

## Importance of Cylindrospermopsin Determination

Most of the world's population relies on surface freshwaters as its primary source for drinking water. The drinking water industry is constantly challenged with surface water contaminants that must be removed to protect human health. Toxic cyanobacterial blooms are an emerging issue worldwide due to increased source water nutrient pollution caused by eutrophication. Cylindrospermopsin is a toxin produced by several different types of cyanobacteria (blue-green algae) and has been found in fresh water throughout the world. Certain strains of *Cylindrospermopsis raciborski* (found in Australia, Hungary, and the United States), *Umezakia natans* (found in Japan), and *Aphanizomenon ovalisporum* (found in Australia and Israel) have been found to produce Cylindrospermopsin. The production of Cylindrospermopsin seems to be strain specific rather than species specific.

Acute poisoning of humans and animals constitutes the most obvious problem from toxic cyanobacterial blooms and, in several cases, has led to death. Human and animal exposure to these toxins can occur through the ingestion of contaminated water, through drinking or during recreational activities in which water is swallowed, or food, such as fish. Dermal contact with Cylindrospermopsin may occur during showering or bathing, or during recreational activities such as swimming or boating. These toxins mediate their toxicity by inhibiting liver function and are potent inhibitors of protein synthesis and glutathione, leading to cell death.

To protect against adverse health effects, the U.S. Environmental Protection Agency (EPA) has established health advisories for Cylindrospermopsin in drinking water:

- For children pre-school age and younger (less than six years old), 0.7 µg/L (ppb)
- For school-age children and adults, 3.0 µg/L (ppb)

## Performance Data

**Test sensitivity:** The Eurofins Abraxis Cylindrospermopsin Strip Test for fresh water will detect Cylindrospermopsin at 0.5 ng/mL or higher. At this level, the test line exhibits moderate intensity. At levels greater than 10 ng/mL the test line is not visible. When compared with samples of known Cylindrospermopsin concentration, it is possible to obtain a semi-quantitative result.

**Selectivity:** The assay exhibits very good cross-reactivity with Cylindrospermopsin and Deoxy-Cylindrospermopsin.

**Cell Lysing:** A sample correlation between the QuikLyse™ reagents and the 3 cycle freeze/thaw method showed a good correlation.

**Samples:** A sample correlation between the Eurofins Abraxis Strip Test and ELISA methods showed a good correlation.

**General Limited Warranty:** Eurofins Abraxis warrants the products manufactured by the Company, against defects and workmanship when used in accordance with the applicable instructions for a period not to extend beyond the product's printed expiration date. **Eurofins Abraxis makes no other warranty, expressed or implied. There is no warranty of merchantability or fitness for a particular purpose.**

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R08122020

# Cylindrospermopsin Strip Test



Abraxis

Immunochromatographic Strip Test for the Detection  
of Cylindrospermopsin in Drinking and Recreational Waters

QuikLyse™ reagents may be used in a method of U.S. Patent 9,739,777

**Product No. 520029 (5 Test), 520030 (20 Test)**

## 1. General Description

The Eurofins Abraxis Cylindrospermopsin Strip Test for Water is a rapid immunochromatographic test designed solely for use in the qualitative screening of Cylindrospermopsin in fresh water. A rapid cell lysis step (QuikLyse™) performed prior to testing is required to measure total Cylindrospermopsin (dissolved, or free, plus cell-bound). The Eurofins Abraxis Microcystins Strip Test provides only preliminary qualitative test results. If necessary, positive samples can be confirmed by ELISA, HPLC or other conventional methods.

## 2. Safety Instructions

Discard samples according to local, state and federal regulations.

## 3. Storage and Stability

The Cylindrospermopsin Strip Kit should be stored between 2-30°C. The test strips, test vials, and water samples to be analyzed should be at room temperature before use. Reagents may be used until the last day of the month as indicated by the expiration date on the box.

## 4. Test Principle

The test is based on the recognition of Cylindrospermopsin by specific antibodies. The toxin conjugate competes for antibody binding sites with Cylindrospermopsin that may be present in the water sample. The test device consists of a vial containing specific antibodies for Cylindrospermopsin labeled with a gold colloid and a membrane strip to which a conjugate of the toxin is attached. A control line, produced by a different antibody/antigen reaction, is also present on the membrane strip. The control line is not influenced by the presence or absence of Cylindrospermopsin in the water sample and, therefore, should be present in all reactions.

