Cylindrospermopsin Lateral Flow Kit (Recreational Water)

Catalog Number: AU2058

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

1. Intended Use

For the screening of Cylindrospermopsin in freshwater source water, drinking water and recreational water samples at 30 and 3,000 ppt. Samples requiring action should be confirmed by ELISA (see Attogene Cylindrospermopsin ELISA catalog number EL2047).

2. Introduction

Attogene' s Cylindrospermopsin Lateral Flow Kit can be used to detect Cylindrospermopsin in source water samples.

Format: Rapid-Water - Run Time: 30 Minutes, enough to run 5 samples undiluted and with a 10-fold dilution and 5 negative controls.

Cylindrospermopsin (CYN) is a potent cyanotoxin synthesized by select species of cyanobacteria, prominently including Cylindrospermopsin raciborskii. It belongs to the tricyclic alkaloid class, exhibiting a molecular weight of approximately 415 Da. Structurally, cylindrospermopsin features an uracil ring fused with a hydantoin moiety, alongside a guanidino group, attributes that render it highly soluble and polar in aqueous environments.

Cylindrospermopsin is notorious for its profound toxicity towards aquatic organisms and its potential threat to human health through exposure via contaminated water and food sources. Consequently, rigorous monitoring protocols are essential in regions prone to cyanobacterial blooms, where cylindrospermopsin can accumulate in freshwater reservoirs and other aquatic habitats. In recognition of these risks, regulatory bodies such as the United States Environmental Protection Agency (EPA) have implemented an action level guideline:

Do not Drink – 0.7 μg/L for bottle fed infants and preschool children, pregnant and nursing woman, elderly immunocompromised and liver conditions
Do not Drink – 3.0 μg/L for school age children to adults
Do Not Use – 20 μg/L
EPA Draft Human Health Recreational Ambient Water Quality Criteria to protect human health: 8 μg/L

3. Kit Contents

Component Name	Volume	Storage
Cylindrospermopsin Cassette	15	RT
Syringe	5	RT
Sample Filter	5	RT
Sample, Dilution Buffer	5	RT
10-fold Sample, Dilution Buffer	5	RT
Negative Control	5	RT
Water Collection Tube	5	
Sample Collection Tube	5	RT
200ul fixed volume pipette	5	RT

4. Storage and Stability

- The kit should be stored at 2°C - 30°C until ready to use.
- The test must remain in the sealed pouch until use.

5. Required Materials Not Supplied

- Timer - For timing use
- Marker – for labeling

6. Precautions

- The Cylindrospermopsin Lateral Flow Kit provides only preliminary qualitative test results. Use another, more quantitative, analytical method such as ELISA or instrumental analysis to obtain a confirmed quantitative analytical result.
- Prior to use, ensure that the product has not expired by verifying that the date of use is prior to the expiration date on the label.
- The test cassettes are individually packaged in a foil pouch with a desiccant & disposable pipette.
- Avoid cross-contamination of samples by using a new bottle for each sample.

- Use only Cylindrospermopsin Lateral Flow Kit reagents from one kit lot, as they have been calibrated in combination.
- It is good laboratory practice to use positive and negative controls to ensure proper test performance. Samples which do not contain Cylindrospermopsin (negative controls) as well as samples containing known quantities of Cylindrospermopsin (positive controls) should be analyzed with each lot of test strips to provide a reference for line intensity to be expected.

7. Water Collection and Storage

- Using gloves, collect water samples into the 125 ml Water Sample Bottle and store refrigerated for up to 5 days (label the water collection bottle). If samples must be stored for a period greater than 5 days, samples should be stored frozen.
- Allow the test cassettes, running buffer, and samples to reach room temperature before testing.

8. Procedure

- Using gloves, remove each lateral flow cassette from the foil pouch. A marker may be used to write on the plastic cassette if desired.

Perform A, B, C and D for each sample evaluation starting with the negative control first.

A. Negative Control (perform first):

- Use the syringe to transfer 200ul (denoted as 0.2 on the side of the syringe) from the Negative Control tube directly into the sample port of the cassette.
- Set a timer for 30 minutes.

B. Sample Preparation:

- Next, using the same emptied syringe, transfer 1mL of sample from the 125mL water sample bottle into the syringe.
- Add the debris filter onto the end of the syringe.
- Collect filtered sample into the sample collection tube.
- Remove the debris filter.
- Transfer an additional 1mL of sample from the 125mL water sample bottle into the syringe.
- Add the debris filter onto the end of the syringe.

