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Staphylococcus aureus Real Time PCR Kit

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For Use with LightCycler 1.0/LightCycler2.0/LightCycler480 (Roche)

Real Time PCR Systems

(Pls ignore the Internal Control if you use the LightCycler 1.0)

For in vitro Diagnostic use only

User Manual

1. Intended Use

Staphylococcus aureus real time PCR kit is used for the detection of Staphylococcus aureus in stool, sputum, C.S.F., urine, gargle, food or water samples by the real time PCR systems.

2. Introduction

Staphylococcus aureus is a major human pathogen that causes both nosocomial and community-acquired infections worldwide. Expression of most virulence factors is controlled by the accessory gene regulator (agr) locus. The agr locus regulates virulence factors such as Staphylococcal enterotoxins, toxic shock syndrome toxin-1 (tst), -toxin, -hemolysin and etc.

3. Principle of Real-Time PCR

The principle of the real-time detection is based on the fluorogenic 5' nuclease assay. During PCR reaction, the DNA polymerase cleaves the probe at the 5' end and separates the reporter dye from the quencher dye only when the probe hybridizes to the target DNA. This cleavage results in the fluorescent signal generated by the cleaved reporter dye, which is monitored real-time by the PCR detection system. The PCR cycle at which an increase in the fluorescence signal is detected initially (Ct) is proportional to the amount of the specific PCR product. Monitoring the fluorescence intensities during Real Time allows the detection of the accumulating product without having to re-open the reaction tube after the amplification.

4. Product Description

Staphylococcus aureus real time PCR kit contains a specific ready-to-use system for the detection of Staphylococcus aureus by polymerase chain reaction (PCR) in the real-time PCR system. The master contains reagents and enzymes for the specific amplification of Staphylococcus aureus DNA. Fluorescence is emitted and measured by the real time systems' optical unit during PCR. The detection of amplified Staphylococcus aureus DNA fragment is performed in fluorimeter **channel FAM** with the fluorescent quencher BHQ1. DNA extraction buffer is available in the kit and sputum sample are used for DNA extraction. In addition, the kit contains a system to identify possible PCR inhibition by measuring the VIC/JOE fluorescence of the internal control (IC). An external positive control (1×10^7 copies/ml) is supplied allows the determination of the gene load. For further information, please refer to section 10.3 Quantitation.

5. Kit Contents

Ref.	Type of Reagent	Presentation 25rxns
1	DNA Extraction Buffer	1 vial, 1.8ml
2	S.A. Reaction Mix	1 vial, 450µl
3	PCR Enzyme Mix	1 vial, 12µl
4	Molecular Grade Water	1 vial, 400µl
5	Internal Control (IC)	1 vial, 30µl
6	S.A. Positive Control (1×10^7 copies/ml)	1 vial, 30µl

6. Storage

- All reagents should be stored at -20°C. Storage at +4°C is not recommended.
- All reagents can be used until the expiration date indicated on the kit label.
- Repeated thawing and freezing (>3x) should be avoided, as this may reduce the sensitivity of the assay.
- Cool all reagents during the working steps.
- Reaction Mix should be stored in the dark.

7. Additionally Required Materials and Devices

- Biological cabinet
- Real time PCR system
- Desktop microcentrifuge for "eppendorf" type tubes (RCF max. 16,000 x g)
- Vortex mixer
- Real time PCR reaction tubes/plates

- Cryo-container
- Trypsin digestive Solution(for sputum sample)
- Pipets (0.5 µl – 1000 µl)
- Sterile filter tips for micro pipets
- Sterile microtubes
- Disposable gloves, powderless
- Biohazard waste container
- Refrigerator and freezer
- Tube racks

8. Warnings and Precaution

Carefully read this instruction before starting the procedure.

