Microcystin ELISA Kit

Item No. 502000

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GENERAL INFORMATION

Materials Supplied

Item Number	Item Name	96 wells Quantity/Size	Storage Temperature
502001	Microcystin-HRP Tracer	1 vial/100 dtn	-20°C
502002	Microcystin ELISA Monoclonal Antibody	1 vial/100 dtn	-20°C
502003	Microcystin ELISA Standard	1 vial	-20°C
401703	Immunoassay Buffer C Concentrate (10X)	1 vial/10 ml	4°C
400062	Wash Buffer Concentrate (400X)	1 vial/5 ml	RT
400035	Polysorbate 20	1 vial/3 ml	RT
400008/400009	Goat Anti-Mouse IgG-Coated Plate	1 plate	4°C
400074	TMB Substrate Solution	2 vials/12 ml	4°C
10011355	HRP Stop Solution	1 vial/12 ml	RT
400040	ELISA Tracer Dye	1 vial	RT
400042	ELISA Antiserum Dye	1 vial	RT
400012	96-Well Cover Sheet	1 ea	RT

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

This kit may not perform as described if any reagent or procedure is replaced or modified.

When compared to quantification by LC/MS or GC/MS, it is not uncommon for immunoassays to report higher analyte concentrations. While LC/MS or GC/MS analyses typically measure only a single compound, antibodies used in immunoassays sometimes recognize not only the target molecule, but also structurally related molecules, including biologically relevant metabolites. In many cases, measurement of both the parent molecule and metabolites is more representative of the overall biological response than is the measurement of a short-lived parent molecule. It is the responsibility of the researcher to understand the limits of both assay systems and to interpret their data accordingly.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Email: techserv@caymanchem.com

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored as directed in the Materials Supplied section (see page 3) and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. A plate reader capable of measuring absorbance at 450 nm
2. Adjustable pipettes; multichannel or repeating pipettor recommended
3. An orbital microplate shaker
4. A source of ultrapure water is recommended. Pure water - glass-distilled or deionized - may not be acceptable *NOTE: UltraPure Water is available for purchase from Cayman (Item No. 400000).*
5. Materials used for **Sample Preparation** (see page 12)

INTRODUCTION

Background

Microcystins are cyclic heptapeptide hepatotoxins released during the degradation of cyanobacteria, also known as blue-green algae, which are found in bodies of marine and freshwater.[1,2] Cyanobacteria accumulate based on a variety of factors, including nutrient availability, pH, and weather conditions, and form blooms on the water's surface.[1] These cyanobacterial blooms affect the smell and taste of the water, and cyanobacterial degradation releases toxic microcystins that affect the water's safety.

The toxicity of microcystins is due to their direct inhibition of serine/threonine protein phosphatases and the induction of oxidative stress.[2,3] Acute exposure of mice to purified microcystins induces severe liver damage and death within hours, while chronic sublethal exposure induces hepatic steatosis that can progress to non-alcoholic steatohepatitis (NASH), as well as lung damage and disruptions in lipid metabolism and the cell cycle, among other effects.[1,2] Purified microcystins are lethal to mice *via* intraperitoneal injection and inhalation, with the 50% lethal dose ranging from 50 to >1,200 µg/kg, i.p., in a congener-specific manner, but oral ingestion is less toxic.[2] Exposure to microcystins affects fish growth and physiological functions, and its accumulation in fish and crustaceans can lead to toxicity in the animals, including humans, that use them as a food source.[4] Furthermore, microcystins can accumulate in food plants irrigated with contaminated water.[1]

The presence of microcystins in drinking water sources is positively correlated with the incidence of colorectal cancer.[5] The microcystin congener microcystin-LR (MC-LR) induces migration and invasion of cancer cells in a mouse xenograft model, and serum MC-LR levels are an independent risk factor for hepatocellular carcinoma.[6,7] Microcystin-contaminated water can be treated through a variety of processes to reduce or remove microcystins, including activated carbon adsorption, ozonation, or chlorination.[1,4] The World Health Organization (WHO) recommends a lifetime safe consumption level for microcystins of 1 µg/L (1.0 part per billion (ppb)).[4] Determining the presence of microcystins in water and other samples is the first step to identifying and eliminating the contamination.

About This Assay

Cayman's Microcystin ELISA Kit is a competitive assay that can be used for quantification of microcystin in various natural water samples, serum, and tissue homogenates. Often, environmental reports involving testing of natural sources such as water samples report numbers in parts per billion as the unit of concentration. In this kit, we report in ng/ml, since 1 ng/ml equals 1 ppb, the units can be used interchangably. The assay has a range of 0.027-8 ng/ml (ppb) with an average sensitivity (80% B/B_0) of 0.196 ng/ml (ppb), and a lower limit of detection (LLOD) of 0.021 ng/ml (ppb).

