Product Information

MBPCR271 **Hi-PCR® Scrub typhus Detection Probe PCR Kit**

Description

Scrub typhus is an infectious disease caused by the bacterium *Orientia tsutsugamushi*, a Gram-negative parasite from the Rickettsiaceae family. This bacterium is transmitted through mite bites and is characterized by an outer envelope where the outer leaflet is thicker than the inner one, unlike other Rickettsia species. Scrub typhus poses a major public health threat across the Asia-Pacific region, impacting countries like Korea, Japan, China, Taiwan, India, Indonesia, Thailand, Sri Lanka, and the Philippines. Approximately two billion people globally are at risk of *O. tsutsugamushi* infection, with an estimated one million new cases reported annually. Patients with scrub typhus often present with nonspecific symptoms, such as fever, headache, myalgia, cough, and abdominal pain, which overlap with other systemic infections. While the disease is usually mild and self-limiting, delayed treatment in severe cases can lead to complications like renal failure, myocarditis, meningoencephalitis, and even death. If untreated, the mortality rate can be as high as 30-35%. As *O. tsutsugamushi* is a leading cause of acute undifferentiated febrile illness (AUFI) in endemic regions of the Asia-Pacific zone, timely and accurate diagnosis is essential for effective treatment. The probe-based Real-Time PCR provides a faster and simpler alternative to traditional PCR and other diagnostic methods for detecting the pathogen. The Hi-PCR® Scrub Typhus Detection Probe PCR Kit offers a highly sensitive and specific solution to detect *O. tsutsugamushi*, the bacterium responsible for scrub typhus.

NOTE: HiMedia's Hi-PCR® Scrub typhus Detection probe PCR Kit is for *in vitro* use only.

Intended Use

Hi-PCR® Scrub typhus Probe PCR Kit is intended for use by qualified clinical laboratory personnel trained in the techniques of real-time PCR and *in vitro* diagnostic procedures. The kit is recommended for sensitive and specific detection of *Orientia tsutsugamushi* in clinical samples.

Principle

The Hi-PCR® Scrub Typhus Detection Probe PCR Kit is a multiplex real-time PCR assay based on the amplification of targeted nucleic acid sequences using hydrolysis probes. These probes are short oligonucleotides with a fluorescent reporter dye attached to the 5' end and a quencher dye attached to the 3' end. Hi-PCR® Scrub typhus Detection Probe PCR Kit includes primer-probe sets specific for detecting the *Orientia tsutsugamushi* gene in the CY5 channel and a human endogenous internal control (IC) gene in the ROX channel. Additionally, synthetic positive controls are provided to validate the test.

Controls

Positive control

A Positive control (PC) is a control reaction which contain the target DNA sequence that the PCR is designed to amplify. It is usually used to ensure proper and intended functioning of all the PCR reagents and is recommended to be used in every run to assess optimal assay performance.

Negative Template Control

A Negative Template Control (NTC) is essential to verify that the reagents, equipment, and environment used in the assay are free from contamination with target nucleic acid. In this control reaction, nuclease-free water is used as the template. It is recommended to include at least one negative template control reaction per run to ensure the reliability of the results.

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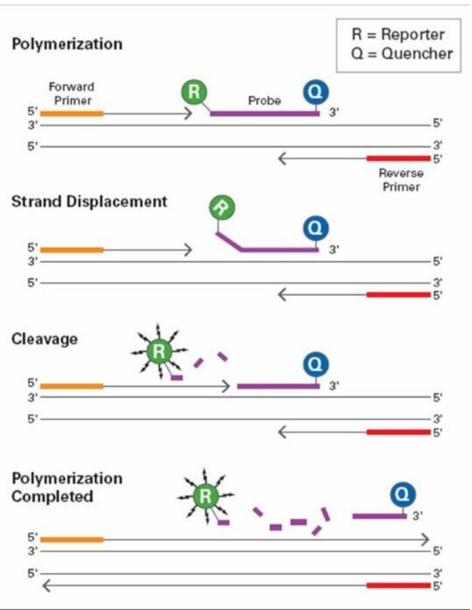
Internal Control

This is a control sequence that should amplify in all clinical samples which indicates the presence of sufficient DNA from human gene indicating the specimen is of acceptable quality. An internal control is often used to detect the failure of amplification in cases where the target sequence is not amplified.

