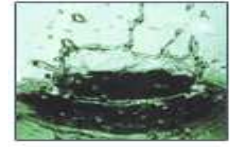




Strategic Diagnostics Inc.

Envirogard™ Microcystins QuantiTube Test Kit

Part Number 7700000 (10 Test)



Intended Use

The EnviroGard Microcystins QuantiTube Test Kit is a quantitative test for the detection of microcystins residues in water.

Test Principles

The EnviroGard Microcystins QuantiTube Kit is calibrated with a non-toxic microcystin-LR surrogate at levels equivalent to 0.2, 0.8, and 4.0 parts per billion (ppb) microcystin-LR. The kit uses polyclonal anti-bodies which bind either microcystins or a microcystin-enzyme conjugate. These antibodies are immobilized to the walls of the test tubes. Microcystins in the sample compete with the microcystin-enzyme conjugate for a limited number of antibody binding sites. In the assay procedure you will:

1. Add a sample containing microcystins to a test tube, incubate, then add microcystin-enzyme conjugate. The enzyme conjugate competes with the microcystins for the antibody binding sites.
2. Wash away any unbound molecules.
3. Add a clear solution of chromogenic substrate to the test tube. In the presence of bound microcystin-enzyme conjugate, the substrate converts to a compound which turns blue. One enzyme molecule can convert many substrate molecules.

Since the same number of antibody binding sites are available on every test tube, and each test tube receives the same number of microcystin-enzyme conjugate molecules, a sample which contains a low concentration of microcystins allows the antibody to bind many microcystin-enzyme conjugate molecules. The result is a dark blue solution. Conversely, a high concentration of microcystins allows fewer microcystin-enzyme conjugate molecules to be bound by the antibodies, resulting in a lighter blue solution.

Note: Color is *inversely proportional* to microcystins concentration.

Darker color = Lower concentration

Lighter color = Higher concentration

Performance Characteristics

Sensitivity

The sensitivity of the EnviroGard Microcystins QuantiTube Test Kit was established by determining the variation in response to three microcystins-free environmental water samples.

The average %B₀* for the three samples was 91.4 and the standard deviation was 1.84. Using three standard deviations, the sensitivity equals $91.4 - (3 \times 1.84) = 85.8\%$ B₀. When this %B₀ is read from the average standard curve of these assays, it corresponds to a sensitivity of 0.10 ppb microcystins.

*%B₀ = average optical density (OD) of the calibrator or sample divided by the average OD of the Negative Control multiplied by 100.

Precision

Intra-assay Precision

Intra-assay precision was determined by running two microcystins control solutions in duplicate. Four assays were run by two operators. The results for signal (OD) and concentration (ppb) are shown below.

	Signal (OD)		Conc. (ppb)	
	Average	%CV	Average	%CV
Control 1	0.66	4.3	0.44	11.1
Control 2	0.31	4.8	2.32	8.4

Inter-assay Precision

Inter-assay precision was determined by running two microcystins control solutions in duplicate. The solutions were run in eight assays by four operators. The results for concentration precision are shown below:

	Average	Std. Deviation	%CV
Control 1	0.46	0.04	9.7
Control 2	2.47	0.27	11.1

Cross-Reactivity

The polyclonal antibody used in the EnviroGard Microcystins QuantiTube Test Kit will not differentiate between microcystin-LR and other microcystins variants, but detects their presence to differing degrees. The following table shows the concentration at 50% inhibition (50% B₀) for these compounds. Concentration is in ppb.

VARIANTS	50% B ₀ (ppb)
MCYST-LR	0.6
MCYST-RR	0.5
MCYST-YR	0.4
NODLN	0.1

Precautions

- Microcystins in environmental samples may be toxic. Use adequate precautions when handling these samples.
- Store all test kit components at 4°C to 8°C (39°F to 46°F) when not in use.
- Do not store test kit components for more than 8 hours at ambient temperatures (18°C to 27°C or 64°F to 81°F).
- Do not expose **substrate** to **direct sunlight**.
- Do not freeze test kit components or expose them to temperatures greater than 37°C (99°F).
- Allow all reagents to reach ambient temperature (18°C to 27°C or 64°F to 81°F) before beginning the test (approximately 30-60 minutes).
- Do not use the test kit components after the expiration date.
- Do not use reagents or test tubes from one test kit with reagents or test tubes from a different test kit.
- Do not dilute or adulterate test reagents or use samples not called for in the test procedure.
- Use approved methodologies to confirm any positive results.

- Stop solution is 1.0 N Hydrochloric acid; handle with care.
- Some solutes and particulates found in untreated ground or surface waters may affect the sensitivity level of this test kit.

