Tuberculosis caused by *Mycobacterium tuberculosis* is a multifaceted disease and challenging public health concern in both industrialized and developing countries. It is estimated that there are 8 million cases of tuberculosis (TB), causing 2.5 million deaths per year, worldwide, making TB the foremost cause of death due to infection. Once thought to be under control or even close to extinction, TB infection levels are rising and the threat is compounded by new, virulent, drug resistant strain. Although most cases (=80%) occur in developing countries, increasing population mobility with ease of transmission means that no country is immune from resurgence of TB. TB control programs are facing a number of constraints. Absence of timely and accurate test of diagnosis of mycobacterial disease is of utmost concern. Early diagnosis is crucial for the prevention of further spread of disease.

Furthermore, mycobacterial infections due to non-tuberculosis mycobacteria (NTM) such as *Mycobacterium avium* complex (MAC), *M. fortuitum* and *M. chelonae* are also increasing. The increasing number of mycobacterial infections has made it clinically important to quickly identify mycobacteria at species level. The diagnosis of pathogenic versus non-pathogenic species not only has epidemiological implications, but is also relevant for patient management. PCR has proven to be a very useful tool for rapid diagnosis of infectious diseases, including mycobacteriosis.

**NOTE: The Mycobacterium tuberculosis Detection Kit is for in vitro use only.**

**Intended Use:**
The Mycobacterium tuberculosis Detection Kit is designed to detect the specific gene regions of *IS6110* of the *Mycobacterium tuberculosis* complex (MTBC). Recommended for sensitive detection of *M. tuberculosis* in clinical samples.

**Principle:**
HiMedia's Mycobacterium tuberculosis Detection Kit is a qualitative conventional PCR kit which includes the amplification of *M. tuberculosis* gene *IS6110 (542 bp)*, using specific primers. The amplified target is confirmed by using agarose gel electrophoresis. The mpt64 gene is found as a single copy in the genome of MTBC species and has been used for both pulmonary and extra pulmonary TB diagnosis and because of this exclusivity, it has become an important diagnostic tool in the identification of MTBC species. 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.
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 25 to 40 times in each PCR assay.

Gel electrophoresis is used to analyze the amplification of desired gene region for target pathogen based on separation of DNA fragments according to their size.

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

**Sample Source:** Human sputum, urine, blood, pleural and other body fluids, bronchial aspirates.

**Storage:**
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 contains:

<table>
<thead>
<tr>
<th>Components</th>
<th>Product codes</th>
<th>Reagents provided for (reactions)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>2X PCR TaqMixture</td>
<td>MBT061</td>
<td>10R 300 µL 25R 750 µL 50R 1.5 mL</td>
</tr>
<tr>
<td>Primer Mix for <em>M. tuberculosis</em></td>
<td>DS0132</td>
<td>25 µL 62.5 µL 125 µL</td>
</tr>
<tr>
<td>Primer Mix for Internal Control (285 bp)</td>
<td>DS0223</td>
<td>25 µL 62.5 µL 125 µL</td>
</tr>
<tr>
<td>Molecular Biology Grade Water for PCR</td>
<td>ML065</td>
<td>500 µL 1.25 mL 2.5 mL</td>
</tr>
<tr>
<td>6X Gel Loading Buffer</td>
<td>ML015</td>
<td>40 µL 100 µL 200 µL</td>
</tr>
<tr>
<td>100 bp DNA Ladder</td>
<td>MBT049</td>
<td>40 µL 100 µL 200 µL</td>
</tr>
<tr>
<td>Positive Control (<em>M. tuberculosis</em> DNA)</td>
<td>DS0360</td>
<td>15 µL 37.5 µL 75 µL</td>
</tr>
<tr>
<td>Internal Control DNA</td>
<td>DS0123</td>
<td>15 µL 37.5 µL 75 µL</td>
</tr>
</tbody>
</table>

* For a 50µl PCR reaction

**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 samples like sputum, body fluids, bronchial aspirates and other clinical materials, cultured bacteria are routinely examined. For preparation of *M. tuberculosis* DNA, perform the nucleic acid purification protocol using any one of the following kits:

- HiMedia’s HiPurA® Mycobacterium tuberculosis DNA Purification Kit (MB545)
- HiPurA® Fast MTB (Mycobacterium tuberculosis) Genomic DNA Purification Kit (MB579)

