MBPCR111 Hi-PCR® Malaria Probe PCR Kit

Malaria is a major tropical disease and an acute febrile and life-threatening illness caused by *Plasmodium* parasites that are transmitted to people through the bites of infected *Anopheles* mosquitoes called "malarial vectors". Malaria is caused primarily by 4 species of the protozoa *Plasmodium*: *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae* and *Plasmodium ovale*. A fifth *Plasmodium* species, *Plasmodium knowlesi*, is a similar parasite that may be an important source of human infection in some regions of Southeast Asia. Among these five species, *P. vivax* and *P. falciparum* are the most common of which *P. falciparum* is the most deadly as it is typically resistant to many commonly used antimalarial agents such as chloroquine. The transmission of malarial parasites depends on climatic conditions that may affect the number and survival of mosquitoes, such as rainfall patterns, temperature and humidity. In a non-immune individual, symptoms appear seven days or more after the infectious mosquito bite. The symptoms include fever, headache, chills, vomiting, etc. If not treated earlier, it can progress to severe illness often leading to death. Hence, early diagnosis and treatment of malaria is important which can also contribute to reducing malaria transmission.

NOTE: HiMedia's Hi-PCR® Malaria Probe PCR Kit is for *in-vitro* use only.

Intended Use

Recommended for sensitive and specific detection of *Plasmodium* species in clinical samples.

Principle

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 PCR. This technique is used to amplify a targeted DNA sequence by use of hydrolysis probes that are short oligonucleotides that have a fluorescent reporter dye attached to the 5' end and a quencher dye to the 3' end. HiMedia's Hi-PCR® Malaria Probe PCR Kit is designed to detect **Malaria (Plasmodium UNIV) in FAM channel** and **Internal Control in JOE channel** in a single tube reaction. The kit allows sensitive and specific detection of malaria in a single tube reaction.

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.

Negative Control

A Negative control is needed to ensure that the reagents, equipment, and environment used in the assay is not contaminated. In this reaction, Nuclease free water is used as the template. It is recommended to have minimum 1 reaction of negative control per run.

Internal Control

This is a heterologous control (Internal Control, IC) sequence that serves as the in-process control to identify possible PCR inhibition and to confirm the integrity of the reagents of the Kit. It determines the validity of the tests.

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

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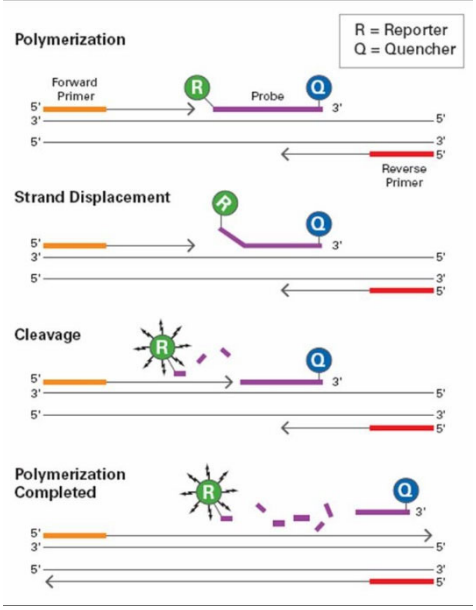
Diagrammatic representation of preferential binding of probe specific to DNA fragments in Real-time PCR



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

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

Sample Source: Blood samples / bacterial cultures / Food samples/ Water samples

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
2X Super Mastermix	DS0900	338	675
Plasmodium Primer-Probe Mix	DS0379	27	54
Internal Control Primer-Probe Mix	DS1117	27	54
Internal Control DNA	DS1096	27	54
Molecular Biology Grade Water for PCR	ML065	200	400
Plasmodium Positive Control	DS0264	25	50

* For a 25 µL 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 Preparation

Various food samples are routinely examined. For extraction and purification of high yield and pure genomic DNA, perform the nucleic acid purification using HiPurA® Blood Genomic DNA Miniprep Purification Kit (MB504).

Materials needed but not provided

- PCR tubes (Product code PW1255) or PCR Strips (Product code: PR17) or PCR Plates (Product code: PR2 / PR3 / PR19) & Sealing film (PR18)
- Insta Q Real Time PCR System (Product Code: LA1012/LA1073/LA1023/LA1024/LA1074)
- Barrier Micropipette Tips (Product Code: LA749 / LA749A / LA751 / LA751A / LA750 / LA750A / LA859 / LA859A)
- Micropipettes
- For Blood Samples: HiPurA® Blood Genomic DNA Miniprep Purification Kit (MB504)

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.

