Plasmodium Genotyping Real Time PCR Kit

IFU

Revision History

No.	Version	Reviser	Revised Sections and Content	Revision Date
1	A/0	李丽丽	First release	2022.03.28
2	A/1	李丽丽	Correct the name of the kit	2022.05.12
3	V1.2	王雪婧	Upgrade IFU to version 1.1 Update the address; Upgrade LOGO.	2022.11.22
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BioPerfectus

INSTRUCTIONS FOR USE

IVD CE

Product Name:
Plasmodium Genotyping Real Time PCR Kit

For use with Bioperfectus STC-96A, STC-96A PLUS, Applied Biosystems 7500, QuantStudio™ 5, Roche LightCycler®480, Bio-Rad CFX96™, QIAGEN Rotor-Gene Q, Analytik Jena qTOWER^3 and other applicable Bioperfectus machines.

REF YJW20601NW -25T/YJW20601NW-50T Σ 25T/50T -15℃ ~ -25℃

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1. Intended Use
The Bioperfectus Plasmodium Genotyping Real Time PCR Kit is an in vitro diagnostic test. based on real-time PCR technology. for the detection and differentiation of DNA from Plasmodium parasites, including *Plasmodium vivax* (Pv), *Plasmodium falciparum* (Pf), *Plasmodium malariae* (Pm), *Plasmodium knowlesi* (Pk) and *Plasmodium ovale* (Po). Samples can be obtained from human whole blood.

2. Kit Components

Components	Vials/Kit	Volume/25T	Volume /50T
PCR Reaction Mix	2	313μL	625μL
A- Detection Mix	1	188μL	375μL
B- Detection Mix	1	188μL	375μL
A- Positive Control	1	25μL	50μL
B- Positive Control	1	25μL	50μL
Blank Control	1	250μL	250μL

3. Storage
- All reagents should be stored at -20±5℃ condition.
- Check expiry date before use and do not use expired reagent.
- Keep detection mix away from light.
- Avoid repeatedly freeze-thaw.
- Manufacturing date and expiry date: see outer packing box.

4. Materials and Devices Required but Not Provided
- Biological safety cabinet or PCR hood.
- Appropriate real time PCR instrument: Bioperfectus STC-96A, STC-96A PLUS, Applied Biosystems 7500, QuantStudio™ 5, Roche LightCycler®480, Bio-Rad CFX96™, QIAGEN Rotor-Gene Q, Analytik Jena qTOWER^3 and other applicable Bioperfectus machines.
- Appropriate Nucleic acid extractor：SSNP-2000B (32 channels), SSNP-3000A (64 channels), SSNP-9600A (96 channels), SMPE-960 (96 channels), SAW-96 (96 channels), SAW-48 (48 channels) and other applicable Bioperfectus machines.
- Magnetic grate for 1.5 mL centrifuge tubes.
- Centrifuge tube shelf.
- Centrifuge with a rotor for 1.5 mL reaction tubes.
- Centrifuge with a rotor for 0.2 mL reaction tubes or plate.
- Vortex mixer.
- Calibrated adjustable pipettes or multi-channel pipette.
- Pipette tips with filters.
- 1.5mL centrifuge tubes.
- 0.2 mL PCR tubes or plates.
- Disposable particle-free gloves and operating grown.
- 10% sodium hypochlorite or pasteurized disinfectant.

5. Background Information
Plasmodium is a unicellular eukaryote, is the pathogen that causes malaria. It is a unicellular parasitic protozoan of the genus Plasmodium in the family Plasmodiidae. There are five common plasmodia: Pv, Pf, Pm, Pk, and Po. Among them, Pk has been identified in recent years and is recognized as the fifth plasmodium that infects humans. Malaria patients may develop anemia and multiple organ damage soon after their infection. Infants or people without immunity may suffer severe malaria and even cerebral malaria and die from the diseases if they do not receive timely and proper treatment. The spread of malaria requires a source of infection, a route of infection, and susceptible populations. Besides, the transmission intensity is subject to natural and social factors. Temperature and rainfall are the most important natural factors that affect the malaria transmission. An increase in temperature and rainfall may cause a proliferation of anopheles, boost their activity, and enable the parasite in the anopheles to grow faster. Malaria is a major parasitosis that causes severe damage to human health and social and economic development. Thus, together with AIDS and tuberculosis, it is listed by the WHO as an urgent global public health challenge.

6. Technical Principle
The Bioperfectus Plasmodium Genotyping Real Time PCR Kit is based on real-time PCR technology. Specific primers and probes are designed based on specific gene areas of Plasmodium (18S rRNA). Probes consist of a reporter dye at 5' and quenching dye at 3'. The fluorescent signals emitted from reporter dye are absorbed by the quencher, so it doesn't emit signals. During amplification, probes bonded to templates are cut off by Taq enzyme (5'→3' exonuclease activity), separating reporter dye from the quencher, generating fluorescent signals, the PCR instrument will then automatically draw a real-time amplification curve based on the signal change, finally realizing the qualitative detection and differentiation of DNA from Plasmodium parasites, including *Plasmodium vivax* (Pv), *Plasmodium falciparum* (Pf), *Plasmodium malariae* (Pm), *Plasmodium knowlesi* (Pk) and *Plasmodium ovale* (Po). In addition, the kit also contains a housekeeping gene (RNase P) as an internal control (IC) for specimen sampling and nucleic acid extraction.

