MBPCR172 Hi-PCR® MTB/NTM Probe PCR Kit

Instructions For Use

Introduction

Tuberculosis (TB) remains a major global health challenge. According to the 2022 WHO report, TB continues to be a significant cause of morbidity and mortality worldwide, with 10.6 million new cases and approximately 1.3 million deaths attributed to the disease. This makes TB the second leading cause of death from a single infectious agent, only behind COVID-19. TB is caused by the bacterium *Mycobacterium tuberculosis* (MTB) complex primarily affecting the lungs, though it can involve other parts of the body. Despite being preventable and curable, TB remains a significant public health issue due to factors like drug resistance, insufficient healthcare infrastructure, and social determinants of health.

In addition to MTB, Nontuberculous mycobacteria (NTM), also known as atypical or environmental mycobacteria, can also cause TB-like symptoms. Both NTM and MTB belong to the *Mycobacteria* genus and can present similarly, especially in pulmonary infections. NTMs are a diverse group of mycobacteria distinct from the *Mycobacterium tuberculosis* complex (MTBC), with over 170 species identified as potential pathogens. The most clinically relevant NTMs in pulmonary disease include *M. avium* complex (MAC), *M. kansasii*, and *M. abscessus*. While pulmonary infections are common, NTMs can also affect lymphatic tissue, skin, and soft tissues. Accurate detection and differentiation between NTM and MTB are crucial to avoid misdiagnosis, which can lead to inappropriate treatments and prolonged illness.

Traditional TB diagnostic methods, such as smear microscopy and culture, are not only time-consuming but also lack the sensitivity and specificity needed to distinguish between MTB and NTM. This can result in diagnostic delays and incorrect treatment regimens. To address these limitations, probe-based real-time PCR assays have been developed. The Hi-PCR® MTB/NTM Probe PCR Kit offers a rapid and reliable solution for detecting and differentiating MTB and NTM in clinical samples, enabling timely and appropriate treatment decisions.

NOTE: Hi-PCR® MTB/NTM Probe PCR Kit is for *in-vitro* use only.

Intended Use

The Hi-PCR® MTB/NTM Probe PCR Kit is an in vitro nucleic acid amplification test designed for the detection of *Mycobacterium tuberculosis* complex and *Nontuberculous mycobacteria* in human biological samples. It is intended for use by qualified clinical laboratory personnel who are trained in real-time PCR techniques and in vitro diagnostic procedures.

Product description

The Hi-PCR® MTB/NTM Probe PCR Kit is a ready-to-use system designed for the detection of *Mycobacterium tuberculosis* (MTB) and non-tuberculous mycobacteria (NTM) through real-time PCR. This kit utilizes hydrolysis probes to amplify target DNA. The probes consist of short oligonucleotides with a fluorescent dye at the 5' end and a quencher dye at the 3' end. During amplification, the probe is cleaved, separating the dye from the quencher, which results in an increase in fluorescence. This fluorescence is monitored in real-time, allowing for accurate detection and identification of MTB and NTM DNA.

The Hi-PCR® MTB/NTM Probe PCR Kit includes two sets of primers and probes: one set targets highly conserved regions specific to the MTB complex (MBP64 and IS6110), while the other amplifies conserved regions common to all mycobacterial species, including both MTB complex (MTBC) and NTM genomes (hsp65), enabling differentiation between the two. The kit is designed to detect MTB complex species in the FAM channel, all mycobacteria species (including NTM) in the ROX channel, and features an internal control detectable in the JOE channel.