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

- Highly sensitive and specific for detection of *Orientia tsutsugamushi* bacteria.
- Fast and Simple – Real-Time PCR within 1 hour
- Includes all reagents and controls
- Synthetic positive controls provided for validity of the test
- Compatible with any 3-channel, 4-channel, 5-channel and 6-channel qPCR cyclers.
- Wet-lab assays validated on the Bio-Rad CFX Opus 96, Applied Biosystems QuantStudio 5, Insta Q96® Plus and Insta Q96® AG Real Time PCR Systems.

Types of Specimen: Blood samples / Eschar swab samples of human origin.

Specimen collection and Handling

Follow appropriate procedures for specimen collection and handling to ensure safety and accuracy. After use, contaminated materials should be sterilized by autoclaving before disposal. Standard precautions, as outlined in established guidelines, must be adhered to when handling clinical specimens and items contaminated with blood or other body fluids. For detailed safety protocols, refer to the relevant safety data sheets.

Storage and Shelf life

The provided kit has a shelf-life of 12 months when stored between -10°C to -20°C. Repeated thawing and freezing of PCR reagents should be avoided, as this may reduce the sensitivity. If the 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 the sensitivity of the assay. HiMedia Laboratories does not recommend using the kit after the expiry date stated on pack.

Kit Contents: The provided PCR kit contains:

Components	Product code	Reagents provided for (reactions)* (µL)		
		25R	50R	100R
Scrub typhus Master mix	DS2010	108	216	424
Scrub typhus Primer Probe mix	DS2011	54	108	212
Scrub typhus Positive Control	DS2012	54	108	216
Molecular Biology Grade water for PCR	ML065	162	324	640

***For 20 µL PCR reaction**

Materials needed but not provided: All 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
Insta Q48® M4 Real time PCR System, 96 well block, 4 channels	LA1023
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 Clinical Multi-purpose Magnetic Nucleic Acid Purification kit (Cartridges)	MB583PC16200
HiPurA® Pre-filled Clinical Multi-purpose Magnetic Nucleic Acid Purification kit (Plates)	MB583MPF16200
HiPurA® DNA/ RNA Purification Kit	MB583
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

Hi-PCR® Scrub typhus Detection probe PCR Kit contains fluorophores that are compatible to the following PCR systems:

Real-Time PCR system	Company	Dye 1	Dye 2
Insta Q96®AG/ Insta Q96®AG 6.0/Insta Q96® - 6.0/Insta Q96® Plus/ Insta Q48® M4	HiMedia Laboratories Pvt. Ltd.	Cy5	ROX
BioRad CFX Opus 96/CFX96 Touch/ CFX384 Touch	Bio-Rad Laboratories, Inc.	Cy5	ROX / Texas Red
QuantStudio™ 5 / Quant Studio™ 6 and 7 Flex Real-Time PCR Systems / QuantStudio™ Dx	Applied Biosystems	Cy5	ROX
ABI® Prism SDS 7500	Applied Biosystems	Cy5	Texas Red/ROX
QIAquant 96 & 384 5plex	QIAGEN	Cy5	Texas Red/ROX
Rotor-Gene®6000 & Q	QIAGEN	Red	Orange
LightCycler® 96	Roche	Cy5	ROX/Texas Red
LightCycler® 480	Roche	Cy5	ROX/Texas Red
qTOWER^3	Analytik Jena	Cy5	ROX/Texas Red

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

Warning and Precautions

Not for Medicinal Use. Read the procedure carefully before beginning the protocol. Wear protective gloves/protective clothing/eye protection/face protection. Follow good clinical laboratory practices while handling clinical samples. Standard precautions should be followed as per established guidelines. Safety guidelines may be referred in safety data sheets of the product.

