HELINI
Plasmodium species
Real-time PCR Kit

Instructions for use

For use with: Agilent, Bio-Rad, Roche Lightcycler-96, Roche-Z480/Cobas-480, Applied Bio systems [ABI], Thermo-Piko-Real, Cepheid Real time PCR machines.

CE IVD

REF 8273

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HELINI Biomolecules, Chennai, INDIA

www.helini.in

Intended Use

HELINI Plasmodium species Real-time PCR is an in vitro nucleic acid amplification test, based on real-time PCR technology, for the detection Plasmodium specie. It detects all species Plasmodium, but, differentiate Plasmodium falciparum and Plasmodium vivax of specific DNA.

Kit components

Components	Volume Per reaction	Number of vials	Volume Per vials
Probe PCR Master Mix	10µl	1	250µl
Plasmodium species PP Mix [Plasmodium PP Mix]	5µl	1	125µl
Positive control Mix	10µl	1	150µl
Water, PCR grade		1	4ml

Storage

- The kit is shipped on Gel ice [Blue ice]. Upon arrival, all components should be stored in -20°C. They are stable until the expiration date stated on the label.
- Repeated thawing and freezing should be avoided, as this might affect the performance of the assay.
- If the reagents are to be used only intermittently, they should be frozen in aliquots. Storage at 2 to 8°C should not exceed a period of 5 hours.

Material and instruments required

- Real-time PCR instrument having FAM/HEX/ROX/Cy5 channels
- Automatic Nucleic acid extraction system or spin column based purification kit for the purification of nucleic acids
- Desktop centrifuge having 13000rpm or above with a rotor for 1.5/2 ml reaction tubes
- Centrifuge with a rotor for PCR strips/tubes and 96 well plates
- Optical cap qPCR tubes or strips or 96 wells
- Micro Pipettes (variables)
- Micro Pipette tips with filters (disposable)
- Powder-free gloves (disposable)

[Please ensure that all instruments used have been installed, calibrated, checked and maintained according to the manufacturer´s instructions and recommendations.]

Product Use Limitations

- All reagents may exclusively be used in molecular diagnosis.
- The product is to be used by personnel specially instructed and trained in Molecular diagnosis.
- Strict compliance with the user manual is required for optimal PCR results.
- Attention should be paid to expiration dates printed on the box and labels of all components. Do not use expired components.
- Wear protective disposable powder-free gloves, a laboratory coat and eye protection when handling specimens and kit components.
- Avoid microbial and nuclease (RNAse/RNase) contamination of the specimens and the components of the kit.
- Always use RNAse/RNase-free disposable pipette tips with aerosol barriers.
- Use separated and segregated working areas for sample preparation, reaction setup and amplification/detection activities.
- The workflow in the laboratory should proceed in unidirectional manner. Always wear disposable gloves in each area and change them before entering a different area.
- Store positive and/or potentially positive material separated from all other components of the kit.
- Do not open the reaction tubes/plates post amplification, to avoid contamination with amplicons.

- Additional controls may be tested according to guidelines or requirements of local, state and/or federal regulations or accrediting organizations.

- Do not autoclave reaction tubes after the PCR, since this will not degrade the amplified nucleic acid and will bear the risk to contaminate the laboratory area.
- Discard sample and assay waste according to your local safety regulations.

Technical Assistance

For technical assistance and more information, please contact;
+91-44-244490433
helinibiomolecules@gmail.com

Product description

HELINI Plasmodium species Real-time PCR Kit constitutes a ready-to-use system for the detection of Plasmodium species DNA using polymerase chain reaction (PCR). It contains reagents and enzymes for the specific amplification of the conserved region of genomes, and for the direct detection of the specific amplicon in FAM, HEX, ROX and Cy5 channel. In addition, it contains an endogenous control amplification system to identify possible PCR inhibition and DNA purification efficiency.

Specificity

The Primer and probe have been designed for the specific and exclusive *in vitro* detection of Plasmodium species (P.falciparum, P.vivax, P.malariae, P.ovale and P.knowlesi.) The target sequences are highly conserved and sequences in this kit have 100% homology with a broad range of clinically relevant reference sequences based on a comprehensive bioinformatics analysis.

Dynamic linear range

The linear range was evaluated by analyzing a logarithmic dilution series of nucleic acids concentrations ranging from 10,00,000 copies/µl to 1000 copies/ml. At least six replicates per dilution were analyzed. The linear range is 1000 – 10,00,000 copies/µl.

