ENVIROLOGIX™

QuantiPlate™ Kit for Microcystins

Highlights:

- *Quantitative laboratory detection of Microcystin toxin in surface water*
- *Detects from 0.16 to 2.5 ppb*
- *High Sensitivity Option for potable water samples (see Appendix) detects from 0.05 to 0.83 ppb*

Contents of Kit:

- *12 strips of 8 antibody-coated wells each, in plate frame*
- *1 vial of Negative Control*
- *1 vial of 0.16 ppb Microcystin LR Calibrator*
- *1 vial of 0.6 ppb Microcystin LR Calibrator*
- *1 vial of 2.5 ppb Microcystin LR Calibrator*
- *1 bottle of Assay Diluent*
- *1 bottle of Microcystin-enzyme Conjugate*
- *1 packet of Wash Solution salts*
- *1 bottle of Substrate*
- *1 bottle of Stop Solution*

Precision

	Recovery (%CV)	OD (%CV)
Intra-Assay	**n=7**	
0.25 ppb	3.9%	1.6%
1.0 ppb	5.8%	5.3%
Inter-Assay	**n=11**	
0.25 ppb	6.0%	n/a
1.0 ppb	3.6%	n/a

Catalog Number EP 022

Intended Use

The EnviroLogix QuantiPlate Kit for Microcystins is designed for the quantitative laboratory detection of Microcystin toxin in surface water samples, with an assay quantitation range from 0.16 to 2.5 parts per billion (ppb). (See the Appendix at the end of this package insert describing an alternate assay protocol, suitable for use with colorless and/or potable water samples, with a quantitation range from 0.05 to 0.83 ppb.)

How the Test Works

This QuantiPlate Kit for Microcystins 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 wells.

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 this Kit is 0.147 ppb. The LOD was determined by interpolation at 81.3% B_0* from a standard curve. 81.3% B_0 was determined to be 3 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). %B_0 = (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 0.175 ppb (quantification between the 0.160 ppb lowest calibrator and 0.175 ppb may be reliable, but has not been validated). The LOQ was determined by fortifying a population of negative water samples at 0.175 ppb. The mean recovery was 108% with a coefficient of variation (CV) [(standard deviation/mean) x 100] of 13.6%.

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

Six surface water samples were fortified with Microcystin to a concentration of 1.0 ppb. The average recovery was 111%, with a CV of 3.6%.

Cross-Reactivity

Compound	50% B_0	LOD 81.3% B_0
Microcystin LR	0.50	0.15
Microcystin LA	0.81	0.24
Microcystin RR	0.92	0.27
Microcystin YR	1.42	0.44
Nodularin	0.73	0.21

Cross-Reactivity

This Kit does not distinguish between the Microcystin toxin variants, but detects their presence to differing degrees. The accompanying table shows the value for 50% B_0 and the value for the 81.3% 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 Needed

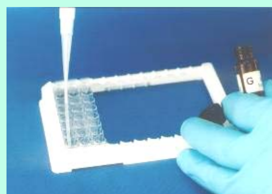
- disposable tip adjustable air-displacement pipette which will measure 20 µL, 100 µL and 125 µL
- marking pen (indelible)
- tape or Parafilm®
- timer (30 minutes)
- distilled water for preparing Wash Solution
- glassware for storing Wash Solution
- wash bottle for washing strips with Wash Solution
- microtiter plate reader or strip reader
- microtiter plate washer (optional)
- twelve-channel pipette that will measure 20 µL, 100 µL and 125 µL (optional)
- racked dilution tubes for loading samples into the plate with a 12-channel pipette (optional)
- orbital plate shaker (optional)

Preparation of Solutions

Wash Buffer:
To make 1 L, add the contents of one packet of phosphate-buffered saline - Tween 20, pH 7.4 (**Wash Solution salts**) to 1 L of distilled water. Mix thoroughly to dissolve the salts. This can be stored at room temperature.

