

Highlights:

- Quantitative laboratory analysis of Microcystin toxin in surface and drinking water samples
- Calibration range from 0.4 to 2.5 ppb (parts per billion, ng/mL)

Contents of Kit:

- 40 antibody-coated test tubes (two bags of 20)
- 1 bottle of Assay Diluent
- 1 vial of Microcystin LR Negative Control
- 1 vial of 0.4 ppb Microcystin LR Calibrator
- 1 vial of 1.0 ppb Microcystin LR Calibrator
- 1 vial of 2.5 ppb Microcystin LR Calibrator
- 1 bottle of Microcystin-enzyme Conjugate
- 1 bottle of Substrate
- 1 bottle of Stop Solution

Precision

	Recovery (%CV)	OD (%CV)
Intra-Assay n=10		
0.7 ppb	8.7%	3.7%
1.75 ppb	10.4%	8.4%
Inter-Assay n=26		
0.7 ppb	12.3%	n/a
1.75 ppb	10.8%	n/a

Catalog Number ET 039

Intended Use

The EnviroLogix QuantiTube Kit for Microcystin is designed for quantitative analysis of Microcystin toxin in water samples. The kit is supplied with calibrators at 0, 0.4, 1.0 and 2.5 ppb. The assay range can easily be extended by dilution of strongly positive samples.

How the Test Works

This Kit is a competitive Enzyme-Linked ImmunoSorbent Assay (ELISA). In the test, Microcystin toxin in the sample competes with enzyme (horseradish peroxidase)-labeled Microcystin for a limited number of antibody binding sites on the inside surface of the test tubes.

After a simple wash step, the outcome of the competition is visualized with a color development step. As with all competitive immunoassays, sample concentration is inversely proportional to color development.

Darker color = Lower concentration

Lighter color = Higher concentration

Limit of Detection

The Limit of Detection (LOD) of the EnviroLogix QuantiTube Kit for Microcystin is 0.18 ppb. The LOD was determined by interpolation at % B₀* from a standard curve. 84.05% B₀ was determined to be 3 standard deviations from the mean of a population of negative water samples.

*100% B₀ equals the maximum amount of Microcystin-enzyme conjugate that is bound by the antibody in the absence of any Microcystin in the sample (i.e. negative control). % B₀ = (OD of Sample or Calibrator/OD of Negative Control) x 100.

Limit of Quantification

The Limit of Quantification (LOQ) of this Kit was validated at 0.7 ppb (quantification between the 0.4 ppb lowest calibrator and 0.7 ppb may be reliable, but has not been validated). The LOQ was determined by fortifying a population of negative water samples at 0.7 ppb. The mean recovery was 78.9% with a coefficient of variation (CV) [(standard deviation/mean) x 100] of 17.6%.

Precision

Microcystin-fortified control solutions were repetitively analyzed both within a single assay, and in different assays on different days. The data is expressed as %CV for both the recovered concentration and for absorbance (OD).

Fortification and Recovery

Eight surface water samples were fortified with Microcystin to a concentration of 1.0 ppb. The average recovery was 109.2%, with a CV of 18.9%.

Cross-Reactivity

Compound	50% B ₀ (ppb)	0.4 ppb– LR equivalent (ppb)
Microcystin LR	0.98	0.40
Microcystin LA	1.82	0.99
Microcystin RR	2.4	0.90
Microcystin YR	2.1	0.95
Nodularin	1.3	0.74

Cross-Reactivity

The QuantiTube Kit for Microcystin does not distinguish between the Microcystin toxin variants, but detects their presence to differing degrees. The table to the left shows the value for 50% B₀ and the concentration equivalent to the 0.4 ppb kit calibrator for four microcystin toxin variants and nodularin toxin. Concentration is in ppb.

Humic acid did not interfere in the assay up to a concentration of 100 ppm.