Analytical Sensitivity

The analytical sensitivity is defined as the concentration of nucleic acids (copies/µl) that can be detected with a positivity rate of 95%. The analytical sensitivity was determined by analysis of dilution series of quantified each bacterial specific pDNA from 100 copies/ml to 10 copies/ml in triplicates. Under optimal PCR conditions, the limit of Detection [LoD] is 60copies/ml

Note:

DNA purification

Purified DNA is the starting material for the Real-time PCR assay. The quality of the purified nucleic acid has a profound impact on the performance of the entire test system. It has to be ensured that the purification system used for Viral nucleic acid purification is compatible with real-time PCR technology.

If you are using a spin column-based sample preparation procedure having washing buffers containing ethanol, it is highly recommended to perform an additional centrifugation step for 3min at approximately 17000 x g (~ 13000 rpm), using a new collection tube, prior to the elution of the DNA.

Detection Protocol

Things to do before starting

- Before use, all kit components need to be thawed completely, mixed by gently inverting and centrifuged briefly.
- Make sure that Positive and Negative control is included in every run.
- Include 0.5 reaction volume for pipetting error while calculating the volume for total number of reactions.

Components	Volume Per Reaction
Probe PCR Master Mix	10µl
PP Mix	5µl
Purified DNA	**10µl**
Final reaction volume	25µl

Negative Control setup [NTC]

Add 10µl of PCR grade water.

Qualitative Positive Control setup

Add 10µl of Positive control

Centrifuge PCR vials briefly before placing into thermal cycler. *[Note: There should not be any bubbles in the reaction mix. Bubbles interfere with fluorescence detection.]*

Programming Thermal cycler

Sample volume	25µl
Fluorescence Dyes	FAM / HEX / ROX/Cy5
Passive reference	None
Ramping rate	Default

Thermal Profile

	Step	Time	Temp
	Taq enzyme activation / Hold	15min	95ºC
40 cycles	Denaturation	20sec	95ºC
	Annealing/Data collection*	20sec**	60ºC
	Extension	20sec	72ºC

Data collection/Acquisition	Targets
FAM	Plasmodium falciparum
HEX	Plasmodium vivax
ROX	Plasmodium universal
CY5	Internal Control

** Some qPCR machines may require minimum 30sec for data collection; in that case, set to 30sec, this will not affect the performance.

Qualitative interpretation of results:

Test Sample	Negative control	Positive control	Endo Control	Interpretation
Positive	Negative	Positive	Positive	Plasmodium specific DNA detected
Negative	Negative	Positive	Positive	No specific Plasmodium species spefific DNA detected. Sample does not contain detectable amounts of specific DNA.
Negative	Negative	Negative	Negative	Experiment fail
Positive	Positive	Positive	Positive	Experiment fail

Qualitative

Observation		Interpretation
Plasmodium	**Endogenous Control**	
<37	<31	DNA detected

Recommendation:

The Ct value beyond 35 is required careful analysis. The analysis may include that the status of NTC amplification curve, threshold adjustment, linear/log scale view assessment, etc.,

Limitations

Good laboratory practice is essential for proper performance of this assay. Strict compliance with the instructions for use is required for optimal results.

Analysts should be trained and familiar with testing procedures and interpretation of results prior to performing the assay.

A false negative result may occur if inadequate numbers of organisms are present in the sample due to improper collection, transport or handling. Appropriate specimen collection, transport, storage and processing procedures are required for the optimal performance of this test.

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Extreme care should be taken to preserve the purity of the components of the kit and reaction setups. All reagents should be closely monitored for impurity and contamination. Any suspicious reagents should be discarded.

The presence of PCR inhibitors may cause under quantification, false negative or invalid results.

Potential mutations within the target regions of the pathogen's genome covered by the primers and/or probes used in the kit may result in under quantification and/or failure to detect.

As with any diagnostic test, the HELINI Plasmodium species Real-time PCR results need to be interpreted in consideration of all clinical and laboratory findings.

Quality Control

In accordance with the HELINI Biomolecules in house Quality Management System, each lot of HELINI Plasmodium species Real-time PCR kit is tested against predetermined specifications to ensure consistent product quality.

Explanations of symbols

In vitro diagnostic medical device

Catalogue number

Pack size – number of tests

Manufacturer

Manufactured by

HELINI *Biomolecules,*
Ohmlina, 26, 2nd Avenue,
Khuthubi Complex, Vettuvankeni,
Chennai 600115, Tamil Nadu, INDIA
www.helini.in
info@helini.in
helinibiomolecules@gmail.com

+91-44-24490433

www.helini.in