MBPCR135 Hi-PCR® Plasmodium species Detection Multiplex Probe PCR Kit

Description

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. Malaria is endemic in almost 106 countries and global death due to the malaria infection is estimated to be 1 million individuals per year. *P. falciparum* is a major cause of severe malaria and approximately 10-20% of the patients with falciparum malaria require urgent detection and intensive medical care. *P. vivax* is the second most harmful parasite of human malaria that cause more than 390 million clinical cases per year and is a chief risk factor for severe anemia among young children in most vivax endemic areas. In general, the distribution of *P. malariae* coincides with that of P. falciparum. Although going undiagnosed in most cases of asymptomatic subclinical conditions, *P. ovale* is a cause of morbidity in many areas.

NOTE: HiMedia's Hi-PCR® Plasmodium species Detection Multiplex Probe PCR Kit is for *in-vitro* use only.

Intended Use

Recommended for sensitive and specific detection of *P. falciparum* and *P. vivax* in clinical samples.

Product Description

HiMedia's Hi-PCR® Plasmodium species Detection Kit includes primer-probe sets specific to detect DNA from the *P. falciparum*, *P. vivax*, and *Plasmodium* species. In addition, internal control (IC) is used for testing successful reactions. The kit also provides synthetic positive controls for validity of the test.

Positive control

This is a control reaction using a known template (target pathogen). A positive control is usually used to check the PCR conditions have been set up correctly.

Negative Control

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

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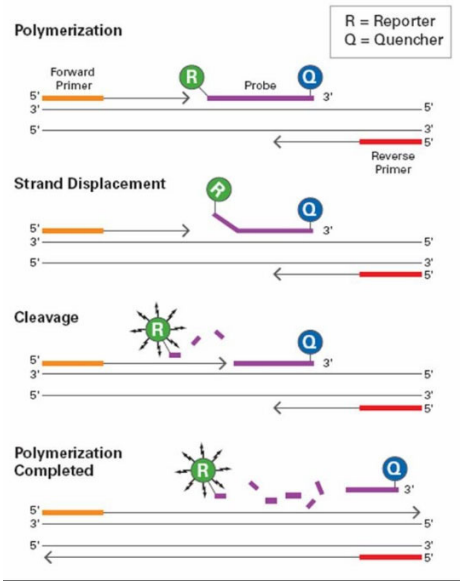
Internal Control

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.

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 target DNA sequence by use of hydrolysis probes (TaqMan probes) that are short oligonucleotides that have a fluorescent reporter dye attached to the 5' end and a quencher dye to the 3' end. This kit is designed to detect *P. falciparum*, *P. vivax*, *Plasmodium* species and Internal Control specific DNA targets in a single reaction.

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 – Real-Time PCR within 1.5 hours
- Highly sensitive and specific for detection of *P. falciparum*, *P. vivax* & *Plasmodium* species infections
- Includes all reagents and controls
- Synthetic positive controls provided for validity of the test
- Guaranteed reproducible results

Types of Specimens: Blood sample

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 other body fluids. Safety guidelines may be referred to in individual 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 RNA specimens can also reduce the sensitivity of the assay. HiMedia Laboratories does not recommend using the kit after the expiry date stated on the pack.

Kit Contents: The provided PCR kit contains:

Components	Product Codes	Reagents provided for (reactions)*		
		25R	50R	100R
Plasmodium Master Mix	DS1621	108 µl	216 µl	424 µl
Plasmodium Primer-Probe Mix	DS1622	54 µl	108 µl	212 µl
Plasmodium Positive Control	DS1623	40 µl	80 µl	160 µl
Water	DS0440	225 µl	450 µl	800 µl

*For a 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 Q48® M4: Real time PCR System, 48 well block, 4 channels	LA1023
Insta Q96® Real time PCR System, 96 well block, 5 channels	LA1012
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
Tubes, plates, and other consumables	
Varivol II Micropipette-10 (Capacity: 0.5 to 10 µl)	LA611
Varivol II Micropipette-100 (Capacity: 10 to 100 µl)	LA615
Varivol II Micropipette-1000 (Capacity: 200 to 1000 µl)	LA614
Q4Pet Autoclavable Micropipette Capacity: 100-1000µL/10-100µL	MBLA008/MBLA011
Q4Pet Autoclavable Micropipette Capacity: 0.5-10µL/20-200µL	MBLA009/ MBLA012
Barrier Tips, Maximum capacity 10 µl	LA749/LA749A
Barrier Tips, Maximum capacity 200 µl	LA751/LA751A
Barrier Tips, Maximum capacity 1000 µl	LA859/LA859A
Micro Centrifuge Tube – B/ Micro Centrifuge Tube-C	PW146/ PW147
8-strip tubes & optically clear flat caps for PCR	PR17
PCR Tubes, 0.2 mL; PCR Plates	PW1255/ PR2/PR3/PR19
Polypropylene Sealing film/ Optical Sealing film/ RNase Kil™	PR21/ PR18/ ML162

