Toxins from Cyanobacteria

Cyanobacteria (blue-green algae) are a diverse group of photo-autotrophic organisms which are found in terrestrial and aquatic environments. 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 (cyllindrospermopsin), dermatoxins (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].

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, desmethylation, and amino acid substitutions. The number of variants/congeners is over 70, creating a challenge for selection/development of robust methods for their detection.

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 accumulation in the liver they are involved in cytoskeletal disorganization, lipid peroxidation, loss of membrane integrity, DNA fragmentation, cell blebbing, apoptosis, cellular disruption, and necrosis.

CONTINUED ON NEXT PAGE
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 1µg/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].

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 complementary tool to microcystins for studying cellular processes.

Cylindrospermopsin

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

Selected Review Articles


www.enzolifesciences.com
The Widest Panel of Microcystins!

<table>
<thead>
<tr>
<th>Produc. No.</th>
<th>Name</th>
<th>Monoisotopic Mass</th>
<th>MW</th>
<th>LD50 (Mouse Intraperitoneal)</th>
<th>Isolated from</th>
<th>X (2)</th>
<th>Y (4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALX-350-096</td>
<td>Microcystin-LA</td>
<td>909</td>
<td>910.1</td>
<td>50</td>
<td>Microcystis aeruginosa</td>
<td>Leu</td>
<td>Ala</td>
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<td>ALX-350-081</td>
<td>Microcystin-LF</td>
<td>985</td>
<td>986.2</td>
<td>toxic</td>
<td>Microcystis aeruginosa</td>
<td>Leu</td>
<td>Phe</td>
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<td>ALX-350-012</td>
<td>Microcystin-LR</td>
<td>994</td>
<td>995.2</td>
<td>50</td>
<td>Microcystis aeruginosa</td>
<td>Leu</td>
<td>Arg</td>
</tr>
<tr>
<td>ALX-350-080</td>
<td>Microcystin-LW</td>
<td>1024</td>
<td>1025.2</td>
<td>not determined</td>
<td>Microcystis aeruginosa</td>
<td>Leu</td>
<td>Trp</td>
</tr>
<tr>
<td>ALX-350-148</td>
<td>Microcystin-LY</td>
<td>1001</td>
<td>1002.2</td>
<td>90</td>
<td>Microcystis aeruginosa</td>
<td>Leu</td>
<td>Tyr</td>
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<tr>
<td>ALX-350-043</td>
<td>Microcystin-RR</td>
<td>1037</td>
<td>1038.2</td>
<td>600</td>
<td>Microcystis aeruginosa</td>
<td>Arg</td>
<td>Arg</td>
</tr>
<tr>
<td>ALX-350-044</td>
<td>Microcystin-YR</td>
<td>1044</td>
<td>1045.2</td>
<td>70</td>
<td>Microcystis aeruginosa</td>
<td>Tyr</td>
<td>Arg</td>
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<tr>
<td>ALX-350-167</td>
<td>Microcystin-WR</td>
<td>1067</td>
<td>1068.3</td>
<td>150-200</td>
<td>Microcystis aeruginosa</td>
<td>Trp</td>
<td>Arg</td>
</tr>
</tbody>
</table>

**TABLE:** Overview on selected microcystin derivatives.

**Microcystin-LR**

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.

Equally potent and selective inhibitor of protein phosphatase 1 (PP1) and 2A (PP2A).

**MS Data Conditions:** ESI + / 70 eV.

**The Widest Panel of Microcystins!**

**Microcystin-LR (desmethylated) [[D-Asp3]microcystin-LR]**

ALX-350-173-C025 25 µg
ALX-350-173-C100 100 µg
Isolated from Microcystis aeruginosa. Microcystin containing a leucine (L) in position 2 and arginine (R) in position 4. Additionally, the D-MeAsp at position 3 is demethylated to Asp. Hepatotoxic.

NEW
The Widest Panel of Microcystins!

**Microcystin-LA**

<table>
<thead>
<tr>
<th>ALX-350-096-C025</th>
<th>25 µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALX-350-096-C100</td>
<td>100 µg</td>
</tr>
</tbody>
</table>

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:**

**Microcystin-LF**

<table>
<thead>
<tr>
<th>ALX-350-081-C025</th>
<th>25 µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALX-350-081-C100</td>
<td>100 µg</td>
</tr>
</tbody>
</table>

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:**

**Microcystin-LW**

<table>
<thead>
<tr>
<th>ALX-350-080-C025</th>
<th>25 µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALX-350-080-C100</td>
<td>100 µg</td>
</tr>
</tbody>
</table>

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 222 nm, whereas most microcystins have a characteristic maximum at 239 nm. 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:**
Microcystin-RR

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.


