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For Research Use Only

Staphylococcus aureus PCR Detection Kit

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Real-time PCR kit for detection of Staphylococcus aureus in various samples.

For 50- Reactions

Store at -20 °C.

INTRODUCTION

Staphylococcus aureus PCR Detection Kit use **Real-time PCR** (qPCR) technology to detect the DNA of Staphylococcus aureus. This detection kit is specially designed to be used on **Real-time PCR** -compatible instrument. The type of experiment and the reagent used in this kit are Quantitation-Comparative CT and SYBR Green, respectively.

The intended users of this kit are aquaculture technicians who have basic laboratory skills. This kit is intended for in vitro use only.

USAGE

The **Staphylococcus aureus PCR Detection Kit** is designed to detect this bacterial species in a variety of samples.
This kit provides Real-time PCR Master Mix and the specific primer sets for rapid testing by Real-time PCR, as well as the positive Control for reliable results.

CONTENTS

This kit is intended for **50** reactions, including positive controls, **table 1**.

Table 1. Kit Contents

Reagent	Volume	Description
Real-time PCR Master mix	500 µl	Ready to use
Staphylococcus aureus F primer	15 µl	Ready to use
Staphylococcus aureus R primer	15 µl	Ready to use
Staphylococcus aureus Positive control	25 µl	DNA
PCR grade DDW	1 ml	PCR Grade water

PRECAUTIONS

· Store extracted positive material (samples and positive controls) away from all other reagents and add it to the reaction mix in a separate area.
· Thaw all components thoroughly on ice before starting experiment.
· When thawed, mix the components and centrifuge briefly.
· Do not use a kit after its expiration date.
· Use disposable gloves, laboratory coats and eye protection while handling samples and reagents. Thoroughly wash hands afterwards.
· Specimens should be considered potentially infectious and handled in biological cabinet in accordance with appropriate biosafety practices.
· Clean and disinfect all sample or reagent spills using a disinfectant such as 0.5% sodium hypochlorite, or other suitable disinfectant.
· Avoid contact of specimens and reagents with the skin, eyes and mucosa. If skin, eyes and mucosa contact immediately flush with water, seek medical attention.
· Use of this product should be limited to personnel trained in the techniques of DNA amplification.

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METHODS AND PROCEDURES

A. Preparing the Real-time PCR mixture

To prevent the risk of contamination with foreign DNA, we recommend that all experiment steps be Performed in a PCR clean room or separated environment area. Filter tips are recommended for each step.
Thawing the kit components on ice. Using ice is recommended during experiment for maintaining the enzyme activity.
Total reaction volume is 20 µl the volume of DNA sample is 2 µl. Prepare a reaction mixture with specific mix primer for each target gene according to table 2.

Table 2: PCR reaction mixture

Composition	Volume
Real-time PCR Master mix	10 µl
Staphylococcus aureus F primer	0/25 µl
Staphylococcus aureus R primer	0/25 µl
PCR-grade water	8/5 µl
Template	1 µl
Total Volume	20 µl

CONTROL + Add 0.5 to 1 µl of Control DNA instead of sample DNA.

CONTROL - Add 1 µl of PCR-grade water instead of sample DNA.

B. Amplification

- Program your PCR instrument according to manufacturer's manual.

Create a temperature time profile on your instrument as follows in Table 3.

Table 3: Temperature Time Profile

Temperature	Time	Cycle
95	10 min	1
95	15 s	40
60	60 s	

Positive control template is expected to amplify usually between Cq 16 and 23. Failure to satisfy this quality control criterion is a strong indication that the experiment has been compromised. The TM of melt curve plot should be around **76** as follows in image 1. If posetive control shows a different TM, the quality of posetive control sample will not be suitable.

Image 1: Melt curve plot