Materials Not Provided

- Test tube rack that can hold at least 20 tubes securely enough to flick out water after wash step (Contact EnviroLogix for information on obtaining an appropriate rack)
- Repeating pipetter with one 2.5 and three 12.5 mL syringe-style tips (Eppendorf™ Repeater® or equivalent)
- disposable tip, adjustable air-displacement pipette to measure 75 µL
- marking pen (indelible)
- timer (15 minutes)
- cool tap or distilled/de-ionized water for rinsing tubes
- photometer with 450 nm filter for reading 12 x 75 mm tubes (Hach Generic 450 Colorimeter™, or equivalent)

How to Run the Assay

- Read all of the instructions before running the kit.
 - Allow all reagents to reach room temperature before beginning (at least 30 minutes with un-boxed tubes and reagents at room temperature - do not remove tubes from bag with desiccant until they have warmed up).
 - Organize all samples and reagents so that steps 2 and 3 can be performed in 10 minutes or less.
 - Do not run any more tubes in a single assay than you can process through steps 2 and 3 in 10 minutes.
 - When adding reagents and samples to the tubes, direct all the liquid to the bottom of the tube; avoid droplets sticking to the sides of the tubes.
1. Place the required number of tubes in the rack and label them in duplicate for each calibrator and sample.
 2. Attach a clean **2.5 mL** syringe tip to the repeating pipetter and set the dispense dial to **3**. Dispense **150 µL Assay Diluent** to all the test tubes.
 3. Immediately add **75 µL** of the **Negative Control**, each of the three **calibrators**, and each **sample** to duplicate tubes, changing tips for each control/calibrator/sample. **Do not add Microcystin-enzyme Conjugate in this step.**
 4. Thoroughly mix the contents of the tubes by moving the tube holder in a rapid circular motion on flat surface for a full 20-30 seconds.
 5. Incubate tubes at ambient temperature for **15 minutes**.
 6. Attach a clean **12.5 mL** syringe tip to the repeating pipetter and set the dispense dial to **1**. Dispense **250 µL Microcystin Enzyme-Conjugate** to all the test tubes. Mix as described in step 4.



7. Incubate tubes at ambient temperature for **15 minutes**.
8. After incubation, vigorously shake the contents of the tubes into a sink or other suitable container. Flood the tubes completely with cool tap water (distilled or de-ionized may be used if clean tap water is not available), then shake to empty. Repeat this wash step four times. Invert the tubes on a paper towel and tap to remove as much water as possible.
9. Attach a clean **12.5 mL** syringe tip to the repeating pipetter and set the dispense dial to **2**. Dispense **500 µL Substrate** to all the test tubes. Mix as described in step 4.
10. Incubate tubes at ambient temperature for **15 minutes**.

NOTE: If blue color does not develop in the Negative Control tube, the assay is invalid and should be repeated.

Caution: Stop Solution is 1.0 N Hydrochloric acid. Handle carefully.

11. Attach a clean **12.5 mL** syringe tip to the repeating pipetter and set the dispense dial to **2**. Dispense **500 µL Stop Solution** to all the test tubes. Mix as described in step 4. This will turn the blue color in the tubes to yellow.

NOTE: Read the tubes within 30 minutes of the addition of Stop Solution.



How to Interpret the Results

Spectrophotometric Measurement

1. Set the wavelength of your photometer to 450 nanometers (nm). If it has dual wavelength capability, use 600, 630 or 650 nm as the reference wavelength.
2. Blank the photometer against 1 mL water in a blank tube. Measure and record the optical density (OD) of each tube's contents.

How to Calculate the Quantitative Results

1. After reading the wells, average the OD of each set of calibrators and samples, and calculate the %B₀ as follows:

$$\%B_0 = \frac{\text{average OD of Calibrator or sample}}{\text{average OD of Negative Control}} \times 100$$

The %B₀ calculation is used to equalize different runs of an assay. While the raw OD values of Negative Controls, Calibrators, and samples may differ from run to run, the %B₀ relationship of calibrators and samples to the Negative Control should remain fairly constant. The %B₀ of each Calibrator should fall within these ranges:

Calibrator	% B ₀
0.4 ppb	60-85%
1.0 ppb	35-60%
2.5 ppb	12-35%

The CV for each pair of Calibrator and sample OD values should not exceed 15%.