Limitations

Although rare, mutations within the highly conserved regions of the targets genes covered by the kit's primers and/or probe may result in under quantitation or failure to detect the presence of the target regions in these cases. Validity and performance of the assay design are revised at regular intervals.

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.

Protocol for PCR Reaction Mix Preparation

1. In the "Master mix Preparation" area, thaw all components from the kit on ice, mix by inverting the tubes and centrifuge the reagents for 5 seconds. Keep on ice for later use.
2. Based on the total number of specimens (including PC and NTC) to be tested (N), calculate the volume of the components to be added as **N X volume of "1R"**
3. Use 1.5 mL Nuclease free centrifuge tube(s) for the preparation of the PCR reaction mix. Refer the following table. After all the reagents are added, mix them thoroughly and centrifuge for 5 seconds.

Components	Product code	Volume for “1R” (one reaction)
Preparation of PCR Reaction Mix		
Scrub typhus Master mix	DS2010	4.0 µL
Scrub typhus Primer Probe mix	DS2011	2.0 µL
Molecular Biology Grade water for PCR	ML065	4.0 µL
Total PCR Reaction Mix	-	**10.0 µL**
Template addition		
Template (Extracted DNA)		10.0 µL
Total reaction volume	**-**	**20.0 µL**

4. Aliquot 10 µL of **PCR reaction mix** into 0.1/0.2mL PCR tube/plate/strips, compatible to the PCR instrument to be used.
5. In the “Nucleic acid handling” area, add 10 µL of extracted nucleic acid of test specimen into the plate/strip to respective wells.
6. For positive and negative control, template nucleic acid is replaced by Positive control mix and nuclease free water respectively. Refer the following table.

Set up of **Positive controls (PC)** for the PCR run		
Components	**Product code**	**Volume for “1R” (one reaction)**
PCR Reaction Mix	-	10.0 µL
Scrub typhus Positive Control	DS2012	10.0 µL
Total reaction volume	**-**	**20.0 µL**

Set up of **Negative Template controls (NTC)** for the PCR run		
Components	**Product code**	**Volume for “1R” (one reaction)**
PCR Reaction Mix	-	10.0 µL
Molecular Biology Grade water for PCR	ML065	10.0 µL
Total reaction volume	**-**	**20.0 µL**

7. Tightly cap the tubes/strips or seal the plate using an optically clear adhesive film.
8. Centrifuge the tube briefly at 6000 rpm for about 10 seconds.
9. Place the tubes in Real-time PCR machine and set the recommended PCR program (mentioned below).

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	2 minutes 30 seconds	---	1
2.	Denaturation	95°C	15 seconds	---	40
3.	Annealing & Extension	58°C	30 seconds	YES	

Selection of channels

Target	Channels	Quencher
O. tsutsugamushi (Scrub typhus)	Cy5	None
Internal Control (IC)	ROX /Texas Red	None

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

Threshold value set up

For the Hi-PCR® Scrub typhus Detection probe PCR Kit, the threshold value for some popular thermal cyclers are as follows:

Sr. No.	Real-Time PCR instrument	Threshold range
1.	HiMedia Insta Q96	500
2.	Applied Biosystems QuantStudio 5	10,000-15000
3.	Bio-Rad CFX-96	100

The threshold range value varies between different instruments depending upon the age, model and the calibration. Please contact our technical team for any queries.

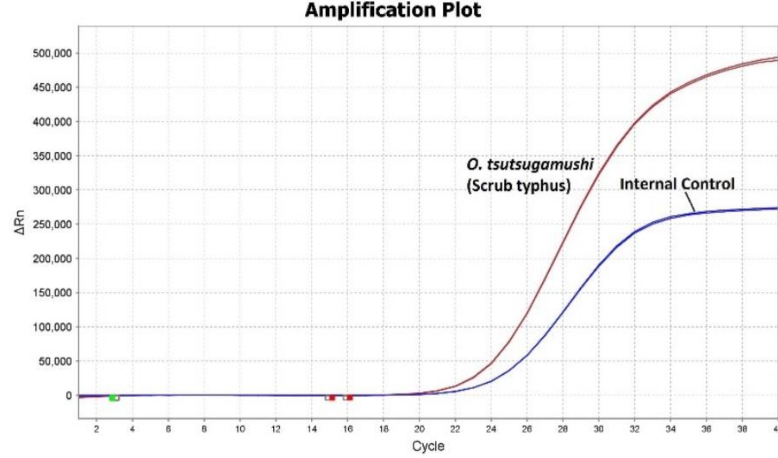
Data Analysis

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

Control	Detection channel	
	CY5 (*O. tsutsugamushi*)	ROX (Internal Control)
Positive Control	+	+
Negative Template Control	-	-