7. Warnings and Precautions
- For *in vitro* diagnostic use only. For professional use only.
- Operators should be trained in real-time PCR techniques.
- Nucleic acid extraction should be manually carried out in biosafety cabinet or by automatic nucleic acid extraction system.
- Wear personal protective equipment (PPE), including (but not limited to) disposable clean powder-free gloves, mask, goggles.
- Working zones in laboratory should be strictly separated. Use separated and segregated working areas for (i) Reagent preparation, (ii) Specimen preparation and (iii) Amplification. The workflow in the laboratory should proceed in unidirectional manner. The experiment processes shall comply with the Good Clinical Laboratory Practice (GCLP) for Molecular Based Tests Used in Diagnostic Laboratories.
- Work benches should be cleaned immediately after use. Amplicon contamination should be avoided.
- Clean work benches, pipettes and centrifuge by using 10% sodium hypochlorite and 70% ethanol.
- The use of sterile disposable pipettes and nuclease-free pipette tips is recommended.
- Use applicable real-time PCR instrument and nucleic acid extraction system to ensure optimal test performance.
- Use reagents before expiry date. DON'T replace or interchange reagents from different batches or manufactures.
- Discard specimens and assay waste according to your local safety regulations.

8. Sample Preparation
8.1 Sample collection method
For blood
- For adults, a minimum volume of 4 mL whole blood is preferable.
- For pediatric samples, a minimum of 1 mL whole blood should be collected in pediatric-sized collection tubes.
- Blood must be collected in plastic collection tubes.
- Whole blood preserved with EDTA is preferred, but whole blood preserved with sodium polyanethol sulfonate, citrate or with clot activator is also acceptable

Other types of specimens should be collected according to clinical laboratory guidelines.

8.2 Specimen transport and storage
- Specimen preserves at 2-8°C up to 72 hours after received.
- Specimen preserves at -70°C or colder if extraction is arranged after 72 hours.
- Extracted DNA preserves at -70°C or colder.

9. Procedure
9.1 DNA Extraction
For reproducible isolation of nucleic acid, the following nucleic acid extraction systems and kits are recommended:

Manufacturer	Nucleic Acid Isolation Kit	Cat. No.
Bioperfectus	Whole Blood DNA Extraction Kit (Magnetic Beads Method)	SDK60110
	Viral Nucleic Acid Extraction Kit (Silica-Based Spin Column)	SDK60102
	Viral Nucleic Acid Extraction Kit (Magnetic Bead Method)	SDK60104
	Nucleic Acid Extraction Rapid Kit (Magnetic Bead Method)	SDKF60101
	Bacteria DNA Extraction Kit (Magnetic Bead Method)	SDK60108
Qiagen	QIAamp DNA Mini Kit	51304
		51306

9.2 Master Mix Preparation
The Master Mix A and B volume for each reaction should be pipetted as follows:

Components	Master Mix A Volume	Master Mix B Volume
PCR Reaction Mix	12.5μL	12.5μL
A- Detection Mix	7.5μL	-
B- Detection Mix	-	7.5μL
Total Volume (Master Mix)	20μL	20μL

A sample must be tested simultaneously by Master Mix A and Master Mix B.
Determine the number of extracted specimens to be tested, thaw the components.

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For maximal recovery of contents, briefly spin vials in the centrifuge before opening. Mix carefully and thoroughly by pipetting up and down.

9.3 PCR Set-up Procedure

Place your samples on ice. Follow the procedure below to prepare the PCR Master Mix.

a. Pipette 20μL of the Master Mix into each required reaction tube/plate.
b. Add 5μL isolated DNA or 5μL Control (Positive Control or Blank Control).
c. Make sure that every run including at least one Positive Control and one Blank Control.
d. Cap or seal the reaction tubes/plate and centrifuge using an appropriate centrifuge for 30 seconds at approximately 2,000 rpm.
e. Ensure that all liquid is at the bottom of the tubes/plate.
f. Perform the following protocol in the instrument.

Step		Temperature	Time	Cycle
1	Initial denaturation	95°C	5 min	1 cycle
2	Denaturation	95°C	10 sec	40 cycles
	Annealing, extension and fluorescent signal collection*	58°C	30 sec	

* Fluorescent signal should be collected during this step through the FAM, VIC, ROX, CY5channels.

10. Real Time PCR System Operation

The following amplification protocol was developed for use on the Bioperfectus STC-96A, STC-96A PLUS. See the instrument operator's manual for detail. Other appropriate real time PCR instruments refer to the corresponding instrument operator's manual.