Principle Of This Assay

This assay is based on the competition between free microcystin and a Microcystin-Horseradish Peroxidase (HRP) conjugate (Microcystin-HRP Tracer) for a limited amount of Microcystin Monoclonal Antibody. Because the concentration of the Microcystin-HRP Tracer is held constant while the concentration of free microcystin varies, the amount of Microcystin-HRP Tracer that is able to bind to the Microcystin Monoclonal Antibody will be inversely proportional to the concentration of free microcystin in the well. This antibody-microcystin complex binds to goat anti-mouse IgG that has been previously attached to the well. The plate is washed to remove any unbound reagents and TMB Substrate Solution (which contains the substrate to HRP) is added to the well, followed by the HRP Stop Solution. The product of this enzymatic reaction has a distinct yellow color that can be measured at 450 nm. The intensity of this color, determined spectrophotometrically, is proportional to the amount of Microcystin-HRP Tracer bound to the well, which is inversely proportional to the amount of free microcystin present in the well during the incubation, as described in the equation:

$$\textbf{Absorbance} \propto \textbf{[Bound Microcystin-HRP Tracer]} \propto \textbf{1/[microcystin]}$$

A schematic of this process is shown in Figure 1, on page 8.

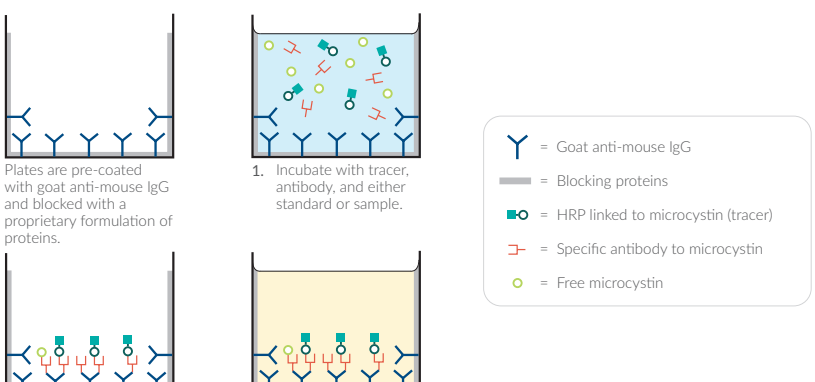


Figure 1. Schematic of the Microcystin ELISA

Definition of Key Terms

Blk (Blank): background absorbance caused by TMB Substrate Solution and the HRP Stop Solution. The blank absorbance should be subtracted from the absorbance readings of all the other wells, including non-specific binding (NSB) wells.

TA (Total Activity): total enzymatic activity of the microcystin HRP-linked tracer.

NSB (Non-Specific Binding): non-immunological binding of the tracer to the well. Even in the absence of specific antibody a very small amount of tracer still binds to the well; the NSB is a measure of this low binding.

B_0 (Maximum Binding): maximum amount of the tracer that the antibody can bind in the absence of free analyte.

%B/B_0 (%Bound/Maximum Bound): ratio of the absorbance of a sample or standard well to the average absorbance of the maximum binding (B_0) wells.

Standard Curve: a plot of the %B/B_0 values *versus* concentration of a series of wells containing various known amounts of analyte.

Determination (Dtn): one determination is the amount of reagent used per well.

Cross Reactivity: numerical representation of the relative reactivity of this assay towards structurally related molecules as compared to the primary analyte of interest. Biomolecules that possess similar epitopes to the analyte can compete with the assay tracer for binding to the primary antibody. Substances that are superior to the analyte in displacing the tracer result in a cross reactivity that is greater than 100%. Substances that are inferior to the primary analyte in displacing the tracer result in a cross reactivity that is less than 100%. Cross reactivity is calculated by comparing the mid-point (50% B/B_0) value of the tested molecule to the mid-point (50% B/B_0) value of the primary analyte when each is measured in assay buffer using the following formula:

$$\% \text{ Cross Reactivity} = \left[\frac{50\%\ B/B_0 \text{ value for the primary analyte}}{50\%\ B/B_0 \text{ value for the potential cross reactant}} \right] \times 100\%$$

Lower Limit of Detection (LLOD): the smallest measure that can be detected with reasonable certainty for a given analytical procedure. The LLOD is defined as a concentration two standard deviations higher than the mean zero value.

PRE-ASSAY PREPARATION

Buffer Preparation

Store all diluted buffers at 4°C; they should be stable for approximately two months. NOTE: It is normal for the concentrated buffers to contain crystalline salts after thawing. These will completely dissolve upon dilution with ultrapure water.

1. **Immunoassay Buffer C (1X) Preparation**

   Dilute the contents of one vial of Immunoassay Buffer C Concentrate (10X) (Item No. 401703) with 90 ml of ultrapure water. Be certain to rinse the vial to remove any salts that may have precipitated.