A. Protocol for PCR Master Mix Preparation

Components	Product Code	Volume to be added for 1R (for a 25 µL reaction)
2X Super Mastermix	DS0900	12.5 µL
Plasmodium Primer-Probe Mix	DS0379	1 µL
Internal Control Primer-Probe Mix	DS1117	1 µL
Internal Control DNA	DS1096	1 µL
Molecular Biology Grade Water for PCR	ML065	4.5 µL
Template DNA/Negative Control/Positive Control	-	5 µL
Total volume	-	25 µL

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

B. Recommended PCR program

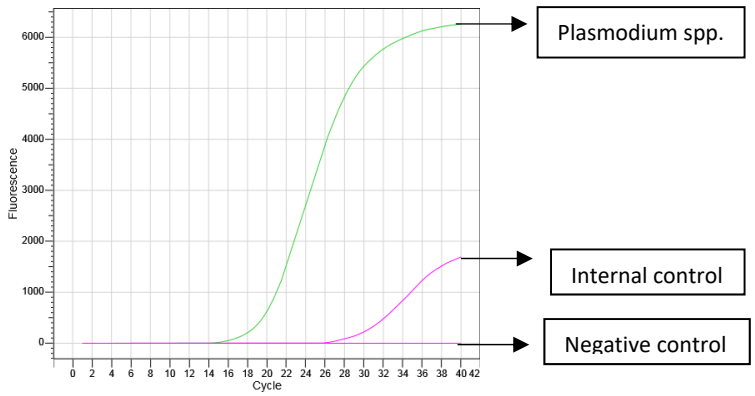
1.	Initial denaturation	:	95°C for 10 minutes	
2.	Denaturation	:	95°C for 15 seconds	No. of cycles: 40
3.	Annealing & Extension	:	60°C for 1 minute (Plate Read)	
	Sampling	:	FAM/JOE	
4.	Hold	:	4°C for ∞	

Data Analysis

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

Ct value	Result
≤ 35	Detected (+)
> 35 or N/A	Not detected (-)

Amplification Data



Sr. No.	Sample	C_t value	
		PC	NC
1.	Plasmodium spp. Positive Control	20.21	-
2.	Internal Control	30.04	30.05

Image representing amplification plot of Plasmodium spp. DNA with Ct values using HiMedia's Hi-PCR® Malaria Based PCR Kit. The results completely depend upon sample types

Data Interpretation

Ct value	Result
≤ 35	Detected (+)
> 35 or N/A	Not detected (-)

Target		Result Interpretation
Plasmodium spp.(FAM)	Internal Control (JOE)	
+	+/-*	Positive for Plasmodium spp.
-	+	Negative for Plasmodium spp.
-	-	PCR inhibition or reagent failure. Repeat PCR or repeat extraction from original sample

*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.

Analytical Performance

Limit of Detection (LoD) - Analytical Sensitivity

Sensitivity for the Hi-PCR® Malaria Probe PCR Kit was conducted using clinical specimens on InstaQ96® Real Time PCR system and Bio-Rad CFX96™ C1000 Real Time PCR system. The detectable limit of the Hi-PCR® Malaria Probe PCR Kit on both instruments was determined to be 1 copies/reaction (**40 copies/mL**).

Inclusivity

In silico analysis for the assessment of inclusivity for the Hi-PCR® Malaria Probe PCR Kit was conducted by mapping the primers and probe against the available Plasmodium sequences in GenBank. The Hi-PCR® Malaria Probe PCR Kit targets 100% of the known Plasmodium strains.

Cross-reactivity - Analytical Specificity

In silico analysis was performed using NCBI nucleotide and Primer BLAST. The primers and probe specific for Plasmodium species were analyzed against the Acute Febrile Illness panel.

Warning
Certified for *in vitro* Diagnostic Use (IVD). Not for Medicinal Use.

Precautions
Read the procedure carefully before starting the experiment. 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.

Performance and Evaluation
Each lot of HiMedia's Hi-PCR® Malaria Probe PCR Kit is tested against predetermined specifications to ensure consistent product quality.

Quality Control
Each lot of HiMedia's Hi-PCR® Malaria Probe PCR 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 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_t values of replicates can show increased variation due to poor laboratory technique 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.
4.	No signal with positive controls	Incorrect programming of the temperature profile of the thermocycler	Compare the temperature profile to the manual.

Safety Information
HiMedia's Hi-PCR® Malaria 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 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.

IVD	In vitro diagnostic medical device
CE	CE Marking
-10°C -20°C	Storage temperature
	Do not use if package is damaged
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