ISO 9001-2015 CERTIFIED WHO GMP CERTIFIED ISO 13485-2016 CERTIFIED

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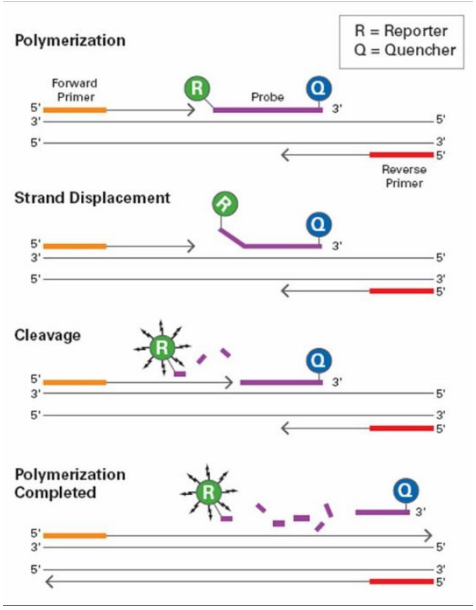
Controls

Internal control (IC): An endogenous internal control (GAPDH), a DNA sequence that is naturally present in the test samples, is amplified in the same tube with a different set of primers and probe which may aid in identification of possible PCR inhibition, DNA purification efficiency and assessing the quality of the sample.

Positive control (PC): A positive control mimics a sample which contains all the target DNA sequences that the PCR is designed to amplify. It is included in a PCR assay to check proper and intended functioning of all the PCR reagents.

No template control (NTC): A No Template Control is a PCR reaction that contains all the PCR reagents but does not include any DNA template. PCR grade water is used as the template to confirm that any observed amplification in the test samples is not due to contamination or non-specific amplification.

Diagrammatic representation of preferential binding of probe specific to DNA fragments in Real-time PCR



Polymerization: A fluorescent reporter (R) dye and a quencher (Q) are attached to the 5' and 3' end of the probe respectively

Strand displacement: When the probe is intact, the report dye emission is quenched.

Cleavage: During each extension cycle,the DNA polymerae cleaves the reporter dye from the probe

Polymerization completed: Once separated from the quencher, the reporter dye emits its characteristic fluorescence

While the probe is intact, the proximity of the quencher dye greatly reduces the fluorescence emitted by the reporter dye by fluorescence resonance energy transfer (FRET). The probes are designed such that they anneal within a DNA region amplified by a specific set of primers. During PCR amplification, these probes will hybridize to the target sequences located in the amplicon i.e. the DNA. As the *Taq* DNA polymerase replicates the template with the bound probe, the 5'- nuclease activity of the polymerase enzyme cleaves the fluorescent probe. The end result in cleavage of the probe is separation of the reporter dye from the quencher dye and increasing the reporter dye signal. As the probe is removed from the target strand, primer extension continues to the end of the template strand. Hence, fluorescence detected in the quantitative PCR thermal cycler is directly proportional to the fluorophore released and the amount of DNA template present in the PCR. Thus, inclusion of the probe does not inhibit the overall PCR process.

Features

Molecular Features:

- Simultaneous detection of MTB and NTM in a single assay.
- Highly sensitive – 1 copy for MTB; 10 copies for NTM.
- Highly specific – No cross reactivity with pathogens with similar clinical presentation.

Technology features:

- Fast and reliable results within 70 minutes.
- Includes all reagents & controls for validity of the test.
- Open system – Compatible with 4-channel and 5-channel qPCR cyclers.
- Wet-lab assays validated on the Bio-Rad CFX Opus 96, Applied Biosystems QuantStudio 5 and Insta Q96® Plus Real Time PCR Systems.

Sample Type: Bacterial DNA extracted from sputum of human origin.

Storage and Shelf life

The provided kit has a shelf life of 12 months when stored between -10°C and -20°C. To maintain optimal sensitivity, avoid repeated thawing and freezing of PCR reagents, as more than five freeze-thaw cycles may reduce performance. For multiple uses, we recommend aliquoting the reagents to prevent repeated freeze-thaw cycles. Exposure to light, heat, or humidity can affect the stability of some kit components and should be avoided. Additionally, degradation of specimens or extracted DNA can impact the assay's sensitivity. HiMedia Laboratories does not recommend using the kit after the expiration date indicated on the packaging.