2. **Wash Buffer (1X) Preparation**

   Dilute the contents of one vial of Wash Buffer Concentrate (400X) (Item No. 400062) with ultrapure water to a total volume of 2 L and add 1 ml of Polysorbate 20 (Item No. 400035). Smaller volumes of Wash Buffer (1X) can be prepared by diluting the Wash Buffer Concentrate (400X) 1:400 and adding Polysorbate 20 to an end concentration of 0.5 ml/L. *NOTE: Polysorbate 20 is a viscous liquid and cannot be measured by a regular pipette. A positive displacement pipette or a syringe should be used to deliver small quantities accurately.*

Sample Preparation

Testing for Interference

This assay has been validated using natural water samples, including pond, marsh, river, lake, brackish, and sea water, as well as serum and tissue homogenates. Other sample types should be tested for interference to evaluate the need for sample purification before embarking on a large number of sample measurements. To test for interference, dilute one or two test samples to obtain at least two different dilutions of each sample within the linear portion of the standard curve. If the two different dilutions of the sample show good correlation (differ by 20% or less) in the final calculated microcystin concentration, sample purification is not required. If you do not see good correlation of the different dilutions, purification is advised. Purification methods will need to be determined by the end user and tested for compatibility in the assay.

All Water Samples

It is recommended that all water samples be centrifuged at 1,000 x g for 5 minutes to remove any particulates. Water samples should then be diluted in Immunoassay Buffer C (1X) prior to testing in the assay.

General Precautions

- All samples must be free of organic solvents prior to assay.
- Samples should be assayed immediately after collection; samples that cannot be assayed immediately should be stored at -80°C.

Serum

Collect blood in vacutainers without an anticoagulant. Allow samples to clot undisturbed for 30-60 minutes at room temperature. To obtain serum, centrifuge at 1,000-2,000 x g for 15-30 minutes. Collect the serum layer for testing. Samples should be assayed immediately after collection; samples that cannot be assayed immediately should be stored at -80°C. Serum can be tested after dilution with Immunoassay Buffer C (1X) at least 1:2.

Muscle Tissue

1. Homogenize tissue in Immunoassay Buffer C (1X) (1 ml of buffer per 50 mg of tissue is recommended).
2. Centrifuge to pellet insoluble material at 3,000 x g for 10 minutes.
3. Collect supernatant for testing. To fall within the range of the standard curve, it may be necessary to dilute samples with Immunoassay Buffer C (1X) prior to performing the assay.

Liver Tissue

1. Homogenize tissue in 90% methanol in ultrapure water (1 ml per 100 mg of tissue is recommended).
2. Centrifuge to pellet insoluble material at 3,000 x g for 10 minutes.
3. Transfer the supernatant to a clean test tube, dry under a gentle stream of nitrogen, and reconstitute in 1 ml of Immunoassay buffer C (1X) for testing. To fall within the range of the standard curve, it may be necessary to dilute samples with Immunoassay Buffer C (1X) prior to performing the assay. Alternatively, the supernatant can be tested without drying if diluted at least 1:50 with Immunoassay Buffer C (1X) prior to the assay.

Sample Matrix Properties

Linearity

Water samples were spiked with 125 ng/ml Microcystin ELISA Standard, serially diluted with Immunoassay Buffer C (1X), and evaluated for linearity using the Microcystin ELISA Kit. The results are shown in the table below.

Dilution Factor	Concentration (ng/ml)	Dilutional Linearity (%)
Pond		
50	103.44	100
100	114.42	111
200	109.11	105
Marsh		
80	101.54	100
160	99.80	98
320	101.47	100
River		
80	101.96	100
160	101.18	99
320	94.51	93
Lake		
40	87.47	100
80	85.70	98
160	83.54	96
Brackish		
80	112.56	100
160	109.29	97
320	110.1	98
Sea Water		
80	106.31	100
160	106.76	100
320	108.58	102

Table 1. Dilutional linearity of freshwater samples

Parallelism

To assess parallelism, water samples were spiked with the Microcystin ELISA Standard, serially diluted, and evaluated using the Microcystin ELISA Kit. Concentrations were plotted as a function of the sample dilution. The results are shown below.