Remove unneeded strips

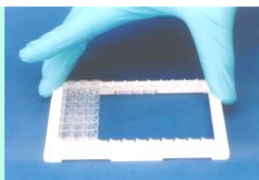
Select Calibrators and Control



Add controls/calibrators/sample

How to Run the Assay

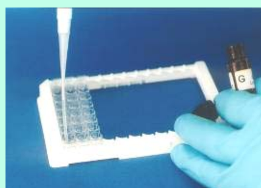
- Read all of these instructions before running the kit.
- Allow all reagents to reach room temperature before beginning (at least 30 minutes with un-boxed strips and reagents at room temperature - do not remove strips from bag with desiccant until they have warmed up).
- Organize all samples, reagents and pipettes so that steps 1 and 2 can be performed in 10 minutes or less.
- If more than three strips are to be run at one time, the 10 minutes is likely to be exceeded, and the use of a multi-channel pipette is recommended (see "Note" below).
- If three or fewer strips are to be run, use a disposable-tip air-displacement pipette and a clean pipette tip to add each Calibrator and sample to the wells. Assay Diluent, Conjugate, Substrate, and Stop Solution may be added in the same manner; alternatively, use a repeating pipette with a disposable tip on the end of the Combitip for these three reagents.
- If fewer than all twelve strips are used, reseal the unneeded strips and the desiccant in the plastic bag provided.
- Use the well identification markings on the plate frame to guide you when adding the samples and reagents. Two strips may be used to run the Negative Control (NC), three Calibrators (C1-C3) and four samples, in duplicate. More samples require more strips. For an example plate layout see Figure 1.


Mix plate

Incubate

Bottle Wash method

Strip Plate Wash option


Complete protocol and add Stop Solution

Read plates in a Plate Reader within 30 minutes of the addition of Stop Solution

1. Rapidly add **125 µL** of **Microcystin Assay Diluent** to each well that will be used, preferably with a repeating or multi-channel pipetter.
2. Immediately add **20 µL** of **Negative Control** (NC), **20 µL** of each **Calibrator** (C1-C3) and **20 µL** of each **sample** (S1-S8) to their respective wells, as shown at left. (Follow this same order of addition for all reagents.) **Do not add Microcystin-enzyme Conjugate in this step.**
3. Thoroughly mix the contents of the wells by moving the strip holder in a rapid circular motion on the benchtop for a full 20-30 seconds. Be careful not to spill the contents!

NOTE: In order to minimize setup time it is recommended that a multi-channel pipette be used in steps 1, 2, 5, 8 and 10 when more than 3 strips are used.

4. Cover the wells with tape or Parafilm to prevent evaporation and incubate at ambient temperature for 30 minutes. If an orbital shaker is available shake at 200 rpm.
5. Add **100 µL** of **Microcystin-enzyme Conjugate** to each well. Do not empty the well contents or wash the strips at this time.
6. Thoroughly mix the contents of the wells as in step 3. Cover the wells with tape or Parafilm and incubate at ambient temperature for 30 minutes. Use orbital shaker if available.
7. After incubation, carefully remove the covering and vigorously shake the contents of the wells into a sink or other suitable container. Flood the wells completely with **Wash Solution**, then shake to empty. Repeat this wash step four times. Slap the plate on a paper towel to remove as much Wash Solution as possible. Alternatively, use a microtiter plate washer with **Wash Solution** for the wash step.
8. Add **100 µL** of **Substrate** to each well.
9. Thoroughly mix the contents of the wells, as in step 3. Cover the wells with <u>new</u> tape or Parafilm and incubate for 30 minutes at ambient temperature. Use orbital shaker if available.

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

10. Add **100 µL** of **Stop Solution** to each well and mix thoroughly. This will turn the well contents yellow.

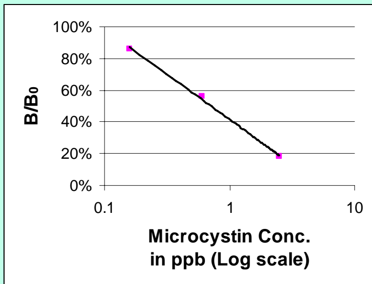
NOTE: Read the plate within 30 minutes of the addition of Stop Solution.

