MBPCR017 Mycobacterium tuberculosis Detection Kit (Real-Time)

Description:
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 resistance 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 programmers are facing a number of constraints. Absence of timely and accurate tests for diagnosis of mycobacterial disease is of utmost concern. Early diagnosis is crucial for the prevention of further spread of the disease.

The resurgence of TB in industrialized countries since the mid-1980s, primarily due to both the increased incidence of immunocompromised patients with AIDS, and the emergence of MDR-strains of *M. tuberculosis* has increased the need for rapid diagnosis of this disease. Rapid detection of active TB infection is critical for the prompt detection of new cases, effective patient management and implementation of infection control measures, and to institute appropriate anti-mycobacterial therapy.

The FDA-approved nucleic acid amplification based assays for MTB display high specificity but variable sensitivity, several stages are required in the amplification and detection steps involving user manipulations at each point of the assays that have the potential for error and sample contamination. The Real time PCR technique is considerably simple and fast with respect to the standard PCR technique. This technique has been successfully used for the rapid detection and identification of a variety of microorganisms.

NOTE: The Mycobacterium tuberculosis Detection Kit (Real-Time) is for *in vitro* use only.

Principle:
The Mycobacterium tuberculosis Detection Kit (Real time) is designed to detect the specific gene regions of *IS6110 gene* (130bp), an insertion element found exclusively within the members of the Mycobacterium tuberculosis complex (MTBC), and because of this exclusivity, it has become an important diagnostic tool in the identification of MTBC species.

Real-time polymerase chain reaction, also called quantitative Polymerase Chain Reaction (qPCR) or kinetic Polymerase Chain Reaction, is a laboratory technique based on the principle of Polymerase Chain Reaction. This technique is used to amplify and simultaneously quantitate a targeted DNA sequence. Real-time PCR systems based on SYBr Green assays have increasingly been used for accurate, reliable detection and quantitation of various food-borne pathogens. HiMedia’s Mycobacterium tuberculosis Detection kit (Real-time), is one such SYBr green based qPCR technique which allows amplification of IS6110 gene.
A. Diagrammatic representation of preferential binding of SYBr Green Dye to specific DNA fragments in real-time PCR.

\[ \text{SYBr Green dye cycles between an unbound (Denaturation step) and a bound (Annealing through Extension) state as the reaction progresses. Signal intensity increases as the quantity of amplicons increase in later cycles indicating amplification. During elongation, more and more dye molecules bind to the newly synthesized DNA. If the reaction is monitored continuously, an increase in fluorescence is viewed in real-time. Upon denaturation of the DNA for the next heating cycle, the dye molecules are released and the fluorescence signal falls.} \]

**Keys:**
- SYBr
- Forward primer
- Reverse primer
- DNA Strand

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

**Kit Contents:**

The provided PCR kit contains:

<table>
<thead>
<tr>
<th>Components</th>
<th>Reagents provided for 10R (reactions)</th>
<th>Reagents provided for 25R (reactions)</th>
<th>Reagents provided for 50R (reactions)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hi-SYBr master mix (2X master mix containing SYBr Green, Assay buffer, Taq DNA Polymerase, MgCl₂, dNTPs) (MBT074)</td>
<td>150 µl</td>
<td>400 µl</td>
<td>700 µl</td>
</tr>
<tr>
<td>Primer Mix</td>
<td>25 µl</td>
<td>60 µl</td>
<td>120 µl</td>
</tr>
<tr>
<td>Nuclease free water (ML065)</td>
<td>1 ml</td>
<td>2 ml</td>
<td>4 ml</td>
</tr>
</tbody>
</table>

* For a 20µl PCR reaction
**General Preparation Instructions:**

- Before use all PCR components should be completely thawed on ice (4°C).
- Perform the amplification reactions in a clean area, preferably in a biosafety cabinet.
- Use of aerosol barrier pipette tips is recommended to reduce contamination risks from extraneous DNA templates.
- Extract and store positive control sample (if used) separately from all other reagents to avoid contamination and add it to the reaction mix in a separate area.

**Sampling and Handling:**

**Sample Preparation:**
Various clinical samples and cultured bacteria are routinely examined.

For extraction and purification of high yield and pure bacterial DNA, perform the nucleic acid purification using HiMedia’s HiPurA Mycobacterium tuberculosis DNA Purification Kit (MB545) as instructed in the protocol.

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**Flow Chart for setting up PCR Reaction**

1. Add 10µl Hi-SYBr master mix (MBT074) in a PCR tube
2. Add 2 µl the Primer mix (Final concentration 10 pmoles provided)
3. Add 1-2 µl template DNA (upto 50 ng of extracted DNA)
4. Add nuclease free water (ML065) to make the final volume to 20 µl
5. Centrifuge the tube briefly at 6000 rpm for about 10 seconds.
6. Place the tubes in real-time PCR machine and set the recommended PCR program (mentioned below)
7. Interpret the data from the amplification plot (observe the Ct values)

**C. Recommended PCR program:**

1. Initial denaturation : 95°C for 10 minutes
2. Cycling Parameters (No. of cycles: 30)
   - Denaturation : 95°C for 1 min
   - Annealing : 60°C for 1 min
   - Extension : 72°C for 1 min
3. Final Extension : 72°C for 10 minutes.
Amplification Data:

Image representing real-time amplification data of *Mycobacterium tuberculosis* with C\textsubscript{t} values (provided in table)

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Sample</th>
<th>C\textsubscript{t} value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Negative control</td>
<td>N/A</td>
</tr>
<tr>
<td>2</td>
<td>1 µl of template DNA (amplicon of <em>Mycobacterium tuberculosis</em>)</td>
<td>13.36</td>
</tr>
<tr>
<td>3</td>
<td>1 µl of template DNA (amplicon of <em>Mycobacterium tuberculosis</em>)</td>
<td>13.29</td>
</tr>
</tbody>
</table>

Sensitivity: Detectable up to 100-1000 CFU/ml (mg).

Storage:
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. HiMedia does not recommend using the kit after the expiry date stated on pack.

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

Troubleshooting Guide:

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Problem</th>
<th>Cause</th>
<th>Solution</th>
</tr>
</thead>
</table>
| 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 a large volume master mix, vortex thoroughly and aliquot into reaction tubes. |
|         |         | Air bubbles in reaction mix | Briefly centrifuge reaction samples/plate prior to running on a real-time PCR instrument. |
|         |         | Pipetting error | C\textsubscript{t} values of replicates can show increased variation due to poor laboratory technique or imprecise pipettes. |

Please refer disclaimer Overleaf.
Safety Information

The Mycobacterium tuberculosis Detection Kit (Real-time) 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 Mycobacterium tuberculosis Detection Kit (Real-time) in the manner described in the product literature. The kit is designed, sold for research and for 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.

<table>
<thead>
<tr>
<th>3.</th>
<th>Amplification in negative control</th>
<th>Reagents contaminated</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Replace all critical solutions.</td>
<td></td>
<td></td>
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<tr>
<td>2. Repeat the analysis of all tests with fresh aliquots of critical reagents.</td>
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