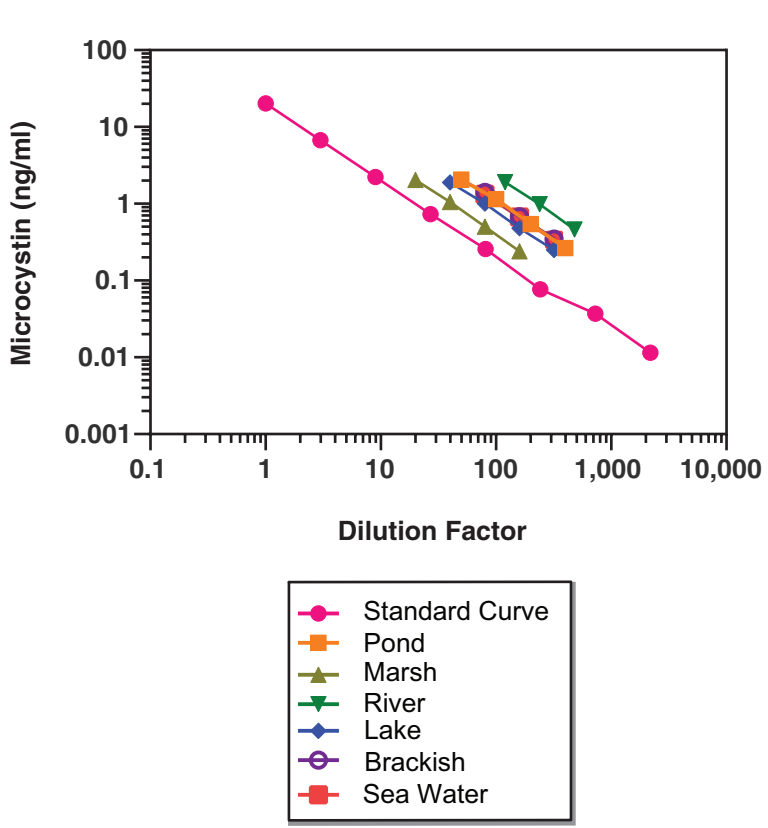


Figure 2. Parallelism of water samples

Spike and Recovery

Water samples were spiked with the Microcystin ELISA Standard, centrifuged as described in the **Sample Preparation** section (see page 12), diluted, and analyzed using the Microcystin ELISA Kit. The results are shown below. The y-intercept corresponds to the amount of native microcystin in the sample. The error bars represent standard deviations obtained from multiple dilutions of each sample.

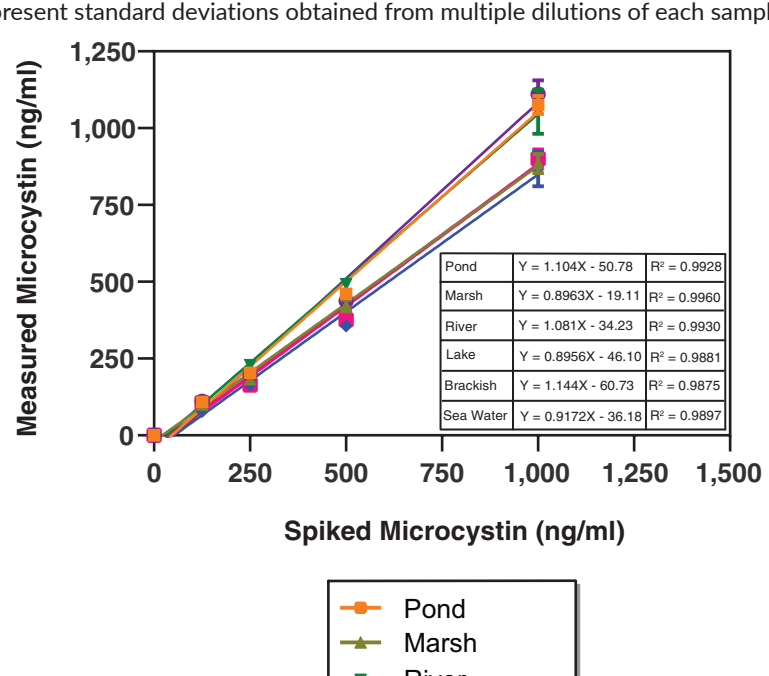


Figure 3. Spike and recovery in water samples

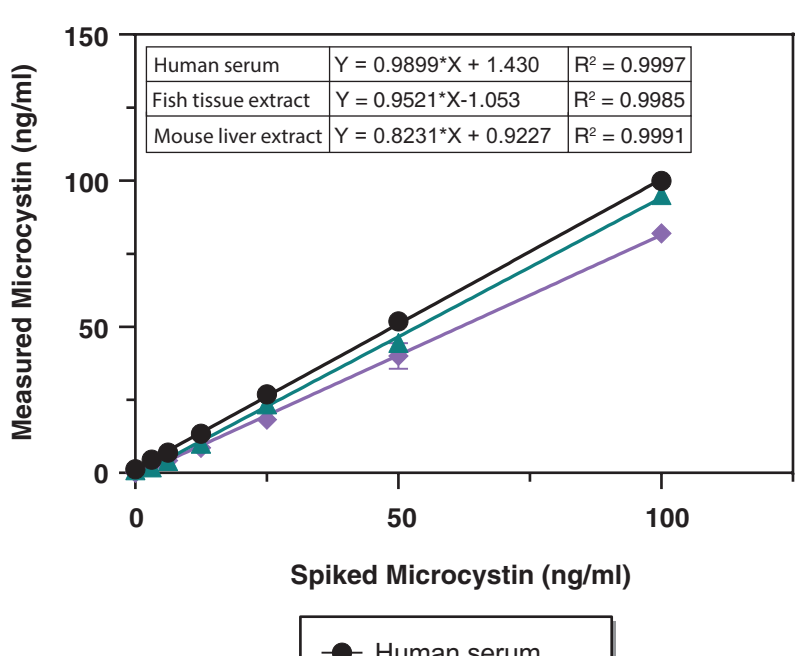


Figure 4. Spike and recovery in serum and tissue samples. Human serum, fish muscle tissue homogenate, and mouse liver extract (prepared as described in the Sample Preparation section on page 12) were spiked with microcystin, serially diluted with Immunoassay Buffer C (1X), and tested in the assay.

