



# Product Manual

## **Microcystins-ADDA ELISA (Microtiter Plate)**

Catalog #: ALX-850-319

Enzyme-linked immunosorbent assay for the congener-independent\*  
determination of Microcystins and Nodularins in water samples



# Product Manual

## **USE FOR RESEARCH PURPOSES ONLY**

Unless otherwise specified expressly on the packaging, all products sold hereunder are intended for and may be used for research purposes only and may not be used for food, drug, cosmetic or household use or for the diagnosis or treatment of human beings. Purchase does not include any right or license to use, develop or otherwise exploit these products commercially. Any commercial use, development or exploitation of these products or development using these products without the express written authorization of Enzo Life Sciences, Inc. is strictly prohibited. Buyer assumes all risk and liability for the use and/or results obtained by the use of the products covered by this invoice whether used singularly or in combination with other products.

## **LIMITED WARRANTY; DISCLAIMER OF WARRANTIES**

These products are offered under a limited warranty. The products are guaranteed to meet all appropriate specifications described in the package insert at the time of shipment. Enzo Life Sciences' sole obligation is to replace the product to the extent of the purchasing price. All claims must be made to Enzo Life Sciences, Inc., within five (5) days of receipt of order. THIS WARRANTY IS EXPRESSLY IN LIEU OF ANY OTHER WARRANTIES OR LIABILITIES, EXPRESS OR IMPLIED, INCLUDING WARRANTIES OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, AND NON-INFRINGEMENT OF THE PATENT OR OTHER INTELLECTUAL PROPERTY RIGHTS OF OTHERS, AND ALL SUCH WARRANTIES (AND ANY OTHER WARRANTIES IMPLIED BY LAW) ARE EXPRESSLY DISCLAIMED.

## **TRADEMARKS AND PATENTS**

Several Enzo Life Sciences products and product applications are covered by US and foreign patents and patents pending. Enzo is a trademark of Enzo Life Sciences, Inc.

**FOR RESEARCH USE ONLY.  
NOT FOR USE IN DIAGNOSTIC PROCEDURES.**



Please read entire booklet before proceeding with the assay.



Carefully note the handling and storage conditions of each kit component.



Please contact Enzo Life Sciences Technical Support if necessary.

## TABLE OF CONTENTS

1. Description.....	1
2. Precautions.....	1
3. Storage and Stability.....	1
4. Test Principle.....	1
5. Importance.....	2
6. Limitations .....	3
7. Materials Supplied .....	4
8. Other Materials Needed.....	4
9. Sample Collection and Handling.....	4
10. Test Preparation .....	5
11. Working Scheme .....	6
12. Assay Procedure .....	7
13. Calculation of Results .....	8
14. References .....	8
15. Performance Data.....	9
16. Microcystin Plate Elisa Procedure.....	10
17. Contact Information .....	13

## DESCRIPTION

The Enzo Life Sciences Microcystins-ADDA ELISA is an immunoassay for the quantitative and sensitive congener-independent\* 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.



Handle  
with care

## PRECAUTIONS

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

## STORAGE AND STABILITY

The Microcystins-ADDA 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 the proper disposal of all reagents.

## TEST PRINCIPLE

The test is an indirect competitive ELISA for the congener-independent detection of Microcystins and Nodularins. It is based on the recognition of Microcystins, Nodularins, and the congeners by specific antibodies. Toxin, when present in a sample, and a Microcystins-protein analog immobilized on the plate compete for the binding sites of the anti-Microcystins/Nodularins antibodies in solution. The plate is then washed and a second antibody-HRP label is added. After a second 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.

## 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 ( $\mu\text{g/l}$ ) in drinking water.

## **LIMITATIONS OF THE MICROCYSTINS-ADDA 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 may be found in water samples, test interferences caused by matrix effects cannot be completely excluded.

Samples containing methanol must be diluted to a concentration <5% methanol to avoid matrix effects.

Seawater samples must be diluted to a concentration  $\leq 2.5\%$  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. 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, and 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.

