Plasmodium Nucleic Acid Detection Kit (Fluorescent PCR)

【Product Name】Plasmodium Nucleic Acid Detection Kit (Fluorescent PCR)

【Packaging Specifications】48 Test / Box

【Expected Use】

This kit is used for qualitative detection of Plasmodium nucleic acid in human serum and plasma from individuals suspected of Malaria infections.

Malaria is an acute febrile illness caused by Plasmodium parasites, which are spread to people through the bites of infected female *Anopheles mosquitoes*. There are 5 parasite species that cause malaria in humans, including *Plasmodium falciparum*, *Plasmodium malariae*, *Plasmodium ovale*, and *Plasmodium vivax*. and *Plasmodium knowlesi*, and 2 of these which *P. falciparum* and *P. vivax* are the greatest threat. The first symptoms of Malaria are fever, headache and chills, usually appear 10–15 days after the infective mosquito bite and may be mild and difficult to recognize as malaria. Left untreated, *P. falciparum* malaria can progress to severe illness and death within a period of 24 hours.

The test results of this kit are for clinical reference only and cannot be used alone as the basis for confirming or excluding cases.

This kit is intended for use by qualified and trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures.

【The Principle of Inspection】

The kit uses real-time fluorescence PCR technology for detection of Plasmodium. Probes have a fluorescent reporter and a quencher at their 5' and 3' ends, respectively. During PCR amplification, the proximity of fluorescent reporter with the quencher prevents the reporter form fluorescing. When the Taq DNA polymerase (5'→3' exonuclease activity) reaches the dual-labeled probe, its 5'→3' exonuclease activity cleaves the fluorescent reporter from the probe. The amount of free reporter accumulates as the number of PCR cycles increases. The fluorescent signal from the free reporter is measured in real time and allows qualitative of the amount of target sequence.Specific primers and probes are designed to detect the highly conservative regions of Plasmodium, and a pair of primers and a probe for detecting human ribonuclease P gene (RNase P) are included as an internal control to monitor the whole test process to avoid false negative results. The specific probes of Plasmodium are labeled with FAM and the probe of internal control is labeled with VIC.In addition, the introduction of UNG enzyme + dUTP anti-pollution measures into the PCR detection system can effectively degrade the aerosol pollution of amplification products and avoid false positives.

【The Main Components】

Serial Number	Label	Ingredient	48 Tests / Box
1	Preservation solution	Hanks liquid, calf serum, antibiotics, protectene, et al	48 Tubes
2	Pla Mix	Taq DNA enzyme, primer probe, et al	6×8 Strip Tubes
3	Pla PC	TE solution containing the target gene	1× 500μL
4	Pla NC	ddH_2O	1× 500μL

Possible Accessories: Pipette, Pipette Tips, Vortex Mixer, and mini centrifugal, DNA Extraction reagent.

Warning: DO NOT interchange, mix, or combine reagents from kits with different master lot numbers.

【Storage Conditions and Expiration Date】

1 The kit should be kept away from light and sealed at 2-8℃. Valid for 12 months.

2 Transportation conditions: ≤37℃, stable for 1 months.

3 Date of production and duration of use: see label.

【Applicable Instruments】

This kit is suitable for Maverick MQ series fluorescent PCR instruments, ABI7500, QuantStudio 3/5, Biorad CFX96, Roche Cobas Z480, etc.

【Sample Requirements】

Sample types: Whole blood

The collected specimens should be sent for testing immediately. Specimens should be tested within 3 days if stored at 2-8°C. Multiple freeze/thaw cycles should be avoided. Specimens should be transported in a sealed frozer with ice or in a sealed foam box with ice.

【The Test Method】

1 Sample processing

1.1 If use a third party’s Nucleic Acid extraction or Purification Reagent (Spin column or magnetic bead), the exaction should be operated in strict accordance with the instructions.

1.2 If use the recommended sample preservation solution, proceed as follows: Add **10~20uL** whole blood sample to the preservation solution, vortex to mix well, and place at room temperature for 5 minutes. Take out, then invert and mix well to obtain the DNA of each sample.

2 PCR Reaction Preparation

2.1 Take out several 8-tubes-strips that containing Pla Mix from the kit based on the total number of samples (including patient specimen(s), Pla PC and Pla NC. ***PCR tube(s) can be cut down from the strip.***

2.2 Open the “Pla Mix” PCR tube caps, then add ***25μL*** sample (including extracted DNA from patient specimen(s), Pla PC and Pla NC) to the PCR tube.

2.3 Cover the tubes with the Caps of 8-strip Tube provided, mix and transient centrifuge at 2000-6000 rpm, then put them into the PCR instrument.

