
# Microcystin qPCR detection Kit

***Real-time quantitative analysis of the Microcystis toxin gene region MycE***

## Catalog Number: NA2024

**For Research Use Only. Not for use in Diagnostic Procedures.**

## 1. Background

Microcystis are a class of hepatotoxins produced by blue-green algae such as *Microcystis aeruginosa*. Microcystin-LR is the most common of the over 50 different congeners. Cyanobacteria can produce microcystin in large quantities during an algal bloom, which then poses a major threat to our aquatic ecosystems and sources of food, as there is a well-documented phenomenon called "bioaccumulation" that describes the increase in consumed toxin quantities as trophic levels increase.

## 2. Test Principle

Attogene's PCR kit for Microcystin is designed for the In vitro analysis of the MycE gene region responsible for assembling part of the Microcystis peptide. The MycE gene region specific primer and probe mix is provided to be detected through the FAM channel on a qPCR machine. A sample of algae is obtained and washed to extract a clean algal gDNA sample. A reaction mixture is assembled from primers, probe, master mix, and gDNA samples as required. The qPCR machine of choice is set up and loaded as needed and the mixture undergoes PCR amplification. The Primer mix provided exploits the Taq polymerase to amplify the gene region of interest; while the DNA probe mixture is cleaved during amplification to release its FAM fluorophore. The resulting FAM release can be detected on a variety of qPCR platforms.

## 3. Applications

This kit can be used for specific analysis of the Microcystis MycE gene region in liquid gDNA samples such as water, wastewater, and algal cultures.

## 4. Equipment and Reagents Needed (not provided)

- Real-time PCR Instrument
- qPCR 2X Master Mix
- DNA extraction kit/ gDNA sample
- PCR reaction tubes/plate
- Vortex and centrifuge

- Polystyrene centrifuge tube: 50mL, 2mL
- Micropipettes & Tips

## 5. Components Provided in This Kit

- MycE region specific primer mixture
- MycE region specific fluorescent probe
- MycE region specific positive control template (Standard curve)
- RNase/DNase free water (resuspension of positive control template and standard curve preparation)

## 6. Reagents Preparation

### Master Mix (not Provided)

-Store at -20C. Master Mix is stable when stored at -20C. Freeze and thaw cycles should be minimized to 6 cycles to increase the shelf life.

-If required aliquots of Master Mix should be made and stored at -20C to minimize freeze thaw.

### Primer/ Probe Mixture

-Precautions: these reagents are light sensitive and should only be handled in a clean area

-Store at -20C. Primer/Probe is stable when stored at -20C. Freeze and thaw cycles should be minimized to 6 cycles to increase the shelf life.

-If required aliquots of Primer/Probe should be made and stored at -20C in the dark to minimize freeze thaw.

## 7. Control Preparation

### Negative extraction control (NEC)

-Prepare at least one NEC each time extracting DNA from your sample.

-RNase/DNase free water is used in place of algae in the extraction system to create a negative for the DNA isolation method.

- The NEC will serve as a contamination control method for the isolation.

**No Template control (NTC)**

-If necessary, a NTC can made by replacing gDNA in the PCR reaction with RNase/DNase free water

-The NTC is used to check for contamination during PCR plate set up

**Positive control template (PCT)**

-The MycE Positive control template contains a high number of copy templates and should be handled away from specimen samples and kit components.

-The PCT contains synthetic Microcystin DNA representing the MycE genomic region of interest.

-To ensure PCR run validity, the PCT should see amplification in the FAM channel.

-Store at -20C. PCT is stable when stored at -20C. Freeze and thaw cycles should be minimized to 6 cycles to increase the shelf life.

## 8. Assay Set Up

-gDNA isolation will need to be done before starting an experiment. For optimal results use >10ng/uL of gDNA with a ratio of >1.80 in your experiment. IEC multiplexing can also be done to ensure proper DNA extraction.

