

MBT118

Multiplex PCR Kit

| Product Name | Product Code | Kit Packing** |
|-------------------|--------------|---------------|
| Multiplex PCR Kit | MBT118-25R | 25r (625 µl) |
| | MBT118-100R | 100r (2.5 ml) |

**** The product is supplied with a vial of Molecular Biology Grade Water.**

Description:

The Multiplex PCR Kit is specially designed for parallel amplification of a multiple fragments in a single PCR assay. The mix is recommended for use in routine PCR reactions and highly suitable for multiple target gene amplification in a single tube.

The provided multiplex PCR master mix is a 2X ready-to-use solution containing an optimized composition of polymerase, dNTPs, MgCl₂ and reaction buffers for efficient amplification of DNA templates by PCR.

Multiplex PCR Protocol

The key to successful multiplex PCR is the ability to define a single set of reaction parameters (reagent concentrations and cycling parameters) that allows for all primers to anneal with high specificity to their target sequences and be extended with the same efficiency. Primer design, as well as the enzyme and buffer system, are critical factors in this challenge.

The performance of the HiMedia's Multiplex PCR Master kit over a wide range of amplicon sizes permits the amplification of templates up to 2kb.

A) Protocol:

1. Thaw the Multiplex PCR Master Mixture on ice. Vortex the master mix, then spin it briefly in a microcentrifuge to collect the material at the bottom of the tube.
2. Prepare one of the following reaction mixes on ice:

| Sr. No. | Components | Amount to be added | | Final Concentration |
|---------|-------------------------------|--------------------|-------------------|---------------------|
| | | For 25µl reaction | For 50µl reaction | |
| 1 | 2X Multiplex PCR Master Mix | 12.5 µl | 25 µl | 1X |
| 2 | Stock Primer mix, 10µM | Variable | Variable | 0.1–1.0µM |
| 3 | Template DNA | Variable | Variable | <250ng |
| 4 | Molecular Biology Grade Water | Upto 25 µl | Upto 50 µl | - |

3. Perform PCR using your standard parameters.
- B) After amplification, the products can be kept at 4°C overnight or frozen at -20°C for long-term storage.

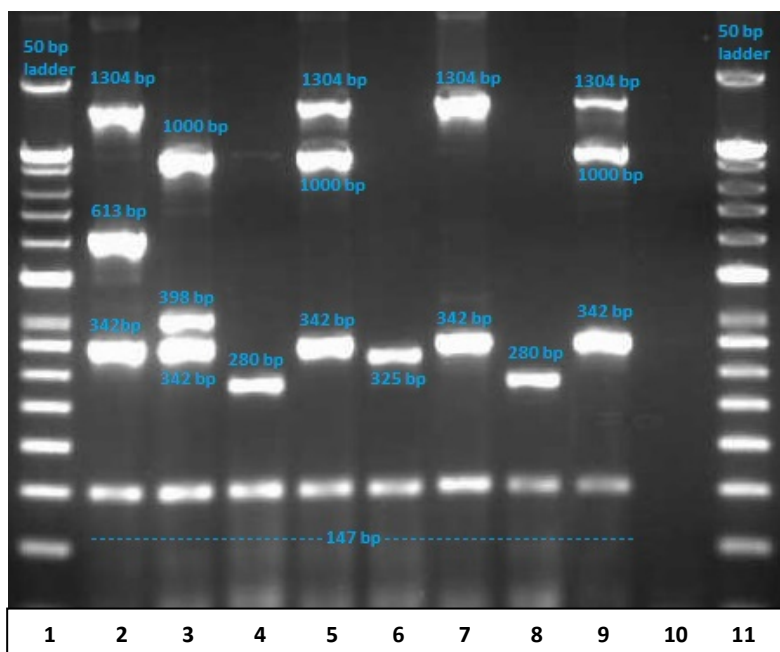
C) Results Interpretation

- For analysis of the PCR data, load 10 µl of amplicon on a 2% Ultra resolution Agarose (Product code: MB073) gel along with 1 µl of 6X DNA loading dye (ML015).
- Load 3 µl of DNA marker in a separate well.

D) EtBr-staining to check results

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

Gel image representing amplification of SCCmec types from clinical samples using 8 sets of primers



| Lanes | 1 & 11 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|----------------|------------------|-------------|----|-----|-----|---|----|-----|----|------------------|
| Identification | 50 bp DNA Ladder | SCCmec type | | | | | | | | Negative control |
| | | I | II | III | IVa | V | VI | III | IV | |

Quality Control:

Each lot of Multiplex PCR Kit is functionally tested for performance in semi-qPCR; free of endo-, exo- deoxyribonuclease, ribonuclease and nicking activities.

Storage and shelf-life:

Multiplex PCR Kit should be stored at -20°C and is stable for 6 months when stored under proper conditions.

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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