MBPCR022  Multi-Drug Resistant Mycobacterium tuberculosis Detection Kit (Multiplex)

Description:

Tuberculosis caused by *Mycobacterium tuberculosis* remains a global scourge, causing 8.8 million new infections and 1.45 million deaths in 2010. The emergence of multi-drug resistant tuberculosis (MDR-TB), defined as resistance of *Mycobacterium tuberculosis* to the first-line drugs isoniazid (INH) and rifampin (RIF) poses a serious threat to global TB control. MDR-TB requires second-line drugs, which are less effective, more expensive and more toxic. Accordingly, it is more associated with treatment failure and increased mortality and transmission.

Thus, rapid diagnosis of MDR-TB plays a vital role in guiding standardized treatment regimen. Traditional drug susceptibility test (DST) takes 6-8 weeks. Even with the aid of liquid cultures and radiometry measures, the bacteriological cultures and DST still takes 2-3 weeks. Hence, to identify MDR-TB with the advance techniques like PCR becomes critical.

Rifampin (RIF), one of the principle first line drugs, inhibits DNA-directed RNA synthesis of *M. tuberculosis* by binding to the subunit of RNA polymerase. Mutations in *rpoB* gene, which codes for the beta subunit of RNA polymerase, have been shown to be strongly associated with RIF-resistant phenotypes.

Isoniazid (INH) is one of the most effective antmycobacterial agents available for the treatment of TB, which is a prodrug that is converted to its active form *in vivo* by *katG*-encoded *M. tuberculosis* catalase peroxidase *katG*. Resistance to INH is predominantly associated with mutations in *katG gene*.

NOTE: The Multi-Drug Resistant Mycobacterium tuberculosis Detection Kit (Multiplex) Is for *in vitro* use only.

Intended Use:

The Multi-Drug Resistant Mycobacterium tuberculosis Detection Kit (Multiplex) is designed to detect mutations in *rpoB* (180 bp) and *katG* (230 bp) gene from sputum specimens of MDR-TB by PCR.

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 Mycobacteria Detection Kit is a qualitative conventional PCR kit which contains the amplification *rpoB* and *katG* gene 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
- Extremely sensitive and specific
- Low cost per reaction
- Guaranteed reproducible results
- Highly specific detection profile
- 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:**

Store 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.

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

<table>
<thead>
<tr>
<th>Components</th>
<th>Reagents provided for 10R (reactions)</th>
<th>Reagents provided for 25R (reactions)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2X PCR Taq Mixture</td>
<td>275 µl</td>
<td>650 µl</td>
</tr>
<tr>
<td>Primer Mix 1 (rpoB gene)</td>
<td>25 µl</td>
<td>60 µl</td>
</tr>
<tr>
<td>Primer Mix 2 (katG gene)</td>
<td>25 µl</td>
<td>60 µl</td>
</tr>
<tr>
<td>Nuclease free water (ML065)</td>
<td>1 ml</td>
<td>2 ml</td>
</tr>
<tr>
<td>6X Loading Dye (ML015)</td>
<td>100 µl</td>
<td>200 µl</td>
</tr>
<tr>
<td>50 bp DNA Ladder (MBT084)</td>
<td>40 µl</td>
<td>90 µl</td>
</tr>
</tbody>
</table>

**Sample Material Preparation:**
Various samples like sputum, Body fluids, Bronchial aspirates and other clinical material, cultured bacteria are routinely examined. For preparation of bacterial DNA perform the nucleic acid purification using HiMedia’s HiPurA Mycobacterium tuberculosis DNA Purification Kit (MB545) as described in the protocol.
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.