Specimen Handling

When handling specimens for MTB testing, always follow proper techniques to ensure safety and prevent contamination. All contaminated materials must be properly sterilized by autoclaving before disposal. Adhere to standard precautions as outlined in established guidelines for handling clinical specimens, as well as items contaminated with other body fluids. This includes using appropriate personal protective equipment (PPE) and working in designated biosafety cabinets when necessary.

For specific safety measures and disposal instructions, consult individual safety data sheets (SDS). It is essential to comply with all relevant safety protocols to minimize risk of exposure and ensure safe laboratory practices.

Kit Contents:

The provided PCR kit contains:

Components	Product code	Reagents provided for (reactions)* (µL)		
		25R (µL)	50R (µL)	100R (µL)
MTB/NTM Mastermix	DS1757	135	270	530
MTB/NTM Primer-Probe Mix	DS1758	81	162	318
Water (PCR grade)	DS0440	216	432	848
MTB/NTM Positive Control	DS1759	27	54	106

***** For a 20 µL PCR reaction

Materials needed but not provided

- Appropriate real-time PCR instrument
- Appropriate nucleic acid extraction system or kit
- Centrifuge with a rotor for 1.5ml - 2 ml reaction tubes
- Centrifuge with a rotor for microtiter plates, if using 96 well reaction plates
- Vortex mixer
- PCR tubes (0.1ml or 0.2ml) or 96 well reaction plates with corresponding (optical) closing material or lid
- Pipettes (Capacity: 0.5 - 10 µL/10 - 100 µL/20 - 200 µL/100 - 1000 µL)
- Pipette tips with filters (As per pipette capacity)
- Powder-free gloves (disposable)

All these materials are available through www.himedialabs.com

Product name	Product Code
Real-Time PCR Instrument and equipment	
Insta Q96®AG Real time PCR System, 96 well block, 5 channels	MBLA027
Insta Q96®AG 6.0 Real time PCR System, 96 well block, 6 channels	MBLA028
Insta Q96® Plus Real time PCR System, 96 well block, 5 channels	LA1073
Insta Q96® - 6.0 Real time PCR System, 96 well block, 6 channels	LA1074
Insta Q96® Real time PCR System, 96 well block, 5 channels	LA1012
TabSpin™ Microcentrifuge	LA1089/LA1090
Automated nucleic acid extraction system and materials	

Insta NX® Instrument - fully automated nucleic acid purification system utilizing the Innovative Super -S membrane column method	LA1056
Insta NX® Mag16, Insta NX® Mag16^{Plus}	LA1118, MBLA018
Insta NX® Mag32, Insta NX® Mag32^{Plus}	LA1096, MBLA019
Insta NX® Mag96	LA1097
Extraction Kits	
HiPurA® Pre-filled Plates for MTB DNA Purification	MB579MPF16
HiPurA® Pre-filled Cartridges for MTB DNA Purification	MB579PC16
HiPurA® Pre-filled Plates for MTB DNA Purification	MB579MPF32
HiPurA® Fast MTB (Mycobacterium tuberculosis) DNA Purification Kit	MB579
HiPurA® Mycobacterium tuberculosis Decontamination Kit	MB545D
Tubes, plates and other consumables	
Varivol II Micropipettes (Capacity: 0.5 to 10 µL/10 to 100 µL/200 to 1000 µL)	LA611/LA614/LA615
µPet Autoclavable Micropipettes (Capacity: 0.5 - 10 µL/10 - 100 µL/20 - 200 µL/100 - 1000 µL)	LA955/LA958/LA959/LA960
Q4Pet Autoclavable Micropipette (Capacity: 0.5 to 10 µL/10 to 100 µL/100 - 1000 µL)	MBLA009/MBLA011/MBLA008
Barrier Tips, Maximum capacity 10 µL	LA749A
Barrier Tips, Maximum capacity 200 µL	LA751A
Barrier Tips, Maximum capacity 1000 µL	LA859A
8-strip tubes & optically clear flat caps for PCR	PR17, PR22, PR23
PCR Tubes, 0.1mL, 0.2 mL PCR Plates	PW1255/PR2/PR3/PR19
Optical Sealing film	PR18