Before You Start

The EnviroGard Microcystins Quantitube Kit contains the following items:

- 40 antibody-coated test tubes
- 1 vial of Negative Control
- 1 vial of 0.2 ppb Microcystins-LR Calibrator
- 1 vial of 0.8 ppb Microcystins-LR Calibrator
- 1 vial of 4 ppb Microcystins-LR Calibrator
- 1 vial of Microcystins-Enzyme Conjugate
- 1 vial of Substrate
- 1 vial of Stop Solution
- 1 Test Tube Rack
- 2 Packets of Phosphate Buffered Saline

You will also need several other items:

- 10cc syringes
- Millex HV₁₃ filter units (0.45 micron, 13mm, non-sterile)
- Disposable-tip air displacement pipette that will measure 250 microliters (μL)
- Eppendorf™ Repeater® Pipettor with three 12.5 mL Combitips® or equivalent
- Indelible marking pen for test tubes
- Stopwatch or wristwatch with second hand
- Spectrophotometer capable of reading 12 millimeter (mm) x 75 mm test tubes at 450 nanometers (nm) minus 600 nm
- Solid phase extraction devices (optional)

Sample Preparation

- If samples are to be stored for later assay, they should be filtered to remove particulates. A syringe-operated Millex-HV₁₃ filter is suitable for this purpose.
- If a lower sensitivity for microcystins is required (<0.2 ppb), a solid-phase extraction device such as a C₁₈ reverse phase SepPak® is necessary to concentrate the sample (see “References”, #2).

Perform the Test

Allow all reagents to reach room temperature before beginning the test (at least 30 minutes). In addition, organize all samples, other required reagents, and equipment so that the set-up procedure can be performed from beginning to end in the minimum amount of time. *The total time required to perform steps 2 and 3 should not exceed 15 minutes.* Label Combitips “Conjugate,” “Substrate,” and “Stop”.

1. Remove the test tubes (no more than 20 tubes per assay) from the plastic bag and label them in duplicate for: Negative Control, 0.2 ppb, 0.8 ppb, 4.0 ppb calibrators and each sample.

- Using an air displacement pipette with disposable tips, pipette 250 μL of each calibrator and sample directly into the bottom of the appropriate tubes. Use a clean tip for each.
- Incubate for 15 minutes at ambient temperature.
- Attach a clean **12.5 mL** Combitip labeled “Conjugate” to the Eppendorf pipette and set the dispense dial to **1**. Immediately add 250 μL of Enzyme Conjugate to all test tubes.
- Swirl the test tubes to mix the contents and incubate for 15 minutes at ambient temperature. During the incubation period, prepare PBS wash solution by mixing 1 PBS packet into 1 L of distilled water. If distilled water is not available, tap water is acceptable. Each PBS packet yields enough to wash 20 tubes.
- Decant and vigorously shake the tube contents. Fill the test tubes to overflowing with PBS wash solution, then decant and vigorously shake out the remaining liquid.
Repeat this wash step three more times, being certain to shake out as much liquid as possible on each wash. After the final wash, tap the inverted test tubes on absorbent paper.
- Attach a clean **12.5 mL** Combitip labeled “Substrate” to the Eppendorf pipette and set the dispense dial to **2**. Add 500 μL of Substrate to each test tube.
- Swirl the test tubes to mix the contents and incubate for 15 minutes at room temperature.
- Attach a clean **12.5 mL** Combitip labeled “Stop” to the Eppendorf pipette and set the dispense dial to **2**. Add 500 μL of Stop Solution to each test tube.
WARNING: Stop Solution is 1.0 N Hydrochloric Acid.
The addition of Stop Solution causes the color to change from blue to yellow.
- Read the test tubes at 450 nm minus 600 nm against a water blank within 30 minutes after the Stop Solution is added.

Assay Modifications

The instructions under “Perform the Test” are for the recommended basic assay and will yield optimal results. Depending on the individual requirements for data quality, modifications can be made (see the following sections). Individual laboratories should establish the exact effect of these modifications in their own laboratories.

NOTE: Calibrators must *always* be run in duplicate.

Curve Storage

Some spectrophotometers are capable of curve storage (i.e., where standard curves can be used to quantitate results from subsequent assays using the same reagents and protocol). The EnviroGard Microcystins QuantiTube assay is compatible with curve storage where the absolute absorbance from assay to assay are normalized by using $\%B_0$ to convert absorbances.

If the calculations are done manually, $\%B_0$ is plotted versus the concentration on semi-log graph paper and a straight line is drawn between the points. In subsequent assays, only the Negative Control is run along with the control(s) and sample(s). The $\%B_0$ is then calculated for each control and sample, and the concentration read from the curve. It is recommended that a control with an established range be run in each assay to ensure that a given assay was run correctly.

Interpret the Results

If a blue color does not develop in the Negative Control test tube within 15 minutes after the substrate has been added, the test is invalid and must be repeated.

Read the tubes in a dual-wavelength spectrophotometer, which has been set to read at 450 nm minus 600 nm.

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Samples that fall below the 0.2 ppb calibrator should be reported as < 0.2 ppb. Those falling above the 4.0 ppb calibrator should be reported as > 4.0 ppb. Samples greater than 4.0 ppb should be diluted and run again.

References: (reprints may be obtained from Strategic Diagnostics Inc.)

1. Chu et al. (1989) Appl. Environ. Microbiol. 55 : 1928-1933.
2. Chu et al. (1990) J. Assoc. Off. Anal. Chem. 73 : 451-456.

Ordering Information

Description	Catalog Number
EnviroGard Microcystins QuantiTube Test Kit	7700000

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