In vitro Diganostic medical device

Asan qPCR Test® Malaria

1. LOT number and Expiry date
Refer to external labelling

2. Intended use
The Asan qPCR Test® Malaria is an in vitro diagnostic medical device designed to qualitatively detect malaria infection by identifying the 18S rRNA gene extracted from whole blood (EDTA) of individuals suspected of malaria infection. Using Real-time RT-PCR, this test enables simultaneous or individual detection of malaria, as well as differentiation between P. falciparum and P. vivax, assisting in the diagnosis of infection.

3. How to use
(1) Product description
This product is a One-step RT-PCR product that uses Real-time reverse transcription polymerase chain reaction (Real-time RT-PCR). One-Step RT-PCR uses materials for reverse transcription reaction and PCR reaction mixed in one tube. In this process, cDNA is synthesized from RNA extracted from the specimen in the same tube, and PCR for the target gene is performed from the synthesized cDNA.

The real-time PCR method uses a primer and probe, wherein the amplification product amplified by the primer binds to the probe, the polymerase decomposes the probe, and the fluorescence signal of the generated reporter dye is measured in real time. In this process, the fluorescence signal increases according to the amount of amplification product. The Human RNase P gene, which is an internal positive control (IC), can check the RNA extraction process, whether PCR reaction inhibitors are mixed in the extracted RNA, and whether the sample was properly collected and extracted.

(2) Kit Components
The Asan qPCR Test® Malaria kit contains sufficient reagents and quality control samples. The kit contains the following:

	Components	Volume	Quantity
1	2x Onestep qRT-PCR Master Mix	1 mL	1 tube
2	Primer/Probe Mix	500 µL	1 tube
3	Positive Control	250 µL	1 tube
4	Negative Control	250 µL	1 tube

※ Material and devices required but not provided
CFX 96™ Real-Time PCR Dectection system(Bio-Rad)
- Nucleic acid extraction system or kit
- Benchtop centrifuge with a rotor for 1.5/2.0 mL reaction tubes
- Centrifuge with a rotor for microtiter plates
- Vortex mixer
- Appropriate reaction plate or tube
- Pipettes
- Pipette tips with filters
- Disposible latex gloves

(3) Specimen preparation and storage
1) The acceptable specimen is human whole blood (EDTA)
2) Use a tube containing an anticoagulant, and the specimen remains stable for up to 72 hours when stored at 2–8℃ (refrigerated).

※ All clinical specimens are handled as potentially infectious.

4. Procedure
(1) Nucleic acid extraction
It is recommended to use the QIAamp DNA Blood Mini Kit as a nucleic acid extraction reagent, and follow the manufacturer's instructions.

(2) How to test
[Preparation of reagent]
1) Open the box, and thaw each components
2) Lightly voltex each completely melted component and spin-down.

[Mix the components]
1) After considering the number of samples N (number of specimens + Positive Control 1EA + Negative Control 1EA + Extra 1EA) to be tested, prepare 15 ㎕ x N of reaction solution using Onestep qPCR Master Mix and Primer/Probe Mix as shown in the table below.

	Components	1 Test Volume	N Test Volume
1	2x Onestep qRT-PCR Master Mix	10 ul	10 ul x N
2	Primer/Probe Mix	5 ul	5 ul x N
3	Sample	5 ul	5 ul x N
	Total	20 ul	20 ul x N

2) Voltex the prepared reaction solution lightly, being careful not to create bubbles, and then spin down.
3) Dispense 15 µl of the prepared reaction solution into the PCR tube.
4) Dispense 5 µl of the extracted Viral RNA Sample, Positive Control (PC), and Negative Control (NC) into each PCR Tube. (To minimize contamination, work in the following order: NC > Sample > PC.

[Amplication of Real-time PCR]
1) After dispensing, spin down the PCR tube and place it into the PCR well.
2) Operate the equipment following the user manual instructions.
3) Refer to the RT-PCR equipment settings in the following table.

<Setting of CFX96™ Dx System fluorescent>

Target	***Plasmodium* spp.**	***P. falciparum***	***P. vivax***	**IC**
Fluorophore	ROX	FAM	HEX	Cy5

<Setting of Real-time PCR condition>

	Steps	Temperature	Time	Cycle
1	cDNA synthesis	50℃	5 min	1
2	RTase Inactivation	95℃	3 min	1
3	Pre-Denaturation	95℃	5 sec	5
4	Pre-Annealing/ Extension	60℃	40 sec	
5	Denaturation	95℃	1 sec	40
6	Annealing / Extension	60℃	5 sec	

5. Result analysis

After the test is completed, open the experiment data in the analysis program, perform result analysis, check the cut-off (Ct value) for the target, and set the threshold accordingly.

