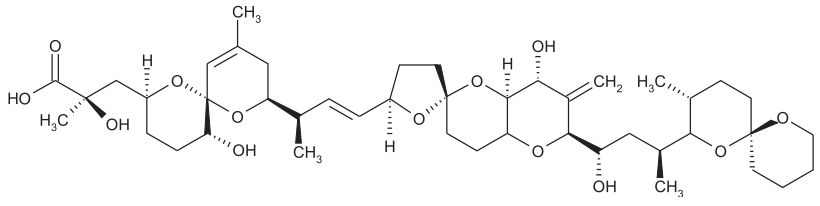
Okadaic acid (high purity)

Okadaic acid (high purity)

ALX-350-003-C025	25 µg
ALX-350-003-C050	50 µg
ALX-350-003-C100	100 µg
ALX-350-003-M001	1 mg

Isolated from *Prorocentrum concavum*. Potent inhibitor of protein phosphatases 1 (PP1) and 2A (PP2A) in numerous cell types. Does not affect activity of acid phosphatases, alkaline phosphatases and tyrosine phosphatases. Non-phorbol type tumor promoter. Induces apoptosis in human breast carcinoma cells (MB-231 and MCF-7) and in myeloid cells, but inhibits glucocorticoid-induced apoptosis in T cell hybridomas. Has shown contractile effect on smooth and heart muscles.

LIT: For a comprehensive bibliography please visit our website.



Okadaic Acid Salt Forms

Salt form of okadaic acid (Prod. No. ALX-350-003), with slightly greater stability than the free acid after it is put into stock solution (in organic solvents).

Okadaic acid . ammonium salt (high purity)

ALX-350-010-C025	25 µg
ALX-350-010-C100	100 µg
ALX-350-010-M001	1 mg

Okadaic acid . potassium salt (high purity)

ALX-350-063-C050	50 µg
ALX-350-063-C100	100 µg
ALX-350-063-M001	1 mg

Okadaic acid . sodium salt (high purity)

ALX-350-011-C025	25 µg
ALX-350-011-C100	100 µg
ALX-350-011-M001	1 mg

New Antibody

PAb to Okadaic Acid

CVL-PAB0021-1	200 µl

From rabbit. **IMMUNOGEN:** Okadaic acid conjugated to ovalbumin. **SPECIFICITY:** Recognizes okadaic acid. **APPLICATION:** ELISA (1:5'000-1:20'000).

Detection of Microcystins – ELISA Kit

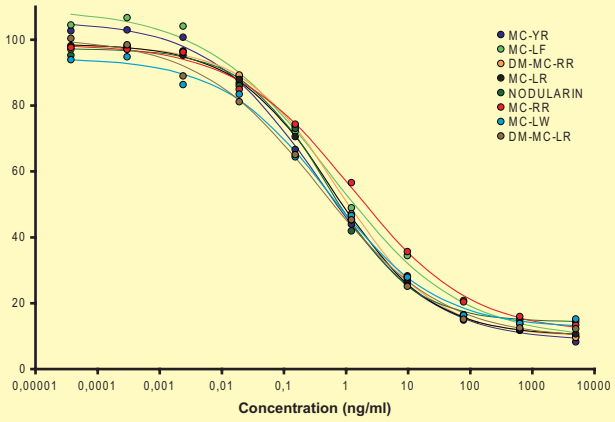


FIGURE: Cross-reactivity pattern against microcystins and nodularin congeners.

NEW

Microcystins (Adda specific) ELISA Kit

ALX-850-319-KI01 **1 Kit**

- ***Enzyme-linked immunosorbent assay for the congener-independent determination of microcystins and nodularins in water samples.***
- ***Detection Limit: 0.1 µg/l (range 0.15 – 5 µg/l)***
- ***Does not cross-react with other non-related toxins or compounds.***
- ***No pre-sample preparation required.***
- ***96-well microplate format with ready-to-use reagents.***
- ***Total time for measurement is less than 2.5 hours.***
- ***Enables simultaneous measurement of multiple samples at reasonable costs.***

U.S. PATENT 6,967,240

WOLDWIDE PATENT PCT WO 01/18059 A2

Manufactured by Abraxis, LLC.

LIT: Congener-independent immunoassay for microcystins and nodularins: W.J. Fischer, et al.; Environ. Sci. Technol. **35,** 4849 (2001) ▪ A review of analytical methods for assessing the public health risk from microcystin in the aquatic environment: P.R. Hawkins, et al.; J. Water Supply **54,** 509 (2005)

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