Preparation of Assay-Specific Reagents

Microcystin ELISA Standard

Equilibrate a pipette tip by repeatedly filling and expelling the tip with the Microcystin ELISA Standard (Item No. 502003) several times. Using the equilibrated pipette tip, transfer 100 µl of the standard into a clean test tube, then dilute with 900 µl ultrapure water. The concentration of this solution (the bulk standard) will be 200 ng/ml. The bulk standard should be stored at 4°C and used within two weeks.

To prepare the standard for use in ELISA: obtain eight clean glass test tubes and label them #1-8. Aliquot 960 µl Immunoassay Buffer C (1X) to tube #1 and 500 µl Immunoassay Buffer C (1X) to tubes #2-8. Transfer 40 µl of the bulk standard (200 ng/ml) to tube #1 and mix thoroughly. Serially dilute the standard by removing 400 µl from tube #1 and placing in tube #2; mix thoroughly. Next, remove 400 µl from tube #2 and place it into tube #3; mix thoroughly. Repeat this process for tubes #4-8. These diluted standards should not be stored for more than one hour at room temperature. ***NOTE: The units of the standard curve are provided in ng/ml but can be easily converted to ppb. The conversion is 1 ng/ml = 1 ppb. Therefore, the range of the standard curve is 0.027-8 ppb.***

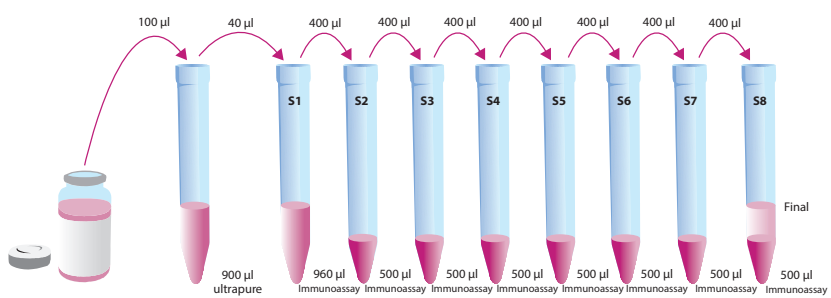


Figure 5. Preparation of the microcystin standards

Microcystin-HRP Tracer

Dilute the Microcystin-HRP Tracer (Item No. 502001) with 5 ml of Immunoassay Buffer C (1X). Transfer diluted Microcystin HRP Tracer into a polypropelene tube, store it at 4°C, and use within 2 weeks. A 20% surplus of tracer has been included to account for any incidental losses.

Tracer Dye Instructions (optional)

This dye may be added to the tracer, if desired, to aid in visualization of tracer-containing wells. Add the dye to the diluted tracer at a final dilution of 1:100 (add 60 µl of dye to 6 ml tracer). *NOTE: Do not store tracer with dye for more than 1 week at 4°C.*

Microcystin ELISA Monoclonal Antibody

Reconstitute the Microcystin Monoclonal Antibody (Item No. 502002) with 6 ml of Immunoassay Buffer C (1X). Store the reconstituted Microcystin Monoclonal Antibody at 4°C (*do not freeze!*). It will be stable for at least 3 weeks. A 20% surplus of antibody has been included to account for any incidental losses.

Antibody Dye Instructions (optional)

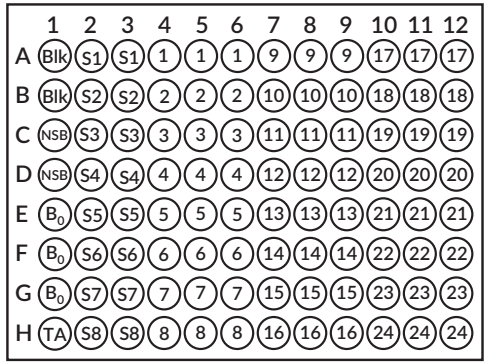
This dye may be added to the antibody, if desired, to aid in visualization of antibody-containing wells. Add the dye to the reconstituted antibody at a final dilution of 1:100 (add 60 µl of dye to 6 ml antibody). *NOTE: Antibody with dye will be stable for at least 3 weeks if stored at 4°C.*