Kit compatibility with Real-Time PCR Systems

The Hi-PCR® MTB/NTM Probe PCR Kit contains fluorophores compatible with following PCR systems. However, its performance has been specifically validated on the Bio-Rad CFX Opus 96, Applied Biosystems QuantStudio 5, and Insta Q96® Plus Real-Time PCR Systems.

Real-Time PCR system	Company	Dye 1	Dye 2	Dye 3
Insta Q96®AG/ Insta Q96®AG 6.0/Insta Q96® - 6.0/Insta Q96® Plus/Insta Q48® M4	HiMedia Laboratories Pvt. Ltd.	FAM	JOE	ROX
BioRad CFX Opus 96/CFX96 Touch/ CFX384 Touch	Bio-Rad Laboratories, Inc.	FAM	JOE	ROX
QuantStudio™ 5	Applied Biosystems	FAM	JOE	ROX
ABI® Prism SDS 7500	Applied Biosystems	FAM (Filter A)	JOE (Filter B)	ROX (Filter D)
Rotor-Gene®6000 & Q	QIAGEN	FAM (Green)	JOE (Yellow)	ROX (Orange)
LightCycler® 96 / LightCycler® 480	Roche	FAM	JOE	ROX
qTOWER^3	Analytik Jena	FAM	JOE	ROX

Note: Ensure that the Real-Time PCR system is calibrated for dyes mentioned above and maintained according to the manufacturer´s instructions and recommendations.

General Preparation Instructions

- Prior to use, ensure all PCR components are fully thawed on ice (4°C).
- Perform amplification reactions in a clean area, preferably within a biosafety cabinet.
- To minimize the risk of contamination from extraneous DNA templates, the use of aerosol barrier pipette tips is strongly recommended.

- If using a positive control sample, extract and store it separately from other reagents to prevent contamination. Add the positive control to the reaction mix in a designated, separate area.

Protocol for PCR Reaction Mix Preparation

1. In the "Master Mix Preparation" area, thaw all kit components on ice. Mix by gently inverting the tubes, then centrifuge the reagents for 5 seconds. Keep the components on ice for later use.
2. Based on the number of specimens to be tested (N), calculate the required volume for each component by multiplying N by the volume of "1X" needed.
3. Use 1.5 mL nuclease-free centrifuge tube(s) to prepare the PCR reaction mix. Refer to the table below for the correct volumes. After adding all reagents, mix thoroughly and centrifuge for 5 seconds.

Components	Product code	Volume for "1X" (one reaction)
Preparation of PCR Reaction Mix		
MTB/NTM Mastermix	DS1757	5.0 µL
MTB/NTM Primer-Probe Mix	DS1758	3.0 µL
Water (PCR grade)	DS0440	7.0 µL
Total PCR Reaction Mix	-	**15.0 µL**
Template addition		
Template/ Purified Bacterial DNA	-	5.0 µL
Total reaction volume	-	**20.0 µL**

4. Aliquot 15.0 µL of the PCR reaction mix into the appropriately labeled 0.1/0.2 mL PCR tubes, plates, or strips, compatible with the PCR instrument being used.
5. In the designated "Nucleic Acid Handling" area, add 5.0 µL of extracted DNA from the test specimen to the respective wells of the plate/strip.
6. For the positive control and no-template control (NTC) tubes, replace the template bacterial DNA with the MTB/NTM Positive Control and PCR-grade water, respectively. Refer to the table below for further details.