## MATERIALS SUPPLIED

1. **Microtiter Plate** (12x8 strips) coated with an analog of Microcystins conjugated to a protein
2. **Standards (6) and Control (1):**  
0.0, 0.15, 0.40, 1.0, 2.0, 5.0 ppb; Control at  $0.75 \pm 0.185$  ppb
3. **Sample Diluent** (for dilution of samples above the range of the curve)
4. **Antibody Solution**
5. **Anti-Sheep-HRP Conjugate Solution**
6. **Wash Solution (5x) Concentrate**  
Must be diluted prior to use, see Test Preparation, Section D
7. **Substrate (Color) Solution (TMB)**
8. **Stop Solution**

## OTHER MATERIALS NEEDED

1. Micro-Pipettes with disposal 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 450nm)
8. Microtiter plate washer (optional)

## 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 period 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, etc.) must be performed prior to analysis.

## TEST PREPARATION

Micro-pipetting equipment and pipette tips for pipetting the standards and the 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 use only 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 sealed).
3. The standards, control, sample diluent, antibody, enzyme conjugate, 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  $\text{H}_2\text{SO}_4$ .



## 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-Std5:** Standards
- Ctrl:** Control
- Smp1, Smp2, etc.:** Samples

	A	B	C	D	E	F	G	H
1	Std0	Std1	Std2	Std3	Std4	Std5	Std0	Std1
2	Std2	Std3	Std4	Std5	Ctrl	Ctrl	Smp1	Smp2
3	Smp3	Smp4	etc.	etc.				
4								
5								
6								
7								
8								
9								
10								
11								
12								

## ASSAY PROCEDURE

1. Add **50  $\mu$ 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 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.
3. Remove the covering and decant the contents of the wells in to 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.
4. Add **100  $\mu$ L of the enzyme conjugate 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 **30 minutes** at room temperature.
5. 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 washing step. Remaining buffer in the wells should be removed by patting the plate dry in a stack of paper towels.
6. Add **100  $\mu$ 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.
7. Add **50  $\mu$ 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.
8. Read the adsorbance at 450nm using a microplate ELISA photometer within 15 minutes after the addition of the stopping solution.

## CALCULATION OF RESULTS

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 adsorbance value for each of the standards. Calculate the %B/Bo for each standard by dividing the mean adsorbance value for each standard by the Zero Standard (Standard 0) mean adsorbance. Construct a standard curve by plotting the %B/Bo for each standard on the vertical linear (y) axis versus the corresponding Microcystins concentration on the horizontal logarithmic (x) axis on graph paper. %B/Bo 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 **Assay Blaster! Data Analysis Software** available from Enzo Life Sciences, or a similar data analysis program.

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 <0.15ppb of Microcystins. Samples showing a higher concentration than standard 5 (5.0ppb) must be diluted to obtain accurate results. The concentration of the positive control provided should be  $0.75 \pm 0.185$  ppb.

