Zebra & Quagga mussel qPCR detection Kit

Real-time quantitative analysis of the Zebra & Quagga mussel gene region 16S

Catalog Number: NA2053-S

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

1. Background

Zebra mussels (Dreissena polymorpha) and quagga mussels (Dreissena rostriformis bugensis) are highly invasive freshwater bivalves native to the Ponto-Caspian region. Both species have spread globally, causing severe economic and ecological damage by fouling infrastructure, outcompeting native species, and altering aquatic ecosystems. Effective surveillance is crucial for management. The 16S ribosomal RNA (rRNA) gene, a region of the mitochondrial genome, is a common molecular target for environmental DNA (eDNA) detection. It is favored due to its multi-copy nature, which increases sensitivity, and its conserved sequence, which allows for the design of primers that can distinguish between these dreissenid mussels and other aquatic life.

2. Test Principle

Attogene's qPCR kit for Zebra & Quagga mussel is designed for the in vitro analysis of the crucial genetic marker 16S. A gene-specific primer mix targeting the 16S region is provided for amplification and detection using SYBR Green dye on a qPCR instrument. samples are collected and processed to extract purified genomic DNA (gDNA). A reaction mixture is prepared using provided primers, SYBR Green master mix, and extracted gDNA samples as required. The assembled reaction is then loaded onto the qPCR instrument for amplification. The primer mix utilizes Taq polymerase to amplify the gene region of interest, with the SYBR Green dye binding specifically to double-stranded DNA during PCR amplification. This allows for real-time fluorescence detection across a wide range of qPCR platforms.

3. Applications

- This kit can be used for specific analysis of the 16S gene region in liquid gDNA samples.

4. Equipment and Reagents Needed (not provided)

- Real-time qPCR Instrument
- qPCR 2X Master Mix 2X SYBR green
- 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 16S region specific primer pairs (150 reactions)
- 1.5ml PCR clean water

6. Reagents Preparation

Master Mix (not Provided)

-Master mix with 2x SYBR green is necessary to use this kit.

-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 Mixture 5uM

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

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

-If required aliquots of Primers 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 sample 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

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 (not included).

-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.

-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 2X SYBR	10uL
PCR Water	8uL
Dual Primer set	1uL
Final Volume	19uL

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

3. 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.

10. qPCR Amplification Protocol

Amplification conditions using 2x qPCR Master Mix:

Steps	Time	Temperature	Cycles	Detection Format
Initial Denaturation (Taq Activation)	2 min.	94C	1	16S = SYBR (518-530)
Denaturation	20 sec.	94C	35	
Annealing*	30 sec.	52C		
Extension	45 sec.	74C		

*Data should be read during this step through the SYBR 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 SYBR (518-530) channel.

b. NTC is free from amplification in the SYBR (518-530) channel.

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:

Contact Us

Attogene

3913 Todd Lane, Suite 310 Austin, TX 78744

Phone: 512-333-1330

Email: sales@attogene.com

Web: www.attogene.com