Anatoxin qPCR detection Kit

Real-time quantitative analysis of the Anatoxin gene region AnaC

Catalog Number: NA2025

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

1. Background

Anatoxin is a class of hepatotoxin produced by blue-green algae such as *Anabaena flos-aquae*. Cyanobacteria can produce Anatoxin 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 Anatoxin is designed for the In vitro analysis of the AnaC gene region responsible for assembling part of the Anatoxin peptide. The AnaC 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 Anatoxin C 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
- PCR clean 1mL tube

- Micropipettes & Tips

5. Components Provided in This Kit

- 170ul AnaC region specific primer mixture (150 reactions)
- 170ul AnaC region specific fluorescent probe (150 reactions)
- 50ul AnaC region positive control template 1E8 copies/ul (Standard curve)
- 1.5ml PCR clean water

6. Reagents Preparation

Master Mix (not Provided)

-Caution this reagent is sensitive to contamination and should only be handled in a clean area away from positive control template.

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

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

Primer/ Probe Mixture

-Caution these reagents are sensitive to contamination and should only be handled in a clean area away from positive control template.

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

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

7. Control Preparation

Negative extraction control (NEC)

-If necessary, prepare 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 AnaC 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 Anatoxin DNA representing the AnaC 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 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 19uL of this reaction mixture and 1ul of isolated gDNA/NTC/PCT/NEC 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	7uL
AnaC Probe	1uL
Dual AnaC Primer set	1uL
Final Volume	19uL

2. Pipette 19uL of this mixture into each well according to your qPCR experimental plate set up.

3. Prepare your DNA templates for each reaction

4. Pipette 1uL of sample gDNA into each well, according to your experiment. For negative controls replace the gDNA sample with 1uL of RNase/DNase free water to bring the total volume to 20uL.

5. To prepare a standard curve dilution series.

1) Pipette 90uL of RNase/DNase free water into 8 separate tubes and label
2) Pipette 10uL of Positive Control Template (1E8 copies/uL) into tube 1
3) Vortex thoroughly
4) Pipette 10ul from prior dilution into the next tube
5) Vortex thoroughly
6) Repeat steps 4-5 six more times

Standard Curve	Copy Number
Tube 1	1E7 per uL
Tube 2	1E6 per uL
Tube 3	1E5 per uL
Tube 4	1E4 per uL
Tube 5	1E3 per uL
Tube 6	1E2 per uL
Tube 7	1E1 per uL
Tube 8	1 per uL

6. Use 1ul 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	AnaC = FAM (456-510)
Denaturation	20 sec.	94C	35	
Annealing*	30 sec.	52C		
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 5-30

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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