ENVIROLOGIX™

QualiTube™ Kit For Microcystin

Highlights:

- *Semi-quantitative field screening of Microcystin toxin in surface water*
- *Detects from 0.5 to 3 ppb*

Contents of Kit:

- *36 antibody-coated test tubes*
- *1 vial of 0.5 ppb Microcystin LR Calibrator*
- *1 vial of 3 ppb Microcystin LR Calibrator*
- *1 dropper bottle of Assay Diluent*
- *1 dropper bottle of Microcystin-enzyme Conjugate*
- *1 dropper bottle of Substrate*
- *1 bottle of Stop Solution*
- *36 sample pipettes*

Optional Accessory Item:

- *ACC 062 – 1 vial of 1.5 ppb Microcystin LR Calibrator*

Precision

Intra-Assay Precision (n=7)	
	%CV (OD)
Negative Control	8.4%
1.0 ppb Control	8.1%
Inter-Assay Precision (n=8)	
	%CV (B/B_0)
1.0 ppb Control	9.6%

Cross-Reactivity

Compound	**50% B_0**	**81.5% B_0 LOD**
Microcystin LR	0.94	0.30
Microcystin LA	0.78	0.43
Microcystin RR	1.53	0.65
Microcystin YR	2.53	0.69
Nodularin	1.44	0.53

Catalog Number ET 022

Intended Use

The EnviroLogix QualiTube Kit for Microcystin is designed for semi-quantitative field screening of Microcystin toxin in surface water samples. The kit is supplied with calibrators at 0.5 and 3 ppb. The assay range can easily be extended.

How the Test Works

The QualiTube Kit for Microcystin 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 Microcystin Tube Kit is 0.3 ppb. The LOD was determined by interpolation at 81.5% B_0* from a standard curve. 81.5% B_0 was determined to be 2 standard deviations from the mean of a population of negative water samples.

*100% B_0 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). %Bo = (OD of Sample or Calibrator/OD of Negative Control) x 100.

Precision

Microcystin-fortified control solutions were repetitively analyzed within a single assay. The data is expressed as %CV for absorbance (OD) and %B_0.

False Positive/False Negative Rate

Six surface water samples were fortified with Microcystin to a concentration of one half and twice the 0.5 ppb low calibrator. All six samples (6/6) fortified with 0.25 ppb resulted in absorbances greater than the 0.5 ppb microcystin calibrator, for a 0% false positive rate. All six samples (6/6) fortified with 1.0 ppb produced absorbances between 0.5 and 3.0 ppb in microcystin concentration, for a 0% false negative rate.

Cross-Reactivity

The QualiTube Kit for Microcystin does not distinguish between the Microcystin toxin variants, but detects their presence to differing degrees. The table (left) shows the value for 50% B_0 and the value for the 81.5% B_0 limit of detection 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

- marking pen (indelible)
- timer (5, 20 and 10 minutes)
- cool tap or distilled water for rinsing tubes, in a wash bottle
- photometer for reading tubes (optional)
- test tube rack that can hold at least 6 tubes securely enough to flick out water after wash step (Contact EnviroLogix for information on obtaining an appropriate rack)
- disposable tip, adjustable air-displacement pipette which will measure 0.7 mL (optional)

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 1 and 2 can be performed in 3 minutes or less.
- Do not run more than 6 tubes at a time.

1. Rapidly add **5 drops of Microcystin Assay Diluent** to each tube in the assay.

2. Using the sample pipette provided, immediately add two drops of **0.5 ppb Microcystin Calibrator** to the first tube. Add two drops of **3.0 ppb Microcystin Calibrator** to the second tube. Add **two drops** of sample to each of the subsequent tubes, up to a total of 4 samples. **Do not add Microcystin-enzyme Conjugate in this step**.

3. 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.

4. Incubate tubes at ambient temperature for 5 minutes.

5. Add **5 drops** of **Microcystin-enzyme Conjugate** to each tube. Do not empty the tube contents or wash the tubes at this time. Thoroughly mix the contents of the tubes as in step 3.

6. Incubate tubes at ambient temperature for 20 minutes.

7. After incubation, vigorously shake the contents of the tubes into a sink or other suitable container. Flood the tubes completely with cool tap water, then shake to empty. Repeat this wash step three times. Invert the tubes on a paper towel and tap to remove as much water as possible.

8. Add **10 drops** of **Substrate** to each tube. Thoroughly mix the contents of the tubes, as in step 3. Incubate substrate in tubes for 10 minutes at ambient temperature.

NOTE: If blue color does not develop in the 0.5 ppb Calibrator tube, the assay is invalid and should be repeated.

TABLE 1
The following table illustrates results interpretation of water samples read visually:

Samples with blue color …	Contain …
Darker than the blue color of 0.5 ppb Calibrator	Less than 0.5 ppb Microcystins
Between the blue color of 0.5 ppb and 3.0 ppb Calibrator	Between 0.5 and 3.0 ppb Microcystins
Lighter than the blue color of 3.0 ppb Calibrator	More than 3 ppb Microcystins

TABLE 2
The following table illustrates results interpretation of water samples using a tube photometer:

Samples with OD values …	Contain …
Greater than OD of 0.5 ppb Calibrator	Less than 0.5 ppb Microcystins
Between OD of 0.5 ppb and 3.0 ppb Calibrator	Between 0.5 and 3.0 ppb Microcystins
Less than OD of 3.0 ppb Calibrator	More than 3 ppb Microcystins

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

9. This assay is designed to be read visually with un-stopped tubes (blue solution). If tubes are to be read using a tube photometer, pipette 0.7 mL of Stop Solution into each tube and mix thoroughly. This will turn the tube contents yellow.

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

10. **Interpret the results of un-stopped tubes immediately following the 10 minute substrate incubation.**

How to Interpret the Results

Reading Tubes Visually

1. Compare the intensity of the blue color of each sample tube to the intensity of the blue color in the 0.5 and 3.0 ppb calibrator tubes.

2. Score each sample tube as having less than, more than or equal color to the two calibrator tubes.

3. Use Table 1 (left) to determine the level of microcystin in the samples.

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. If the photometer does not auto-zero on air, zero the instrument against 1 mL water in a blank tube. Measure and record the optical density (OD) of each tube's contents. Alternatively, measure and record the OD in every tube, then subtract the OD of the water blank from each of the readings.

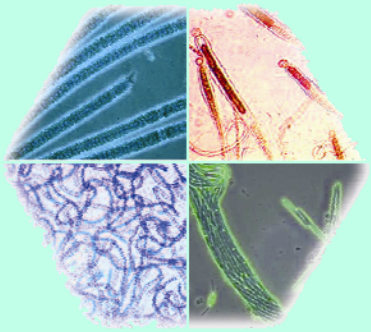
3. Use Table 2 (left) to determine the level of Microcystin in the sample.

4. For information on a field portable differential photometer contact EnviroLogix Technical Support. Contact information is at the end of these instructions.

Figure 1. Illustrative results interpretation using tube photometer

Well Contents	OD	Microcystin Concentration (ppb)
0.5 ppb Calibrator	0.984	NA
3.0 ppb Calibrator	0.306	NA
Sample	1.332	< 0.5 ppb
Sample	0.604	> 0.5 ppb, < 3.0 ppb

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



Precautions and Notes

- **While dropping solutions into tubes from dropper bottles, hold the top of each tube between your thumb and index finger. This will prevent the drops from adhering to the sides of the tube, allowing the drops to fall to the bottom of the tube.**
- **Hold pipette bulbs and dropper bottles vertically over the tube opening while dropping.**
- 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.

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