## Plasmodium Nucleic Acid Detection Kit (Fluorescent PCR)

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

**【Packaging Specifications】**50 Test / Box

**【Expected Use】**

This kit is used for qualitative detection of Plasmodium nucleic acid in samples. 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 detects the conserved regions of Plasmodium with high sensitivity and specificity.

**【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' exonulease 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】**

| Components | Ingredient | Quantity |
|---|---|---|
| PCR Master Mix | Contains nucleotides of triphosphate, magnesium ions, DNA polymerase, $ddH_2O$,etc | 1× 625μL |
| Pla Mix | Contains primers, probes, $ddH_2O$,etc | 1× 375μL |
| Pla PC | TE solution containing the target gene | 1× 200μL |
| Pla NC | $ddH_2O$ | 1× 200μL |

**Possible Accessories**: Pipette, Pipette Tips, Vortex Mixer, and mini centrifugal (instantly), 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 are stable when stored at -20±5° C for 12 months

2 Transportation conditions: must be sealed with dry ice and ice bags, and transported under refrigerated conditions no more than 5 days.

3 Freezing and Thawing Stability: Freezing and thawing cycle is limited to 5 times without impacting performance or reliability.

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

**【Applicable Instruments】**

This kit is suitable for Anitoa labs MQ/Z/F series fluorescent PCRs, 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. Specimens that cannot be tested should be stored at -20±5°C for 4 months, stored at-70°C for 12 months.

Multiple freeze/thaw cycles should be avoided. Specimens should be transported in a sealed frozenpitcher with ice or in a sealed foam box with ice.

**【The Test Method】**

**1 Reagent Preparation (Reagent Preparation Area)**

Unpacking all reagents from Kit, place at room temperature for thawing, all reagents shall be vortexed briefly before use. According to the specimen number to prepare reaction buffers, and it is recommended to set negative control and positive control for each test.

| Reaction Components | Volume（μL）/Test |
|---|---|
| PCR Master Mix | 12.5 |
| Pla Mix | 7.5 |
| Total Volume | 20 |

Calculate the volume as described above, add reagents to a sterile microcentrifuge tube, mix well, and then distribute 20 μL to each PCR reaction tubes, transfer all PCR tubes to Sample Preparation Area.

**2.Sample Preparation（Sample Preparation Area）**

2.1 Nucleic Acid Extraction

Refer to the product manual of the nucleic acid extraction or purification kit for operation.

2.2 Sampling

Add 5 μL specimen, 5 μL of negative control and 5 μL of positive control to distributed reaction tubes respectively, total volume 25 μL/tube, caped the tube, and centrifuge at low speed instantaneously. The experimental shall be carried out on ice as far as possible.

**3 PCR Amplification (PCR Amplification Area)**

Place the PCR reaction tube in the Anitoa labs MQ/Z/F series fluorescent PCR instrument and set the cycle parameters as follows:

| Steps | Number of cycles | Temperature | Reaction time |
|---|---|---|---|
| 1 | 1 | 37℃ | 2min |
| 2 | 1 | 95℃ | 1min |
| 3 | 40 | 95℃ | 3s |
| | | 58℃(collecting fluorescent) | 20s |

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

**【Explanation of The Test Results】**

After the reaction, the Anitoa labs MQ/Z/F series fluorescent PCR 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 |
|---|---|---|
| Negative Control | Negative | Negative or Ct>38 |
| Positive Control | Ct≤32 | Ct≤32 |

**2 Result determination**

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

| Channels | Targets | Result determination |
|---|---|---|
| FAM | Plasmodium（target gene） | 1.Positive: Ct≤40 and With Typical S-curve;<br>2.Negative：Ct＞40 or negative |
| VIC | RNase P（Internal control） | When testing human samples, Ct≤40, otherwise resampling. If the FAM channel is positive, Ct＞40 or negative, the result is reliable. |

**【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 sub 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.

**【Basic Information】**

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