- Collect remaining sample into the sample collection tube.

C. Undiluted Sample:

a. Using the 200ul fixed volume pipette, transfer 200ul of sample from the sample collection tube into the sample, dilution buffer tube.
b. Mix gently up and down 3 times using the fixed volume pipette.
c. Collect 1mL of sample into the syringe.
d. Add the debris filter onto the end of the syringe.
e. Add the full contents of the syringe directly into the sample port of the cassette.
f. Set a timer for 30 minutes.

D. 10X Diluted Sample:

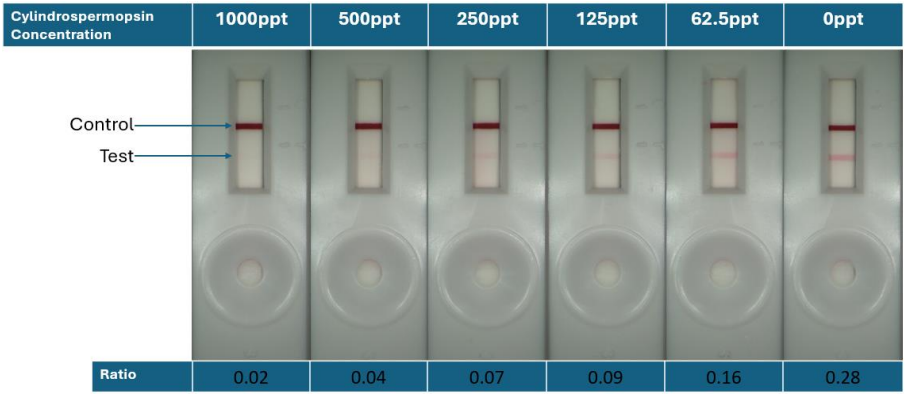
a. Using the 200ul fixed volume pipette, transfer 200ul of sample from the sample collection tube into the sample, 10X dilution buffer tube.
b. Mix gently up and down 3 times using the fixed volume pipette.
c. Collect 1mL of sample into the syringe.
d. Add the debris filter onto the end of the syringe.
e. Add the full contents of the syringe directly into the sample port of the cassette.
f. Set a timer for 30 minutes.

9. Interpretation of Results

For samples prepared as described above, screening concentrations are determined by comparison of the intensity of the test line to the intensity of the control line on parallel test strips. Although the control line intensity may vary, a visible control line must be present for results to be considered valid. Test strips with a test line which is darker than or of equal intensity to the test line of the control are indicative of results which are below the limit of detection of the test. Test strips with a test line which is lighter than the control strip indicates a result which is $\geq$ 30 ppt. Test strips with no test line visible (only the control line is visible) indicate a result which is $\geq$ 1 ppb. Results should be determined within 5 minutes after completion of the strip test procedure. Determinations made using strips which have dried outside of the required time may be inaccurate, as line intensities may vary with drying time.

When interpreting the results of your sample, the dilution factor must be taken into account. The EPA offers HA in drinking water levels for both children under 6 years of age and for anyone older.

The levels for children under the age of 6 years old are 700ppt. For adults it is 3000ppt (3ppb). Therefore, if you have a sample with a starting concentration of 3000ppt, for the first dilution (10-fold) you should expect to see a faint test line present although not completely absent from the test. For the second dilution (100-fold) expect to see *almost* no visually discernable drop in test line strength.



The appearance of test strips may also be compared to the illustration to determine approximate sample concentration ranges. Please note that the illustration is intended for the demonstration of test line to control line intensity only. Results should not be determined by comparing the intensity of test lines from test strips to the test line intensity of the illustration, as the overall intensity of test strips may vary slightly with different lots of reagents. To obtain semi-quantitative results in the range of 0 – 1 ppb, solutions of known Cylindrospermopsin concentrations (control solutions) must be tested concurrently with samples. Sample test line intensities can then be compared with control solution test line intensities, yielding approximate sample concentrations. Do not use strips run previously to determine semi-quantitative sample concentrations, as test line intensities may vary once strips are completely dry.

10. Additional Analysis

If necessary, positive samples can be confirmed by ELISA, HPLC, or other conventional methods. A lateral flow reader may also be employed to generate numerical readings from the visual result. Contact us if you have any questions at sales@attogene.com.