Version 22-04/24

User's Manual

Microcystin ELISA Kit

REF **DEIA-NS2306-3**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

CD's Microcystin ELISA Kit is a competitive assay that can be used for quantification of microcystin in various natural water samples. The assay has a range of 0.027-8 ng/ml (0.027-8 ppb) with a midpoint of approximately 0.658ng/ml(0.658 ppb)(50%B/B_0)and asensitivity(80%B/B_0)of approximately 0.196 ng/ml(0.196 ppb). Often environmental reports involving testing of natural sources such as water samples report numbers in part per billion (ppb) 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.

General Description

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. Cyanobacteria accumulate based on a variety of factors, including nutrient availability, pH, and weather conditions, and form blooms on the water's surface. 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. 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. Purified microcystins are lethal to mice via intraperitoneal injection and inhalation, with the 50% lethal dose ranging from 50 to >1,200 µug/kg, i.p., in a congener-specifc manner, but oral ingestion is less toxic. 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. Furthermore, microcystins can accumulate in food plants irrigated with contaminated water.

The presence of microcystins in drinking water sources is positively correlated with the incidence of colorectal cancer. 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. Microcystin-contaminated water can be treated through a variety of processes to reduce or remove microcystins, including activated carbon adsorption, ozonation, or chlorination. The World Health Organization(WHO) recommends a lifetime safe consumption level for microcystins of 1 µg/L (1.0 part per billion (ppb)). Determining the presence of microcystins in water and other samples is the first step to identifying and eliminating the contamination.

Principles of Testing

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 lgG 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:

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

Note:

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-specifc 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.

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.

Reagents And Materials Provided

1. Microcystin-HRP Tracer, 1 vial/100 dtn, -20°C
2. Microcystin ELLISA Monoclonal Antibody, 1vial/100 dtn, -20°C
3. Microcystin ELISA Standard, 1 vial, -20°C
4. Immunoassay Buffer C Concentrate (10×), 1vial/10 ml, 4°C
5. Wash Buffer Concentrate(400×), 1 vial/5 ml, RT
6. Polysorbate 20, 1vial/3 ml, RT
7. Goat Anti-Mouse lgG-Coated Plate, 1 plate, 4°C
8. TMB Substrate Solution, 2 vials/12 ml, 4°C
9. HRP Stop Solution, 1 vial/12 ml, RT
10. ELISA Tracer Dye, 1 vial, RT
11. ELISA Antiserum Dye, 1 vial, RT
12. 96-Well Cover Sheet 1ea RT

Materials Required 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
5. Materials used for Specimen Collection And Preparation

Storage

This kit will perform as specifed if stored as directed in the Reagents And Materials Provided section and used before the expiration date indicated on the outside of the box.

Specimen Collection And Preparation

1. Testing for Interference

This assay has been validated using natural water samples, including pond, marsh, river, lake water, brackish, and sea water. Other sample types should be tested for interference to evaluate the need for sample purification before embarking on alarge number of sample measurements. To test for interference, dilute one or two test samples to obtain at least two different dilutions of each sample between approximately 300 and 1,800 pg/ml(i.e., between 70-25% B/B_0, which is 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.

2. 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(1×) prior to testing in the assay.

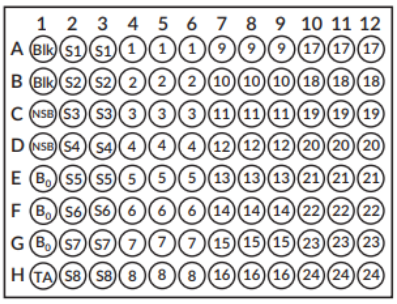
3. General Precautions

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.

Plate Preparation

The 96-well plate(s) included with this kit must be pre-washed five times with Wash Buffer(1×)(~300 µl/well) prior to use in the ELISA. **NOTE: If you donot 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 Bp 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.**



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

Reagent 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(1×) Preparation

Dilute the contents of one vial of Immunoassay Buffer C Concentrate (10×) with 90 ml of ultrapure water. Be certain to rinse the vial to remove any salts that may have precipitated.

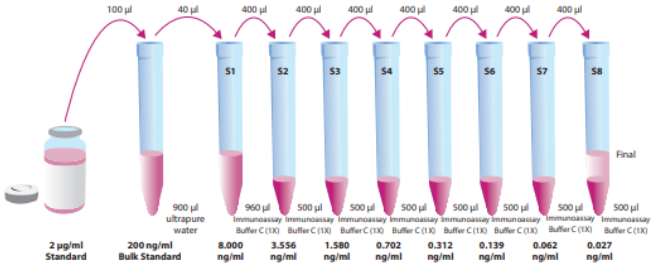
2. Wash Buffer(1×) Preparation

Dilute the contents of one vial of Wash Buffer Concentrate (400×) with ultrapure water to a total volume of 2 L and add 1 ml of Polysorbate 20. Smaller volumes of Wash Buffer(1×) can be prepared by diluting the Wash Buffer Concentrate (400×) 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.**

3. Microcystin ELISA Standard

Equilibrate a pipette tip by repeatedly filling and expelling the tip with the Microcystin ELISA Standard 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 4C 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(1×) to tube#1 and 500 µl Immunoassay Buffer C(1×) 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.**



4. Microcystin-HRP Tracer

Dilute the Microcystin-HRPTracer with 5 ml of Immunoassay Buffer C(1×). Transfer diluted Microcystin HRP Tracer into a polypropelene tube, store it at 4°Cand 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.**

5. Microcystin ELISA Monoclonal Antibody

Reconstitute the Microcystin Monoclonal Antibody with 6 ml of Immunoassay Buffer C(1×). 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.**

Assay Procedure

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 (1×).

Addition of the Reagents:

a. Immunoassay Buffer C(1×)

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

b. Microcystin ELISA Standard

Add 50µl from tube #8 to both of the lowest standard wells (S8). Add 50µl 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.

c. Samples

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

d. Microcystin-HRP Tracer

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

e.Microcystin ELISA Monoclonal Antibody

Add 50µl 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 Plate Cover Sheet and incubate 2 hours at room temperature on an orbital shaker.

Development of the Plate

a.Empty the wells and rinse five times with ~300 µl Wash Buffer (1×) per well.

b. Add 175 µl of TMB Substrate Solution to each well.

c. Add 5 µl of the diluted tracer to the TA wells.

d.Cover the plate with plastic film. Optimum development is obtained by using an orbital shaker at room temperature for 30 minutes.

e. 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.**

f. DO NOT WASHTHE PLATE. Add 75 µl of HRP Stop Solution 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

a. Wipe the bottom of the plate with a clean tissue to remove fingerprints, dirt, etc

b. Read the plate at a wavelength of 450 nm.

ANALYSIS

Manyplate readers come with datareduction softwarethat 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.

Calculation

1. 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.**

a.Average the absorbance readings from the NSB wells.

b.Average the absorbance readings from the B_0 wells.

c.Subtract the NSB average from the Bo average. This is the corrected Bo or corrected maximum binding.

d.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. Erratic absorbance values could indicate the presence of organic solvents in the buffer or other technical problems. 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.

2. 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 pg/ml but can be easily converted to parts per billion (ppb). The conversion is 1ng/ml=1ppb**

3. 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 70% or less than 25% 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 errorin the Bo wells it is possible to calculate sample concentrations by plotting the absorbance values and back calculating sample absorbance off the standard curve.

Specificity

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

Precautions

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.

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 modifed. 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.