Congener-Independent Microcystin ELISA Kit

Competitive enzyme immunoassay kit for quantitative analysis of Microcystins and Nodularins

Catalog Number: EL2024-05

For Research Use Only. Not for use in Diagnostic Procedures.

1. Background

Microcystins are a class of hepatotoxins produced by blue-green algae such as *Microcystis aeruginosa*. Microcystin-LR is the most common of the over 50 different congeners. Cyanobacteria can produce microcystin in large quantities during an algal bloom, which then poses a major threat to our aquatic ecosystems and sources of food, as there is a well-documented phenomenon called "bioaccumulation" that describes the increase in consumed toxin quantities as trophic levels increase.

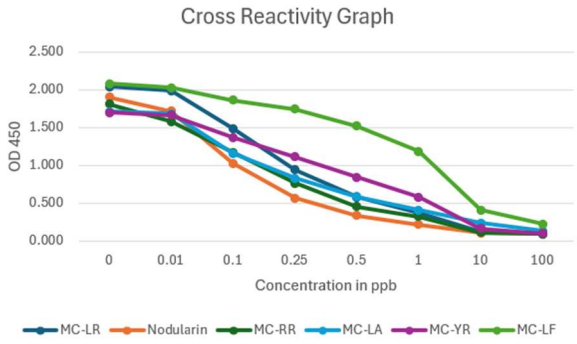
2. Test Principle

The Microcystin ELISA kit is a congener-independent competitive enzyme-labeled immunoassay. The test samples and standards are pipetted into the test wells followed by the anti-Microcystin antibody into the test wells to initiate the reaction. Microcystin from the sample and Microcystin antigen compete for binding to the antibodies. The Microcystin antibody is captured in the test well. Following the 30-minute incubation, the contents of the wells are removed and the wells are washed to remove any unbound Microcystin and free Microcystin antibody. After washing step, HRP-conjugated Antibody#2 is added for another 30-minute incubation. The wells are washed afterwards, and an HRP substrate is then added to the wells and any bound enzyme conjugate causes the production of a blue color. After 15 minutes, the reaction is stopped and the amount of color in each well is measured using a plate reader. The color of the test samples is compared to the color of the standards, and the Microcystin concentration of the samples is derived.

3. Applications

This kit can be used to rapidly test for a broad spectrum of Microcystin congeners in liquid samples such as drinking water and ambient water.

4. Cross Reactivity



5. Equipment and Reagents Not Provided

5.1 Equipment

- ELISA Reader (450nm)
- Distilled or deionized water
- Methanol
- Vortex mixer
- Timer
- Tape or parafilm
- Wash bottle
- Polystyrene centrifuge tube: 50mL, 2mL
- Micropipettes: 20μL-200μL, 100μL-1000μL
- 300μL-multichannel pipette

6. Components Provided in This Kit

- Microtiter plate with 96 wells coated with conjugated Microcystin
- Microcystin (LR) Standards (6 vials × 0.8mL/vial): 0ppb (green cap), 0.15ppb (purple cap), 0.4ppb (yellow cap), 1ppb (blue cap), 2ppb (orange cap), 5ppb (red cap)
- Microcystin Antibody#1: 11mL
- 100X HRP-Conjugated Antibody#2: 0.25mL
- Antibody#2 Diluent: 20mL
- 20X Wash Solution: 28mL
- TMB Substrate Solution: 12mL
- Stop Solution: 14mL
- Sample Diluent: 25 mL

Additional Quality Control, Calibration Verification, and Spiking Solution materials can be obtained separately in optional Supplemental Pack for **Method 546** (catalog # EL2024-Q).

7. Reagents Preparation

- 1X Wash solution: combine one volume of the 20X Wash Solution with 19 volumes of deionized water. Mix well.
- 1X HRP-conjugated Antibody#2: combine one volume of the 100X HRP-Conjugated Antibody#2 with 99 volumes of Antibody#2 Diluent. Vortex for 10 seconds to mix.
  ☛ Prepare this solution fresh before each test.

8. Notice and Precautions Before Operation

- Please use a fresh tip in the process of experiment and change the tips when absorbing different reagent.
- If running more than two strips at once, the use of a multichannel pipette is recommended.

- Make sure that all experimental instruments are clean.
- Stop Solution and TMB Solution are dangerous to touch with direct skin contact, and nitrile gloves are recommended.

9. Sample Preparation

9.1. Liquid (drinking water, ambient water)

- Make sure sample is free of particles and adjusted to a neutral pH.
- All samples should be tested within 24 hours or stored refrigerated (<5 day storage) or frozen at -20°C or under (>5 day storage).
- If necessary, centrifuge to pellet insoluble material (3000g / 5 minutes / at room temperature or filter using a 1.2µm syringe filter). Use 50µL of the supernatant for assay.

10. Assay Process

10.1 Instructions Prior to Beginning Assay

1. Ensure that all reagents and microwells are at room temperature (20-25°C).
2. Refrigerate all reagents immediately after their use.
3. Wash the microwells correctly; this is a vital factor in the reproducibility of the ELISA analysis.
4. Avoid direct sunlight during incubation.