Materials needed but not provided:

- PCR tubes (Product code PW1255) or PCR Strips (Product code: PR17) or PCR Plates (Product code: PR2 / PR3 / PR19)
- Thermal Cycler (Product Code: LA948/LA949/LA950/LA1006/LA1015/LA1059/LA1060/LA1066)
- Barrier Micropipette Tips
- Micropipettes

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 for PCR Master Mix Preparation:

Perform PCR reactions for each DNA sample as per the following table:

<table>
<thead>
<tr>
<th>Components</th>
<th>Recommended volume to be added per reaction (μL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2X PCR TaqMixture (MBT061)</td>
<td>25 μL</td>
</tr>
<tr>
<td>Primer Mix for <em>M. tuberculosis</em> (DS0132)</td>
<td>2 μL</td>
</tr>
<tr>
<td>Primer Mix for Internal Control (285 bp) (DS0223)</td>
<td>2 μL</td>
</tr>
<tr>
<td>Template DNA</td>
<td>2 μL</td>
</tr>
<tr>
<td>Internal Control DNA (DS0123)</td>
<td>1 μL</td>
</tr>
<tr>
<td>Molecular Biology Grade Water for PCR (ML065)</td>
<td>Up to 50 μL</td>
</tr>
</tbody>
</table>

NOTE: (Optional) – The user can also set up an additional PCR reaction containing 1μL of Positive control DNA (provided) in a separate tube.

Centrifuge the tube briefly at 6000 rpm for about 10 seconds and place the tubes in the PCR machine and set the recommended PCR program (mentioned below). Interpret the data using Agarose Gel Electrophoresis.

B. Recommended PCR program:

- Initial denaturation : 94°C for 10 minutes  No. of cycles: 1
- Denaturation : 94°C for 1 minute  No. of cycles: 30
- Annealing : 60°C for 1 minute
- Extension : 72°C for 1 minute
- Final Extension : 72°C for 10 minutes  No. of cycles: 1
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 Gel Loading Buffer (ML015).
   - Load 4 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 minutes.
   - Confirm the expected amplicon size comparing with 50 bp DNA marker.

F. Quality Control:
   Each lot of HiMedia’s Mycobacterium tuberculosis (MTB) Detection Kit is assayed for contaminating endonuclease, exonuclease and non-specific DNase activities. Functionally tested in DNA amplification.

G. Amplification Data:

<table>
<thead>
<tr>
<th>Lane no.</th>
<th>Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100 bp DNA ladder</td>
</tr>
<tr>
<td>2</td>
<td>Positive control amplicon of <em>Mycobacterium tuberculosis</em> (542 bp)</td>
</tr>
<tr>
<td>3,4</td>
<td>Test sample amplicon of <em>Mycobacterium tuberculosis</em> (572 bp) with internal control (285 bp)</td>
</tr>
<tr>
<td>5,6</td>
<td>Negative control</td>
</tr>
</tbody>
</table>

Gel image representing amplification of mpt64 gene using target sample of *Mycobacterium tuberculosis* with positive control (542 bp) and internal control (285 bp)

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 Mycobacterium tuberculosis Detection Kit is tested against predetermined specifications to ensure consistent product quality.
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></td>
<td>Error in protocol setup</td>
<td>Verify that the correct reagent volumes, dilutions and storage conditions have been used.</td>
</tr>
<tr>
<td>2.</td>
<td>Variability between replicates</td>
<td>Error in reaction setup</td>
<td>Prepare large volume reaction mix, vortex thoroughly and aliquot appropriately into reaction tubes.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Air bubbles in reaction mix</td>
<td>Briefly centrifuge reaction samples/plate prior to running on a PCR machine.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pipetting error</td>
<td>Replicates can show increased variation due to poor laboratory techniques or imprecise pipettes.</td>
</tr>
<tr>
<td>3.</td>
<td>Amplification in negative control</td>
<td>Reagents contaminated</td>
<td>1. Replace all critical solutions.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2. Repeat the analysis of all tests with fresh aliquots of critical reagents.</td>
</tr>
</tbody>
</table>

Safety Information

The Mycobacterium tuberculosis (MTB) Detection Kit 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.
In vitro diagnostic medical device

CE Marking

Storage temperature

Do not use if package is damaged

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