Set up of controls for the PCR run		
Components	**Product code**	**Volume for "1X" (one reaction)**
Total PCR Reaction Mix	-	15.0 µL
Positive Control		
MTB/NTM Positive Control	DS1759	5.0 µL
No Template Control		
Water (PCR grade)	DS0440	5.0 µL
Total reaction volume	-	**20.0 µL**

7. Tightly cap the tubes/strips or seal the plate with an optically clear adhesive film.
8. Briefly centrifuge the tubes at 6000 rpm for approximately 10 seconds.
9. Place the tubes in the real-time PCR machine and set the recommended PCR program (outlined below).
10. Interpret the data from the amplification plot (observe the Ct values).

Recommended PCR program

Sr. No	Step	Temperature	Time	Sampling	No. of cycles
1.	Initial denaturation	95°C	10 minutes	---	1
2.	Denaturation	95°C	15 seconds	---	40
3.	Annealing & Extension	62°C	30 seconds	YES	

Data Analysis
Selection of channels:

Detection/ Target	Channel/ Reporter	Quencher
Mycobacterium tuberculosis complex specific DNA	FAM	None
Mycobacterium genus specific DNA	ROX	None
Endogenous Internal Control (IC)	JOE	None

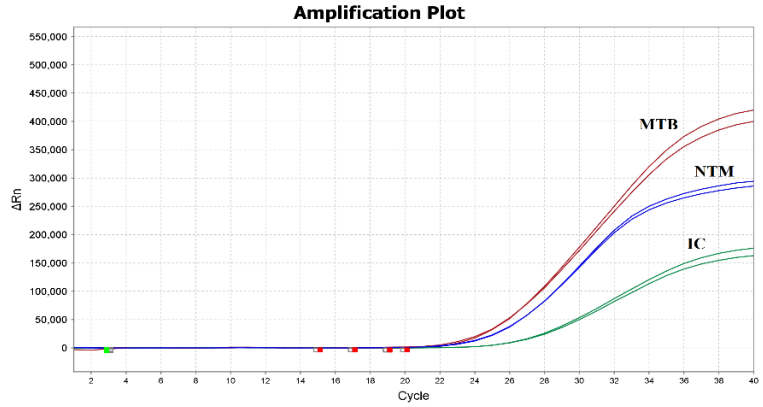
Please select 'Passive reference dye' as 'None' wherever applicable

Data Analysis
The following conditions should be met for a valid diagnostic test:

Control	Detection channel		
	FAM	ROX	JOE
MTB/NTM Positive Control	+	+	+
No Template Control (Water)	-	-	-

Target	Ct value	Result
MTB	≤ 37	Detected (+)
NTM	≤ 36	Detected (+)

Amplification plot:



Note: Image representing probe based Real-Time amplification of MTB, NTM and IC targets run on Applied Biosystems QuantStudio 5.

Data Interpretation:

Target			Result Interpretation
FAM	**ROX**	**JOE**	
+	+/-*	+/-**	Positive for MTB
-	+	+/-**	Positive for NTM
-	-	+	Negative for both MTB and NTM
-	-	-	Inconclusive test*** Likely poor extraction or sample quality. PCR inhibition or reagent failure.

*The presence or absence of a signal in the ROX channel for MTB positive cases is not relevant for the validity of the test.

**The presence or absence of a signal in the JOE channel is not relevant for the validity of the test run due to competition between the test template and Internal Control template.
*** When an inconclusive result is obtained, repeat PCR or re-test the extracted DNA or re-extract the specimen and test the newly extracted DNA.

Performance Evaluation

Analytical Sensitivity
Limit of Detection (LoD)
The Limit of Detection (LoD) is defined as the concentration (copies per µl of the eluate) of target molecule that can be detected at 95% or greater probability according to CLSI EP17-A2. The LoD assay of the Hi-PCR® MTB/NTM Probe PCR kit was determined by testing 20 replicates of dilution series of ATCC standards of *Mycobacterium tuberculosis* strain H37Ra [ATCC 25177DQ] and *Mycobacterium avium* subsp. *paratuberculosis* strain K-10 [ATCC BAA-968D] for MTB and NTM respectively on Biorad CFX Opus 96, Applied Biosystems QuantStudio 5 and Insta Q96® Plus Real Time PCR Systems. The detectable limit of the Hi-PCR® MTB/NTM Probe PCR kit was determined to be 1 copy/µL for MTB (*M. tuberculosis*) and 10 copies/µL for NTM (*M. avium*).