How to Interpret the Results

Spectrophotometric Measurement

1. Set the wavelength of your microtiter plate reader to 450 nanometers (nm). (If it has dual wavelength capability, use 600, 630 or 650 nm as the reference wavelength.)
2. If the plate reader does not auto-zero on air, zero the instrument against 200 µL water in a blank well. Measure and record the optical density (OD) of each well's contents. Alternatively, measure and record the OD in every well, then subtract the OD of the water blank from each of the readings.
3. A semi-log curve fit should be used for the standard curve if the microtiter plate reader you are using has data reduction capabilities. If not, calculate the results manually as described in the next section.

Illustrative standard curve



Precautions and Notes

- *Store all components at 4°-8°C (39°-46°F) when not in use.*
- *Do not expose 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 well strips from one QuantiPlate Kit with reagents or test well strips from a different QuantiPlate Kit.*
- *Do not expose **Substrate** to **sunlight** during pipetting or while incubating in the test wells.*
- *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 when necessary.*
- *Observe any applicable regulations when disposing of samples and kit reagents.*
- *Microcystin LR in aqueous solution will stick to plastics such as polypropylene. Collect and process samples in glass containers. Clear samples free of organic material can be stored refrigerated for up to two weeks before analysis.*

How to Calculate the Quantitative Results

1. After reading the wells, average the OD of each set of calibrators and samples, and calculate the $\%B_0$ as follows:

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

The $\%B_0$ 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 $\%B_0$ relationship of calibrators and samples to the Negative Control should remain fairly constant.

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

2. Graph the $\%B_0$ of each Calibrator against its Microcystin concentration on a semi-log scale (see Illustrative Standard Curve, left).
3. Determine the Microcystin concentration of each sample by finding its $\%B_0$ value and the corresponding concentration level on the graph.
4. Interpolation of sample concentration is only possible if the $\%B_0$ of the sample falls within the range of $\%B_0$'s of the Calibrators.

If the $\%B_0$ of a sample is higher than that of the lowest Calibrator, the sample must be reported as less than 0.16 ppb.

If the $\%B_0$ 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 1:8 in distilled water. Run this dilution in a repeat of the immunoassay. If the result now falls within the range of the $\%B_0$'s of the Calibrators, you must then multiply the concentration measured in the diluted sample by a factor of 8.

Figure 1a. Example of a typical plate setup. (1 x 8 strips)

	1	2	3	4	5	6	7	8	9	10	11	12
A	NC	NC										
B	C1	C1										
C	C2	C2										
D	C3	C3										
E	S1	S1										
F	S2	S2										
G	S3	S3										
H	S4	S4										

Figure 2a. Illustrative quantitative calculations

Well contents	OD	Average OD	%CV	$\%B_0$	Microcystin Concentration (ppb)
Negative Control	1.398 1.347	1.373	2.628	100	NA
0.16 ppb Calibrator	1.184 1.177	1.181	0.419	86	NA
0.6 ppb Calibrator	0.773 0.776	0.775	0.274	56.4	NA
2.5 ppb Calibrator	0.246 0.250	0.248	1.14	18.1	NA
Sample	0.573 0.567	0.570	0.744	41.5	1.01

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

For Technical Support Contact Us At:

EnviroLogix
500 Riverside Industrial Parkway
Portland, ME 04103-1486
USA

Tel: (207) 797-0300
Toll Free: 866-408-4597
Fax: (207) 797-7533

e-mail:
info@envirologix.com

website:
www.envirologix.com

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HIGH SENSITIVITY PROTOCOL

Limit of Detection

The Limit of Detection (LOD) of the High Sensitivity Protocol for this Kit is 0.03 ppb. The LOD was determined by interpolation at 90.9% B_0 from a standard curve. 90.9% B_0 was determined to be 3 standard deviations from the mean of a population of negative water samples.

