Competitive enzyme immunoassay kit for quantitative analysis of Microcystin

Catalog Number: EL2024-01

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

This kit is a product for detection of microcystin in liquid samples based on a one-step competitive enzyme-labeled immunoassay technology. It is fast, simple, accurate and sensitive. And it requires only 1 hour to perform which considerably minimizes work intensity and operation error. Briefly, microcystin enzyme conjugate is pipetted into the test wells followed by calibrators or sample. A microcystin antibody solution is then added into the test wells to initiate the reaction. During a 30-minute incubation period, microcystin from the sample and microcystin enzyme conjugate compete for binding to the microcystin antibody. Following this incubation, the wells are washed, and a colorless substrate is added into the wells and any bound microcystin enzyme conjugate will convert the substrate to a blue color. Following another 30-minute incubation, the reaction is stopped with the addition of stop solution. The color of the unknown sample is compared to the color of the microcystin standards.

3. Applications

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

4. Cross Reactions

Microcystin LR	100%
Microcystin RR	73%
Microcystin YR	58%
Microcystin LA	2%
Microcystin LF	3%
Microcystin LW	4%
Nodularin	126%

5. Equipment and Reagents Needed (not provided)

5.1 Equipment

- ELISA reader (450nm/630nm)
- Deionized water
- 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 antibody
- Microcystin-LR standard solutions (5 bottles×2ml/bottle) 0ppb, 0.1ppb, 0.3ppb, 0.8ppb, 1.0ppb, 2.0ppb
- Spiking solution (1ppb) 2ml
- Microcystin enzyme conjugate 8ml
- Microcystin Antibody solution 8ml
- Substrate solution 14ml
- Stop solution 7ml
- 100×concentrated wash solution 25ml

7. Reagents Preparation

Solution 1: Wash solution

- Dilute the 100X concentrated wash solution with deionized water in the volume ratio of 1:99, which will be used to rinse the plates. The diluted wash solution can be conserved for one month at 4℃.

8. Notice and Precautions Before Operation

- Please use one tip in the process of experiment and change the tips when absorbing different reagent.
- The stop solution is 1 N hydrochloric acid, which is corrosive and an irritant. Avoid contact with skin and mucous membranes. Immediately clean up any spills and wash area with copious amounts of water. If contact should occur, immediately flush with copious amounts of water.
- 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 24h in 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 (20-25°C);
- Take 50μl of the supernatant of the sample for assay.

10. Assay Process

10.1 Instructions Prior to Beginning Assay

- Ensure that all reagents and microwells are at room temperature (20-25℃). Notice: The antibody solution should be stored at 4℃, which will be used immediately after taking out.
- Return all the rest reagents to 2-8℃ immediately after their use.
- Wash the microwells correctly; this is a vital factor in the reproducibility of the ELISA analysis.
- Avoid direct sunlight during the incubation; use the plate cover provided in the kit to cover the plate.

10.2 Steps in the Assay Process

1. Take all reagents out at room temperature (20-25℃) for more than 30min. Shake gently before use. Notice: The antibody solution should be stored at 4℃, which will be used immediately after taking out.
2. Get the microwells needed out and return the rest into the zip-lock bag at 2-8℃ immediately.
3. The diluted wash solution should be brought to room temperature before use.
4. Number: 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 enzyme conjugate into each well.
6. Dispense 50μl of the standards, positive control, or sample into appropriate test wells.
7. Dispense 50μl of antibody solution into each well.
8. Shake the plate gently for 30 seconds using a back and forth motion.
9. Incubate for 30 minutes at room temperature.
10. Decant the contents of the wells into an appropriate waste container.
11. Rinse the microwells with 250μl diluted wash solution at interval of 10 seconds for 4-5 times.
12. Absorb the residual water by inverting with absorbent paper to remove the last if the wash solution.
13. Add 100μl substrate solution to each well, mix gently by shaking the plate manually and incubate for 30min at 25℃ with cover.
14. 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 (2.0 ppb), the concentration of Microcystin is too high and out of range of the standard curve. Dilute the sample in laboratory grade water and rerun. Samples should be diluted to fit into the standard curve (0.1 ppb to 2.0 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±15%

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 regulated to room temperature (20-25℃). The antibody solution should be stored at 4℃, which will be used immediately after taking out. If the antibody solution is return to room temperature before assay, the OD values will be higher, and the result of the assay will not be right.

13.2 Microwells

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

13.3. Shaking of Reagents

- Shake each reagent gently before use.

13.4. Skin Protection

- Keep your skin away from the stop solution for it is the 1 N HCl solution.

13.5 Out of Date Kits

- Don't use kits that are out of date. 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℃, do not freeze. Seal rest microwell plates, 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 needs 30min after the addition of solution A and solution B, 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 (20-28℃). Higher or lower temperature on day of testing will lead to experiment-to-experiment changes.

14. Storage

- Storage condition: 2-8℃
- Storage period: 12 months

Who we are

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Our mission is to:

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