



Revision No.: ZJ0008
Issue Date: Jan 30th, 2012

**Malaria Parasite Real Time PCR Kit User Manual
For In Vitro Diagnostic Use Only**

REF ED-0066-02



For use with **ABI Prism®7000/7300/7500/7900/Step One Plus; iCycler iQ™4/iQ™5; Smart Cycler II; Bio-Rad CFX 96; Rotor Gene™ 6000; Mx3000P/3005P; MJ-Option2/Chromo4; LightCycler®480 Instrument**

1. Intended Use

The malaria parasite real time RT-PCR kit is used for the detection of malaria parasites in whole blood sample by using real time PCR systems.

2. Principle of Real-Time PCR

The principle of the real-time detection is based on the fluorogenic 5' nuclease assay. During the PCR reaction, the DNA polymerase cleaves the probe at the 5' end and separates the reporter dye from the quencher dye only when the probe hybridizes to the target DNA. This cleavage results in the fluorescent signal generated by the cleaved reporter dye, which is monitored real-time by the PCR detection system. The PCR cycle at which an increase in the fluorescence signal is detected initially (Ct) is proportional to the amount of the specific PCR product. Monitoring the fluorescence intensities during Real Time allows the detection of the accumulating product without having to re-open the reaction tube after the amplification.

3. Product Description

Malaria is one of the leading causes of disease and death in the world. It is estimated that there are 300-500 million new cases every year, with 1.5 to 2.7 million deaths worldwide. Malaria is a potentially fatal tropical disease that is caused by a parasite known as Plasmodium. Four kinds of malaria parasites can infect humans: *Plasmodium falciparum*, *P. vivax*, *P. ovale*, and *P. malariae*. It is spread through the bite of an infected female mosquito.

The malaria parasites real time PCR Kit contains a specific ready-to-use system for the detection of the malaria parasites (for *P. vivax*, *P. falciparum*, *P. malariae* and *P. ovale*) through polymerase chain reaction (PCR) in the real-time PCR system. The master contains reagents and enzymes for the specific amplification of the malaria parasites DNA. Fluorescence is emitted and measured by the real time systems' optical unit during the PCR. The detection of amplified malaria parasites DNA fragment is performed in fluorimeter channel **FAM** with the fluorescent quencher **BHQ1**. DNA extraction buffer is available in the kit and whole blood samples are used for the extraction of the DNA. In addition, the kit contains a system to identify possible PCR inhibition by measuring the **HEX/VIC/JOE** fluorescence of the internal control (IC). An external positive control defined as 1×10^7 copies/ml is supplied which allow the determination of the gene load.

4. Kit Contents

Ref.	Type of Reagent	Presentation 25rxns
1	DNA Extraction Buffer	2 vials, 1.5ml
2	Malaria Parasites Reaction Mix	1 vial, 950µl
3	PCR Enzyme Mix	1 vial, 12µl
4	Molecular Grade Water	1 vial, 400µl
5	Internal Control	1 vial, 30µl
6	Malaria Parasites Positive Control (1×10^7 copies/ml)	1 vial, 30µl

Analysis sensitivity: 1×10^3 copies/ml

5. Storage

- All reagents should be stored at -20°C. Storage at +4°C is not recommended.
- All reagents can be used until the expiration date indicated on the kit label.
- Repeated thawing and freezing (>3x) should be avoided, as this may reduce the sensitivity of the assay.
- Cool all reagents during the working steps.
- Reaction mix should be stored in the dark.

6. Additionally Required Materials and Devices

- Biological cabinet
- Real time PCR system
- Desktop microcentrifuge for "ependorf" type tubes (RCF max. 16,000 x g)
- Vortex mixer
- Real time PCR reaction tubes/plates
- Cryo-container
- Pipets (0.5µl – 1000µl)
- Sterile filter tips for micro pipets
- Sterile microtubes
- Disposable gloves, powderless
- Biohazard waste container
- Refrigerator and Freezer
- Tube racks

7. Warnings and Precaution

- Carefully read this instruction before starting the procedure.
- For in vitro diagnostic use only.
- This assay needs to be carried out by skilled personnel.
- Clinical samples should be regarded as potentially infectious materials and should be prepared in a laminar flow hood.
- This assay needs to be run according to Good Laboratory Practice.
- Do not use the kit after its expiration date.
- Avoid repeated thawing and freezing of the reagents, this may reduce the sensitivity of the test.
- Once the reagents have been thawed, vortex and centrifuge briefly the tubes before use.
- Quickly prepare the reaction mix on ice or in the cooling block.
- Set up two separate working areas: 1) Isolation of the RNA/ DNA and 2) Amplification/ detection of amplification products.
- Pipets, vials and other working materials should not circulate among working units.
- Use always sterile pipette tips with filters.
- Wear separate coats and gloves in each area.

