MBPCR064  Staphylococcus aureus Detection Kit (Real-time PCR Based)

Description

*Staphylococcus aureus* is gram-positive cocci, non-motile, non-spore forming commonly found on the skin or in the nose of even healthy individuals. Most of the time, these bacteria cause no problems or result in relatively minor skin infections. However, staph infections can turn deadly if the bacteria invade deeper into your body, entering into the bloodstream, joints, bones, lungs or heart. Treatment usually involves antibiotics and drainage of the infected area. However, some staph infections no longer respond to common antibiotics. MRSA strains are common if infection is acquired in a health care facility. Staph infections can range from minor skin problems to endocarditis.

The Real time PCR technique is considerably simple and fast with respect to the standard PCR technique. This technique has been successfully used for the rapid detection and identification of a variety of microorganisms.

**NOTE:** The *Staphylococcus aureus* Detection Kit is for *in vitro* use only.

Intended Use:

Recommended for sensitive detection in clinical samples.

Product characteristics

The Staphylococcus aureus Detection Kit is designed for fast detection of specific sequence of *femA* gene for *S. aureus* from various clinical material samples. This kit also contains Positive control.

**Positive control:** This is a control reaction using a known template (target pathogen). A positive control is usually used to check that the primers have been designed properly and the PCR conditions have been set up correctly.

**Principle:**

Real-time Polymerase Chain Reaction, also called quantitative Polymerase Chain Reaction (qPCR) or kinetic Polymerase Chain Reaction, is a laboratory technique based on the principle of Polymerase Chain Reaction. This technique is used to amplify and simultaneously quantitate a targeted DNA sequence. Real-time PCR systems based on SYBr Green assays have increasingly been used for accurate & reliable detection and quantitation of various food-borne pathogens. HiMedia’s Staphylococcus aureus Detection Kit (Real-time) is one such SYBr green based qPCR technique which allows amplification of specific sequence *femA* gene.
Diagrammatic representation of preferential binding of SYBr Green Dye to specific DNA fragments in real-time PCR.

**Features:**
- Fast and simple
- Sensitive and specific results
- Guaranteed reproducible results
- Rapid detection of all relevant clinical pathogens

**Sample Source:** Clinical samples

**Storage and Shelf-life:**
The provided kit has a shelf-life of **12 months** when stored at -20°C. Repeated thawing and freezing of PCR reagents should be avoided as this may reduce the sensitivity. If reagents are to be used multiple times, we recommend storing reagents as aliquots to avoid repeated freeze and thaw. Degradation of
sample DNA specimens can also reduce sensitivity of the assay. HiMedia does not recommend using the kit after the expiry date stated on pack.

**Kit Contents:**
The provided PCR kit contains:

<table>
<thead>
<tr>
<th>Components</th>
<th>Product code</th>
<th>Reagents provided for 10R (reactions)*</th>
<th>Reagents provided for 25R (reactions)*</th>
<th>Reagents provided for 50R (reactions)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hi-SYBr master mix (2X master mix containing SYBr Green, Assay buffer, Taq Polymerase, MgCl₂, dNTPs)</td>
<td>MBT074</td>
<td>150 µl</td>
<td>400 µl</td>
<td>700 µl</td>
</tr>
<tr>
<td>Primer Mix for <em>S. aureus</em></td>
<td>DS0156</td>
<td>25 µl</td>
<td>60 µl</td>
<td>120 µl</td>
</tr>
<tr>
<td>Positive control (<em>S. aureus</em> DNA)</td>
<td>DS0310</td>
<td>15 µl</td>
<td>35 µl</td>
<td>65 µl</td>
</tr>
<tr>
<td>Molecular Biology Grade Water for PCR</td>
<td>ML065</td>
<td>1 ml</td>
<td>2 ml</td>
<td>4 ml</td>
</tr>
</tbody>
</table>

* For a 20µl PCR reaction

**Materials needed but not provided**
- Micropipette & tips
- Micro centrifuge (for spinning down the reaction mix)
- HiMedia's HiPurA Bacterial DNA Purification Kit (MB505)

**Specimen collection and Handling:**
Follow appropriate techniques for handling specimens; after use, contaminated materials must be sterilized by autoclaving before discarding. Standard precautions as per established guidelines should be followed while handling clinical specimens and items contaminated with blood and other body fluids. Safety guidelines may be referred in individual safety data sheets.

**Sample Material Preparation:**
Various clinical materials and cultured bacteria can be examined. For preparation of bacterial DNA, perform the nucleic acid purification using HiMedia’s HiPurA Bacterial DNA Purification Kit (MB505) as described in the protocol. The purified DNA is free of any inhibitors and can be used directly for PCR.