Protocol:

A. Preparation of PCR Reaction Mixture:

<table>
<thead>
<tr>
<th>Components</th>
<th>Recommended volume to be added for 50 µl reaction</th>
<th>Final concentration in the reaction mixture</th>
</tr>
</thead>
<tbody>
<tr>
<td>2X PCR Taq Mixture</td>
<td>25 µl</td>
<td>-</td>
</tr>
<tr>
<td>Primer Mix</td>
<td>2 µl</td>
<td>10 pmoles each</td>
</tr>
<tr>
<td>Template</td>
<td>2-5 µl of Enriched product or upto 50 ng of extracted DNA (1-2 µl of control DNA)</td>
<td>-</td>
</tr>
<tr>
<td>Nuclease free water (ML065)</td>
<td>Upto 50 µl</td>
<td>-</td>
</tr>
</tbody>
</table>

B. Recommended PCR program:

For *rpoB* gene: (180 bp)

1. Initial denaturation: 94°C for 3 minutes

2. Cycling Parameters (No. of cycles: 35)
   - Denaturation: 94°C for 1 min
   - Annealing: 68°C for 1 min
   - Extension: 72°C for 1 min

3. Final Extension: 72°C for 5 minutes

For *katG* gene: (230 bp)

4. Initial denaturation: 94°C for 3 minutes

5. Cycling Parameters (No. of cycles: 35)
   - Denaturation: 94°C for 1 min
   - Annealing: 60°C for 1 min
   - Extension: 72°C for 1 min

6. 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. Mycobacteria PCR Assay Results Interpretation

- For analysis of the PCR data, load 10 µl of amplicon on a 2% Agarose gel along with 1 µl of 6X DNA loading dye (ML015).
- Load 5 µl of 50 bp DNA ladder (MBT084) in separate well.

E. 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 100 bp DNA marker

Specifications:

Sensitivity: Detectable upto 10-100 CFU / ml (mg)
Specificity: 100% exclusivity for about 100 non-target strains.

Quality Control:

Each lot of HiMedia Multi-Drug Resistant Mycobacterium tuberculosis Detection Kit (Multiplex) is assayed for contaminating endonuclease, exonuclease, and non-specific DNase activities. Functionally tested in DNA amplification.

Gel image showing result interpretation for MDR-TB detection

Lane1: 50 bp DNA Ladder
Lane2&3: katG+ (273bp)
Lane6&7: rpoB+ (180bp)
Lane10&11: katG+ and rpoB+
Lane12: Non template control
Lane No. 4, 5, 8, 9: contains no sample loaded
## 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>Low or no desired PCR product</td>
<td>Check template quantity and quality</td>
<td>1. Increase the template DNA amount. 2. Check the quality of template DNA by Agarose Gel Electrophoresis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Check the concentration of Taq Polymerase</td>
<td>Increase the Taq polymerase concentration in increments of 0.25 units per 50µl reaction</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Check the annealing temperature</td>
<td>Lower the annealing temperature in 2°C decrements</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Carry out &quot;Hot start&quot; PCR</td>
<td>Carry out modified “hot start” PCR by assembling the reaction on ice and setting up the reaction in pre-heated thermal cycler</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Increase extension time</td>
<td>Increase extension time, generally 1 minute for every kilobase of product</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Increase number of cycles</td>
<td>Increase number of cycles in increments of 5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Redesign the Primers</td>
<td>Design primers with higher annealing temperature and that do not form hairpin loops or anneal to each other</td>
</tr>
<tr>
<td>2.</td>
<td>Multiple product bands or smear detected</td>
<td>Increase the annealing temperature</td>
<td>Increase the annealing temperature in 2°C increments</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Carry out “Hot start” PCR</td>
<td>Carry out modified “hot start” PCR by assembling the reaction on ice and setting up the reaction in pre-heated thermal cycler</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Decrease extension time</td>
<td>Reduce extension time in decrements of 1 minute</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Decrease number of cycles</td>
<td>Decrease number of cycles in decrements of 5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Redesign the Primers</td>
<td>Design primers with higher annealing temperature and that do not form hairpin loops or anneal to each other</td>
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</table>

Please refer disclaimer Overleaf.
### Safety Information

The Multi-Drug Resistant Mycobacterium tuberculosis 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. Multi-Drug Resistant Mycobacterium tuberculosis 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.