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 cyanobacteria (blue-green algae) blooms are an emerging issue in the U.S. and the world because of increased source water nutrient pollution caused by eutrophication. Microcystins and Nodularins are cyclic toxin peptides. Microcystins (several structural variants or congeners are found) have been found in fresh water throughout the world, they are produced by the genus *Microcystis, Anabaena, Oscillatoria, Nostoc, Anabaenopsis,* and terrestrial *Hapalosiphon.* Nodularins are produced by the genus Nodularia and they are found in marine and brackish water. To date, approximately 80 variants of microcystins have been isolated, the most common variant is microcystin-LR. Other common microcystin variants include LA, YR, RR, and LW.

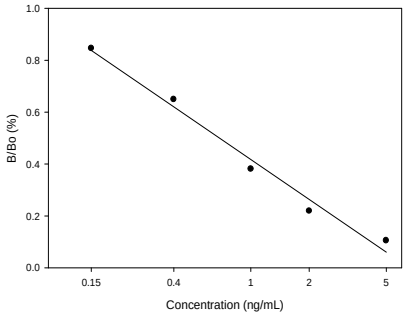
Acute poisoning of humans and animals constitutes the most obvious problem from toxic cyanobacterial blooms, and in several cases has lead to death. These toxins mediate their toxicity by inhibiting liver function and are potent inhibitors of the serine/threonine protein phosphatases, therefore they might act as tumor promoters. To protect consumers from adverse health effects caused by these toxins, the WHO has proposed a provisional upper limit for microcystin-LR of 1.0 ppb (ng/L) in drinking water.

Performance Data

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



Specificity

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

Compound	LDD (ppb)	50% B/Bo (ppb)	X-reactivity (%)
Microcystins LR	0.093	0.66	100
Microcystins LW	0.080	0.65	102
Microcystins LF	0.110	0.92	72
Microcystins YR	0.120	1.03	76
Microcystins RR	0.193	1.24	67
Microcystins 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: A sample correlation between the ELISA and HPLC showed a good correlation.

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R111410

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. A pre-sample concentration is not required. If necessary, positive samples can be confirmed by HPLC, protein phosphatase assay or other conventional methods.

2. Safety Instructions

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 the skin, wash with water.

3. Storage and Stability

The microcystins ELISA should be stored in the refrigerator (4–8°C). Solutions should 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 that allows the detection of microcystins and nodularins. It is based on the recognition of microcystins, nodularins and their congeners by a monoclonal antibody. Microcystins, nodularins and their congeners when present in a sample, and a microcystins-HRP analogue compete for the binding sites of anti-microcystins antibodies in solution. The microcystins antibodies are then bound by a second antibody (goat anti-mouse) immobilized in the 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 the microcystins present in the sample. The color reaction is stopped after a specified time and the color is evaluated using an ELISA reader.

5. Limitations of the Microcystins 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 can't be completely excluded.
The presence of the following substances up to 10,000 ppm were found to have no significant effect on the Microcystins Assay results: calcium sulfate, magnesium sulfate, sodium chloride, magnesium chloride, sodium nitrate, phosphate, calcium chloride, manganese sulfate, aluminum oxide. Copper chloride, sodium fluoride, sodium thiosulfate, ferric sulfate, zinc sulfate up to 1,000 ppm. Humic acid up to 10 ppm. Lugol's solution up to 0.01%. Salt water up to 100%.

Mistakes in handling the test can also cause errors. Possible sources for such errors can be:
Inadequate storage conditions of the test kit, wrong 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). The assay procedure should be performed away from direct sun light.

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

A. Materials Provided

1. Microtiter plate 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 ppb
3. Antibody solution (monoclonal anti-Microcystins), 6 mL
4. Microcystins-HRP Conjugate, 6 mL
5. Diluent/zero, 25 mL. Use to dilute samples with concentration above 5 ppb.
6. Wash Solution 5X Concentrate, 100 mL
7. Color Solution (TMB), 16 mL
8. Stop Solution, 2 X 6 mL

B. Test Preparation

Micro-pipetting equipment and pipette tips for pipetting the standards and samples are necessary. We recommend using a multi-channel pipette or a stepping pipette for adding the antibody, enzyme conjugate, substrate solution, and the stop solution in order to equalize the incubation periods of the standard solutions and the samples 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. Read and understand the instructions and precautions given in this insert before proceeding.