Analytical Specificity
Inclusivity – In silico
The analytical specificity of the Hi-PCR® MTB/NTM Probe PCR Kit was confirmed through in silico analysis of the oligonucleotides (primers and probes). The oligonucleotide sequences of all targets were compared against the sequences of MTB complex and NTM strains available in the GenBank database (listed below), showing 100% specificity.

MTB
Mycobacterium tuberculosis H37Ra, H37Rv, H37RvSiena, (Zopf 1883) Lehmann and Neumann 1896, *Mycobacterium tuberculosis* complex sp. N0072, *Mycobacterium tuberculosis* strain 1-19, 2.2.1, H-19-0008, H-20-0024, N1015
Mycobacterium bovis AF2122/97, *BCG* str. Tokyo 172, *BCG* strain Moreau PL, BCG SL 222 Sofia, *BCG strain* Russia 368, *BCG* str. Moreau RDJ, *BCG* str. Tokyo 172
Mycobacterium africanum strain 25, UT307, GM041182,
Mycobacterium microti strain 12, OV254, Reed 1957
Mycobacterium caprae strain Allgaeu
Mycobacterium canetti strain ET1291
Mycobacterium orygis strain NIAB_BDWBCSHFL_1, strain MUHC/MB/EPTB/Orygis/51145
NTM
Mycobacterium avium complex (MAC), *M. abscessus, M. kansasii, M. intracellulare, M. scrofulaceum, M. fortuitum* complex*, M. malmoense , M. interjectum, M. gordonae, M. flavescens, M. chelonae, M. simiae, M. gastri, M. smegmatis, M. avium, M. celatum, M. terrae complex, M. xenopi, M. marinum, M. phlei, M. vaccae, M. ulcerans, M. tusciae, M. triplex, M. septicum, M. mucogenicum, M. asiaticum, M. intermedium, M. chimaera, M. senegalense, M. parascrofulaceum, M. toakiense, M. haemophilum, M. aurum, M. thermoresistable, M. aichiense, M. thermophilum, M. neoaurum, M. kubicae, M. bohemicum, M. shimoidei, M. rhodesia, M. florentinum, M. hiberniae, M. mucogenicum, M. colombiense, M. wolinsky , M. longobardum, M. nonchromogenicum*

Analytical Reactivity
The analytical reactivity of the Hi-PCR® MTB/NTM Probe PCR Kit was validated through wet lab testing of the oligonucleotides (primers and probes) against commercial controls of MTB and NTM. The MTB controls included quantitative genomic DNA from *Mycobacterium tuberculosis* strain H37Ra (ATCC 25177DQ), quantitative genomic DNA from *Mycobacterium tuberculosis variant bovis* BCG strain TMC 1011 (ATCC 35734D), and quantitative genomic DNA from *Mycobacterium microti* (ATCC 19422DQ). The NTM controls included genomic DNA from *Mycobacterium avium subsp. paratuberculosis* strain K-10 (ATCC BAA-968D), genomic DNA from *Mycobacterium abscessus* strain L948 (ATCC 19977D-5), genomic DNA from *Mycobacterium gordonae* strain TMC 1327 (ATCC 35760D-5), genomic DNA from *Mycobacterium marinum* strain M (ATCC BAA-535D-5), and genomic DNA from *Mycobacterium smegmatis* strain mc(2)155 (ATCC 700084D-5).