-Plate set up will vary with the quantity of samples you need to run on your plate. A NEC is preferably included in each plate set up. NTCs should be included in each plate set up. A PCT must be included in each plate set up. The PCT should be added after all other reagents and samples have been added to the plate.

-Determine the number of reactions to set up in your assay (including NEC, PCT and any NTCs for your plate). It is necessary to make extra reaction mixture to allow for pipetting error.

-For convenience a large solution of PCR components will be mixed shortly before starting a reaction and subsequently aliquoted into your plate or tubes. Each PCR run will use 18uL of this reaction mixture and 1ul of isolated gDNA/NEC/PCT/NTC based on the experiment set up.

## 9. qPCR detection protocol

1. For each DNA sample prepare a reaction mix according to the table below:
(Include sufficient reactions for positive and negative controls)

| Reagent | Quantity |
|---|---|
| 2X Master Mix | 10uL |
| PCR Water | 6uL |
| Mc Probe | 1uL |
| Dual Mc Primer set | 1uL |
| Final Volume | 18uL |

2. Pipette 15uL of this mixture into each well according to your qPCR experimental plate set up.

3. Prepare your DNA templates for each reaction

4. Pipette 2uL of sample gDNA into each well, according to your experiment. For negative controls replace the gDNA sample with 2uL of RNase/DNase free water to bring the total volume to 20uL.

5. To prepare a standard curve dilution series.
   1) Pipette 40uL of RNase/DNase free water into 5 tubes and label 2-6
   2) Pipette 10uL of Positive Control Template into tube 2
   3) Vortex thoroughly
   4) Pipette 10ul from tube 2 into tube 3
   5) Vortex thoroughly
   6) Repeat steps 4-5

| Standard Curve | Copy Number |
|---|---|
| Tube 1 (Positive Control Template) | 2x10^5 per uL |
| Tube 2 | 4x10^4 per uL |
| Tube 3 | 8x10^3 per uL |
| Tube 4 | 1600 per uL |
| Tube 5 | 320 per uL |
| Tube 6 | 64 per uL |

6. Use 2ul of standard curve template into each well according to your experiment and plate set up.

Final well volume should be 20uL.

## 10. qPCR Amplification Protocol

Amplification conditions using 2x qPCR Master Mix:

| Steps | Time | Temperature | Cycles | Detection Format |
|---|---|---|---|---|
| Initial Denaturation (Taq Activation) | 2 Minutes | 94C | 1 | MycE = FAM (456-510) |
| Denaturation | 20 sec. | 94C | 40 | |
| Annealing* | 30 sec. | 50C | | |
| Extension | 60 sec. | 74C | | |

*Fluorogenic data should be read during this step through the FAM channel

## 11. Expected Performance

Before Interpreting results, it is necessary to verify the integrity of the reaction. If the following criteria are not satisfied, then testing needs to be repeated.

a. NEC is free from amplification in the FAM (465-510) channel.

b. NTC is free from amplification in the FAM (465-510) channel.

c. PCT produces amplification in the FAM (465-510) channel between CT 16-25

Manually inspect amplification criteria are fulfilled for all samples to verify the integrity of the results.

## 12. Interpreting the test results

If all the data analysis criteria are fulfilled, then each sample can be assessed with the following metric:

| Target | Positive control | Negative control | Result |
|---|---|---|---|
| + | + | - | Positive Quantitative result (calculate quantity) |
| - | + | - | Negative result |
| +/- | + | CT<35 | Experiment failed (contamination) |
| +/- | + | CT>35 | * |
| +/- | - | +/- | Experiment failed |

*The sample must be reinterpreted based on relative signal of the Target vs. Negative control.

## 13. General Instructions

### 13.1 Shaking of Reagents

- Shake each reagent gently before use.

### 13.2 Out of Date Kits

- Don't use kits that are expired. Don't exchange the reagents of different batches, or else it will drop the sensitivity.

## 14. Storage

- Storage condition: -20°C
- Storage period: 12 months

## Customer Notes:

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