Target	Ct value	Result/Interpretation
O. tsutsugamushi (Scrub typhus)	≤ 36	Detected (+)

Amplification plot



Sr. No	Targets	Ct value	
		PC	NTC
1	*O. tsutsugamushi* (Scrub typhus)	21.65	--
2	Internal control	23.48	--

Note: Image representing probe based Real-Time amplification of *O. tsutsugamushi* target with internal control (Ct values provided in table are for representation) run on Quantstudio5.

Data Interpretation

Detection Channel		Result Interpretation
CY5 (*O. tsutsugamushi*)	**ROX (Internal Control)**	
+	+/- *	Positive for Scrub typhus
-	+	Negative for Scrub typhus
-	-	PCR inhibition or reagent failure. Repeat PCR or repeat extraction from original sample

*The presence or absence of a signal in the ROX channel is not relevant for the validity of the test run due to competition between the test template and Internal Control template.

Kindly correlate the results with clinical findings. Diagnosis generally relies on combination of clinical symptoms, PCR results, serological tests, etc.
A positive PCR result indicates the presence of *Orientia tsutsugamushi* DNA in the sample, suggesting an active Scrub typhus infection.
A negative result suggests the absence of detectable *O. tsutsugamushi* DNA, but it doesn't definitively rule out Scrub typhus infection, especially in early infection stages. It may be crucial to consider other diagnostic methods such as serology and clinical findings.
Note: Data on the accuracy of specimen type recommended for diagnosis is limited for *O. tsutsugamushi* detection, therefore a negative sample specimen should be interpreted with caution.

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® Scrub typhus Detection Probe PCR Kit was performed using 20 replicates each on Biorad CFX Opus 96, Applied Biosystems QuantStudio 5 and Insta Q96® Plus Real Time PCR Systems using Quantitative nucleic acids for *O. tsutsugamushi* target. The detectable limit of the Hi-PCR® Scrub typhus Detection Probe PCR Kit was determined to be 5 copies/µL for the target gene.

Analytical Specificity

Inclusivity

The ability of the Hi-PCR® Scrub typhus Detection Probe PCR Kit to detect a wide range of related target organisms has been assessed in the inclusivity parameter by two ways (i) *in silico* analysis of the oligonucleotides (primers and probes) and (ii) wet lab testing using nucleic acids of related target organisms. The oligonucleotide sequences of all the targets were checked by sequence comparison against all the relevant sequences of *O. tsutsugamushi* available in the GenBank database.

Exclusivity / Cross-Reactivity Analysis

The ability of the Hi-PCR® Scrub typhus Detection Probe PCR Kit to distinguish the target organisms from similar but genetically distinct non-target organisms has been assessed by (i) *in silico* analysis of the oligonucleotides (primers and probes) and (ii) wet lab testing using nucleic acids of non-related target organisms.

Wet lab testing of the Hi-PCR® Scrub typhus Detection Probe PCR Kit for potential cross-reactivity was performed using DNA/RNA from various pathogens available in the laboratory, on InstaQ 96 system. None of the pathogens listed in the table below exhibited any reactivity with the primers and probes of the Hi-PCR® Scrub typhus Detection Probe PCR Kit.

