

### Importance of Microcystins/Nodularins Determination

Most of the world's population relies on surface freshwaters as its primary source for drinking water. The drinking water industry is constantly challenged with surface water contaminants that must be removed to protect human health. Toxic cyanobacterial blooms are an emerging issue worldwide due to increased source water nutrient pollution caused by eutrophication. Microcystins and Nodularins are cyclic toxin peptides. Microcystins (of which there are many structural variants, or congeners) have been found in fresh water throughout the world. To date, approximately 80 variants of Microcystin have been isolated. The most common variant is Microcystin-LR. Other common Microcystin variants include YR, RR, and LW. These toxins are produced by many types of cyanobacteria (blue-green algae), including *Microcystis*, *Anabaena*, *Oscillatoria*, *Nostoc*, *Anabaenopsis*, and terrestrial *Hapalosiphon*. Nodularins are produced by the genus *Nodularia* and are found in marine and brackish water.

Acute poisoning of humans and animals constitutes the most obvious problem from toxic cyanobacterial blooms, and in several cases has led to death. Human and animal exposure to these toxins occurs most frequently through ingestion of water, through drinking or during recreational activities in which water is swallowed. These toxins mediate their toxicity by inhibiting liver function and are potent inhibitors of the serine/threonine protein phosphatases, and therefore may act as tumor promoters.

To protect consumers from adverse health effects caused by these toxins, the World Health Organization (WHO) has proposed a provisional upper limit for Microcystin-LR of 1.0 ppb (µg/L) in drinking water.

### Performance Data

Test sensitivity: The detection limit for this assay, based on MC-LR, is 0.10 ppb (µg/L).

Test reproducibility: Coefficients of variation (CVs) for standards: <10%; for samples: <15%.

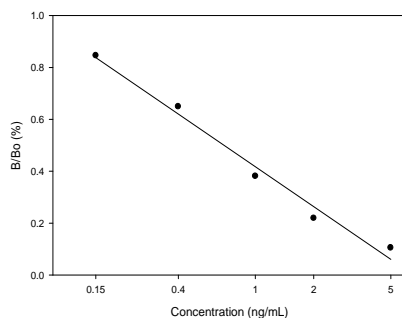
Selectivity\*: The assay exhibits very good cross-reactivity with all cyanobacterial cyclic peptide toxin congeners tested to date (see Specificity table below).

#### Specificity

The cross-reactivity of the Abraxis Microcystins-DM ELISA for various Microcystins congeners can be expressed as the least detectable dose (LDD) (90% B/Bo), or as the dose required for 50% absorbance inhibition (50% B/Bo).

Compound	LDD (ppb)	50% B/Bo (ppb)	X-reactivity (%)
Microcystin-LR	0.093	0.66	100
Microcystin-LW	0.080	0.65	102
Microcystin-LF	0.110	0.92	72
Microcystin-YR	0.120	1.03	76
Microcystin-RR	0.193	1.24	67
Microcystin-LA	0.210	1.39	66
Nodularins	0.05	0.87	78
N-hemi-ADDA	0.105	1.80	38
ADDA	0.62	4.85	15
D-Phenylalanine	NR	NR	NR
L-Phenylalanine	NR	NR	NR
DL-Phenylalanine	NR	NR	NR

NR = no reactivity up to 1000 ppb



Samples: Sample correlation between the ELISA and HPLC showed a good correlation.

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## Microcystins-DM ELISA (Microtiter Plate)

Enzyme-Linked Immunosorbent Assay for the Determination of Microcystins and Nodularins in Water Samples



Product No. 522015

### 1. General Description

The Abraxis Microcystins-DM (direct monoclonal) ELISA is an immunoassay for the quantitative and sensitive detection of Microcystins and Nodularins in water samples. No additional sample preparation is required prior to analysis. If necessary, positive samples can be confirmed by HPLC, protein phosphatase assay, or other conventional methods.

### 2. Safety Instructions

The standard solutions in the test kit contain small amounts of Microcystins. The substrate solution contains tetramethylbenzidine (TMB) and the stop solution contains diluted sulfuric acid. Avoid contact of the TMB and stopping solution with skin and mucous membranes. If these reagents come in contact with skin, wash with water.

### 3. Storage and Stability

The Microcystins-DM ELISA kit should be stored in the refrigerator (4–8°C). The solutions must be allowed to reach room temperature (20–25°C) before use. Reagents may be used until the expiration date on the box. Consult state, local, and federal regulations for proper disposal of all reagents.