**Enrichment of pathogens (if required):**
In order to ensure sensitive detection of pathogens from different variety of samples by PCR, the pathogens need to be enriched in broth.
General Preparation Instructions:

- Before use, suitable amount of all PCR components should be completely thawed on ice (4°C).
- Perform the amplification reactions in a clean area.
- Use of aerosol barrier pipette tips is recommended to reduce contamination risks from extraneous DNA templates.
- Extract and store positive control material (if used) separately from all other reagents to avoid contamination and add it to the reaction mix in a separate area.
- Centrifuge the components briefly once thawed.

A) Protocol:

**Flow Chart for setting up PCR Reaction**

1. Add 10µl Hi-SYBr master mix (MBT074) in a PCR tube
2. Add 2 µl the Primer mix (Final concentration 10 pmoles provided)
3. Add 1-2 µl template DNA (upto 50 ng of extracted DNA)
4. Add Molecular Biology Grade Water for PCR (ML065) to make the final volume to 20 µl
5. Centrifuge the tube briefly at 6000 rpm for about 10 seconds.
6. Place the tubes in real-time PCR machine and set the recommended PCR program
7. Interpret the data from the amplification plot (observe the Ct values)

**NOTE: (Optional) – The user can also set up an additional PCR reaction containing Positive control DNA (provided) in a separate tube.**

B) Recommended PCR program:

1. Initial denaturation :  94°C for 5 minutes
2. Cycling Parameters (No. of cycles: 30)
   - Denaturation :  94°C for 30 seconds
   - Annealing :  56°C for 30 seconds
   - Extension :  72°C for 30 seconds
3. Final Extension :  72°C for 5 minutes
C) After amplification, the products can be kept at 4°C overnight or frozen at –20°C for long-term storage.

Warning
Certified for *in vitro* Diagnostic Use (IVD). Not for Medicinal Use.

Precautions
Read the procedure carefully before starting the experiment.

Performance and Evaluation
Each lot of HiMedia’s Staphylococcus aureus Detection Kit (Real-time) is tested against predetermined specifications to ensure consistent product quality.

Quality Control
Each lot of HiMedia’s Staphylococcus aureus Detection Kit (Real-time) is assayed for contaminating endonuclease, exonuclease and non-specific DNase activities. Functionally tested in DNA amplification.

Amplification Data:

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Sample</th>
<th>C&lt;sub&gt;f&lt;/sub&gt; value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Negative control</td>
<td>N/A</td>
</tr>
<tr>
<td>2</td>
<td>1 µl of template DNA (amplicon of <em>S. aureus</em>) in duplicates</td>
<td>8.62</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>8.73</td>
</tr>
</tbody>
</table>

Figure: Data representing real-time amplification data of *S. aureus* with C<sub>f</sub> values (provided in table)

Troubleshooting Guide

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Problem</th>
<th>Cause</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>No amplification</td>
<td>Degraded samples</td>
<td>1. Check the integrity of DNA using agarose gel electrophoresis.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2. Use freshly prepared DNA to ensure the availability of intact template sequence for efficient amplification.</td>
</tr>
<tr>
<td></td>
<td>Error in protocol setup</td>
<td></td>
<td>Verify that the correct reagent volumes, dilutions and storage conditions have been used.</td>
</tr>
</tbody>
</table>
2. Variability between replicates

<table>
<thead>
<tr>
<th>Error in reaction set-up</th>
<th>Prepare large volume reaction mix, vortex thoroughly and aliquot appropriately into reaction tubes.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air bubbles in reaction mix</td>
<td>Briefly centrifuge reaction samples/plate prior to running on a PCR machine.</td>
</tr>
<tr>
<td>Pipetting error</td>
<td>Replicates can show increased variation due to poor laboratory techniques or imprecise pipettes.</td>
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</tbody>
</table>

3. Amplification in negative control

| Reagents contaminated | 1. Replace all critical solutions.  
<table>
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<tbody>
<tr>
<td></td>
<td>2. Repeat the analysis of all tests with fresh aliquots of critical reagents.</td>
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</tbody>
</table>

**Safety Information**

The Staphylococcus aureus Detection Kit (Real-time) is for laboratory use only, not for drug, household or other uses. Take appropriate laboratory safety measures and wear gloves when handling.

**Disposal**

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed off in accordance with current laboratory techniques.

**Technical Assistance**

At HiMedia, we pride ourselves on the quality and availability of our technical support. For any kind of technical assistance, mail at mb@himedialabs.com.