Plate Set Up

The 96-well plate(s) included with this kit must be pre-washed five times with Wash Buffer (1X) (~300 µl/well) prior to use in the ELISA. *NOTE: If you do not need to use all the strips at once, place the unused unwashed strips back in the plate packet and store at 4°C. Be sure the packet is sealed with the desiccant inside.*

Each plate or set of strips must contain a minimum of two Blk, two NSB, and three B_0 wells, and an eight-point standard curve run in duplicate. *NOTE: Each assay must contain this minimum configuration in order to ensure accurate and reproducible results. Each sample should be assayed at a minimum of two dilutions and each dilution should be assayed in duplicate. For statistical purposes, we recommend assaying samples in triplicate.*

A suggested plate format is shown in Figure 6, on page 21. The user may vary the location and type of wells present as necessary for each particular experiment. The plate format provided below has been designed to allow for easy data analysis using a convenient spreadsheet offered by Cayman (see page 24 for more details). We suggest recording the contents of each well on the template sheet provided (see page 33).



Blk - Blank
TA - Total Activity
NSB - Non-Specific Binding
B_0 - Maximum Binding
S1-S8 - Standards 1-8
1-24 - Samples

Figure 6. Sample plate format

Performing the Assay

Pipetting Hints

- Use different tips to pipette each reagent.
- Before pipetting each reagent, equilibrate the pipette tip in that reagent (*i.e.*, slowly fill the tip and gently expel the contents, repeat several times).
- Do not expose the pipette tip to the reagent(s) already in the well.

Pre-Wash the Plate

Rinse the plate (or strips to be used) five times with ~300 µl Wash Buffer (1X).

Addition of the Reagents

1. **Immunoassay Buffer C (1X)**

   Add 100 µl Immunoassay Buffer C (1X) to NSB wells. Add 50 µl Immunoassay Buffer C (1X) to B_0 wells.

2. **Microcystin ELISA Standard**

   Add 50 µl from tube #8 to both of the lowest standard wells (S8). Add 50 µl from tube #7 to each of the next standard wells (S7). Continue with this procedure until all the standards are aliquoted. The same pipette tip should be used to aliquot all the standards. Before pipetting each standard, be sure to equilibrate the pipette tip in that standard.

3. **Samples**

   Add 50 µl sample per well. Each sample should be assayed at a minimum of two dilutions. Each dilution should be assayed in duplicate (triplicate recommended).

4. **Microcystin-HRP Tracer**

   Add 50 µl to each well except the TA and Blk wells.

5. **Microcystin ELISA Monoclonal Antibody**

   Add 50 µl to each well except the TA, NSB, and Blk wells within 15 minutes of addition of the tracer.

Incubation of the Plate

Cover each plate with a 96-Well Cover Sheet (Item No. 400012) and incubate 2 hours at room temperature on an orbital shaker.

Development of the Plate

1. Empty the wells and rinse five times with ~300 µl Wash Buffer (1X) per well.
2. Add 175 µl of TMB Substrate Solution (Item No. 400074) to each well.
3. Add 5 µl of the diluted tracer to the TA wells.
4. Cover the plate with 96-Well Cover sheet and protect from light. Optimum development is obtained by using an orbital shaker at room temperature for 30 minutes.
5. Remove the plate cover being careful to keep TMB Substrate Solution from splashing on the cover. *NOTE: Any loss of TMB Substrate Solution will affect the absorbance readings.*
6. DO NOT WASH THE PLATE. Add 75 µl of HRP Stop Solution (Item No. 10011355) to each well of the plate. Blue wells should turn yellow and colorless wells should remain colorless. *NOTE: The stop solution in this kit contains an acid. Wear appropriate protection and use caution when handling this solution.*

Reading the Plate

1. Wipe the bottom of the plate with a clean tissue to remove fingerprints, dirt, *etc.*
2. Read the plate at a wavelength of 450 nm.

ANALYSIS

Many plate readers come with data reduction software that plot data automatically. Alternatively, a spreadsheet program can be used. The data should be plotted as either %B/B_0 *versus* log concentration using a four-parameter logistic fit or as logit B/B_0 *versus* log concentration using a linear fit. *NOTE: Cayman has a computer spreadsheet available for data analysis. Please contact Technical Service or visit our website (www.caymanchem.com/analysis/elisa) to obtain a free copy of this convenient data analysis tool.*

Calculations

Preparation of the Data

The following procedure is recommended for preparation of the data prior to graphical analysis.

NOTE: If the plate reader has not subtracted the absorbance readings of the blank wells from the absorbance readings of the rest of the plate, be sure to do that now.

1. Average the absorbance readings from the NSB wells.
2. Average the absorbance readings from the B_0 wells.
3. Subtract the NSB average from the B_0 average. This is the corrected B_0 or corrected maximum binding.
4. Calculate the B/B_0 (Sample or Standard Bound/Maximum Bound) for the remaining wells. To do this, subtract the average NSB absorbance from the S1 absorbance and divide by the corrected B_0 (from Step 3). Repeat for S2-S8 and all sample wells. (To obtain %B/B_0 for a logistic four-parameter fit, multiply these values by 100.)