Target	Threshold	Ct Value	Result
Plasmodium spp.	Auto	Ct ≤ 36	Malaria Positive
P. falciparum	Auto	Ct ≤ 36	Malaria-P. falciparum Positive
P. vivax	Auto	Ct ≤ 36	Malaria-P. vivax Positive
IC	Auto	Ct ≤ 36	Internal control Positive

Case	P. spp.	*P. f*	*P. v*	IC	Interpretation
1	+	-	-	+/-	*Plasmodium* spp. Positive (*P. falciparum, P. vivax* Negative)
2	+	+	-	+/-	*P. falciparum* Positive
3	+	-	+	+/-	*P. vivax* Positive
4	-	+	-	+/-	*P. falciparum* Positive
5	-	-	+	+/-	*P. vivax* Positive
6	+	+	+	+/-	*P. falciparum* Positive, *P. vivax* Positive
7	-	+	+	+/-	*P. falciparum* Positive, *P. vivax* Positive
8	-	-	-	+	Negative
9	-	-	-	-	Invalid/Re-test

- Even if the internal control material (IC) shows a negative result, it may still be judged positive. If each detected gene is strongly positive, the IC result value may be impaired due to the high concentration of the detected gene. Therefore, a positive judgment is made even if the IC is negative.
- Invalid/Re-test: If the test is invalid, a retest is performed.

6. Quality control and validity of results

A minimum of one Negative Control and one Positive Control must be included in each run. All control wells must pass for the Real-time RT-PCR plate to be considered valid.

Target	P. spp	P. f	P. v	IC
PC	Ct ≤ 36	Ct ≤ 36	Ct ≤ 36	Ct ≤ 36
NC	N/A	N/A	N/A	N/A

7. Performance

(1) Analytical sensitivity – Limit of detection (LoD)

To determine the limit of detection (LoD) for Malaria, the standard materials were serially diluted and tested in two separate lots. Each test was performed five times per run, twice a day, for three days, resulting in a total of 60 repetitions per concentration. The lowest concentration detected with ≥95% accuracy was determined.

	Target	Limit of detection (LoD)
		Detection concentration (Copies/μL)
1	*P. falciparum*	1.06 copies/μL
2	*P. vivax*	3.7 copies/μL
3	*Plasmodium* spp.	0.96 copies/μL

(2) Precision - Repeatability

Three samples of high concentration (100xLoD), medium concentration (10xLoD), and low concentration (2xLoD) were tested as a set by the same experimenter, twice daily, three times per test, over five days using one lot. The test confirmed that the Ct values detected within each test, between tests, and across test dates had a coefficient of variation (CV) of less than 5%, indicating effective repeatability.

(3) Precision – Reproducibility

Three samples of high concentration (100xLoD), medium concentration (10xLoD), and low concentration (2xLoD) were used as a set to assess reproducibility across locations, testers, and lots. Two testers across two laboratories tested two different lots twice daily for five days, with each test repeated three times. The results consistently showed 100% agreement between negative and positive outcomes, and the coefficient of variation (CV) for detected Ct values was less than 5%. This confirmed effective reproducibility.

(4) Analytical specificity - cross-reactivity

Samples containing 25 different microorganisms and viruses that could potentially be present were tested to evaluate cross-reactivity. Each sample was tested three times on a single instrument using a single lot of the product. The results showed no cross-reactivity at the analyzed concentrations.

No	Pathogen name	Concentration	Dection or not
1	Dengue virus type 1	7.3 ng/ul	N.D.
2	Dengue virus type 2	5.8 ng/ul	N.D.
3	Dengue virus type 3	6.0 ng/ul	N.D.
4	Dengue virus type 4	6.9 ng/ul	N.D.
5	Zika virus	6.5 ng/ul	N.D.
6	Chikungunya virus	11.4 ng/ul	N.D.
7	West Nile Virus	$10^{3.8}TCID_{50}$/mL	N.D.
8	Yersinia enterocolitica	25 ng/ml	N.D.
9	Japanese Encephalitis virus	13.9 ng/ul	N.D.
10	Bacillus cereus	5 ng/ul	N.D.
11	Clostridium difficile	$1x10^5$ CFU/ml	N.D.
12	Enterococcus faecalis	5.12 ng/ml	N.D.
13	Escherichia coli	$1x10^5$ CFU/ml	N.D.
14	Japanese Encephalitis virus	10^3 pfu/ml	N.D.
15	Klebsiella pneumoniae	$1x10^5$ CFU/ml	N.D.
16	Listeria monocytogenes	$1x10^5$ CFU/ml	N.D.
17	Mycobacterium fortuitum	$8.053x10^7$ copies/ul	N.D.
18	Mycobaterium goodii	$1x10^5$ CFU/ml	N.D.
19	Proreus vulgaris	$1x10^6$ CFU/ml	N.D.
20	Proteus mirabilis	$1x10^5$ CFU/ml	N.D.
21	Rotavirus A	$1x10^5$ copies/ul	N.D.
22	Salmonella Typhimurium	$1x10^5$ CFU/ml	N.D.
23	Salmonella Typhi	$1x10^5$ CFU/ml	N.D.
24	Staphylococcus aureus	$1x10^5$ CFU/ml	N.D.
25	Streptococcus pneumoniae	$2.318x10^7$ copies/ul	N.D.