Corynebacterium diphtheriae strain NCTC 13129 (ATCC: 700971D-5)	*Mycoplasma pneumoniae* strain M129-B7 (ATCC: 29342DQ)
Leptospira interrogans serovar Copenhageni strain Fiocruz L1-130 (ATCC: 1198D-5)	Quantitative Genomic RNA from Enterovirus 68 strain Fermon (ATCC: 1826DQ)
Quantitative Genomic DNA from Legionella pneumophila subsp. Pneumophila (ATCC: 33152DQ)	Human parainfluenza virus 1 strain C35 (ATCC: VR-94DQ)
Human coronavirus 229E (ATCC: VR-740DQ)	Quantitative Genomic DNA from Escherichia coli (ATCC: 10798DQ)
Quantitative Genomic DNA from Chlamydophila pneumoniae strain CM-1 (ATCC: 1360DQ)	*Salmonella typhi* (ATCC: 14028)
Plasmodium falciparum strain 3D7 (ATCC: 405D)	*Candida albicans* strain SC5314 (ATCC: MYA-2876DQ)
Influenza B virus (ATCC: VR-1804DQ)	Quantitative Genomic RNA from Human respiratory syncytial virus strain 18537 (ATCC: 1580DQ)
Influenza A virus (H3N2) strain A/ Wisconsin/15/2009 (ATCC: VR-1882DQ)	Quantitative Genomic DNA from Human adenovirus 1 strain Adenoid 71 (ATCC: 1DQ)
Quantitative Genomic RNA from Influenza A virus (H1N1) strain A/Virginia/ATCC1/2009 (ATCC: 1726DQ)	Human herpesvirus 2 (ATCC: 540DQ)
Genomic DNA from Giardia intestinalis strain Portland-1 (ATCC: 30888D)	Measles virus strain Edmonston (ATCC: VR-24D)
Quantitative Genomic DNA from Bordetella pertussis (ATCC: 9797DQ)	Quantitative Genomic DNA from Neisseria meningitidis strain FAM18 (ATCC: 700532DQ)
Quantitative Genomic RNA from Zika virus strain PRVABC59 (VR-1843DQ)	Quantitative Genomic DNA from Staphylococcus aureus subsp. Aureus (ATCC: 43300DQ)
Quantitative Genomic DNA from Streptococcus pyogenes strain Bruno (ATCC: 19615DQ)	

Evaluation

Each lot of HiMedia's Hi-PCR® Scrub typhus Detection Probe PCR Kit is tested against predetermined specifications to ensure consistent product quality.

Quality Control

Each lot of HiMedia's Hi-PCR® Scrub typhus Detection Probe PCR Kit is functionally tested in amplification assay.

The Hi-PCR® Scrub typhus Detection Probe PCR Kit provides controls- Scrub typhus Positive Control mix (PC) and a No Template Control (NTC) which are to be included in every run.

Disclaimers

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 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® Scrub typhus Detection Probe PCR Kit need to be interpreted in consideration of all clinical and laboratory findings.

The performance of this kit in monitoring the treatment of scrub typhus infection has not been evaluated.

Troubleshooting Guide

Sr. No.	Problem	Possible Cause	Solution
1.	No amplification in test and/or control wells	Degraded samples or poor-quality DNA template	Use freshly extracted, high-quality DNA. Check DNA concentration and purity (e.g., A260/280 ratio). Avoid repeated freeze-thaw cycles for stored DNA.
		Missing or incorrect addition of reagents	Verify all reagents were added in the correct volumes and order. Recheck reaction setup steps.
		Incorrect thermal cycling conditions	Cross-check the PCR cycling profile with the IFU.
		Expired or improperly stored reagents	Confirm the expiry date and proper storage. Avoid using reagents that have undergone multiple freeze-thaw cycles.
		Instrument malfunction	Verify that the real-time PCR instrument is functioning properly and calibrated.
2.	Variability between replicates	Inconsistent pipetting or error in reaction setup	Prepare a single master mix for all replicates to minimize variation. Vortex thoroughly and aliquot carefully. Use calibrated pipettes and consistent technique.
		Air bubbles in reaction mix	Briefly centrifuge PCR tubes or plate before placing in the instrument to eliminate air bubbles.
		Uneven mixing of reagents	Ensure all reagents are fully thawed and mixed by vortexing before use. Spin down before pipetting.
		Edge effect (thermal variation across plate)	Avoid using outer wells in PCR plates if not temperature-uniform; use a plate seal and consistent plate layout.
3.	Amplification of pathogen targets in Negative control	Reagent contamination	Replace all critical reagents. Clean workspace, pipettes, and repeat analysis with fresh aliquots. Use filter tips and maintain a unidirectional workflow.
		Cross-contamination or aerosol contamination during reaction setup	Maintain strict unidirectional workflow. Set up reactions in a PCR hood or clean bench. Use aerosol-resistant filter tips for all pipetting steps. Avoid fast, forceful pipetting. Regularly clean work surfaces and equipment with DNA-decontaminating agents. Minimize opening of positive control tubes and avoid splashing.
		Template contamination in workspace or pipettes	Decontaminate work surfaces, pipettes, and equipment using DNA/RNA decontamination solutions. Perform regular cleaning.
		Improper sealing of PCR plate/tubes	Ensure plates/tubes are properly sealed to prevent cross-well contamination during thermal cycling. Use optical-grade seals if required.
4.	No signal in positive control or partial target amplification	Degradation of the positive control material due to improper storage or	Use a fresh aliquot of positive control. Ensure storage conditions follow IFU and avoid repeated freeze-thaw. Discard expired or compromised controls.