### 4. Test Principle

The test is a direct competitive ELISA for the detection of Microcystins and Nodularins. It is based on the recognition of Microcystins, Nodularins, and their congeners by a monoclonal antibody. Toxin, when present in a sample, and a Microcystins-HRP analogue compete for the binding sites of anti-Microcystins antibodies in solution. The anti-Microcystins antibodies are then bound by a second antibody (goat anti-mouse) immobilized on the wells of the microtiter plate. After a washing step and addition of the substrate solution, a color signal is generated. The intensity of the blue color is inversely proportional to the concentration of Microcystins present in the sample. The color reaction is stopped after a specified time and the color is evaluated using an ELISA reader. The concentrations of the samples are determined by interpolation using the standard curve constructed with each run.

### 5. Limitations of the Microcystins-DM ELISA, Possible Test Interference

Numerous organic and inorganic compounds commonly found in water samples have been tested and found not to interfere with this test. However, due to the high variability of compounds that might be found in water samples, test interferences caused by matrix effects cannot be completely excluded.

The presence of the following substances were found to have no significant effect on the Microcystins-DM assay results: calcium sulfate, magnesium sulfate, sodium chloride, magnesium chloride, sodium nitrate, potassium phosphate, calcium chloride, manganese sulfate, and aluminum oxide up to 10,000 ppm; copper chloride, sodium fluoride, sodium thiosulfate, ferric sulfate, and zinc sulfate up to 1,000 ppm; humic acid up to 10 ppm; Lugol's solution up to 0.01%.

Samples containing methanol must be diluted to a concentration ≤ 20% methanol to avoid matrix effects.

Seawater samples must also be diluted to a concentration ≤ 20% to avoid matrix effects. Alternately, if a lower detection limit is required, interfering compounds can be removed from seawater or brackish water samples prior to analysis. Please see the Microcystins in Brackish Water or Seawater Sample Preparation for the Microcystins-DM ELISA Technical Bulletin (available upon request).

Removal of chlorine from tap water samples prior to analysis is not necessary. Also, no matrix effects have been observed with samples which have been treated with sodium thiosulfate at concentrations up to and including 1 mg/mL.

Mistakes in handling the test can cause errors. Possible sources for such errors can include: inadequate storage conditions of the test kit, incorrect pipetting sequence or inaccurate volumes of the reagents, too long or too short incubation times during the immune and/or substrate reaction, extreme temperatures during the test performance (lower than 10°C or higher than 30°C).

As with any analytical technique (GC, HPLC, etc.), positive results requiring regulatory action should be confirmed by an alternative method.

### A. Materials Provided

1. Microtiter plate (12 X 8 strips) coated with a second antibody (goat anti-mouse)
2. Standards (6) and Control (1): 0, 0.15, 0.40, 1.0, 2.0, 5.0 ppb; Control at  $0.75 \pm 0.185$  ppb
3. Sample Diluent, 25 mL (for dilution of samples above the range of the curve)
4. Microcystins-HRP Conjugate Solution, 6 mL
5. Microcystins-DM Antibody Solution (monoclonal anti-Microcystins), 6 mL
6. Wash Solution (5X) Concentrate, 100 mL, must be diluted prior to use, see Test Preparation (Section D)
7. Substrate (Color) Solution (TMB), 16 mL
8. Stop Solution, 12 mL

### B. Additional Materials (not delivered with the test kit)

1. Micro-pipettes with disposable plastic tips (20-200  $\mu$ L)
2. Multi-channel pipette (50-300  $\mu$ L) or stepper pipette with plastic tips (50-300  $\mu$ L)
3. Deionized or distilled water
4. Paper towels or equivalent absorbent material
5. Timer
6. Tape or parafilm
7. Microtiter plate reader (wavelength 450 nm)
8. Microtiter plate washer (optional)

### C. Sample Collection and Handling

Collect water samples in glass containers and test within 24 hours. If samples must be held for longer periods (up to 5 days), samples should be stored refrigerated. For storage periods greater than 5 days, samples should be stored frozen.

If total Microcystins concentration (free and cell bound) is required, an appropriate cell lysing procedure (freeze and thaw, sonication, QuikLyse™, etc.) must be performed prior to analysis.

### D. Test Preparation

Micro-pipetting equipment and pipette tips for pipetting the standards and samples are necessary. A multi-channel pipette or a stepping pipette is recommended for the addition of the antibody, enzyme conjugate, substrate, and stop solutions in order to equalize the incubation periods on the entire microtiter plate. Please only use the reagents and standards from one kit lot in one test, as they have been adjusted in combination.

1. Allow the reagents and samples to reach ambient temperature before use.
2. Remove the number of microtiter plate strips required from the resealable pouch. The remaining strips are stored in the pouch with the desiccant (tightly closed).
3. The standards, control, sample diluent, enzyme conjugate, antibody, substrate, and stop solutions are ready to use and do not require any further dilutions.
4. Dilute the Wash Solution (5X) Concentrate at a ratio of 1:5 with deionized or distilled water. If using the entire bottle (100 mL), add to 400 mL of deionized or distilled water and mix thoroughly.
5. The stop solution must be handled with care as it contains diluted  $H_2SO_4$ .