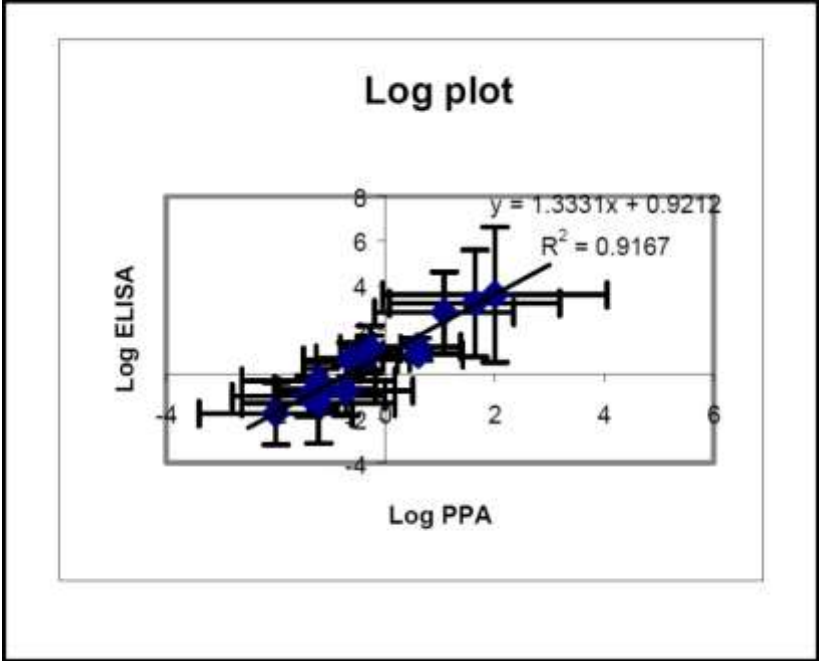
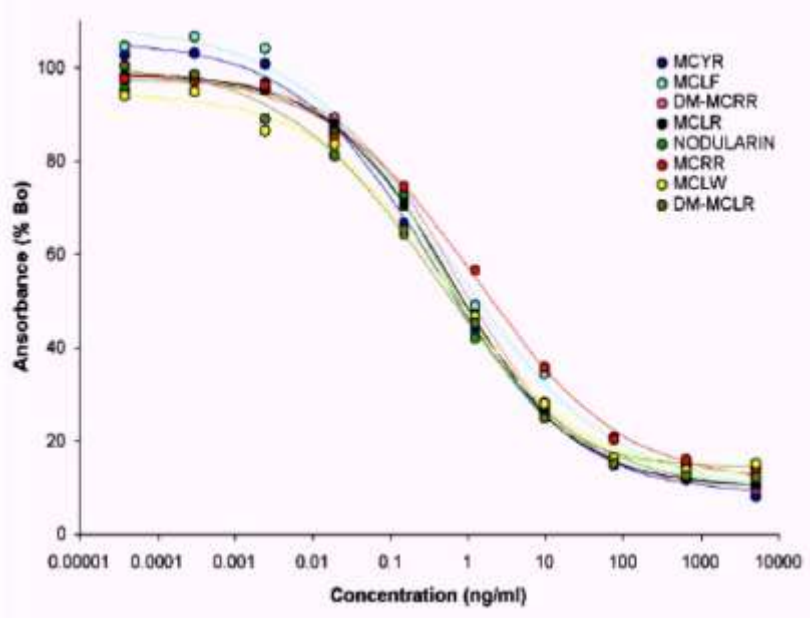
Semi-quantitative results can be derived by simple comparison of the sample adsorbances to the adsorbance of the calibrators. Samples with lower adsorbances than a calibrator will have concentrations of Microcystins greater than that of the calibrator. Samples which have higher adsorbances than a calibrator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

## REFERENCES

1. W.J. Fischer, I. Garthwaite, C.O. Miles, K.M. Ross, J.B. Aggen, A.R. Chamberlin, N.A. Towers, and D.R. Dietrich. Congener-Independent Immunoassay for Microcystins and Nodularins. *Environ. Sci. Technol.* 35, 2001, 4849-4858.
2. Worldwide Patenting PCT WO 01/18059 A2.
3. U.S. Patent Number 6,967,240.

## PERFORMANCE DATA

- Test sensitivity: The detection limit for the 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 cross-reactivity illustration below)

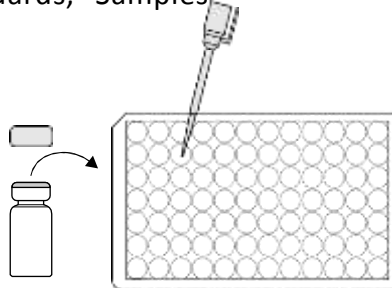


- Samples: Sample correlation between HPLC, PPA, and ELISA methods showed a good correlation (see ELISA and PPA correlation above).

## Microcystin Plate, Concise ELISA Procedure

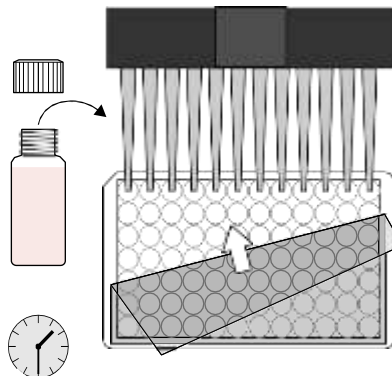
### 1. Addition of Standards, Samples

Add 50  $\mu$ L of the standards, control or samples.