		repeated freeze-thaw cycles	
		Incorrect thermal cycling conditions	Cross-verify cycler settings with IFU. Ensure annealing/extension temperature and time are as specified.
		Improper mixing of PC before use	Vortex and briefly spin down the positive control before adding it to the PCR mix. Mix well to ensure homogeneity.
5.	Early Ct value with non-sigmoidal amplification of targets	Instrumental noise, background fluorescence, or non-specific amplification	Visually inspect amplification plots. Disregard flat or atypical (non-sigmoidal) curves that show a sudden rise in fluorescence with very early Ct values (e.g., <6). These may not indicate true amplification. Repeat the assay if necessary. Adjust threshold setting in software within exponential phase. Refer to instrument-specific guidance.
6.	Non-specific amplification of pathogen targets in samples and/or negative control	Improper threshold setting in the real-time PCR software	Visually inspect all amplification plots. Do not rely on Ct values alone. Manually adjust the threshold as recommended in the threshold setting table. Refer to the instrument-specific guidelines or user manual for proper threshold adjustment.
		Contamination of reagents or workspace	Use fresh aliquots of reagents. Prepare reactions in a contamination-free environment using separate areas for pre- and post-PCR steps. Use aerosol-resistant filter tips and routinely decontaminate surfaces and pipettes.
7.	No Internal Control (IC) amplification in target-positive sample	High load of target may suppress IC amplification	If valid Ct for target is observed, it is still considered a valid result.
8.	High Ct values in positive samples (late amplification)	Low target/pathogen load or suboptimal sample	Results near the cut-off should be interpreted with caution. Repeat test using freshly extracted nucleic acid. Confirm with orthogonal test if clinically significant.
9.	Inconclusive result (no amplification of target or IC)	Sample inhibition, extraction failure, or expired reagents	Repeat extraction or test with fresh sample. Check IC amplification to rule out PCR inhibition. Use validated extraction method and fresh reagents.
		Instrument malfunction	Verify that the real-time PCR instrument is functioning properly and calibrated.
10.	Signal in only one replicate (of duplicate or triplicate reactions)	Pipetting error or borderline positivity	Repeat the test. If consistent upon retesting, interpret cautiously in context of clinical findings. Samples with Ct values near to detection Ct cutoff may require repeat sampling or orthogonal testing (e.g., NGS). Use calibrated pipettes and proper technique.

Please refer disclaimer Overleaf.

Safety Information

Hi-PCR® Scrub typhus Detection Probe PCR 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 of in accordance with current laboratory.

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
LOT	Batch code		Temperature limit
	Date of manufacture (YYYY-MM)		Consult instructions for use
	Use-by date (YYYY-MM)	REF	Catalogue number

Identification No.: PIMBPCR271
Rev.No.:04
Date of Issue: 2025-07

Disclaimer :

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