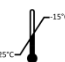
A Real-Time PCR (qPCR) Assay for Detection of Malaria

Product Insert

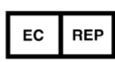
*ab*TES™ Malaria qPCR I Kit
Kit Version: 1.0

300221 (50 Reactions)
300222 (100 Reactions)



Store at -25°C to -15°C

AITbiotech Pte Ltd
25 Pandan Crescent #05-15
TIC TECH Centre
Singapore 128477



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For use on Bio-Rad-CFX96 and ABI 7500 Fast.

1. Pathogen Information

Malaria is a vector-borne disease caused by protozoan parasite of the genus *Plasmodium*. It is widespread in tropical and subtropical regions such as Sub-Saharan Africa, Asia and the America, resulting in annual 500 million clinical cases and 2.7 million deaths worldwide. While *Anopheles* mosquito is being the main vector of transmission, other transmission pathways such as blood transfusions, hypodermic needle sharing among intravenous drug users and transplantation remain eminent. Five species of *Plasmodium* were identified as human pathogens, namely *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae*, and *P. knowlesi*. The disease results from the multiplication of malaria parasites within red blood cells, causing symptoms that typically include headaches, lassitude, nausea and fever, in severe cases progressing to coma and death.

2. Test Description

The *ab*TES™ Malaria qPCR I Kit is a real-time polymerase chain reaction (qPCR) kit for the detection of all *Plasmodium spp*. affecting humans (*P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae*, and *P. knowlesi*). The kit contains all the necessary PCR reagents for rapid, sensitive and reproducible real-time detection of the all five *Plasmodium spp*. affecting humans using highly specific primer pairs and double-dye hydrolysis probes. The recommended sample type is EDTA blood (heparin blood is not recommended). The *ab*TES™ Malaria qPCR I Kit enables detection of a human single-copy housekeeping gene, β2-microgobulin as an **Internal Control (IC)** to identify possible PCR inhibitions. In addition, an Uracil-D-glycosylase (UDG)/dUTP contamination control system is incorporated in the kit to eliminate PCR carryover contamination.

3. Storage Conditions

The components of *ab*TES™ Malaria qPCR I Kit should be stored in the dark, at -20ºC in a **NON**-frost-free freezer. Frost-free freezers go through freeze-thaw cycles to remain frost-free and may cause accelerated degradation of enzymes and nucleic acids. Avoid repeated thawing and freezing (max 3 times) as this may lower the sensitivity. If reagents are used intermittently, it is suggested to keep the reagents frozen in aliquots.

4. Kit Components

Tubes	Items	300221 (50 rxns)	300222 (100 rxns)
1	Primer/Probe Mix	1x 50 µL	2x 50 µL
2	5x Enzyme/Reaction Mix	1x 200 µL	2x 200 µL
3	Nuclease-free Water	1x 600 µL	2x 600 µL
4	Malaria Positive Control	1x 100 µL	1x 100 µL

5. Additional Materials Required but not Provided

- Disposable powder-free gloves
- Nucleic acid extraction Kit
- Vortex mixer
- Pipettes and pipette tips with filter
- Desktop centrifuge with rotor
- Real-time thermal cycler
- 0.2 mL PCR tubes/ 96-well PCR plates
- Ice box/ cooling block

6. Limitations and General Precautions

- The use of this product and its data interpretation are intended for personnel trained in real-time PCR techniques and *in vitro* diagnostics procedures only.
- It is advisable to analyze the real time PCR graph at the end of the run to determine the validity of the Ct data.
- Appropriate specimen collection, transport, storage and nucleic acid extraction procedures are required for reliable results.
- Wear disposable gloves, laboratory coats and eye protection when handling samples and reagents. Wash hands thoroughly thereafter.
- Use sterile pipette tips with filters and replace the tip for every procedure.
- Store and extract positive materials (specimens, controls and amplicons) separately from all other reagents and add to the reaction mix in a separate facility, if possible.
- Thaw all the components thoroughly at room temperature before starting an assay.
- When the components are thawed, mix the components and centrifuge briefly.
- Do not use the kit after its expiration date or mix components from different lots.

7. Procedures

7.1. Nucleic Acids (NA) Extraction

Standard NA extraction kits are compatible with this assay. Please carry out NA extraction as per instructed in the manufacturer’s Extraction Kit manual.

7.2. PCR Reaction Setup

Thoroughly thaw all components, mix and spin briefly. Keep all components and samples on ice. Prepare your PCR reaction based on the following pipetting scheme:

Reagent	Volume per reaction		
	Test sample reaction	Positive control reaction	Non template control reaction
Primer/Probe Mix	1 µL	1 µL	1 µL
Enzyme /Reaction Mix	4 µL	4 µL	4 µL
Nuclease-Free Water	10 µL	10 µL	15 µL
Positive Control	-	5 µL	-
Extracted Test Sample	5 µL	-	-
Total Volume	**20 µL**	**20 µL**	**20 µL**

7.3. PCR Cycling Conditions

The following cycling conditions were established and validated on Bio-Rad CFX96 and ABI 7500 Fast. You may need to adjust these conditions for other real-time platforms. **FAM** (Malaria) and **HEX/VIC** (IC) channels should be chosen and the fluorescence is measured at the end of annealing-extension phase of each cycle.