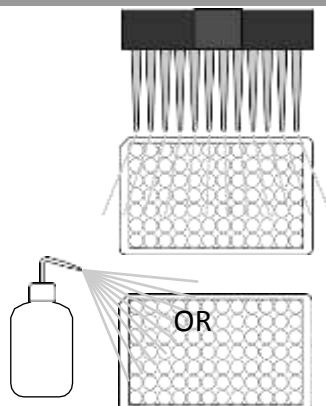
### 2. Addition of Antibody Solution

Add 50  $\mu$ L of antibody solution. Cover and mix for 30 seconds by rotating on benchtop. Incubate for 90 minutes at room temperature.



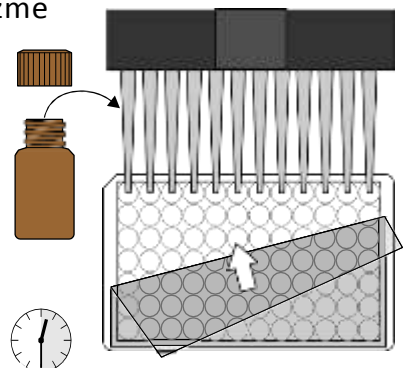
### 3. Washing of Plates

Wash plates three times with 250  $\mu$ L of 1X washing buffer.



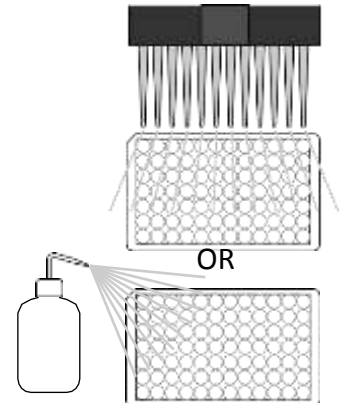
### 4. Addition of Enzyme Conjugate

Add 100  $\mu$ L of enzyme conjugate. Incubate for 30 minutes at room temperature.



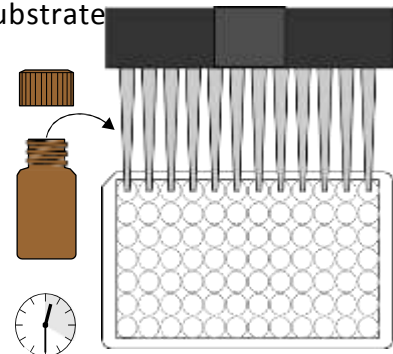
### 5. Washing of Plates

Wash the plates three times with 250  $\mu$ L of 1X washing buffer.



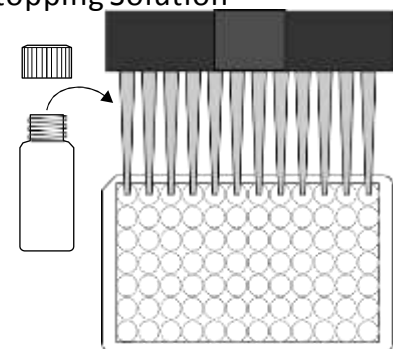
### 6. Addition of Substrate

Add 100  $\mu$ L of substrate solution. Incubate 20-30 minutes at room temperature and away from direct sunlight.



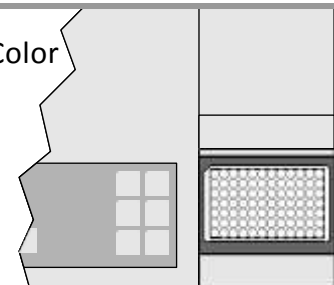
### 7. Addition of Stopping Solution

Add 50  $\mu$ L of stop solution.



### 8. Measurement of Color

Measure color at 450 nm. Calculate results.





# Product Manual

## NOTES



# Product Manual

## NOTES



# Product Manual

## **GLOBAL HEADQUARTERS**

Enzo Life Sciences Inc.  
10 Executive Boulevard  
Farmingdale, NY 11735  
Toll-Free: 1.800.942.0430  
Phone: 631.694.7070  
Fax: 631.694.7501  
info-usa@enzolifesciences.com

## **EUROPE/ASIA**

Enzo Life Sciences (ELS) AG  
Industriestrasse 17  
CH-4415 Lausen  
Switzerland  
Phone: +41/0 61 926 89 89  
Fax: +41/0 61 926 89 79  
info-ch@enzolifesciences.com

For local distributors and detailed product information visit us online:  
**[www.enzolifesciences.com](http://www.enzolifesciences.com)**