Cross-Reactivity and Interference with Other Microorganisms

Wet testing was conducted using commercial genomic or synthetic DNA/RNA of the pathogens (from ATCC) listed in the table below on the Applied Biosystems QuantStudio 5, to evaluate any potential cross-reactivity. None of the tested pathogens demonstrated any reactivity with the primers and probes of the Hi-PCR® MTB/NTM Probe PCR Kit.

Influenza A virus (H3N2) strain A/Wisconsin/15/2009 (VR-1882DQ)	*Corynebacterium diphtheriae* strain NCTC 13129 (700971D-5)
Human coronavirus 229E (ATCC VR-740DQ)	*Haemophilus influenzae* (51907DQ)
Human metapneumovirus hMPV RNA (ATCC VR-3250SD)	*Pseudomonas aeruginosa* strain PAO1-LAC (47085DQ)
Enterovirus 68 strain Fermon (ATCC VR-1826)	*Staphylococcus aureus* subsp. *aureus* (43300DQ)
Human parainfluenza virus 1 strain C35 (ATCC VR-94DQ)	*Chlamydophila pneumoniae* strain CM-1 (1360DQ)
Human parainfluenzavirus 3 strain C 243 (ATCC VR-93DQ)	*Mycoplasma pneumoniae* strain M129-B7 (29342DQ)
Human respiratory syncytial virus strain 18537 (ATCC VR-1580DQ)	*Legionella pneumophila* subsp. *pneumophila* (33152DQ)
Influenza B virus (ATCC VR-1804DQ)	*Bordetella pertussis* (9797DQ)
Influenza A virus (H1N1) strain A/PR/8/34 (ATCC VR-1469DQ)	*Candida albicans* strain SC5314 (MYA-2876DQ)
Human coronavirus NL63 RNA (ATCC VR-3263SD)	*Aspergillus niger* strain A1144 3528.7 (1015DQ)
Measles virus strain Edmonston (VR-24D)	*Aspergillus flavus* strain SN 3 (9643DQ)
Human adenovirus 1 strain Adenoid 71 (VR-1DQ)	*Streptococcus pyogenes* strain Bruno (19615DQ)
Human parainfluenza virus 2 strain Greer (VR-92DQ)	

Cross-Reactivity Analysis – *in silico*

The oligonucleotide sequences (primers and probes) used in the Hi-PCR® MTB/NTM Probe PCR Kit were subjected to BLAST (Basic Local Alignment Search Tool) analysis against the organisms listed in the table below. No significant cross-reactivity was observed for any of the sequences during the in-silico BLAST analysis.

Epstein Barr virus (taxid:10376)	*Klebsiella pneumoniae* (taxid:573)
Human bocavirus (taxid:329641)	*Streptococcus pyogenes* (taxid:1314)
Rhinoviruses (taxid:12059)	*Streptococcus group G* (taxid:1320)
Cytomegalovirus (taxid:10358)	*Escherchia coli* (taxid:562)
VZV (taxid:10335)	*Moraxella catarrhalis* (taxid:480)
Herpes simplex virus 1 (taxid:10298)	*Haemophilus parainfluenzae* (taxid:729)
Herpes simplex virus 2 (taxid:10310)	*Corynebacterium diphtheriae* (taxid:1717)
Severe acute respiratory syndrome coronavirus (taxid:694009)	*Corynebacterium ulcerans* (taxid:65058)
MERS-CoV (taxid:1335626)	*Salmonella* (taxid:590)
Human Parainfluenza Virus-4 (taxid:2560526)	*Bordetella pertussis* (taxid:520)
Measles morbillivirus (taxid:11234)	*Legionella pneumophila* (taxid:446)
SARS-CoV-2 (taxid:2697049)	

Extraction kit compatibility:

The following HiMedia kits are suitable for nucleic acid extraction:

- MTB DNA Purification Nucleic Acid Kits: MB579, MB579MPF16, MB579PC16, MB579MPF32.

Alternative nucleic acid extraction systems and kits may also be suitable. However, the user is responsible for validating the suitability of the nucleic acid extraction procedure for use with the Hi-PCR® MTB/NTM Probe PCR Kit.