Limit of Quantification

The Limit of Quantification (LOQ) of this Kit's High Sensitivity Protocol was validated at 0.06 ppb (quantification between the 0.05 ppb lowest calibrator and 0.06 ppb may be reliable, but has not been validated). The LOQ was determined by fortifying a population of negative water samples at 0.06 ppb. The mean recovery was 88% with a coefficient of variation (CV) [(standard deviation/mean) x 100] of 9.3%.

APPENDIX

Instructions for Assay Protocol with Increased Sensitivity

The following assay protocol will produce an assay with calibrator values of 0.05, 0.20 and 0.83 ppb. This protocol is suitable only for colorless and/or potable water samples; surface waters containing visible organic matter will likely cause interference in the assay

NOTE: All of the precautions and notes discussed under **HOW TO RUN THE KIT** apply to this assay format.

In addition to the items listed above, these additional items will be needed for this assay protocol:

- disposable tip adjustable air-displacement pipette which will measure 50 and 200 µL
- glass test tubes in which to dilute the calibrators

Dilution of Calibrators

Dilute the Negative Control and the 3 Calibrators 1:3 in distilled water by adding 100 µL of calibrators supplied with this kit to 200 µL of distilled water. Label these dilutions Negative Control, 0.05, 0.20 and 0. 83 ppb. Mix thoroughly.

Assay Protocol

1. Rapidly add **50 µL** of **Microcystin Assay Diluent** to each well that will be used, preferably with a repeating or multi-channel pipetter.

2. Immediately add **50 µL** of **Negative Control** (NC), **50 µL** of each diluted **Calibrator** (C1-C3) and **50 µL** of each **sample** (S1-S8) to their respective wells, as shown in Figure 1. (Follow this same order of addition for all reagents.) **Do not add Microcystin-enzyme Conjugate in this step.**

3. Thoroughly mix the contents of the wells by moving the strip holder in a rapid circular motion on the benchtop for a full 20-30 seconds. Be careful not to spill the contents!

NOTE: In order to minimize setup time it is recommended that a multi-channel pipette be used in steps 1, 2, 5, 8 and 10 when more than 3 strips are used.

4. Cover the wells with tape or Parafilm to prevent evaporation and incubate at ambient temperature for 30 minutes. If an orbital shaker is available shake at 200 rpm.

5. Carefully remove tape or Parafilm and then add **100 µL** of **Microcystin-enzyme Conjugate** to each well. Do not empty the well contents or wash the strips at this time.

6. Thoroughly mix the contents of the wells as in step 3. Cover the wells with tape or Parafilm and incubate at ambient temperature for 30 minutes. Use orbital shaker if available.

7. After incubation, carefully remove the covering and vigorously shake the contents of the wells into a sink or other suitable container. Flood the wells completely with **Wash Solution**, then shake to empty. Repeat this wash step four times. Slap the plate on a paper towel to remove as much **Wash Solution** as possible. Alternatively, use a microtiter plate washer with **Wash Solution** for the wash step.

8. Add **100 µL** of **Substrate** to each well.

9. Thoroughly mix the contents of the wells, as in step 3. Cover the wells with <u>new</u> tape or Parafilm and incubate for 30 minutes at ambient temperature. Use orbital shaker if available.

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

10. Add **100 µL** of **Stop Solution** to each well and mix thoroughly. This will turn the well contents yellow.

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

Assignment of Calibrator Values

In this assay format, assign the low, middle and high calibrators microcystin concentrations of 0.05 ppb, 0.2 ppb and 0.83 ppb, respectively.

For Interpretation and Calculation of Results see the sections on **How to Interpret the Results** and **How to Calculate the Results** above. The information contained in those sections is applicable to this more sensitive assay format, with the exception that the calibrator values are different.