1. Adjust the microtiter plate and the reagents to room temperature before use.
2. Remove the number of microtiter plate strips required from the foil bag. The remaining strips are stored in the foil bag and zip-locked closed. Store the remaining kit in the refrigerator (4-8°C).
3. The standard, control, antibody solution, enzyme conjugate, substrate and stop solutions are ready to use and do not require any further dilutions.
4. The wash solution is a 5X concentrated solution and needs to be diluted with deionized water. In a 1L container dilute the 5X solution 1:5 (i.e. 100 mL of the 5X wash solution plus 400 mL of deionized water). The diluted solution is used to wash the microtiter wells.
5. The stop solution has to be handled with care as it contains diluted H_2SO_4.
6. If testing water sources other than drinking water and a total microcystins concentration (free and cell bound) is needed then an appropriate cell lysing procedure (freeze and thaw, sonication, QuickLyse, etc.) should be performed prior to the ELISA.

C. Assay Procedure

1. Add 100 µL of the standard solutions, control or samples into the wells of the test strips according to the working scheme given. We recommend using duplicates or triplicates.
2. Add 50 µL of the enzyme conjugate solution to the individual wells successively using a multi- channel pipette or a stepping pipette.
3. Add 50 uL 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 rapid circular motion on the benchtop for 30 seconds. Be careful not to spill contents. Incubate for 90 minutes at room temperature.
4. After incubation, remove the covering and vigorously shake the contents of the wells into a sink. Wash the strips three times using the 1X washing buffer solution. Please use at least a volume of 250 µL of washing 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 µL of color solution to the wells using a multi- channel pipette or a stepping pipette. The strips are incubated for 20-25 minutes at room temperature. Protect the strips from sunlight.
6. Add 100 µL of stop solution to the wells in the same sequence as for the 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 stopping the reaction.

C. Evaluation

The evaluation of the ELISA can be performed using commercial ELISA evaluation programs (4-parameters, Logit/Log or alternatively point to point). For a manual evaluation, calculate the mean absorbance value for each of the standards. Then, 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 horizontal logarithmic (x) axis on graph paper. $\%B/B_0$ for controls and samples will then yield levels in ppb of microcystins by interpolation using the standard curve.

The concentrations of the samples are determined using this standard curve. Samples showing a lower concentration than 0.10 ppb of microcystins are considered to be negative. Samples showing a higher concentration than standard 6 (5.0 ppb) must be diluted to obtain more accurate results. The concentration of the positive control should be in the range given on the bottle (±25%).

D. Additional Materials (not delivered with the test kit)

1. Micro-pipettes with disposable plastic tips (50-250 µL)
2. Multi-channel pipette (50-250 µL) or stepper pipette with plastic tips (50-250 µL)
3. Reagent reservoir for multichannel pipettes
4. Microtiter plate washer (optional)
5. Microtiter plate reader (wavelength 450 nm)
6. Shaker for microtiter plates (optional)

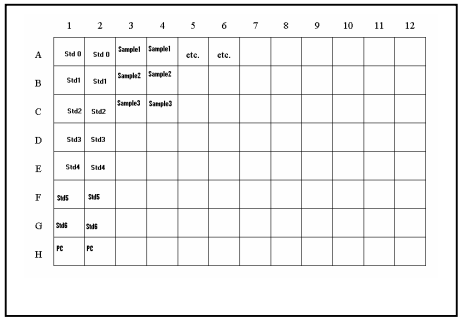
E. Working Scheme

The microtiter plate consists of 12 strips of 8, 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-Sd6: Standards

PC (Positive Control): 0.75 ppb

Sample1, Sample2, Sample3, etc.: Samples



Recovery

Four (4) groundwater samples were spiked with various levels of Microcystins LR and then assayed using the Abraxis Microcystins-DM Assay. The following results were obtained:

Amount of Microcystins LR Added (ppb)	Recovery Mean (ppb)	Recovery S.D. (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

The following results were obtained:

Control	1	2	3
Replicates	5	5	5
Days	3	3	3
n	15	15	15
Mean (ppb)	0.25	0.99	2.86
% CV (within)	5.3	3.6	3.0
% CV (between)	6.5	5.4	4.0

Sensitivity

The Abraxis Microcystins-DM ELISA has an estimated minimum detectable concentration, based on 90% B/Bo of 0.10 ppb.

G. 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.

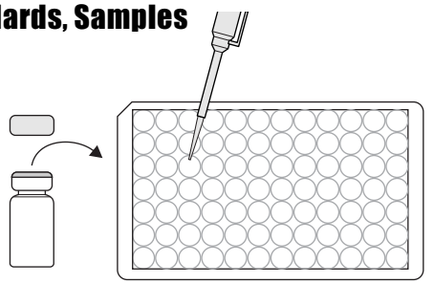
(2) Worldwide Patenting PCT WO 01/18059 A2.