3 On-machine detection (PCR amplification zone).

Place the PCR reaction tube in the Maverick MQ series fluorescent PCR instruments and set the cycle parameters as follows:

Steps	Number of cycles	Temperature	Reaction time
1	1	50℃	2min
2	1	95℃	1min
3	40	95℃	3s
		58℃(collecting fluorescent)	13s

Fluorescent signals are collected as FAM and VIC, and the data is collected at 58℃.

【Explanation of The Test Results】

After the reaction, the Maverick MQ series fluorescent PCR instruments automatically saves the results.

1 Quality Control

The kit provides positive control and negative control. A complete assay is required all conditions showed in the following table at once, otherwise, experiment results will be regarded as invalid， please retesting one more time.

Channels / Control	FAM	VIC
Pla NC	Negative	Negative or Ct>38
Pla PC	Ct≤35	Negative or Ct>38

2 Result determination

Select each fluorescent channel to read the Ct value, and determine against the following table:

Situation	Ct Vaule		Interpretation
	Pla (FAM)	IC (VIC)	
1	Ct≤38.5	Ct＜40 or negative	Positive for Malaria
2	Ct＞38.5 or negative	Ct≤38.5	Negative for Malaria
3	Ct＞38.5 or negative	Ct＞38.5	Invalid test, it is recommended to retest or re-sample before retest

【Limitations of The Test Method】

1 This kit is only used for aid clinical diagnosis, not as the sole criteria of clinical diagnosis. Therefore, the clinical symptoms/signs, disease history, other laboratory tests and therapeutic response of the patients should be considered comprehensively.

2 Possibility of false negative results:

2.1 False negative results may be caused by incorrect specimen collection, transportation and treatment, and low pathogen content in the specimen.

2.2 The mutation or various of target sequence related to unknown factors can cause false negative results.

2.3 Other unverified interferences or PCR inhibitors may cause false negative results.

3 If there is a contamination of specimen preparation, false positive results may occur.

4 For kits with inclusions, failure to amplify the inner label can result when the sample concentration is too high.

【Product Performance Indicators】

1 Limit of detection (LOD): 1000 copies/mL.

2 Specificity: No cross-reaction with human genomic DNA and total leukocyte nucleic acid.

3 Precision: The CV of In-batch inspection, batch inspection, and operational difference between two operators are less than 5%.

【Note】

1. FOR IN VITRO DIAGNOSIS USE.

2. This kit is only for the use of professionals, and the operators shall have skilled training and experience.

3. The operator shall collect, transport and store the samples in strict accordance with the instructions, and conduct the test within the specified time.

4. The experiment should be strictly operated in different areas, the articles and work clothes in each area are dedicated, and they should not be cross used to avoid pollution. Please clean the working table immediately after the experiment.

5. In operation, should always take care to avoid RNase and DNase pollution, should use non-fluorescent substances disposable gloves (often replaced), disposable thin-walled 200 uL PCR tube (or 96-hole PCR plate plus optical film), pipette head (with filter dump), can not touch the reaction tube directly by hand.

6. Negative control and positive control shall be set for each test. Reagents of different batches shall not be mixed, and kits

shall be used within the validity period.

7. When the reaction liquid is re-packed, try to avoid bubbles. Check whether the reaction pipes are tightly covered before operation to avoid leaking and polluting the instrument.

8. The treatment of specimens should use biosecurity cabinets to ensure operator safety and prevent environmental pollution.

9. Harmful and toxic specimens and reagents in the experiment should be properly placed and kept by special persons; Instruments such as operator stations, pipettes, centrifuges, amplifiers, etc. should often be wiped and disinfected with 1.0% sodium hypochlorite and/or 70% ethanol. Experiment room, ultra-clean workbench should be regularly and after each experiment with UV lamp treatment.

10. Pay attention to the timely cleaning of medical waste.

【Approval and modification date of the specification】

February 25,2022. V01

【Basic Information】

Registrant / Manufacturer Enterprise /After-sale Company: Anitoa Biotechnology（Hangzhou）Co.,LTD.

Address: Room 210,2/F,Building 2,No.658 binan Road,Changhe Street,Binjiang District,Hangzhou City,Zhejiang Provice

Tel: +86 0571-85155309

CE

European Authorized Representative:	**Riomavix S.L.(ES-AR-000001202)**
Address:	**Calle de Almansa 55,1D,Madrid 28039 Spain**
E-mail:	

【Description of Symbols】

Symbol	Descriptions	Symbol	Descriptions
LOT	Batch Code		Date of Manufacture
	Consult Instructions For Use	IVD	In Vitro Diagnostic Medical Device
	Keep Dry	EC REP	Authorized Representative in The European Community
	Do Not Re-use		Do Not Use If The Package Is Damaged
	Temperature limit	Σ	Contains Sufficient for <n> Tests
	Use-by Date	CE	CE Mark
	Manufacturer		