MBPCR008 Methicillin Resistant Staphylococcus aureus (MRSA) Detection Kit (Uniplex)

Description:

Nosocomial transmission of Methicillin Resistant Staphylococcus aureus (MRSA) has increased dramatically in the last few years. Quick and reliable methods are required to diagnose and prevent transmission of the resistance gene. The Methicillin Resistant Staphylococcus aureus (MRSA) Detection Kit is designed for detection of Staphylococcus aureus (MRSA) by Polymerase Chain Reaction (PCR) method. The Methicillin Resistant Staphylococcus aureus (MRSA) produces penicillin binding protein which is coded by mecA gene.
Thus, mecA gene can be used for detection of DNA sequence specific for Methicillin Resistant Staphylococcus aureus (MRSA).

NOTE: The MRSA Detection Kit (Uniplex) is for *in vitro* use only.

Intended Use:

The MRSA Detection Kit is designed for detection of the specific sequence of **mecA (293 bp**) gene in MRSA from various sources. Conventional PCR testing can provide rapid, sensitive and specific detection of MRSA. This kit also contains **Internal control** and **Positive control.**

Internal control: This is a control sequence, which is amplified in the same reaction tube along with the target sequence (target pathogen) but detected with a different primer (i.e. Multiplex PCR). An internal control is often used to detect the failure of amplification in cases where the target sequence is not amplified.

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.

This diagnostic kit assures very high sensitivity of detection in clinical samples. The kit is designed for *in vitro* diagnostics and provides qualitative detection.

Principle:

HiMedia's MRSA Detection Kit (Uniplex) is a qualitative conventional PCR kit which allows the amplification of MRSA specific gene, **mecA (293 bp**) using specific primers. The amplified target is confirmed by using agarose gel electrophoresis.

Polymerase Chain Reaction (PCR) is a very sensitive and specific method for amplification based detection of 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 between 30 to 40 times in each PCR assay.

Features:

- Fast and simple
- Sensitive and specific results

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- 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 provided kit has a shelf-life of 6 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. 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 **(MBT061)**	260 µl	650 µl	1.5ml
Primer Mix for mec A	25 µl	60 µl	120 µl
Primer Mix for Internal Control	25 µl	60 µl	120 µl
Nuclease free water **(ML065)**	1 ml	2 ml	4ml
6X Loading Dye **(ML015)**	100 µl	200 µl	400 µl
100 bp DNA Ladder **(MBT049)**	40 µl	90 µl	180 µl
Positive control (MRSA DNA)	15 µl	35 µl	65 µl
Internal Control DNA	15 µl	35 µl	65 µl

Sample Material Preparation:

Various food and environmental samples, clinical materials, cultured bacteria and human fecal specimens can be examined. For preparation of bacterial DNA, perform the nucleic acid purification using HiMedia's HiPurA Bacterial DNA Extraction kit (MB505) as described in the protocol.

Enrichment of pathogens (if required):

- In order to ensure sensitive detection of pathogens from different variety of food products 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:

<u>Preparation of PCR Reaction Mixture</u>

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

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In the same tube, add 2 µl of mecA primer mix + 2 µl internal control primer mix (10 pmoles concentration provided)

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Add 1-2 µl of **template DNA** (upto 50 ng of extracted DNA) and add 1µl of **Internal Control DNA** (provided)

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

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

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

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Interpret the data using Agarose gel electrophoresis

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 10 minutes
2. Cycling Parameters (No. of cycles: 30)
   Denaturation : 94°C for 1 minute
   Annealing : 60°C for 1 minute
   Extension : 72°C for 1 minute
3. Final Extension : 72°C for 10 minutes

C. After amplification, the products may be kept at 4°C overnight or frozen at –20°C for long-term storage.

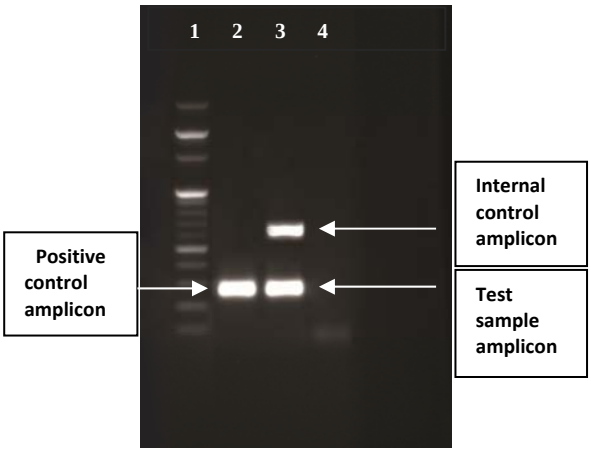
D. MRSA PCR Assay Results Interpretation

- For analysis of the PCR data, load 10 µl of amplicon on a 1.5% agarose gel along with 1 µl of 6X DNA loading dye (ML015).

- Load 3 µl of 100 bp DNA ladder (MBT049) in separate well.

E. EtBr-staining staining to check results

- Incorporate EtBr in the agarose gel or stain the agarose gel with EtBr for 10-15 mins.
- Confirm the expected amplicon size comparing with 100 bp DNA marker.



Lane no.	Samples
1	100 bp ladder
2	Positive control amplicon MRSA sample (293bp)
3	Test sample amplicon MRSA sample (293bp) with internal control (685 bp)
4	Negative control

Gel image representing amplification of mecA gene using target MRSA sample with positive control (293 bp) and internal control (685 bp).

Specifications:

Sensitivity: Detectable upto 10-1000 cfu/ml (mg) before pre-enrichment.

Quality Control:

Each lot of HiMedia's Methicillin Resistant Staphylococcus aureus Detection Kit 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	1. Replace all critical solutions 2. Repeat the analysis of all tests with fresh aliquots of critical reagents.

Safety Information

The Methicillin Resistant Staphylococcus aureus (MRSA) Detection Kit (Uniplex) 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 (Uniplex) 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.

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