In the absence of toxin in the water sample, the colloidal gold labeled antibody complex moves with the water sample by capillary action to contact the immobilized Cylindrospermopsin conjugate. An antibody-antigen reaction occurs forming a visible line in the 'test' area. The formation of two visible lines of similar intensity indicates a negative test result, meaning the test did not detect the toxin at or above the cut-off point established for the toxin. If Cylindrospermopsin is present in the water sample, it competes with the immobilized toxin conjugate in the test area for the antibody binding sites on the colloidal gold labeled complex. If a sufficient amount of toxin is present, it will fill all of the available binding sites, thus preventing attachment of the labeled antibody to the toxin conjugate, therefore preventing the development of a colored line. If a colored line is not visible in the test line region, or if the test line is lighter than the control line, Cylindrospermopsin is present at a level of concern. Semi-quantitative results in the range of 0-10 ppb can be obtained by comparing the sample test strip appearance to the appearance of test strips from solutions of known Cylindrospermopsin concentrations (control solutions). Concentrated Cylindrospermopsin standards which can be used to prepare Cylindrospermopsin controls are available through Eurofins Abraxis (PN 300626).

## 5. Limitations of the Cylindrospermopsin Strip Test, Possible Test Interference

Numerous organic and inorganic compounds commonly found in water samples have been tested and found not to interfere with this test. However, due to the high variability of compounds that might be found in water samples, test interferences caused by matrix effects can't be completely excluded.

Mistakes in handling the test can also cause errors. Possible sources for such errors include:

Inadequate storage conditions of the test strip, too long or too short incubation times, extreme temperatures during the test performance (lower than 10°C or higher than 30°C).

The test is designed for use with fresh water only. The use of the test with brackish or seawater samples will produce inaccurate results. The Cylindrospermopsin Strip Test provides only a preliminary qualitative test result. Use another more quantitative analytical method such as ELISA or instrumental analysis to obtain a quantitative analytical result. Apply good judgement to any test result, particularly when preliminary positive results are observed.

## 6. Warnings and Precautions

- Cylindrospermopsin Strip Test is for the screening of fresh water samples for total Cylindrospermopsin content (free and cell-bound). To screen chlorinated source drinking water or finished drinking water samples for total Cylindrospermopsin content, samples must be preserved (quenched) with sodium thiosulfate at the time of collection and should be manually lysed (freeze/thaw method, etc.) prior to testing.
- QuikLyse™ reagents **must** be used with the Cylindrospermopsin Test Strips for all samples, including positive and negative controls and samples which have been subjected to manual (freeze/thaw) lysing. Use of the Cylindrospermopsin Test Strips **without** the QuikLyse™ reagents will adversely affect the performance of the test, producing inaccurate results.
- Use only the Cylindrospermopsin test strips and QuikLyse™ reagents from one kit lot, as they have been adjusted in combination.
- All reagents and samples should be allowed to reach room temperature before testing.
- 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.
- For test strips packaged in a desiccated vial, the vial should be kept completely closed except for opening to remove test strips. When re-closing, snap lid firmly.
- Avoid cross-contamination of water samples by using a new sample vial and disposable pipette for each sample.
- Samples containing unusually large amounts of algal blooms or very thick algal scums should be diluted 1:1 with deionized or distilled water prior to lysis, as overly viscous samples may not allow for uniform cell lysis or proper capillary flow up the test strip. Diluted samples will have a cut-off of 20 ppb.
- Use reasonable judgment when interpreting the test results.
- Results should be interpreted within 5-10 minutes after completion of the test.

## 7. Sample Collection and Handling

- Collect water samples in glass, polyethylene terephthalate (PETG), high density polyethylene (HDPE), polycarbonate (PC), polypropylene (PP), or polystyrene (PS) containers.
- Samples can be stored refrigerated for up to 5 days. If samples must be held for greater than 5 days, samples should be stored frozen.

### A. Materials Provided

1. Cylindrospermopsin test strips in a desiccated container
2. Sample collection vials
3. Lysis vials
4. Graduated disposable pipettes (calibrated at 1 mL)
5. Forceps
6. Reagent papers
7. Conical test vials
8. Disposable transfer pipettes
9. User's guide

### B. Additional Materials (not provided with the test)

1. Timer
2. Cylindrospermopsin standard, Eurofins Abraxis PN300626, for the preparation of control solutions which can be analyzed with samples, to obtain semi-quantitative sample results (see Section C, Controls, below).