NOTE: The TA values are not used in the standard curve calculations. Rather, they are used as a diagnostic tool. Low or no absorbance from a TA well could indicate a dysfunction in the enzyme-substrate system. Only the linear part of this standard curve should be used in calculations.

Plot the Standard Curve

Plot %B/B_0 for standards S1-S8 *versus* microcystin concentration using linear (y) and log (x) axes and perform a four-parameter logistic fit.

Alternative Plot - The data can also be linearized using a logit transformation. The equation for this conversion is shown below. *NOTE: Do not use %B/B_0 in this calculation.*

$$\text{logit } (B/B_0) = \ln [B/B_0/(1 - B/B_0)]$$

Plot the data as logit (B/B_0) *versus* log concentrations and perform a linear regression fit. *NOTE: The units of the standard curve in this assay are provided in ng/ml but can be easily converted to parts per billion (ppb). The conversion is 1 ng/ml = 1 ppb.*

Determine the Sample Concentration

Calculate the B/B_0 (or %B/B_0) value for each sample. Determine the concentration of each sample by identifying the %B/B_0 on the standard curve and reading the corresponding values on the x-axis. *NOTE: Remember to account for any dilution of the original sample concentration prior to its addition to the well. Samples with %B/B_0 values greater than 80% or less than 20% should be re-assayed as they generally fall out of the linear range of the standard curve. A 20% or greater disparity between the apparent concentration of two different dilutions of the same sample indicates interference, which could be eliminated by purification.*

NOTE: If there is an error in the B_0 wells, it is possible to calculate sample concentrations by plotting the absorbance values and back-calculating sample absorbance off the standard curve.

Performance Characteristics

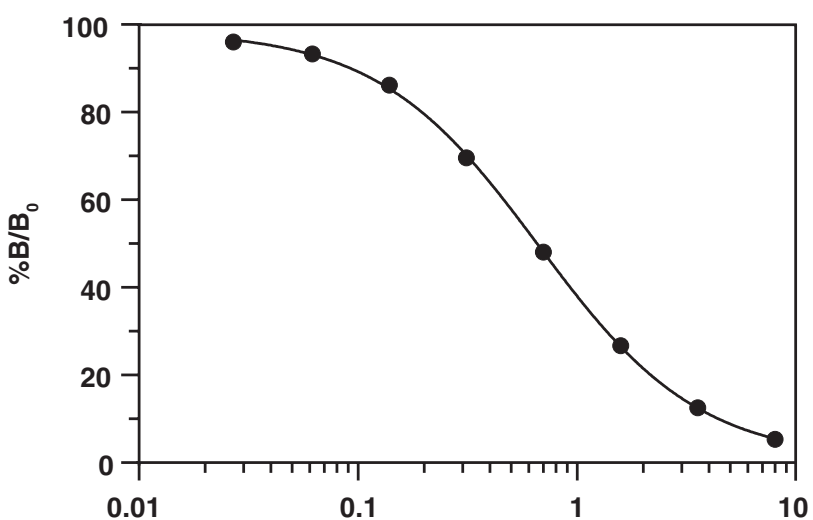
Representative Data

The standard curve presented here is an example of the data typically produced with this kit; however, your results will not be identical to these. You must run a new standard curve. Do not use the data below to determine the values of your samples.

Absorbance at 450 nm (30 minutes)

Analyte Standards (ng/ml)	Blank-subtracted Absorbance	NSB-corrected Absorbance	%B/B_0	%CV* Intra-assay Precision	%CV* Inter-assay Precision
NSB	0.001				
B_0	1.013	1.012			
8.000	0.054	0.053	5.2	4.5	4.2
3.556	0.128	0.127	12.5	6.1	1.6
1.580	0.271	0.270	26.7	7.6	1.9
0.702	0.486	0.485	47.9	8.5	2.5
0.312	0.701	0.700	69.2	9.3	3.1
0.139	0.866	0.865	85.5	11.1	5.5
0.062	0.941	0.940	92.9	25.9**	18.4
0.027	0.967	0.966	95.5	49.0**	25.0**
TA	3.004				

Table 2. Typical results
*%CV represents the variation in concentration (not absorbance) as determined using a reference standard curve
**Evaluate data in this range with caution



Assay Range = 0.027-8.000 ng/ml
Sensitivity (defined as 80% B/B_0) = 0.196 ng/ml
Mid-point (defined as 50% B/B_0) = 0.658 ng/ml
Lower Limit of Detection (LLOD) = 0.021 ng/ml

The sensitivity and mid-point were derived from the standard curve shown above. The standard was diluted in Immunoassay Buffer C (1X).

Figure 7. Typical standard curve

Precision:

Intra-assay precision was determined by analyzing 24 replicates of three matrix controls (marsh water) in a single assay.