N.D: Not detected

(5) Analytical specificity – interference response

Samples were spiked with eight types of endogenous or exogenous substances and tested three times for each sample. It was confirmed that none of the seven substances caused interference at the tested concentrations.

No	Interference material	Concentration	Interference or not
1	EDTA	292 umol/L	N.A
2	Heparin	3000 U/L	N.A
3	Sodium citrate	12.9 pmol/L	N.A
4	Bilirubin, Conjugated	0.05 mg/mL	N.A
5	Albumin	0.24 g/mL	N.A
6	Haemoglobin	2 mg/mL	N.A
7	Biotin	300 ug/mL	N.A
8	Human genomic DNA	10 ug/mL	N.A

N.A: Not available

8. Storage method and period of use

(1) Store the kit in its original sealed packaging at -20℃. or below, away from direct sunlight. Kit contents are stable until the expiration date printed on the outer box.

(2) The number of freeze/thaw cycles is limited to four.

9. Precautions

[General precautions]

(1) This product is for in vitro diagnostic use only.

(2) This test should only be conducted by trained professionals.

(3) Before starting the assay, thoroughly read the user manual and follow the instructions for use.

(4) All specimens collected from humans should be considered potentially infectious. Follow Good Laboratory Practice (GLP) when handling samples to prevent contamination.

(5) Avoid contact of this product with skin, eyes, or mucous membranes. If contact occurs, rinse immediately with plenty of water.

(6) Improper control of sample cross-contamination during handling and processing may lead to false positive results.

[Precautions for storage]

(1) Store the product below -20°C.

(2) Do not use products beyond their expiration date.

[Precautions when test]

(1) Before use, visually inspect each component of the product for leaks. Do not use if a leak is detected.

(2) Do not mix this product with products from other lots during use.

(3) Avoid repeated freezing and thawing of the product, as it may affect test results.

(4) This product is optimized for use with the recommended real-time gene amplification device. Use with other equipment may result in incorrect results.

(5) Take care to prevent contamination of product components by nucleic acid extracts, PCR products, or positive controls. We recommend using filter tips to prevent contamination.

(6) In case of spills onto equipment, clean according to the equipment manual and remove contamination from surfaces.

(7) All samples used in testing are potentially infectious. Use appropriate protective equipment and conduct testing in facilities such as biosafety cabinets of Biosafety Level 2 or higher.

(8) Improper collection, transport, or handling of samples may affect test results. Handle samples with caution.

[Precautions when determining results]

(1) Do not utilize test results obtained with this product for disease diagnosis. Diagnosis should be based on test results, clinical findings, and other clinical data evaluated by a healthcare professional.

(2) A negative result alone may not exclude Malaria infection if the viral concentration in the sample is below the detection limit or if amplification inhibitors are present.

(3) Sensitivity may decrease if there are mutations in the primer and probe binding regions included in the product.

(4) This test does not exclude diseases caused by other bacterial or viral pathogens.

[Precautions for disposal]

(1) Dispose of products and samples following guidelines and methods outlined in the Waste Management Act.

9. Creation and revision date

First creation date : 2025.03

[Product name] High-risk infectious agent genetic testing reagent
[Classification number (grade)] K05030.01(3)
[Model name] Asan qPCR Test® Malaria

ASAN
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Manufactured & Sold by
ASAN PHARMACEUTICAL CO., LTD
Factory1 : 725, Dongtan-daero, Hwaseong-si, Gyeonggi-do, 18462, Korea
Factory2 : 122-26, Gieopdanji-ro, Gongdo-eup, Anseong-si, Gyeonggi-do, 17551, Korea