### E. Working Scheme

The microtiter plate consists of 12 strips of 8 wells, which can be used individually for the test. The standards must be run with each test. Never use the values of standards which have been determined in a test performed previously.

Std0-Sd5: Standards  
Contr.: Control

Samp1, Samp2, etc.: Samples

	1	2	3	4	5	6	7	8	9	10	11	12
A	Std 0	Std 4	Samp2									
B	Std 0	Std 4	Samp2									
C	Std 1	Std 5	etc.									
D	Std 1	Std 5	etc.									
E	Std 2	Contr.										
F	Std 2	Contr.										
G	Std 3	Samp1										
H	Std 3	Samp1										

### F. Assay Procedure

1. Add **100  $\mu$ L of the standard solutions, control, or samples** into the wells of the test strips according to the working scheme given. Analysis in duplicate or triplicate is recommended.
2. Add **50  $\mu$ L of the enzyme conjugate solution** to the individual wells successively using a multi-channel pipette or a stepping pipette.
3. Add **50  $\mu$ L of the antibody solution** to the individual wells successively using a multi-channel pipette or a stepping pipette. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop for 30 seconds. Be careful not to spill the contents. Incubate the strips for **90 minutes** at room temperature.
4. Remove the covering and decant the contents of the wells into a sink. Wash the strips **three times** using the 1X wash buffer solution. Please use at least a volume of **250  $\mu$ L of wash buffer** for each well and each washing step. Remaining buffer in the wells should be removed by patting the plate dry on a stack of paper towels.
5. Add **150  $\mu$ L of substrate (color) solution** to the individual wells successively using a multi-channel pipette or a stepping pipette. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop for 30 seconds. Be careful not to spill the contents. Incubate the strips for **20-30 minutes** at room temperature. Protect the strips from sunlight.
6. Add **100  $\mu$ L of stop solution** to the wells in the same sequence as for the substrate (color) solution using a multi-channel pipette or a stepping pipette.
7. Read the absorbance at 450 nm using a microplate ELISA photometer within 15 minutes after the addition of the stopping solution.

### G. Evaluation

The evaluation of the ELISA can be performed using commercial ELISA evaluation programs such as 4-Parameter (preferred) or Logit/Log. For a manual evaluation, calculate the mean absorbance value for each of the standards. Calculate the  $\%B/B_0$  for each standard by dividing the mean absorbance value of each standard by the Zero Standard (Standard 0) mean absorbance. Construct a standard curve by plotting the  $\%B/B_0$  for each standard on a vertical linear (y) axis versus the corresponding Microcystins concentration on the horizontal logarithmic (x) axis on graph paper.  $\%B/B_0$  for the control and samples will then yield levels in ppb of Microcystins by interpolation using the standard curve. Results can also be determined using a spreadsheet macro available from Abraxis upon request.

The concentrations of the samples are determined using the standard curve run with each test. Samples showing a lower concentration of Microcystins than standard 1 (0.15 ppb) should be reported as containing  $< 0.15$  ppb of Microcystins. Samples showing a higher concentration than standard 5 (5.0 ppb) must be diluted to obtain accurate results. The concentration of the positive control provided should be  $0.75 \pm 0.185$  ppb.

Semi-quantitative results can be derived by simple comparison of the sample absorbances to the absorbances of the calibrators. Samples with lower absorbances than a calibrator will have concentrations of Microcystins greater than that calibrator. Samples which have higher absorbances than a calibrator will have concentrations of Microcystins less than that calibrator.

### H. References

- (1) M. G. Weller, A. Zeck, A. Eikenberg, S. Nagata, Y. Ueno, and R. Niessner, Development of a Direct Competitive Microcystins Immunoassay of Broad Specificity. Analytical Sciences. 17, 2001, 1445-1448.
- (2) Worldwide Patenting PCT WO 01/18059 A2.
- (3) U.S. Patent Number 6,967,240.

### I. Sample Recoveries and Assay Precision

#### Recovery

Four (4) groundwater samples were spiked with various levels of Microcystin-LR and assayed using the Abraxis Microcystins-DM Assay:

Spike Level (ppb)	Recovery		
	Mean (ppb)	Std.Dev. (ppb)	Recovery (%)
0.25	0.237	0.019	95
0.50	0.480	0.036	96
1.0	0.959	0.024	96
2.0	1.919	0.067	96
Average			96

#### Precision

Control	1	2	3
Replicates	5	5	5
Days	3	3	3
n	15	15	15
Mean (ppb)	0.25	0.99	2.9
% CV (within)	5.3	3.6	3.0
% CV (between)	6.5	5.4	4.0