Kit Compatibility with Real-Time PCR systems:

Hi-PCR® Plasmodium species Detection Kit Multiplex Probe PCR Kit contains fluorophores compatible to:

Real-Time PCR system	Company	Dye 1 (*P. falciparum*)	Dye 2 (*P. vivax*)	Dye 3 (*Plasmodium* species)	Dye 4 (IC)
Insta Q96® - 6.0/Insta Q96® Plus/Insta Q48®	HiMedia Laboratories Pvt. Ltd.	FAM	JOE	ROX	Cy5
QuantStudio™ 5	Applied Biosystems	FAM	VIC	ROX	Cy5
Applied Biosystems 7500	Applied Biosystems	FAM	JOE	ROX	Cy5
BioRad CFX Opus 96/CFX96	Bio-Rad Laboratories, Inc.	FAM	HEX/VIC	Texas Red	Cy5
Rotor-Gene® Q/Corbett Rotor-Gene® 6000	QIAGEN	Green	Yellow	Orange	Red
Roche LightCycler® 96	Roche	FAM	HEX/VIC	Texas Red	Cy5
AriaMx	Agilent	FAM	HEX	ROX	Cy5
Alta RT-96E/96S	Athenese-Dx Private Limited	FAM	VIC, HEX, TET, JOE	ROX, Texas Red	Cy5

Note: Ensure that the Real-Time PCR system is calibrated for dyes and is maintained according to the manufactureŕs 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 to in the 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.
- Do not vortex the reagents, instead mix the contents of the vial by gently pipetting.

A. Protocol for PCR Master 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 several seconds. Keep on ice for later use.

2. Based on the number of specimens to be tested (N), including the PTC and NTC, calculate the volume of the components to be added as follows

Components	Volume to be added for 1X (for a 20 µL reaction)
Plasmodium Master Mix	4 µL
Plasmodium Primer-Probe Mix	2 µL
Water	6 µL
Total volume of Master Mix	**12 µL**
Template	8.0 µL
Total reaction volume	**20 µL**

3. Use 1.5 mL Nuclease free centrifuge tube(s) for the preparation of the reaction system. After all the reagents are added, mix them thoroughly and centrifuge for several seconds.
4. Load 12 µL of master mixture into the 0.1/0.2 mL PCR reaction plate/strips, compatible to the instrument to be used.
5. Add 8 µL of nuclease free water to the negative control tube.
6. In the "Nucleic acid handling" area, add 8 µL of Plasmodium Positive Control and extracted test DNA into the respective tubes.
7. Tightly cap the strips or seal the plate using an optically clear adhesive film.
8. Briefly, spin the strips/tubes to settle the reagent to the bottom of the tube.
9. Place the plate/strips in the Real-time PCR machine and set the PCR program.

B. Recommended PCR program:

1. Initial denaturation : 95°C for 10 minutes
2. Denaturation : 95°C for 15 seconds
   Annealing : 60°C for 45 seconds (Plate Read) } No. of cycles: 45
   Channel : FAM/JOE/ROX/Cy5

Note: For ABI and QuantStudio systems select the passive reference dye and Quencher as "NONE".

C. Data Analysis

When the following controls meet the stated requirement the PCR run is considered valid, and the specimens can be considered for interpretation.

Control	Target			
	FAM (*P. falciparum*)	**JOE (*P. vivax*)**	**Texas Red (*Plasmodium* species)**	**Cy5 (IC)**
Positive Template Control (PTC)	**+**	**+**	**+**	**+**
Negative Template Control (NTC)	**-**	**-**	**-**	**-**

D. Cutoff

1. All clinical samples should exhibit IC amplification at or below 35 Ct value, thus suggesting the presence of sufficient DNA from human gene indicating the specimen is of acceptable quality.