Microcystin-LY

ALX-350-043-C025 25 µg
ALX-350-043-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.


International Edition
**Microcystin-WR**

ALX-350-167-C025 25 µg
ALX-350-167-C100 100 µg

Isolated from *Microcystis aeruginosa*.  
**MS Data Conditions:** ESI + / 70 eV.

**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 phosphatases 1 and 2A.**  
**MS Data Conditions:** ESI + / 70 eV.

**Microcystin-HtyR**

ALX-350-174-C025 25 µg
ALX-350-174-C100 100 µg

Isolated from *Microcystis aeruginosa*. Microcystin containing a homotyrosine (Hty) in position 2 and arginine (R) in position 4. Hepatotoxic.

**Microcystin-LR (methylated)**

ALX-350-169-C025 25 µg
ALX-350-169-C100 100 µg

COMING SOON

For a comprehensive bibliography please visit our website.
Detection of Microcystins – ELISA Kit

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: 0.1µg/l (range 0.15 – 5µg/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
Worldwide patent PCT WO 01/18059 A2
Manufactured by Abraxis LLC.

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

Microcystin Coated Tube Kit

ALX-850-333-KI01 1 Kit

SENSITIVITY: 0.15ppb (range 0 to 5ppb).
APPLICATION: For the quantitative detection of microcystins in water samples.
Total time for measurement is less than 2 hours. Enables simultaneous measurement of multiple samples at reasonable costs.
Manufactured by Abraxis LLC.
Detection of Microcystins – Antibodies

Microcystins (Adda specific), mAb (AD4G2)

ALX-804-585-C100 100 µg


With a direct competitive enzyme-linked immunosorbent assay (ELISA) using antibody AD4G2, IC50 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 0.07 µg/l. All microcystin variants show similar IC50 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 1 µg/l were measured and a mean recovery of 113 ± 23% was found.

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


<table>
<thead>
<tr>
<th>Microcystin Derivative</th>
<th>Prod. No.</th>
<th>ALX-804-320 (MC10E7)</th>
<th>ALX-804-585 (AD4G2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microcystin-LR</td>
<td>100</td>
<td>0.006</td>
<td>100</td>
</tr>
<tr>
<td>[Asp³]microcystin-RR</td>
<td>134</td>
<td>0.006</td>
<td>109</td>
</tr>
<tr>
<td>Microcystin-RR</td>
<td>96</td>
<td>0.011</td>
<td>70</td>
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<tr>
<td>Microcystin-YR</td>
<td>68</td>
<td>0.008</td>
<td>129</td>
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<tr>
<td>Nodularin</td>
<td>7</td>
<td>0.095</td>
<td>163</td>
</tr>
<tr>
<td>Microcystin-LY</td>
<td>0.07</td>
<td>29*</td>
<td>103</td>
</tr>
<tr>
<td>Microcystin-LF</td>
<td>&lt;10⁴</td>
<td>&gt;1000</td>
<td>69</td>
</tr>
<tr>
<td>Microcystin-LW</td>
<td>&lt;10⁴</td>
<td>&gt;1000</td>
<td>84</td>
</tr>
<tr>
<td>Microcystin-LA</td>
<td>&lt;10⁴</td>
<td>&gt;1000</td>
<td>66</td>
</tr>
<tr>
<td>Adda</td>
<td>&lt;10⁴</td>
<td>&gt;1000</td>
<td>27</td>
</tr>
<tr>
<td>N-Acetyl-Adda</td>
<td>&lt;10⁴</td>
<td>&gt;1000</td>
<td>25</td>
</tr>
<tr>
<td>N-Acetyl-Adda-methylamide</td>
<td>n.d.</td>
<td>n.d.</td>
<td>99</td>
</tr>
</tbody>
</table>

Microcystin-LR, mAb (MC10E7)

ALX-804-320-C200 200 µg

CLONE: MC10E7. ISO TYPE: 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, IC50 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 IC50 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 7x10¹⁰ 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 0.1 µg/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.

Other Cyanotoxins & Related Products

**Cyanopeptolins**

Cyanopeptolins are cyclic non-ribosomal peptides isolated from various cyanobacteria. Cyanopeptolins have been reported to inhibit serine proteases such as trypsin and chymotrypsin.

**Cyanopeptolin 1007**

- ALX-350-399-C100 100 µg
  - Isolated from *Microcystis aeruginosa*.

**Cyanopeptolin 1041**

- ALX-350-400-C100 100 µg
  - Isolated from *Microcystis aeruginosa*.

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**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.