Phase	Description	No. of Cycles	Temperature	Duration
1	UDG incubation	1	50 °C	2 min
2	*Taq* activation	1	95 °C	2 min
3	Amplification	40	95 °C	5 sec
			*60 °C	25 sec

**Data acquisition at annealing phase*

ABI 7500 Fast Settings	
Ramp speed	Fast
Passive reference	None

8. Performance Characteristics

8.1. Analytical Sensitivity

Analytical sensitivity (limit of detection) is defined as the lowest concentration at which the assay can detect with a positivity rate of at least 95%. The analytical sensitivity of the assay was estimated by analyzing a dilution series of a Gombak A strain of *Plasmodium falciparum* DNA sample in triplicates followed by running 20-replicates at the concentrations near the analytical sensitivity. The DNA sample was quantified based on 1 genomic equivalent of the parasite being 0.02 pg (Goman et al.).

The analytical sensitivity (not in consideration of extraction) for the *ab*TES™ Malaria qPCR I Kit was determined to be at least 0.48 geq/µl.

8.2. Analytical Specificity

The assay was tested for potential cross-reactivity against the following panel of 37 organisms. All organisms were tested in 1000 copies/µl concentration except otherwise stated. No cross-reactivity was observed.

Organisms Tested for Analytical Specificity	
Bacteroides fragilis	*Legionella pneumophila*
Candida albicans	*Mobiluncus mulieris*
Chikungunya virus	*Mycoplasma genitalium*
Chlamydia trachomatis	*Mycoplasma hominis*
Cytomegalovirus	*Neisseria gonorrhoeae*
Enterococcus faecalis	*Neisseria meningitidis*
Escherichia coli (VTEC)	Parvovirus B19
Gardnerella vaginalis	*Proteus mirabilis*
Haemophilus influenzae	*Pseudomonas aeruginosa*
Herpes simplex virus 1	*Staphylococcus aureus*
Herpes simplex virus 2	*Streptococcus agalactiae*
Human herpes virus 6	*Streptococcus pneumoniae*
Human papilloma virus 16	*Streptococcus pyogenes*
Human papilloma virus 18	*Treponema pallidum*
Influenza A Pandemic H1N1/2009 (NIBRG-122)	*Trichomonas vaginalis*
Influenza A H1N1 (A/PR/8/34)	*Ureaplasma urealyticum*
Influenza A H3N2 (A/VICTORIA/3/75)	Varicella zoster virus
Influenza B (B/HONG KONG/5/72)	West Nile Virus
Klebsiella pneumoniae	

8.3. Precision/Reproducibility

The inter-assay and intra-assay precision was determined by performing the assay once per day in triplicates over a period of 6 days for 3 samples with different concentrations (total of 54 reactions per target).

The qualitative results of all 54 reactions were 100% reproducible.

The coefficient of variation (CV) of the cycle threshold (Ct) for the intra- and inter-assay precision are as follows:

	1500 geq/µl	60 geq/µl	2.4 geq/µl
Inter-assay	2.7%	2.1%	2.3%
Intra-assay	0.6%	0.5%	1.5%

8.4. Diagnostic Evaluation

A total of 59 clinical blood samples were used for diagnostic evaluation by comparing the results with peripheral blood film examination. Of the 59 samples, 37 were positive for malaria and 22 were negative for malaria based on peripheral blood film examination. DNA was isolated from the blood samples using the QIAamp DNA blood mini kit (Qiagen) or the MagNA Pure System (Roche) according to manufacturer's instructions.

All 37 of 37 malaria positive samples were detected by the abTES™ Malaria qPCR I Kit and all 22 of 22 malaria negative samples were negative. The diagnostic sensitivity and specificity were both 100% based on peripheral blood film examination as the reference method.

		Peripheral Blood Film	
		Positive	Negative
*ab*TES™	Positive	37	0
	Negative	0	22
		100% Diagnostic Sensitivity	100% Diagnostic Specificity

9. Interpretation of Data

A sample will be considered as having a positive result if the fluorescence level is higher than the threshold value and will be considered negative, otherwise.

As the kit contains an internal control targeted at the human β2-microgobulin gene, all human sourced specimens that are negative for Malaria (FAM) should be positive at the internal control channel (HEX). A negative internal control in this case may indicate a presence of PCR inhibitors in the sample, a problem during the extraction step or a problem with the PCR reaction. The internal control may not necessarily be positive if the sample is positive for Malaria due to competition of reagents.

Result	Malaria Ct (FAM)	Internal Control (HEX/VIC)
Negative	-	+
Positive	+	+ or -
Indeterminate	-	-

10. Troubleshooting

10.1. No signal observed with positive control

- Check programmed temperature settings against the protocol given.
- Affirm if proper storage was done and check the expiry date on the kit; Repeat the run with a new kit if needed.
- PCR inhibition has possibly occurred: re-purify DNA sample to remove inhibitors and repeat PCR, if needed.

10.2. Signal detected for negative control

- A contamination in the reagents or samples is highly possible.
- Repeat experiment protocol and take steps to locate source of contamination.

10.3. Weak or no signal of the internal control and no sign detection in analytical channel as well

- A possible PCR inhibition has occurred. Re-extract the sample to remove inhibitors and repeat PCR, if needed.
- Affirm if proper storage was done and check the expiry date on the kit. Repeat the run with a new kit if needed.

11. Explanation of Symbols

Symbol	Meaning
IVD	*In vitro* diagnostic medical device
REF	Catalogue number
-25°C / -15°C	Store at -25°C to -15°C
	Manufacturer
EC REP	Authorized representative in the European community
LOT	Lot number
	Use by
Σ	Contains sufficient for <n> tests

12. References

- Goman, M., G. Langsley, J. E. Hyde, N. K. Yankovsky, J. W. Zolg, and J. G. Scaife. 1982. The establishment of genomic DNA libraries for the human malaria parasite P. falciparum and identification of individual clones by hybridization. Mol. Biochem. Parasitol. 5:391–400.

Electronic copy of the product insert can be downloaded at http://aitbiotech.com/Malaria/