2. For the interpretation of target gene, follow the below mentioned chart.

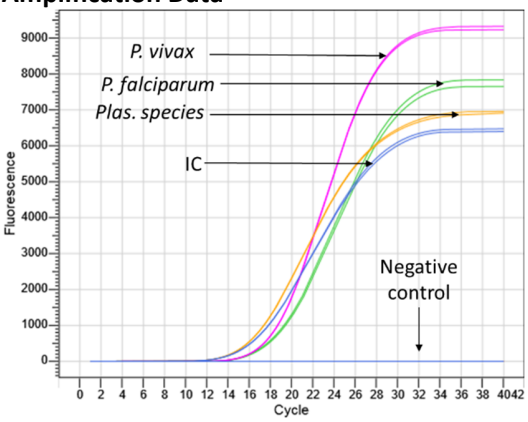
Target gene	Detected (+)
P. falci	Ct value ≤ 35
P. vivax	Ct value ≤ 36
Plasmodium Species	Ct value ≤ 36

E. Data Interpretation

Targets				Assay Interpretation
FAM (*P. falciparum*)	**JOE (*P. vivax*)**	**Texas Red (*Plasmodium* species)**	**Cy5 (IC)**	
+	-	+	+	Positive for *P. falciparum*
-	+	+	+	Positive for P. vivax
-	-	+	+	Positive for Plasmodium species
+	+	+	+	Positive for *P. falciparum* and *P. vivax**
-	-	-	+	Negative for Plasmodium
-	-	-	-	PCR inhibition or reagent failure. Repeat PCR or repeat extraction from original sample

* Note: The detection of 2 or more organisms is uncommon. Retest the sample again then the results can be reported.

Amplification Data



Sr. No	Target	Ct value	
		PTC	NTC
1	*P. falciparum*	18.5	--
2	*P. vivax*	18.26	--
3	*Plas. species*	16.53	--
4	IC	16.57	--

Note: Image representing probe based Real-Time amplification of *P. falci. P. vivax*, Plasmodium species, and IC (Ct values provided in table are for representation).

Performance Evaluation

Limit of Detection (LoD) - Analytical Sensitivity

Analytical sensitivity was defined as the lowest concentration of the target that could be reliably detected with 95% confidence. The analytical sensitivity for the Hi-PCR® Plasmodium species Detection Multiplex Probe PCR Kit was conducted using ATCC Synthetic DNA. The preliminary LoD of each target was determined by testing a 10-fold dilution series in triplicates per concentration, and then confirmed with 20 replicates of the concentration determined to be the LoD. The data revealed that Hi-PCR® Plasmodium species Detection Multiplex Probe PCR Kit detects 1 copy/µL with a confidence ≥95%.

Inclusivity - Analytical Sensitivity

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

Cross-reactivity - Analytical Specificity

Wet testing analysis was performed against the recommended list of organisms for febrile panel. No cross-reaction was observed with any strains mentioned below.

Dengue virus type 1	*Mycoplasma pneumoniae*
Dengue virus type 2	*Streptococcus pyogenes*
Dengue virus type 3	*Bordetella pertussis*
Dengue virus type 4	Enterovirus 68 strain Fermon
Influenza B	Human metapneumovirus hMPV
Human coronavirus NL63	Influenza A virus (H3N2)
Chikungunya virus	*Leptospira interrogans*
Human respiratory syncytial virus strain 18537	*Neisseria meningitidis*
Human adenovirus 1 Adenoid 71	*Staphylococcus aureus*
Zika virus	

Evaluation

Each lot of Hi-PCR® Plasmodium species Detection Multiplex Probe PCR Kit is tested against predetermined specifications to ensure consistent product quality.

Quality Control

Each lot of Hi-PCR® Plasmodium species Detection Kit Multiplex Probe PCR Kit is assayed for contaminating endonuclease, exonuclease, and non-specific DNase activities. Functionally tested in amplification.

Troubleshooting Guide

Sr. No.	Problem	Cause	Solution
1.	No amplification	Degraded samples	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 thermal cycler	Compare the temperature profile to the manual.

Safety Information

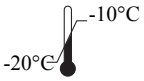
Hi-PCR® Plasmodium species Detection Kit Multiplex Probe PCR Kit is for laboratory use only, not for drug, household, or other uses. Take appropriate laboratory safety measures and wear gloves when handling.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures while disposing the infectious materials. Material that comes into contact with clinical samples 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
	Storage temperature
	Do not use if package is damaged
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