Warning

Not for Medicinal Use.

Precautions

Carefully read the procedure before starting the protocol. Always wear protective gloves, clothing, eye protection, and face protection. Follow good clinical laboratory practices when handling clinical samples.

Adhere to standard precautions as outlined in established guidelines. Safety guidelines may be referred to in safety data sheets of the product.

Quality Control

Every lot of Hi-PCR® MTB/NTM Probe PCR kit would be tested against predetermined specifications to ensure consistent product quality.

The Hi-PCR® MTB/NTM Probe PCR kit provides controls- MTB/NTM Positive Control and a No Template Control (NTC) which are to be included in every run.

Limitations

Strict compliance with the Instructions for Use is required for optimal results and the use of the kit is limited to staff qualified clinical laboratory personnel trained in the techniques of real-time PCR and in vitro diagnostic procedures.

Appropriate specimen collection, transport, storage and processing procedures are required for the optimal performance of this test.

This assay must not be used on the specimen directly. Bacterial DNA should be extracted from human sputum sample using appropriate nucleic acid extraction method.

Presence of PCR inhibitors and other interferences may lead to false negative or invalid results.

Although rare, mutations within the highly conserved regions of the targets genes covered by the kit's primers and/or probe may result in failure to detect the presence of pathogen.

As with any diagnostic test, results of the Hi-PCR® MTB/NTM Probe PCR kit need to be interpreted in consideration of all clinical and laboratory findings.

Performance of the kit in monitoring treatment of MTB/NTM infections has not been evaluated.

Hi-PCR® MTB/NTM Probe PCR kit can detect NTM but cannot speciate NTM species.

Troubleshooting Guide

Sr. No.	Problem	Cause	Solution
1.	No amplification	Degraded samples	Check the integrity of nucleic acid using agarose gel electrophoresis. Use freshly prepared DNA to ensure the availability of intact template for amplification.
		Error in protocol setup	Verify that all components are added in the correct volumes as per the manual.
		Inappropriate storage conditions	Store the reagents at recommended temperature for its optimal performance. Check expiry of the reagents and use new lot of reagents if necessary.
		Incorrect PCR programming	Ensure the correct fluorescence channel is selected as outlined in the manual. Compare the PCR program settings with the manual.
		Low DNA template	Ensure adequate DNA extraction and correct sample input. Quantify DNA concentration for optimal template use.
		PCR inhibitors in the sample	Purify the sample further to remove inhibitors.
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 the reaction samples/plate prior to running them on a Real-Time PCR instrument to remove air bubbles.

		Pipetting error	Ct values of replicates may show increased variation due to poor technique or imprecise pipettes. Use calibrated pipettes and repeat the run.
3.	Amplification in No template control	Cross contamination during handling	Replace all critical solutions. Repeat the analysis of all tests with fresh aliquots of critical reagents. Follow good laboratory practices to avoid contamination.
		Contamination from positive control or reagents	Ensure that separate pipette tips, tubes, and work areas are used for positive controls and sample preparation.
4.	Positive control amplification issues	No amplification of positive control	Ensure that the positive control is stored and handled correctly. Check the integrity of the positive control DNA. Verify that the positive control is not expired.
		Not all targets amplified in positive control	Ensure that the positive control is mixed properly before use. Repeat the assay with a fresh aliquot.

Safety Information

HiMedia's Hi-PCR® MTB/NTM Probe PCR Kit is for laboratory use only and is not intended for drug, household, or other non-laboratory applications. Appropriate laboratory safety precautions should be followed, and gloves should be worn when handling the kit.

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.

Symbols

	Manufacturer		Do not use if package is damaged
	Batch code		Temperature limit
	Date of manufacture (YYYY-MM)		Consult instructions for use
	Use-by date (YYYY-MM)		Catalogue number

Identification No.: PIMBPCR172
Rev.No.:05
Date of Issue: 2025-01