### C. Controls

It is a good laboratory practice to use positive and negative controls to ensure proper test performance. Water samples containing known quantities of Cylindrospermopsin (positive and negative controls) should be analyzed with each lot of test strips to provide a reference for line intensity to be expected. Control samples should be evaluated in the same manner as samples, using the QuikLyse™ reagents, in order to produce accurate results. Analysis of control samples which have not been treated with the QuikLyse™ reagents will produce inaccurate positive and negative control sample results.

### D. Test Preparation

1. Allow the reagents and water sample to reach room temperature before use.
2. Remove the number of test strips required from the package. The remaining strips are stored in the tightly closed desiccated container.

### E. Procedure

When analyzing for total Cylindrospermopsin content (dissolved, or free, and cell-bound), a sample lysis is necessary before analysis. The Eurofins Abraxis QuikLyse™ reagents provide a rapid option for cell lysis.

1. Using a new graduated disposable pipette for each sample, draw the sample to the 1 mL line (graduation mark slightly below bulb) and add 1 mL of sample to the lysis vial.

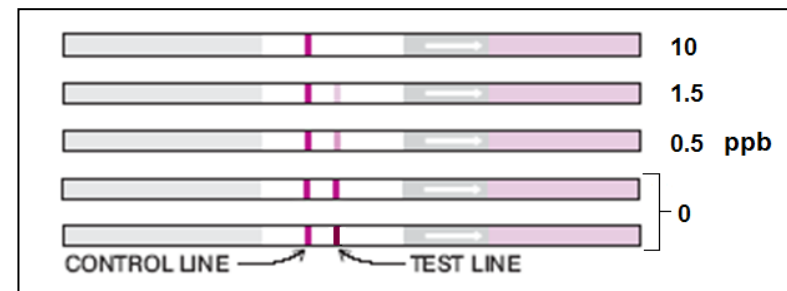
2. Cap the vial and shake for 2 minutes, then allow the sample in the vial to incubate at room temperature for 8 minutes, to begin the cell lysis.
3. Using the forceps provided, add 1 reagent paper to the lysis vial.
4. Cap the vial and shake for 2 minutes, then allow the sample in the vial to incubate at room temperature for 8 minutes.
5. Label conical test vials for each sample to be tested.
6. Using a new disposable transfer pipette for each sample, transfer 7 drops (approximately 200 µL) of the previously lysed water sample (Steps 1-4 above) to the appropriately labeled conical test vial.
7. Close the conical test vial and shake for 30 seconds. Examine the vial to ensure all dried reagents are completely dissolved (dried reagents will dissolve, turning the sample purple).
8. Incubate the conical test vial at room temperature for 10 minutes.
9. Insert test strip (arrows down) into the conical vial.
10. Allow the test to develop for 10 minutes.
11. Remove the test strip. Lay the strip flat and allow to continue developing for 5 minutes.
12. Read the results visually, as explained below in Section F, Interpretation of Results.

### F. Interpretation of Results

Sample concentrations are determined by comparison of the intensity of the test line to the intensity of the control line on the same test strip. Although 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 control line indicates a result which is below the limit of detection of the test. Test strips with a test line which is lighter than the control line indicates a result which is <10 ppb. Test strips with no test line visible (only the control line is visible) indicates a result which is ≥ 10 ppb. Results should be determined within 5-10 minutes after completion of the strip test procedure. Determination made using strips which have dried for more or less than the required time may be inaccurate, as line intensities may vary with drying time.

<u>Control Line</u>	<u>Test Line</u>	<u>Interpretation</u>
No control line present	No test line present	Invalid result
Control line present	No test line present	>10 ng/mL (ppb)
Control line present	Moderate to equal intensity test line present	Between 0 and 10 ng/mL (ppb)

The appearance of test strips may also be compared to the illustration below 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 0-10 ppb, solutions of known Cylindrospermopsin concentration (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.



Alternately, test strips can also be interpreted using the AbraScan test strip reader (PN 475025), which provides objective determination of line intensities for consistent interpretation of results as well as a digital photographic record of all test strips.

### G. Additional Analysis

If necessary, positive samples can be confirmed by ELISA, HPLC or other conventional methods. These services are available from commercial analytical laboratories (list of analytical laboratories available upon request).