



**SPECIAL INSTRUCTIONS
for the QuantiPlate™ Microcystin Kit
with High Sensitivity Calibrators (EP 022 HS)**

Note: The QuantiPlate™ Microcystin Kit (EP 022) comes with the following Calibrators: 0.16, 0.6, and 2.5 ppb.

**This High Sensitivity Kit (EP 022 HS) comes with these Calibrators *pre-diluted*
for the convenience of the end user to 0.05, 0.2 and 0.83 ppb**

Instructions for Assay Protocol with Increased Sensitivity When Pre-diluted Calibrators are Supplied:

NOTE: All of the precautions and notes discussed in the QuantiPlate™ Microcystin Kit apply to this assay format. This protocol is only suitable for potable and/or colorless water samples. Surface waters containing visible organic matter are likely to cause interference in the assay.

Additional materials that you may need to supply:

- a disposable tip adjustable air-displacement pipette which will measure 50 and 200 μL .
- a multi-channel pipette is required to minimize setup time if more than three strips are run at a time.

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.

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.

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!
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** directly 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 at 200 RPM 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 new tape or Parafilm and incubate for **30 minutes** at ambient temperature. Use orbital shaker at 200 RPM 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 immediately, if possible, or 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.

The %B₀ of each Calibrator should fall within these ranges:

<u>Calibrator</u>	<u>% B₀</u>
0.05 ppb	75 - 89%
0.20 ppb	39 - 58%
0.83 ppb	12 - 19%

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