

## MBPCR020 Methicillin Resistant *Staphylococcus aureus* (MRSA) Detection Kit (Multiplex)

### Description:

*Staphylococcus aureus* is one of the most common causes of nosocomial or community-based infections, leading to serious illnesses with high rates of morbidity and mortality. In recent years, the increase in the number of bacterial strains that show resistance to methicillin (MRSA) has become a serious clinical and epidemiological problem. For these reasons, accuracy and promptness in the detection of methicillin resistance is of key importance to ensure correct antibiotic treatment in infected patients as well as control of MRSA isolates in hospital environments, to avoid them spreading.

MRSA strains harbour the *mecA* gene, which encodes a modified PBP2a protein with low affinity for methicillin and all  $\beta$ -lactam antibiotics. Phenotypic expression of methicillin resistance may alter depending on the growth conditions for *S. aureus*, such as temperature or osmolarity of the medium, and this may affect the accuracy of the methods used to detect methicillin resistance. Heteroresistant bacterial strains may evolve into fully resistant strains and therefore be selected in those patients receiving  $\beta$ -lactam antibiotics, thus causing therapeutic failure. From a clinical point of view, they should, therefore, be considered fully resistant. Detection of the *mecA* gene is considered as the reference method for determining resistance to methicillin.

### Intended Use:

The Methicillin Resistant *Staphylococcus aureus* (MRSA) PCR Detection Kit (Multiplex) is focused on simultaneous detection of four targets, *Staphylococcus* genus (16S rRNA gene), methicillin-resistant staphylococci (*mecA* gene), Panton Valentine Leucocidin toxin (*lukPV* gene) and discrimination between *S. aureus* and Coagulase negative Staphylococci - CoNS (*femA* gene).

**NOTE:** The Methicillin Resistant *Staphylococcus aureus* PCR kit is for *in vitro* use only.

### Principle:

HiMedia's Methicillin Resistant *Staphylococcus aureus* (MRSA) PCR Detection Kit (Multiplex) is a qualitative conventional PCR kit which focuses on simultaneous amplification of four targets, *Staphylococcus* genus (16S rRNA gene), methicillin-resistant staphylococci (*mecA* gene), community-acquired MRSA (*lukPV* gene), and discrimination between *S. aureus* and CoNS (*femA* gene). The amplified target is confirmed by using agarose gel electrophoresis. 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.

Polymerase Chain Reaction (PCR) is a very sensitive and specific method for amplification based detection of target genes. The three steps of a successful PCR reaction include Denaturation, Annealing and Extension. The double-stranded DNA melts and forms single stranded DNA at high temperature (Denaturation). Sequence-specific primers bind to the target sequence on single-stranded DNA at lower temperature (Annealing). Taq DNA

Polymerase adds dNTPs onto the single stranded DNA at intermediate temperature (Extension). These 3 steps of PCR are usually repeated upto 30 to 40 times in each PCR assay.

**Features:**

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

**Unit Definition:**

1U is defined as amount of enzyme that is required to catalyze the incorporation of 10 nmoles of dNTP into acid-insoluble material in 30 minutes at 74°C.

**Storage and Shelf-life:**

The kit must be stored on arrival 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. The kit provided is stable for 6 months when stored at mentioned conditions. HiMedia does not recommend using the kit after the expiry date stated on pack.

**Kit Contents:**

Components	Reagents provided for 10R (reactions)	Reagents provided for 25R (reactions)	Reagents provided for 50R (reactions)
2X PCR Master Mix ( <b>MBT061</b> )	260 µL	650 µL	1.5 ml
MRSA Primer Mix	180 µL	420 µL	810 µL
Nuclease free water ( <b>ML065</b> )	1 ml	2 ml	4 ml
6X Loading Dye ( <b>ML015</b> )	30 µL	75 µL	150 µL
100 bp DNA Ladder ( <b>MBT049</b> )	60 µL	150 µL	300 µL
Positive control DNA	55 µL	130 µL	260 µL

**Sample Material Preparation:**

Various clinical isolate either hospital or community based can be examined. Extract bacterial DNA using HiMedia’s HiPurA Bacterial DNA Extraction kit (MB505) or equivalent extraction kit according to manufacturer's instructions.

**Enrichment of pathogens (if required):**

- In order to ensure sensitive detection of pathogens, the pathogens need to be enriched in broth.

**General Preparation Instructions:**

- Before use, 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.