(3) U.S. Patent Number 6,967,240

Microcystin-DM ELISA Kit, Detailed Procedure

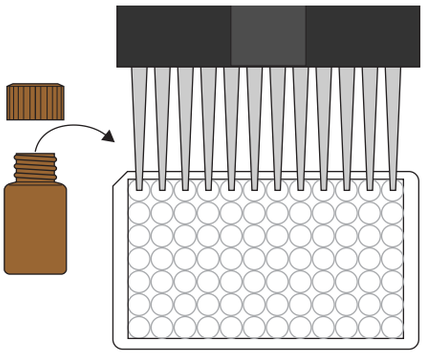
1. Addition of Standards, Samples

Add 100 uL of the standard solutions, control or samples into the wells of the test strips according to the working scheme given. We recommend using duplicates or triplicates.



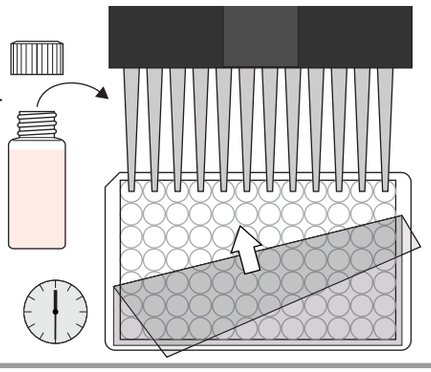
2. Addition of Enyzme Conjugate

Add 50 uL of the enzyme conjugate to the individual wells successively using a multi- channel pipette or a stepping pipette.



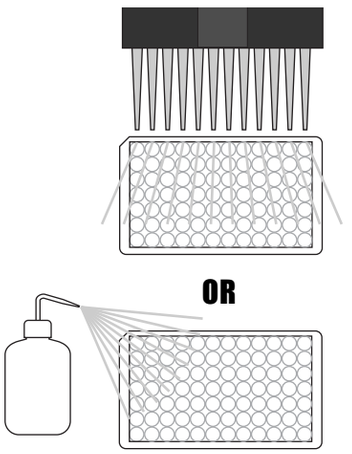
3. Addition of Antibody Solution

Add 50 uL of the Microcystin Monoclonal antibody solution to the individual wells successively using a multi-channel pipette. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a rapid circular motion on the benchtop. Be careful not to spill contents. Incubate the strips for 90 min. at room temperature.



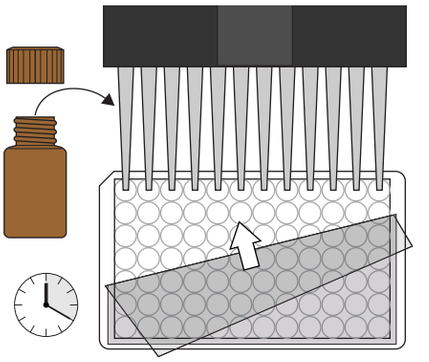
4. Washing of Plates

After incubation, remove the covering and vigorously shake the contents of the wells into a sink. Wash the strips three times with a multi-channel pipette or wash bottle using the diluted 1X washing buffer solution. Please use at least a volume of 250 uL of washing 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.



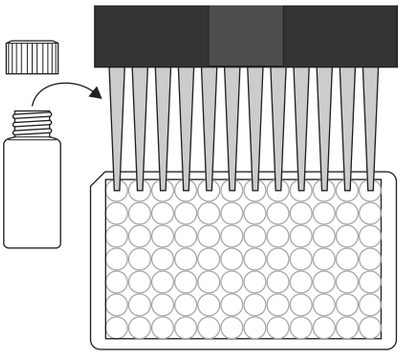
5. Addition of Substrate/Color Solution

Add 150 uL 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 rapid circular motion on the benchtop. Be careful not to spill contents. Incubate the strips for 20 min. at room temperature.



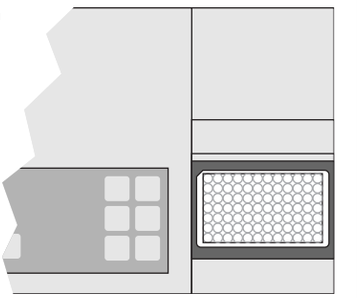
6. Addition of Stopping Solution

Add 100 uL of stop solution to the wells in the same sequence as for the substrate solution using a multi- channel pipette or a stepping pipette.