2. Graph the %B₀ of each Calibrator against its Microcystin concentration on a semi-log scale (see Illustrative Standard Curve, Figure 1). If your

Precautions and Notes

- Dispense samples and reagents into the assay tubes in such a way that all of the liquid settles to the bottom of the tubes, with no droplets clinging to the sides. Avoid splash back from too forceful dispensing.
- Store all Tube Kit components at 4°C to 8°C (39°F to 46°F) when not in use.
- Do not expose Tube Kit components to temperatures greater than 37°C (99°F) or less than 2 °C (36°F).
- Allow all reagents to reach ambient temperature (18°C to 27°C or 64°F to 81°F) before use.
- Do not use kit components after the expiration date.
- Do not use reagents or test tubes from one Tube Kit with reagents or test tubes from a different Tube Kit.
- Do not expose **Substrate** to **sunlight** during pipetting or while incubating in the test tubes.
- Do not dilute or adulterate test reagents or use samples not called for in the test procedure.
- As with all tests, it is recommended that results be confirmed by an alternate method if necessary.
- Microcystin LR in aqueous solution will stick to plastics such as polypropylene. Collect and process samples in glass containers.
- Observe any applicable regulations when disposing of samples and kit reagents.

photometer does not possess the software to do this, please use a data reduction spreadsheet available from EnviroLogix.

3. Determine the Microcystin concentration of each sample by finding its %B₀ value and the corresponding concentration level on the graph.
4. Interpolation of sample concentration is only possible if the %B₀ of the sample falls within the range of %B₀'s of the Calibrators.

If the %B₀ of a sample is higher than that of the lowest Calibrator, the sample must be reported as less than 0.4 ppb.

If the %B₀ of a sample is lower than that of the highest Calibrator, the sample must be reported as greater than 2.5 ppb. If a concentration must be determined for these high level samples, dilute the sample 1:5 in distilled water. Run this dilution in a repeat of the immunoassay. If the result now falls within the range of the %B₀'s of the Calibrators, you must then multiply the concentration measured in the diluted sample by a factor of 5.

Figure 1. Illustrative standard curve

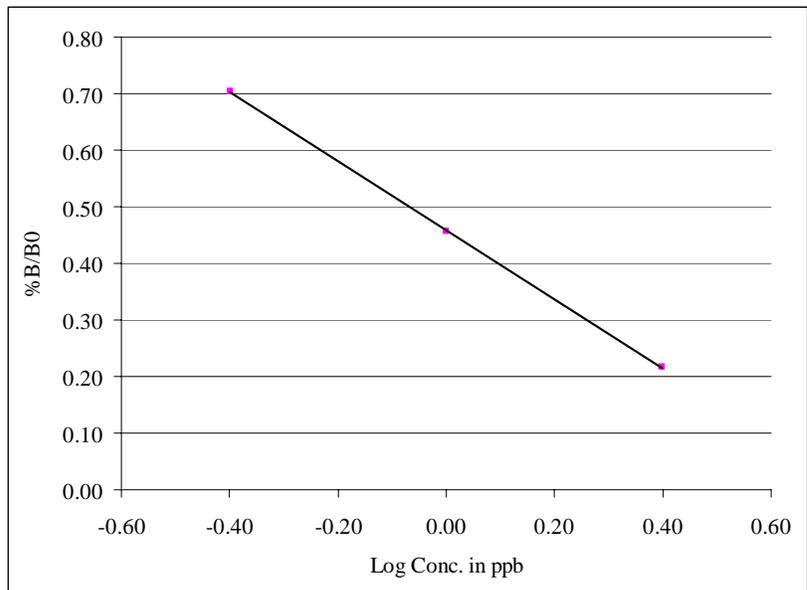


Figure 2. Illustrative quantitative calculations

Tube contents	OD	Average OD	%CV	%B ₀	Microcystin Concentration (ppb)
Negative Control	0.97 1.02	0.99	3.6	NA	NA
0.4 ppb Calibrator	0.70 0.70	0.70	0.0	70.35	NA
1.0ppb Calibrator	0.43 0.48	0.45	7.8	45.23	NA
2.5 ppb Calibrator	0.17 0.26	0.21	29.6	21.60	NA
Sample	0.54 0.57	0.55	3.8	55.78	0.7

**Actual values may vary; this data is for demonstration purposes only.*

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