**Protocol:**

**Preparation of PCR Reaction Mixture**

Add 25 µl of 2X PCR Master Mix (**MBT061**) in a PCR tube



In the same tube, add 16 µl of MRSA primer mix\*\*



Add 3-5 µl of **template DNA** (upto 50 ng of extracted DNA)



Add nuclease free water (**ML065**) to make the final volume to 50 µl



Centrifuge the tube briefly at 6000 rpm for about 10 seconds.



Place the tubes in the PCR machine and set the recommended PCR program  
(mentioned below )



Interpret the data using Agarose gel electrophoresis

**\*\* Primer concentration provided:**

- 16S rRNA – 0.6 pmol
- femA – 0.8 pmol
- mecA – 1.0 pmol
- PVL – 0.6 pmol

**NOTE: (Optional) – The user can also set up an additional PCR reaction containing 5µl Positive control DNA (provided) in a separate tube.**

**A. MRSA detection program:**

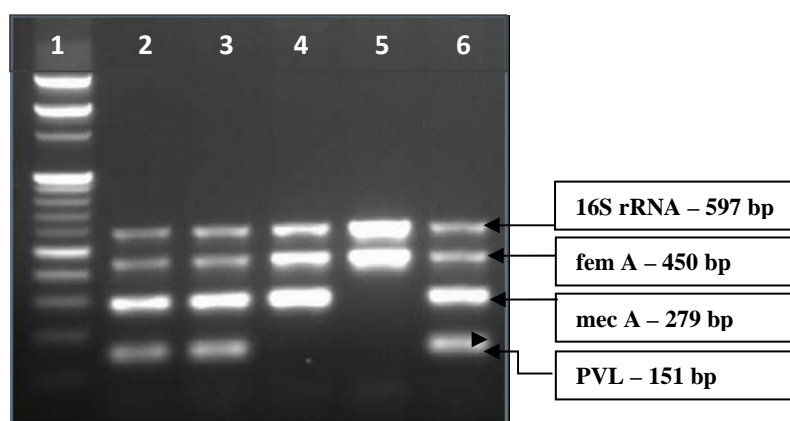
Step	Temperature (°C)	Time (minute : second)	No. of cycles
Initial Denaturation	94	03:00	1
Denaturation	94	00:30	30
Annealing	56	00:30	30
Extension	72	00:30	30
Extra Annealing	60	00:30	1
Final Extension	72	05:00	1
Post run	4	Hold	-

**B. After amplification, store the products at 4°C overnight or -20°C for long-term storage.**

**C. Agarose Gel Electrophoresis**

- 10µL of the amplicons along with 1µL of 6X DNA loading dye (**Product Code: ML015**) are separated on a 1.8% of low EEO agarose gel (**MB002**) prepared in 1X TAE buffer (**Product Code : ML010**) under an electric current of 15V/cm for 45 mins.
- Load 5µL of 100bp DNA ladder (**Product Code : MBT049**) in separate well.

**Gel image representing amplification of MRSA clinical samples**



Lane	1	2	3	4	5	6
Identification	100bp Ladder	MRSA PVL +	MRSA PVL +	MRSA PVL -	MSSA	MRSA PVL +

**Specifications:**

**Sensitivity:** Detectable upto 10<sup>3</sup> cfu/ml

**Quality Control:**

Each lot of HiMedia’s Methicillin Resistant Staphylococcus aureus (MRSA) Detection Kit (Multiplex) is assayed for contaminating endonuclease, exonuclease, and non-specific DNase activities. Functionally tested in DNA amplification.

**Troubleshooting Guide:**

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

2.	Variability between replicates	Error in reaction set-up	Prepare large volume reaction mix, vortex thoroughly and aliquot appropriately into reaction tubes.
		Air bubbles in reaction mix	Briefly centrifuge reaction samples/plate prior to running on a PCR machine.
		Pipetting error	Replicates can show increased variation due to poor laboratory techniques or imprecise pipettes.
3.	Amplification in negative control	Reagents contaminated	<ol style="list-style-type: none"> <li>1. Replace all critical solutions</li> <li>2. Repeat the analysis of all tests with fresh aliquots of critical reagents.</li> </ol>

### Safety Information

The Methicillin Resistant Staphylococcus aureus (MRSA) PCR Detection Kit (Multiplex) is for laboratory use only, not for drug, household or other uses. Take appropriate laboratory safety measures and wear gloves when handling.

### Product Use Limitation & Warranty

HiMedia guarantees the performance of product in the manner described in the product literature. Methicillin Resistant Staphylococcus aureus (MRSA) Detection Kit (Multiplex) is designed and sold for research and *in vitro* purposes only. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

All due care and attention should be exercised in the handling of the products. We recommend all users of HiMedia products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

### 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](mailto:mb@himedialabs.com).

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