- For in vitro diagnostic use only.
- This assay needs to be carried out by skilled personnel.
- Clinical samples should be regarded as potentially infectious materials and should be prepared in a laminar flow hood.
- This assay needs to be run according to Good Laboratory Practice.
- Do not use the kit after its expiration date.
- Avoid repeated thawing and freezing of the reagents, this may reduce the sensitivity of the test.
- Once the reagents have been thawed, vortex and centrifuge briefly the tubes before use.
- Prepare quickly the Reaction mix on ice or in the cooling block.
- Set up two separate working areas: 1) Isolation of the RNA/ DNA and 2) Amplification/ detection of amplification products.
- Pipets, vials and other working materials should not circulate among working units.
- Use always sterile pipette tips with filters.
- Wear separate coats and gloves in each area.
- Do not pipette by mouth. Do not eat, drink, and smoke in laboratory.
- Avoid aerosols

9. Sample Collection, Storage and transport

- Collect samples in sterile tubes;
- Specimens can be extracted immediately or frozen at -20°C to -80°C.
- Transportation of clinical specimens must comply with local regulations for the transport of etiologic agents

10. Procedure

10.1 DNA-Extraction

DNA extraction buffer is supplied in the kit, please thaw the buffer thoroughly and spin down briefly in the centrifuge before use.

10.1.1 Stool or food sample

1) Take about 30mg stool or 500mg food samples to a tube; add 1.0ml normal saline then vortex vigorously. Centrifuge the tube at 13000rpm for 2 minutes, carefully remove and discard supernatant from the tube without disturbing the pellet.

2) Add 50µl DNA extraction buffer, close the tube then resuspend the pellet with vortex vigorously. Spin down briefly in a table centrifuge.

3) Incubate the tube for 10 minutes at 100°C.

4) Centrifuge the tube at 13000rpm for 5 minutes. The supernatant contains the DNA extracted and can be used for PCR template.

10.1.2 Sputum sample

1) Trypsin digestive Solution preparation

Add 10g trypsin to 200ml sterile purified water and mix thoroughly. Adjust the PH value to 8.0 with 2% NaOH solution. Add 2mL 25mmol/L CaCl₂, mix thoroughly and store at 4°C. **Please incubate at 37°C for 10 minutes before use.**

2) Estimate the volume of sputum and add partes aequales of trypsin digestive solution then vortex vigorously. Set at room temperature for 30 minutes. Transfer 0.5ml mixture to a new tube. Centrifuge the tube at 13000rpm for 5 minutes, carefully remove and discard supernatant from the tube without disturbing the pellet.

3) Add 1.0ml normal saline. Resuspend the pellet with vortex vigorously. Centrifuge at 13000rpm for 5 minutes. Carefully remove and discard supernatant from the tube without disturbing the pellet.

4) Repeat step 3)

5) Add 50µl DNA extraction buffer, close the tube then resuspend the pellet with vortex vigorously. Spin down briefly in a table centrifuge.

6) Incubate the tube for 10 minutes at 100°C.

7) Centrifuge the tube at 13000rpm for 10 minutes. The supernatant contains the DNA extracted and can be used for PCR template.

10.1.3 C.S.F, urine, gargle, water samples

1) Take 1.5 ml sample to a tube, Centrifuge the tube at 13000rpm for 2 minutes, carefully remove and discard supernatant from the tube without disturbing the pellet.

2) Add 50µl DNA extraction buffer, close the tube then resuspend the pellet with vortex vigorously. Spin down briefly in a table centrifuge.

3) Incubate the tube for 10 minutes at 100°C.

4) Centrifuge the tube at 13000rpm for 5 minutes. The supernatant contains the DNA extracted and can be used for PCR template.

10.1.4 Other samples

1) Pipet 50µl sample to a new 0.5ml tube, add 50µl DNA extraction buffer, closed the tube then vortex for 10 seconds. Spin down briefly in a table centrifuge.

2) Incubation the tube for 10 minutes at 100°C.

3) Centrifuge the tube at 13000rpm for 10 minutes. The supernatant contains the extracted DNA and can be used for the template of the PCR.

Attention:

A. During the incubation, make sure the tube is not open. Since the vapor will volatilize into the air and may cause contamination if the sample is positive.