Matrix Control (ng/ml)	%CV
607.8	3.4
151.5	4.0
58.9	7.6

Table 3. Intra-assay precision

Inter-assay precision was determined by analyzing replicates of three matrix controls (marsh water) in 8 separate assays on different days.

Matrix Control (ng/ml)	%CV
608.5	7.2
145.3	7.6
56.5	6.5

Table 4. Inter-assay precision

Compound	Cross Reactivity (%)
Microcystin-LR	100.0
Nodularin	155.2
Microcystin-LA	111.5
Microcystin-RR	74.9
Microcystin-LW	68.9
Microcystin-LY	51.4
Microcystin-LF	41.7

Table 5. Cross reactivity

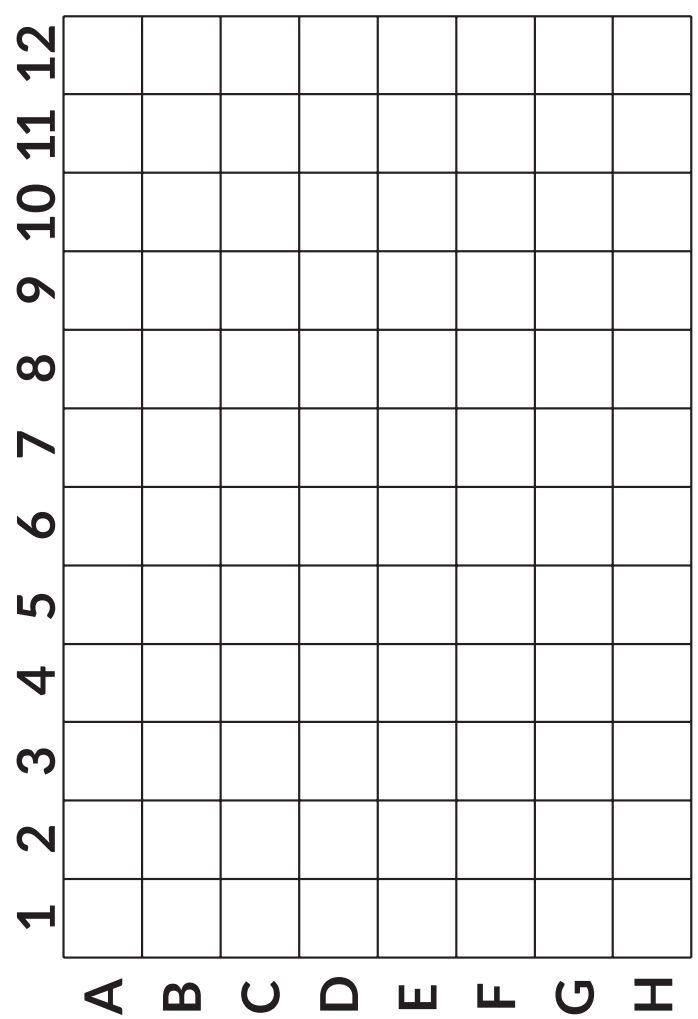
RESOURCES

Troubleshooting

Problem	Possible Causes
Erratic values; dispersion of duplicates	A. Trace organic contaminants in the water source B. Poor pipetting/technique
High NSB (>10% of B_0)	A. Poor washing B. Exposure of NSB wells to specific antibody
Very low B_0	A. Trace organic contaminants in the water source B. Plate requires additional development time C. Dilution error in preparing reagents
Low sensitivity (shift in dose-response curve)	Standard is degraded
Analyses of two dilutions of a biological sample do not agree (*i.e.*, more than 20% difference)	Interfering substances are present
Only TA wells develop	A. Trace organic contaminants in the water source B. The tracer was not added to the well(s)

Procedure	Blk	TA	NSB	B_0	Standards/ Samples
Plate Preparation	Rinse the plate (or strips to be used) five times with ~300 µl Wash Buffer (1X)				
Reconstitute and mix	Mix all reagents gently				
Immunoassay Buffer C (1X)	--	--	100 µl	50 µl	--
Standards/Samples	--	--	--	--	50 µl
Microcystin-HRP Tracer	--	--	50 µl	50 µl	50 µl
Microcystin ELISA Monoclonal Antibody	--	--	--	50 µl	50 µl
Seal and Incubate	Seal and incubate plate for 2 hours at room temperature on an orbital shaker				
Aspirate and Wash	Aspirate wells and wash 5 x ~300 µl with Wash Buffer (1X)				
Apply TMB Substrate	175 µl	175 µl	175 µl	175 µl	175 µl
TA - Apply Tracer	--	5 µl	--	--	--
Development	Seal plate and incubate for 30 minutes at room temperature on an orbital shaker, protected from light				
Apply HRP Stop Solution	75 µl	75 µl	75 µl	75 µl	75 µl
Read	Read optical density at 450 nm				

Table 6. Assay summary



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NOTES

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