Cylindrospermopsin ELISA Kit

Competitive enzyme immunoassay kit for quantitative analysis of Cylindrospermopsin

Catalog Number: EL2047-02

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

1. Background

The Cylindrospermopsin Plate Kit is a competitive ELISA for the quantitative analysis of Cylindrospermopsin in water.

2. Test Principle

The Cylindrospermopsin plate kit is a competitive enzyme-labeled immunoassay. The Cylindrospermopsin sample extract and calibrators are pipetted into the test wells followed by the Cylindrospermopsin antibody into the test wells to initiate the reaction. During the 30 minutes incubation period, Cylindrospermopsin from the sample and Cylindrospermopsin antigen compete for binding to the Cylindrospermopsin antibody. The Cylindrospermopsin 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 Cylindrospermopsin and free Cylindrospermopsin 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 Cylindrospermopsin concentration of the samples is derived.

3. Applications

This kit can be used for rapid testing of Cylindrospermopsin in liquid samples such as water and wastewater.

4. Components Provided in This Kit

- Microtiter plate with 96 wells coated with Cylindrospermopsin
- Cylindrospermopsin Standard Solutions (5 vials × 0.8mL/vial): 0ppb (green cap), 0.03ppb (purple), 0.1ppb (yellow cap), 0.2ppb (blue cap), 2ppb (orange cap)
- Cylindrospermopsin Positive Control 1 vial × 0.8mL: 0.4ppb (red cap)
- Cylindrospermopsin Antibody#1: 11mL
- 100X HRP-Conjugated Antibody#2: 0.25mL

- Antibody#2 Diluent: 20mL
- TMB Substrate Solution: 12mL
- Stop Solution: 14mL

5. Equipment and Reagents Needed (not provided)

- ELISA reader (450nm)
- Deionized or distilled water
- Vortex mixer
- Timer
- Wash bottle
- Absorbent material
- Micropipettes: 20μl-200μl, 100μl-1000μl
- Multi-channel pipette: 8-channel (50 & 100 μl)

6. Specificity

Cylindrospermopsin residues can be detected by this assay. Common cyanotoxins which can be found in water samples were tested in the assay and their reactivity is listed in the table below.

Compound	% Reactivity
Cylindrospermopsin	100%
7-Epi-Cylindrospermopsin	153%
7-Deoxy-Cylindrospermopsin	126%
Microcystin-LR	<1%
Nodularin	<1%

7. 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.

8. Sample Preparation

- Water samples should be free of particles and adjusted to a neutral pH.
- If necessary, centrifuge or filter samples prior to running in the assay.

9. Reagents Preparation

- 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.

10. Assay Procedure

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 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. Number every microwell position and all standards and samples should be run in duplicate. Record the standards and samples positions.
4. Dispense **50μL of Cylindrospermopsin Standards, positive control, or sample** into each well.
5. Dispense **100μL of Antibody#1** into appropriate test wells.
6. Shake the plate gently for 30 seconds using a back-and-forth motion.
7. Cover the plate. Incubate for **30 minutes** at room temperature.

8. Decant the contents of the wells into an appropriate waste container.
9. Rinse the microwells with 250µL of deionized or distilled water 4 times.
10. Absorb the residual solution by inverting with absorbent paper to remove the last of the residual solution.
11. Add **150µL of 1X HRP-Conjugated Antibody#2** (freshly prepared) to each well.
12. Shake the plate gently for 30 seconds using a back-and-forth motion.
13. Cover the plate. Incubate for **30 minutes** at room temperature.
14. Decant the contents of the wells into an appropriate waste container.
15. Rinse the microwells with 250µL of the deionized or distilled water 4 times.
16. Absorb the residual solution by inverting with absorbent paper to remove the last of the residual solution.
17. Add **100µL of TMB Substrate Solution** to each well, mix gently by shaking the plate manually and incubate for **15 minutes** at 25°C with cover.
18. 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 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%.

$$\text{Absorbance (\%)} = B / B_0 * 100$$

B = the mean absorbance value of each standard 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 Cylindrospermopsin 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 (0.03 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 0.1ppb

12.3 Accuracy:

- Water, wastewater ..... 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 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

Who we are

Attogene is a biotechnology company located in Austin, Texas. Our focus is to enhance health and wellness by offering and developing customer focused Life Science Products domestically and internationally.

Our mission is to:

- Enhance detection technologies
- Enable rapid responses
- Enable impactful research discoveries

Contact Us

3913 Todd Lane, Suite 310
Austin, TX 78744

Phone: 512-333-1330

Email: sales@attogene.com

Web: www.attogene.com

EL2047-02.V1_202305012