

MBPCR016

Malaria Detection Kit (Multiplex)

Description:

Malaria is a life-threatening disease caused by *Plasmodium* parasites that are transmitted to people through the bites of infected *Anopheles* mosquitoes called "malarial vectors". Malaria in humans is mainly caused by infection with four *Plasmodium* species: *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae*, *Plasmodium ovale*. Out of which *P. falciparum* and *P. vivax* are the most common and *P. falciparum* is the most deadly.

Malaria is preventable and curable. Transmission depends on climatic conditions that may affect the number and survival of mosquitoes, such as rainfall patterns, temperature and humidity. Malaria is an acute febrile illness. In a non-immune individual, symptoms appear seven days or more (usually 10–15 days) after the infective mosquito bite. The symptoms include fever, headache, chills and vomiting etc. If not treated within 24 hours, *P. falciparum* malaria can progress to severe illness often leading to death. Early diagnosis and treatment of malaria reduces disease and prevents deaths. It also contributes to reducing malaria transmission.

NOTE: The Malaria Detection Kit is for *in vitro* use only.

Intended Use:

The Malaria Detection Kit (Multiplex) is designed for detection of five gene sequences for *Plasmodium spp.*, *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae*, *Plasmodium ovale* in the malarial clinical samples. Conventional PCR testing can provide rapid, sensitive and specific detection of malaria.

This diagnostic kit assures very high sensitivity of detection in clinical samples. The kit is designed for *in vitro* diagnostics and provides qualitative detection.

Principle:

HiMedia's Malaria Detection Kit is a qualitative conventional PCR kit, which contains the amplification of specific gene using specific primers. The amplified target is detected 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 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.

Gel electrophoresis is used to analyze the amplification of desired gene region for target pathogen based on separation of DNA fragments according to their size.

Features:

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

Unit Definition:

1u is defined as amount of enzyme that is required to catalyze the incorporation of 10 nmoles of dNTP into acid-insoluble material in 30 minutes at 74°C.

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	1.5 ml
Primer Mix includes <i>Plasmodium spp.</i> , <i>Plasmodium falciparum</i> , <i>Plasmodium vivax</i> , <i>Plasmodium malariae</i> , <i>Plasmodium ovale</i> genes	120 µl	260 µl	510 µl
Nuclease free water (ML065)	1 ml	2 ml	4 ml
6X Loading Dye (ML015)	100 µl	200 µl	400 µl
100 bp DNA Ladder (MBT049)	40 µl	90 µl	180 µl
<i>Plasmodium spp.</i> Positive control DNA	20 µl	55 µl	110 µl

Sample Collection and Preparation:

For preparation of purified blood DNA, perform blood DNA extraction using HiPurA™ Blood Genomic DNA Miniprep Purification Kit (MB504). The purified DNA is free of any inhibitors and can be used directly for PCR.

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:**Preparation of PCR Reaction Mixture**

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



In the same tube, add 10 µl of given primer mix (10 pmoles concentration provided)



Add 1-2 µl of **template DNA** (upto 50 ng of extracted DNA)



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:

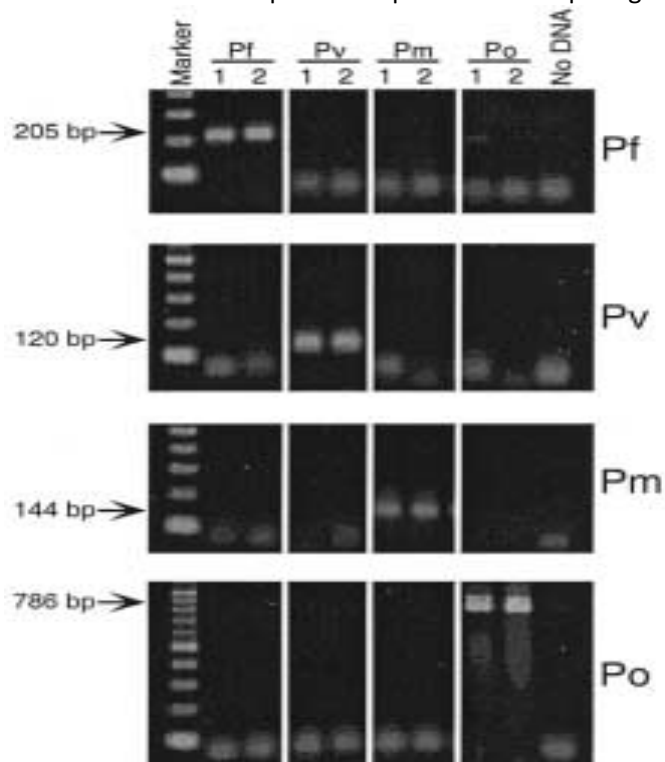
1. Initial denaturation : 94°C for 10 minutes
2. Cycling Parameters (No. of cycles: 30)
 - Denaturation : 94°C for 1 minute
 - Annealing : 60°C for 2 minute
 - Extension : 72°C for 2 minute
3. Final Extension : 72°C for 10 minutes

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

- For analysis of the PCR data, load 10 µl of amplicon on a 2% Agarose gel along with 1 µl of 6X DNA loading dye (ML015).
- Load 3 µl of 100 bp DNA ladder (MBT049) in separate well.

E. EtBr-staining to check results

- Incorporate EtBr in the agarose gel or stain the agarose gel with EtBr for 10-15 min
- Confirm the expected amplicon size comparing with 100 bp DNA marker.



Gel image representing amplification of *Plasmodium* spp. [*P. falciparum* (205 bp), *P. ovale* (786 bp), *P. malariae* (144 bp), *P. vivax* (120 bp)] using malarial blood DNA sample as template

Specifications

Sensitivity: Detectable upto 10-1000 cfu/ml (mg) before pre-enrichment.

Quality Control:

Each lot of HiMedia Malaria Detection Kit (Multiplex) are 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 Malaria Detection Kit (Multiplex) 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. Malaria Detection Kit (Multiplex) 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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