B. The extraction sample should be used in 3 hours or stored at -20°C for one month.

C. DNA extraction kits are available from various manufacturers. You may use your own extraction systems or the commercial kit based on the yield. For the DNA extraction, please comply with the manufacturer's instructions.

10.2 Internal Control

It is necessary to add internal control (IC) in the reaction mix. Internal Control (IC) allows the user to determine and control the possibility of PCR inhibition.

Add the internal control (IC) 1µl/rxn and the result will be shown in the VIC/JOE channel.

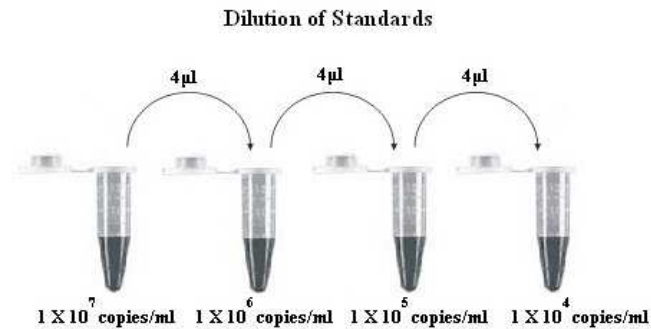
10.3 Quantitation

The kit can be used for **quantitative** or **qualitative** real-time PCR. A positive control (1×10^7 copies/ml) is supplied in the kit.

For performance of quantitative real-time PCR, standard dilutions must be prepared first as follows. Molecular Grade Water is used for dilution.

Dilution is not needed for performance of qualitative real-time PCR.

Take positive control (1×10^7 copies/ml) as the starting high standard in the first tube. Respectively pipette **36 μ l** of Molecular Grade Water into next three tubes. Do three dilutions as the following figures:



To generate a standard curve on the real-time system, all four dilution standards should be used and defined as standard with specification of the corresponding concentrations.

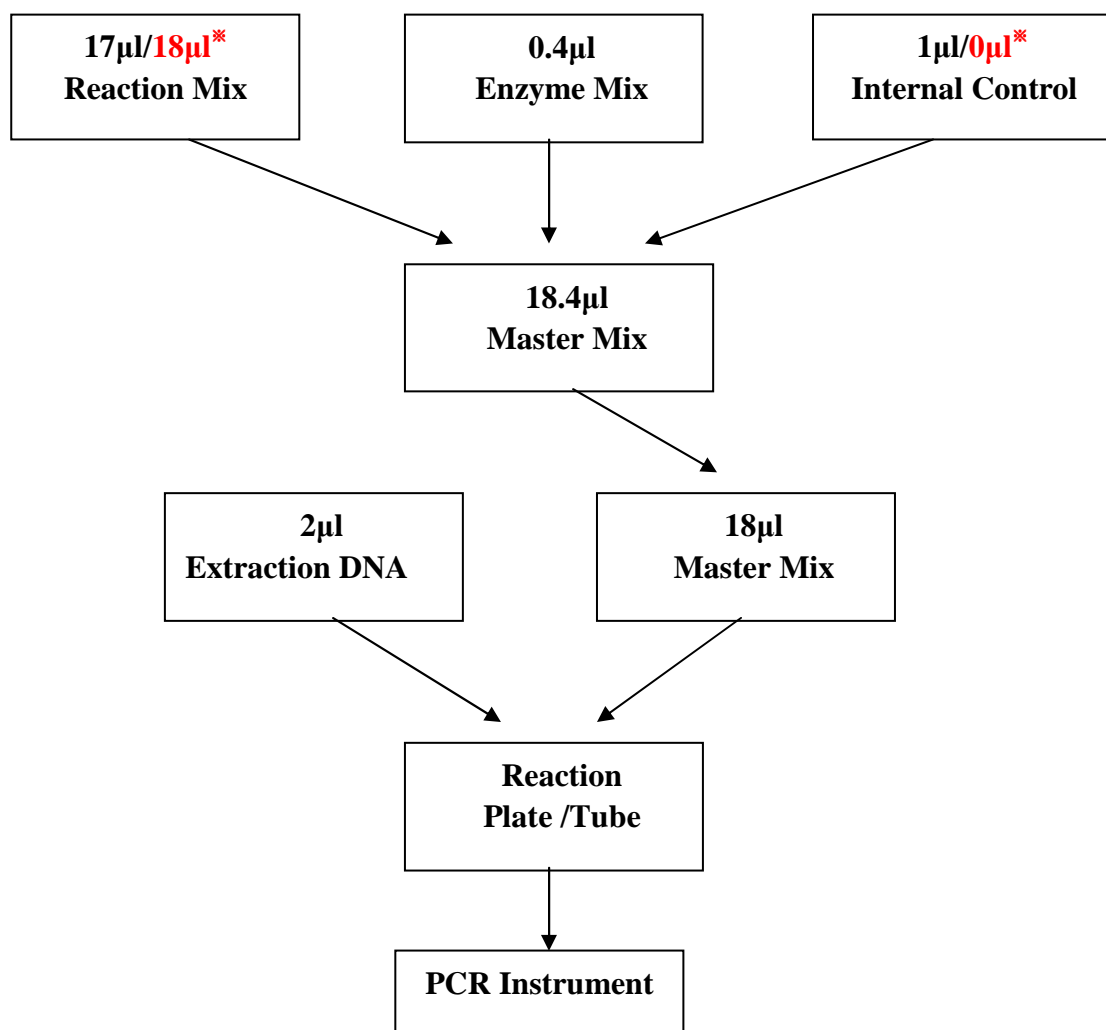
Attention:

A. Mix thoroughly before next transfer.

B. The positive control (1×10^7 copies/ml) contains high concentration of the target DNA. Therefore, be careful during the dilution in order to avoid contamination.

10.4 PCR Protocol

The Master Mix volume for each reaction should be pipetted as follow:



※ Only For Lightcycler 1.0

- 1) The volumes of Reaction Mix and Enzyme Mix per reaction multiply with the number of samples that includes the number of controls, standards, and sample prepared. Molecular Grade Water is used as the negative control. For reasons of unprecise pipetting, always add an extra virtual sample. (**n: the number of reaction**).

Reaction Volume	Master Mix Volume	Master Mix Volume (Only for Lightcycler 1.0)
Reaction Mix	$17\mu\text{l} \times (n+1)$	$18\mu\text{l} \times (n+1)$
Enzyme Mix	$0.4\mu\text{l} \times (n+1)$	$0.4\mu\text{l} \times (n+1)$
Internal control (IC)	$1\mu\text{l} \times (n+1)$	—

Mix completely then spin down briefly in a centrifuge.

- 2) Pipet **18µl** Master Mix with micropipets of sterile filter tips to each *Real time* PCR reaction plate/tubes. Then separately add **2µl** DNA sample supernatant, positive and negative controls to different plates/tubes. Immediately close the plate/tubes to avoid contamination.
- 3) Spin down briefly in order to collect the Master Mix in the bottom of the reaction tubes.
- 4) Perform the following protocol in the instrument:

37°C for 2 min, 1 cycle;



94°C for 2 min, 1 cycle;



93°C for 5 sec, 60°C for 30 sec, 40 cycles.

Fluorescence is measured at 60°C

11. Data Analysis and Interpretation

The following results are possible:

1) A signal is detected in channel FAM. **The result is positive: The sample contains STAPHYLOCOCCUS AUREUS DNA.**

In this case, the detection of a signal in channel VIC/JOE (Internal control) is dispensable, as high initial concentrations of MP DNA can lead to a reduced or absent fluorescence signal of the internal control (competition).

2) In channel FAM no signal is detected, at the same time, a VIC/JOE signal from the Internal Control appears. **The sample does not contain any Staphylococcus aureus DNA. It can be considered negative.**

In the case of a negative STAPHYLOCOCCUS AUREUS PCR the detected signal of the internal control rules out the possibility of PCR inhibition.

3) Neither in channel FAM nor in channel VIC/JOE is a signal detected. **A diagnostic statement can not be made.** Inhibition of the PCR reaction.