10.2 Steps in the Assay Process

1. Take all reagents out at room temperature (20-25°C) for more than 30 minutes. Shake gently before use.
2. Get the microwells needed out and return the rest into the zip-lock bag at 4-8°C immediately.
3. The diluted wash solution should be brought to room temperature before use.
4. Number every microwell position. <u>All standards and samples should be run in duplicate</u>. It is helpful to record the standards' and samples' positions.
5. Dispense **50µL of Microcystin Standards, controls, or samples** into each well.
6. Dispense **100µL of Antibody #1** into appropriate test wells.
7. Shake the plate gently for 30 seconds using a back-and-forth motion.
8. Cover the plate. Incubate for **30 minutes** at room temperature.
9. Decant the contents of the wells into an appropriate waste container.
10. Rinse the microwells **3 times** with 250µL of the diluted (1X) Wash Solution for each well.
11. Absorb the residual water by inverting with absorbent paper to remove the last of the Wash Solution.
12. Add **150µL of 1X HRP-Conjugated Antibody #2** (freshly prepared) to each well.
13. Shake the plate gently for 30 seconds using a back-and-forth motion.
14. Cover the plate. Incubate for **30 minutes** at room temperature.
15. Decant the contents of the wells into an appropriate waste container.
16. Rinse the microwells **3 times** with 250µL of the diluted (1X) Wash Solution for each well.
17. Absorb the residual solution by inverting with absorbent paper to remove the last of the

Wash Solution.

18. Add **100µL TMB Substrate Solution** to each well, mix gently by shaking the plate manually and incubate for **15 minutes** at 25°C with cover.
19. Add **100µL of Stop Solution** to each well. Mix gently by shaking the plate manually and measure the absorbance at 450nm. Read the result within 5 minutes after addition of Stop Solution.

11. Results

11.1 Calculating the Percentage Absorbance

- The mean values of the absorbance values obtained from the standards and the samples are divided by the absorbance value of the first standard (zero standard) and multiplied by 100%.

  Absorbance (%) = B / B_0 *100

  B = mean absorbance value of each standard or each sample

  B_0 = mean absorbance value of zero standard (0 ppb)

11.2 Standard Curve

- To produce the standard curve, make the average absorbance value of standards the y-axis, and make the x-axis the concentration of the standards (ppb) using a logarithmic scale.
- To obtain the actual concentration of your diluted sample(s), the concentration of each sample calculated from the standard curve must then be multiplied by the sample's corresponding dilution factor.
- Sample dilution factor: If the absorbance of a sample is lower than that of the highest calibrator (5 ppb), this means the concentration of microcystin in the sample is too high, exceeding the standard curve's upper limit. Dilute the sample using the Sample Diluent and rerun to obtain accurate results.
  - Sample(s) should be diluted to fit into the standard curve, based on the estimated concentration. The dilution factor should result in a diluted sample concentration near the EC_{50}.
  - Resulting concentration must then be multiplied by the dilution factor used.
- Curve-fitting can be done manually or with data reduction software, fit according to a 4-parameter logistic (4PL) curve:

$$y = d + \frac{a-d}{1+\left(\frac{x}{c}\right)^b}$$

y= absorbance, x= concentration

a= maximum absorbance (B_0), d= minimum absorbance

b= slope at inflection point, c= concentration yielding the inflection point (EC_{50})

11.3 Evaluation

- A sample with a lower absorbance than a standard has more Microcystins in it than the standard does, and the opposite is true with a higher absorbance. This is a way to obtain semi-quantitative results before manually evaluating or running your data through commercial programs for ELISA analysis.

- Samples with less than the 0.15ppb standard should be reported as having <0.15 ppb Microcystins.

12. Sensitivity, Accuracy and Precision

12.1 Test Sensitivity:

- Overall Sensitivity 0.15ppb

12.2 Detection limit:

- Drinking water and ambient water 0.15ppb

12.3 Accuracy:

- Drinking water and ambient water..... 80±10%

12.4 Precision:

- C.V. of the ELISA kit ..... less than 10%

13. General Instructions

13.1 Temperature of Reagents and Samples

- The mean values of the absorbance values obtained for the standards and the samples will be reduced if the reagents and samples have not been restored to room temperature (20-25°C) before use.

13.2 Microwells

- Do not allow microwells to dry between steps to avoid unsuccessful reproducibility and operate the next step immediately after tapping the microwells holder.

13.3. Shaking of Reagents

- Shake each reagent gently before use.

13.4. Skin Protection

- Take extra care to keep the Stop Solution and TMB Solution from contacting your skin. Nitrile or nitrile-blend gloves are recommended.

13.5 Out-of-Date Kits

- Don't use kits that are expired. Don't exchange the reagents of different batches, or it will drop the sensitivity.

13.6 General Comments

- Keep the ELISA kit at 4-8°C, and **do not freeze**. Refrigerate the unused microwell strips in the foil pouch they came in. Avoid straight sunlight during all incubations. Covering the microtiter plates is recommended.

13.7 Special Issues Concerning Solutions and Reagents

- TMB Substrate should be discarded if it turns a blue hue, as it should be clear.
- One or more of the reagents may be bad if the absorbance value of the zero standard is less than 0.5 (A450nm<0.5).

13.8 Incubation Temperatures

- Incubation temperature should be at room temperature (20-25°C). Higher or lower temperature on the day of testing will lead to experiment-to-experiment variability.

14. Storage

- Storage conditions: 4-8°C, without light
- Storage period: 12 months or until expiration date
    - For most accurate results, only use reagents and standards from the same kit. The kit's expiration date is thereby that of the earliest expiring component.

Customer Notes:

Contact Us
Attogene
3913 Todd Lane, Suite 310 Austin, TX 78744
Phone: 512-333-1330
Email: sales@attogene.com
Web: www.attogene.com