7. Measurement of Color

Read the absorbance at 450 nm using a microplate ELISA reader. Calculate results.



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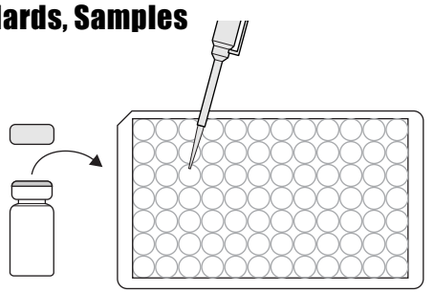
Phone: 215-357-3911 Fax: 215-357-5232
www.abraxiskits.com

Microcystin-DM ELISA Kit, Concise Procedure

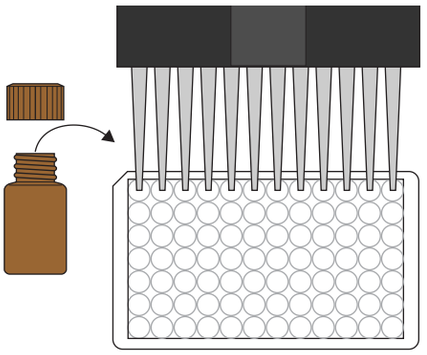
1. Addition of Standards, Samples

Add 100 uL of standard solutions, control or samples.



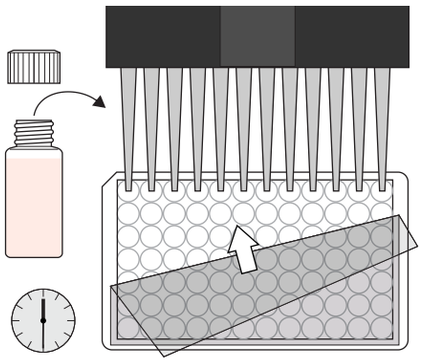
2. Addition of Enyzme Conjugate

Add 50 uL of enzyme conjugate.



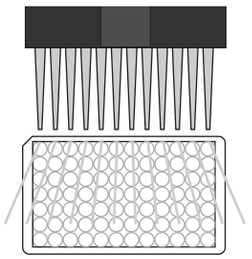
3. Addition of Antibody Solution

Add 50 uL of the antibody solution. Cover and mix for 30 seconds by rotating on benchtop. Incubate for 90 minutes at room temperature.

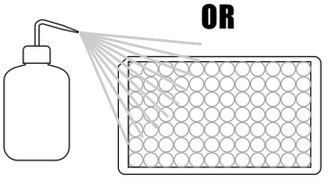


4. Washing of Plates

Wash the plates three times with 250 uL of diluted 1X washing buffer.

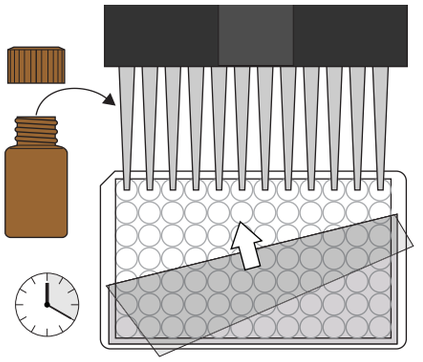


OR



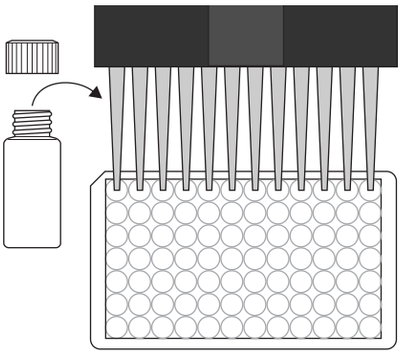
5. Addition of Substrate/Color Solution

Add 150 uL of substrate/color solution. Incubate 20 minutes at room temperature and away from direct sunlight.



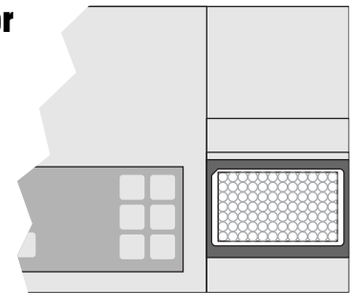
6. Addition of Stopping Solution

Add 100 uL of stop solution.



7. Measurement of Color

Measure color at 450 nm. Calculate results.



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