8. Sample Collection, Storage and transportation

- Collect samples in sterile tubes;

- Specimens can be extracted immediately or frozen at -20°C to -80°C.
- Transportation of clinical specimens must comply with local regulations for the transport of etiologic agents

9. Procedure

9.1 DNA-Extraction

DNA extraction buffer is contained in the kit, please thaw the buffer thoroughly and spin down briefly in the centrifuge before use. You may use your own extraction systems or the commercial kit.

- 1) Pipet 100µl whole blood sample to a 0.5ml tube, add 100µl DNA extraction buffer, close the tube and vortex for 10 seconds. Spin down briefly in a table centrifuge.
- 2) Incubation the tube for 10 minutes at 100°C.
- 3) Centrifuge the tube at 13000rpm for 10 minutes. The supernatant contains the DNA extracted and is used for PCR template.

Attention:

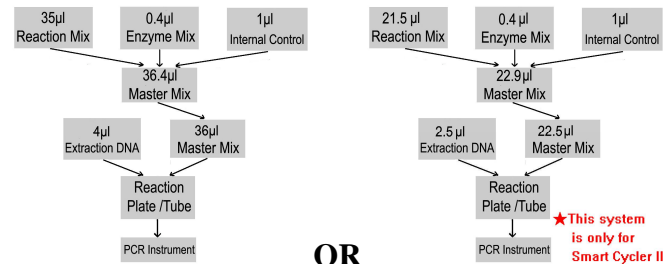
- A. During the incubation, make sure the tube is not open, as the vapor will volatilize into the air and may cause contamination if the sample is positive.
- B. The extraction sample should be used in 3 hours or store at -20°C for one month.
- C. Different brand DNA extraction kits are available. You can also use your own extraction systems or the commercial kit based on the yield. For the DNA extraction, please comply with the manufacturer's instructions.

9.2 Internal Control

It is necessary to add internal control (IC) in the reaction mix. Internal Control (IC) allows the user to determine and control the possibility of PCR inhibition. Add the internal control (IC) 1µl/rxn and the result will be shown in the HEX/VIC/JOE.

9.3 PCR Protocol

The Master Mix volume for each reaction should be pipetted as follows:



OR

✳️ PCR system without HEX/VIC/JOE channel may be treated with 1µl Molecular Grade Water instead of 1µl IC.

- 1) The volumes of Reaction Mix and Enzyme Mix per reaction multiply with the number of samples, which includes the number of the controls, standards and sample prepared. Molecular Grade Water is used as the negative control. For reasons of unprecise pipetting, always add an extra virtual sample. Mix completely then spin down briefly in a centrifuge.
- 2) Pipet 36µl (22.5µl for SmartCycler II) Master Mix with micropipets of sterile filter tips to each Real time PCR reaction plate/tube. Then separately add 4µl (2.5µl for SmartCycler II) DNA sample, positive and negative controls to different reaction plate/tubes. Immediately close the plate/tubes to avoid contamination.
- 3) Spin down briefly in order to collect the Master Mix in the bottom of the reaction tubes.
- 4) Perform the following protocol in the instrument:

37°C for 2min	1cycle
94°C for 2min	1cycle
93°C for 15sec, 60°C for 1min (Fluorescence measured at 60°C)	40cycles

Selection of fluorescence channels	
FAM	Target Nucleic Acid
HEX/VIC/JOE	IC

- 5) ⚠️ If you use ABI Prism® system, please choose "none" as passive reference and quencher.

10. Threshold setting: just above the maximum level of molecular grade water.

11. Quality control:

Negative control, positive control and internal control must be performed correctly, otherwise the sample results is invalid.

Control	Channel	
	FAM	HEX/VIC/JOE
Molecular Grade Water	UNDET	25~33
Positive Control(qualitative assay)	≤38	—

12. Data Analysis and Interpretation

The following sample results are possible:

	Ct value		Result Analysis
	FAM	HEX/VIC/JOE	
1#	UNDET	25~33	Below the detection limit or negative
2#	≤38	—	Positive
3#	38~40	25~33	Re-test; If it is still 38~40, report as 1#
4#	UNDET	UNDET	PCR Inhibition; No diagnosis can be concluded.