10.1 Bioperfectus STC-96A/96A PLUS Real-Time PCR System Amplification Protocol

1. Switch on Bioperfectus STC-96A/96A PLUS Real-Time PCR System.
2. Launch the Bioperfectus STC-96A/96A PLUS Real-Time PCR System software Version 1.0.
3. Click on "Experiment Wizard", and set up proper parameters in "Project" and "Setup".
4. Set up "Plate".
5. Set up "Sample".
6. Starting the PCR
a. Insert the 96 well PCR plate or reaction tubes into the machine.
b. Select the "Start Run" button,
7. Post PCR Analyze the data by pressing the "Analysis" button on left side of the menu and analyze the data using the "Analyze".

11. Quality Control

Prior to evaluating the specimen results, the Positive Control and Blank Control should be interpreted using the table below.

Channels / Controls	Threshold cycle (Ct) value			
	FAM	VIC	ROX	CY5
Blank Control	UNDET	UNDET	UNDET	UNDET
Positive Control	Ct≤30	Ct≤30	Ct≤30	Ct≤30

NOTE: Internal Control is specially designed to detect in fluorimeter channel ROX

- The Positive Control and Blank Control should be included per PCR run.
- If the Positive Control and Blank Control do not meet the criteria, the entire run is invalid and results should not be reported. Repeat the entire process (specimen and control preparation, amplification and detection). If the repeat run is still invalid, please contact Technical Support.
- Viral transport media or previously characterized negative specimen may be used as an external negative control. This must be treated as a patient specimen in every extraction and PCR run.
- Additional controls may be used in accordance with local, state, federal accrediting organizations, as applicable.

12. Data Analysis and Interpretation

Master Mix A				Master Mix B				Results	Report
FAM	VIC	ROX	CY5	FAM	VIC	ROX	CY5		
Pf	Pv	IC	Plasmodium	Pm	Po	IC	Pk		
+	-	+/-	+	-	-	+/-	-	Pf Detected	Pf Positive
-	+	+/-	+	-	-	+/-	-	Pv Detected	Pv Positive
-	-	+/-	+	+	-	+/-	-	Pm Detected	Pm Positive
-	-	+/-	+	-	+	+/-	-	Po Detected	Po Positive
-	-	+/-	+	-	-	+/-	+	Pk Detected	Pk Positive
-	-	+	-	-	-	+	-	Undetected	Plasmodium Negative
-	-	-	-	/	/	/	/	Experiment fail (Master Mix A)	Invalid
/	/	/	/	-	-	-	-	Experiment fail (Master Mix B)	Invalid

Note: For Plasmodium: Ct value ≤38 is considered positive(+) ; Ct value > 38 is considered negative (-). For IC: Ct value ≤38 is considered positive (+); Ct value > 38 is considered negative (-)
1. Reporting positive: Plasmodium is detected.
2. Reporting negative: Plasmodium is not detected.
3. Reporting invalid: It is possible due to low load and should be analyzed by combining clinical sign. Repeat sampling or collect specimen from different parts of the patient and repeat the test when clinical sign and other examinations are high suspected.

13. Limitations

- Negative results do not preclude infection with Plasmodium and should not be the sole basis of a patient treatment decision.
- Reliable results are dependent on the adequate specimen collection, transport, storage and processing procedures.
- Inhibitors present in the sample and/or errors in following the assay procedure may lead to false negative results.
- A trained healthcare professional should interpret assay results in conjunction with the patient's medical history, clinical signs and symptoms, and the results of other diagnostic tests.
- Potential mutations within the target regions of the virus/bacteria genome covered by the tests primers and/or probes may result in failure to detect the presence of the pathogens.
- There is a risk of false positive values resulting from cross-contamination by target organisms, their nucleic acids or amplified product, or from non-specific signals in the assay.

14. Performance Evaluation

14.1 Analytical Sensitivity

The limit of detection of the Plasmodium Genotyping Real Time PCR Kit for the detection of DNA specific for Plasmodium from human whole blood was determined to be 5 copies/reaction.

14.2 Analytical Specificity

No cross-reactivity of the Kit within the following selected microorganisms were observed.

Cross pathogens	
Toxoplasma	Streptococcus
Anaplasma phagocytophilum	Leptospira
Brucella	Salmonella typhi
Neisseria meningitidis	Salmonella paratyphi

14.3 Precision

Precision references were used to evaluate the precision of Plasmodium Genotyping Real Time PCR Kit. The results show that, for the precision references, coefficients of variation (CV%) of the repeatability and within-laboratory precision are less than 5%.

15. Appendix

Index of Symbols

CE	CE certification	EC REP	Authorized representative in the European Community
IVD	In vitro diagnostic Medical device		Use-by date
	Manufacturer		Date of manufacture
REF	Catalogue number	Σ	Contains sufficient for <n> tests
	Consult instructions for use		Temperature limit
LOT	Batch code		This side up

16. Contact and Support

For more information about Bioperfectus Technologies, please visit our web-site at: http:// www.bioperfectus.com or contact at E-mail: info@bioperfectus.com.
For detailed programming instructions regarding the use of the Bioperfectus Technologies Real Time PCR Kits on specific real-time PCR instruments please contact our Technical Support at E-mail: support@bioperfectus.com.