Microcystin ELISA Kit

Competitive enzyme immunoassay kit for quantitative analysis of Microcystin

Catalog Number: EL2024-02

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

1. Background

Microcystin 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 algae bloom which then pose a major threat.

2. Test Principle

The Microcystin plate kit is a competitive enzyme-labeled immunoassay. The Microcystin sample extract and calibrators are pipetted into the test wells followed by the Microcystin antibody into the test wells to initiate the reaction. During the 30 minutes incubation period, Microcystin from the sample and Microcystin antigen compete for binding to the Microcystin antibody. The Microcystin antibody is captured on the walls of the test well. Following this 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 wash, 1X HRP-conjugated Antibody#2 is added for 30 minutes incubation. The wells are washed afterwards, and a clear substrate is then added to the wells and any bound enzyme conjugate causes the conversion to a blue color. Following a 15-minute incubation, the reaction is stopped and the amount of color in each well is read. The color of the unknown samples is compared to the color of the calibrators and the Microcystin concentration of the samples is derived.

3. Applications

This kit can be used for rapid test of Microcystin in liquid samples such as water, wastewater, algal cultures, and solid samples such as fish.

4. Cross Reactions

Microcystin LR	100%
Microcystin RR	162%
Microcystin YR	< 1%
Microcystin LA	< 1%
Microcystin LF	< 1%

5. Equipment and Reagents Needed (not provided)

5.1 Equipment

- ELISA Reader (450nm/630nm)
- Deionized water
- Methanol
- Vortex mixer
- Timer
- Wash bottle
- Polystyrene centrifuge tube: 50mL, 2mL
- Micropipettes: 20μL-200μL, 100μL-1000μL
- 300μL-multipipette

6. Components Provided in This Kit

- Microtiter plate with 96 wells coated with Microcystin
- Microcystin (LR) Standards (6 vials×0.8mL/vial) 0ppb (green cap), 0.05ppb (purple), 0.1ppb (yellow cap), 0.2ppb (blue cap), 0.4ppb (orange cap), 2.0ppb (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

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 required.
- Make sure that all experimental instruments are clean.
- Treated samples can be stored at 2-8°C for 24 hours in the dark.

9. Sample Preparation

9.1. Liquid (water, wastewater, liquid media)

- Make sure sample is free of particles and adjusted to a neutral pH.
- If necessary, centrifuge to pellet insoluble material (3000g / 5min / at room temperature or filter using a 1.2μm syringe filter).
- Take 50μL of the supernatant for assay.

9.2. Solid

- Homogenize with commercial blender for at least 3 minutes to be sure of homogeneity.
- Add 9.0 mL of 90% Methanol/water to 1 g of this homogenate and vortex for two minutes.
- Centrifuge to pellet insoluble material at 3000xg for 10 minutes.
- Dilute the supernatant with Antibody #2 Diluent.
- Use 50μL of the diluted 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. Return all reagents to 2-8°C 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 the incubation.

10.2 Steps in the Assay Process

1. Take all reagents out at room temperature (20-25°C) for more than 30min. Shake gently before use.
2. Get the microwells needed out and return the rest into the zip-lock bag at 2-8°C immediately.
3. The diluted wash solution should be brought to room temperature before use.
4. Number every microwell position and all standards and samples should be run in duplicate. Record the standards and samples positions.
5. Dispense 50μL of the Microcystin Standards, positive control, or sample into each well.
6. Dispense 100μL of the 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 with 250μL of the 1X Wash Solution 3 times.
11. Absorb the residual water by inverting with absorbent paper to remove the last of the wash solution.
12. Add 150μL of freshly prepared 1X HRP-Conjugated Antibody#2 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 with 250μL of the 1X Wash Solution 3 times.
17. Absorb the residual water 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 15min at 25°C with cover.
19. Add 100μL the Stop Solution to each well. Mix gently by shaking the plate manually and measure the absorbance at 450nm (Read the result within 5min 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 = the mean absorbance value of each standards or each sample

  B_0 = absorbance value of zero standard

11.2 Drawing a Standard Curve

- To draw a standard curve, the absorbance value of standards as y-axis, semilogarithmic of the concentration of the standards (ppb) as x-axis.
- The concentration of each sample (ppb), which can be read from the standard curve, is multiplied by the corresponding dilution factor of each sample followed, and the actual concentration of sample is obtained.
- Sample dilution factor: If the absorbance of a sample is lower than the highest calibrator (2 ppb), the concentration of Microcystin is too high and out of range of the standard curve. Dilute the sample and rerun. Samples should be diluted to fit into the standard curve (0.05 ppb to 2 ppb). Results must then be multiplied by the dilution factor used.

12. Sensitivity, Accuracy and Precision

12.1 Test Sensitivity:

- Overall Sensitivity 0.1ppb

12.2 Detection limit:

- Water, wastewater, culture media 0.1ppb

12.3 Accuracy:

- Water, wastewater, culture media ..... 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).

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

- The Stop Solution is 0.75N HCl, keep your skin away from it.

13.5 Out of Date Kits

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

13.6 General Comments

- Keep the ELISA kits at 2-8°C, do not freeze. Store the unused microwell plates back to the foil pouch. Avoid straight sunlight during all incubations. Covering the microtiter plates is recommended.

13.7 Special Issues Concerning Solutions and Reagents

- Substrate solution should be abandoned if it turns colors. The reagents may be turn bad if the absorbance value (450/630nm) of the zero standard is less than 0.5 (A450nm<0.5).

13.8 Special Issues Concerning Color

- The coloration reaction takes 15min after the addition of TMB Substrate, but you can prolong the incubation time ranges to 35min or more if the color is too light to be determined, never exceed 40min, on the contrary, shorten the incubation time properly.

13.9 Incubation Temperatures

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

14. Storage

- Storage condition: 2-8°C
- Storage period: 12 months

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