MBPCR009 Mycobacterium tuberculosis Detection Kit

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 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. fortutium 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, an insertion element found exclusively within the members of the *Mycobacterium tuberculosis*, and because of this exclusivity, it has become in important diagnosis. 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 sensitive detection in clinical samples. The kit is designed for *in vitro* diagnostics and provides qualitative detection.

Principle:

HiMedia's Mycobacterium tuberculosis Detection Kit is a qualitative conventional PCR kit which includes the amplification of ***M. tuberculosis*** **gene [IS6110] (130 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

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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.

Features:

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

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:

The provided PCR contains:

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 *M. tuberculosis*	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
50 bp DNA Ladder **(MBT084)**	40 µl	90 µl	180 µl
Positive control (*M. tuberculosis* DNA)	15 µl	35 µl	65 µl
Internal Control DNA	15 µl	35 µl	65 µl

Sample Material Preparation:
Various samples like sputum, body fluids, bronchial aspirates and other clinical materials, 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.

A) Protocol:

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

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

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

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

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

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

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

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

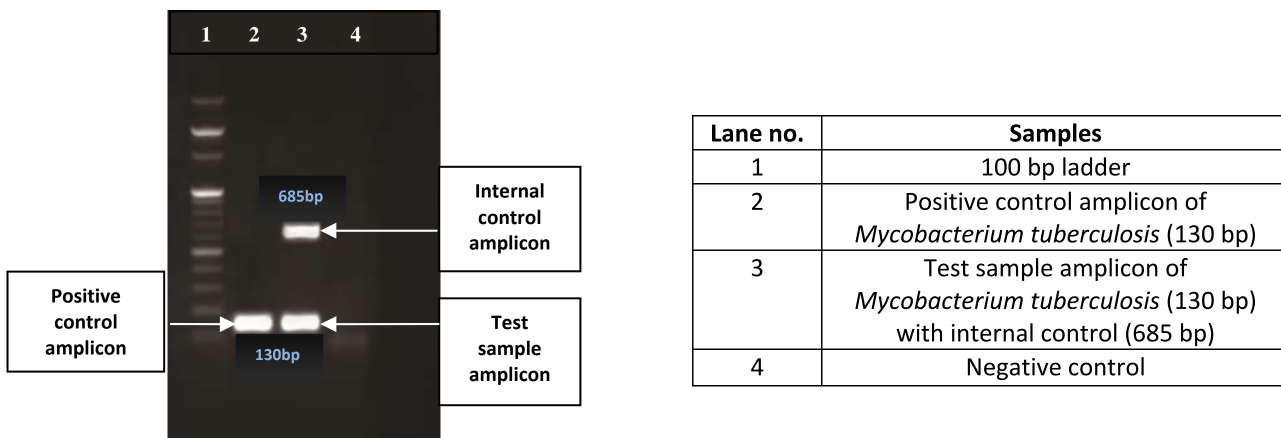
C. After amplification, the products may be kept at 4°C overnight or frozen at –20°C for long-term storage.

D. Mycobacteria 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 50 bp DNA ladder (MBT084) 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



Lane no.	Samples
1	100 bp ladder
2	Positive control amplicon of *Mycobacterium tuberculosis* (130 bp)
3	Test sample amplicon of *Mycobacterium tuberculosis* (130 bp) with internal control (685 bp)
4	Negative control

Gel image representing amplification of IS6110 gene using target sample of *Mycobacterium tuberculosis* with positive control (130bp) and internal control (685bp)

Specifications:

Sensitivity: Detectable upto 10-100 cfu / ml (mg)

Quality Control:

Each lot of HiMedia's Mycobacterium tuberculosis 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 Mycobacterium tuberculosis Detection Kit 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. Mycobacterium tuberculosis Detection Kit 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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