**Cylindrospermopsin ELISA Kit**

- ALX-850-332-KI01 1 Kit
  - No pre-sample preparation required.
  - Total time for measurement is less than 90 minutes.
  - Enables simultaneous measurement of multiple samples at reasonable costs.
  - **SENSITIVITY:** 0.04 ng/ml (range 0.05 to 2 ng/ml).
  - **APPLICATION:** For the quantitative and sensitive detection of cylindrospermopsin in water samples.

*Manufactured by Abraxis LLC.*
Other Cyanotoxins & Related Products

**Domoic Acid**

**BML-EA117-0001 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:**
- For a comprehensive bibliography please visit our website.

**Gonyautoxin 2/3 Epimers**

**ALX-350-307-1 1 Vial**

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

**LIT:**
- For a comprehensive bibliography please visit our website.

**Saxitoxin ELISA Kit**

**ALX-850-334-KI01 1 Kit**

The assay sensitivity allows the determination of saxitoxin in a range of environmental samples (water, mussels, etc.). Total time for measurement is 60 minutes. Enables simultaneous measurement of multiple samples at reasonable costs.

**SENSITIVITY:** 0.015ng/ml (range 0.02 to 4ng/ml).

**APPLICATION:** For the quantitative and sensitive detection of saxitoxin in water or other contaminated samples.

**Manufactured by Abraxis LLC.**

**Nodularin**

**ALX-350-061-C050**

**ALX-350-061-C100**

**ALX-350-061-C250**

**ALX-350-061-M001 1 mg**

Isolated from *Nodularia spumigena*. Inhibitor of protein phosphatase 1 (PP1) (IC50=1.8nM), protein phosphatase 2A (PP2A) (IC50=0.026nM) and to a lesser extent protein phosphatase 2B (PP2B) (IC50=8.7µm). Similar to microcystin-LR (Prod. No. ALX-350-012) but with increased water solubility.

**MS Data Conditions: ESI + / 70 eV.**

**LIT:**
- For a comprehensive bibliography please visit our website.
Okadaic Acid

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 *Prorocentum 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 (MCF-7) and in myeloid cells, but inhibits glucocorticoid-induced apoptosis in T cell hybridomas. Has shown contractile effect on smooth and heart muscles.


Okadaic acid C8-diol ester
ALX-350-392-C100 100 µg

Isolated from *Prorocentrum lima*. Fragment of a precursor to okadaic acid. Inactive against protein phosphatases in vitro. Potent toxin to whole cells, suggesting transport across the cell membrane and cleavage of the ester bond by cellular esterases.


Okadaic acid methyl ester
ALX-350-039-C100 100 µg

Isolated from *Prorocentrum lima*. Okadaic acid derivative which is inactive against protein phosphatases. May be metabolized to okadaic acid in whole cell assays. Semisynthetic derivative of okadaic acid.


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

Okadaic Acid ELISA Kit

ALX-850-335-KI01 1 Kit

Total time for measurement is less than 2 hours. Enables simultaneous measurement of multiple samples at reasonable costs.

**SENSITIVITY:** 0.1 µg/g (range 0.2 to 5 µg/g).

**APPLICATION:** For the quantitative and sensitive detection of okadaic acid in water or shellfish samples.

*Manufactured by Abraxis LLC.*
Microginins

Microginins are a class of small, linear non-ribosomal peptides isolated from various cyanobacteria, primarily Microcystis aeruginosa. The microginins act as inhibitors against zinc metalloproteases such as angiotensin-converting enzyme and leucine aminopeptidase.

<table>
<thead>
<tr>
<th>Product</th>
<th>Prod. No.</th>
<th>Isolated from</th>
<th>Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microginin 527</td>
<td>ALX-350-394-C100</td>
<td>Microcystis aeruginosa</td>
<td>100 µg</td>
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<tr>
<td>Microginin 527 methyl ester</td>
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<td>Microginin 690</td>
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<td>100 µg</td>
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<tr>
<td>Microginin 690 methyl ester</td>
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<td>100 µg</td>
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<td>Microginin 704</td>
<td>ALX-350-398-C100</td>
<td>Microcystis aeruginosa</td>
<td>100 µg</td>
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</tbody>
</table>

Microginin 527

Microginin 527 methyl ester

Microginin 690

Microginin 690 methyl ester

Microginin 704

Hepatotox Set™ 1

ALX-850-325-KI01 1 Set

Set of major microcystins:

- MC-LA (25µg) MC-LY (25µg)
- MC-LF (25µg) MC-PR (50µg)
- MC-LR (50µg) MC-YR (25µg)
- MC-LW (25µg) Nodularin (50µg)