

## **Instructions for use**

# **AltoStar<sup>®</sup> Malaria PCR Kit 1.5**

10/2022 EN



# AltoStar<sup>®</sup>

## Malaria PCR Kit 1.5

For research use only!

(RUO)



AS0341503



96



10 2022



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## 1. Application

The AltoStar® Malaria PCR Kit 1.5 is a reagent system, based on real-time PCR technology, for the qualitative detection of DNA of *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae*, *Plasmodium ovale* and *Plasmodium knowlesi*.

**For research use only (RUO)! Not for use in diagnostic procedures.**

## 2. Kit content

The AltoStar® Malaria PCR Kit 1.5 contains the following components:

**Table 1:** Kit components

| Lid color | Component              | Number of tubes | Nominal volume [µl/tube] |
|-----------|------------------------|-----------------|--------------------------|
| Blue      | Master A <sup>1)</sup> | 8               | 60                       |
| Purple    | Master B <sup>1)</sup> | 8               | 180                      |
| Red       | PC <sup>2)</sup>       | 2               | 250                      |
| White     | NTC <sup>3)</sup>      | 2               | 250                      |

<sup>1)</sup> Contains biological material of animal origin

<sup>2)</sup> Positive Control

<sup>3)</sup> No Template Control (negative control)

The AltoStar® Malaria PCR Kit 1.5 contains enough reagents to perform 96 reactions.

### 3. Storage

- The AltoStar® Malaria PCR Kit 1.5 is shipped on dry ice. The components of the kit should arrive frozen. If one or more components are not frozen upon receipt, or if tubes have been compromised during shipment, contact Altona Diagnostics GmbH for assistance.
- All components should be stored at -25 °C to -15 °C upon arrival.
- Repeated thawing and freezing of master reagents (more than twice) should be avoided, as this might affect the performance of the assay.
- Storage at +2 °C to +8 °C should not exceed a period of 2 hours.
- Protect Master A and Master B from light.

### 4. Product description

The AltoStar® Malaria PCR Kit 1.5 is a reagent system, based on real-time PCR technology, for the qualitative detection of *Plasmodium* spp. (*Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae*, *Plasmodium ovale* and *Plasmodium knowlesi*) specific DNA.

Real-time PCR technology utilizes polymerase chain reaction (PCR) for the amplification of specific target sequences and target specific probes for the detection of the amplified DNA. The probes are labeled with fluorescent reporter and quencher dyes.

In addition to the *Plasmodium* spp. DNA specific amplification and detection systems the AltoStar® Malaria PCR Kit 1.5 includes oligonucleotides for the amplification and detection of an internal control (IC, AltoStar® Internal Control 1.5). For details refer to the instructions for use of the AltoStar® Internal Control 1.5.

Probes specific for *Plasmodium* spp. DNA are labeled with the fluorophore FAM™. The probe specific for the IC is labeled with a fluorophore (JOE™) detectable in the e.g. VIC™ channel.

Using probes linked to distinguishable dyes enables the parallel detection of *Plasmodium* spp. specific DNA and the IC in corresponding detection channels of the real-time PCR instrument.

## 4.1 Component description

Master A and Master B contain all components (PCR buffer, DNA polymerase, magnesium salt, primers and probes) to allow PCR mediated amplification and detection of *Plasmodium* spp. specific DNA and the IC (AltoStar® Internal Control 1.5) in one reaction setup.

The Positive Control (PC) contains *Plasmodium* spp. specific DNA. It is used to verify the functionality of the *Plasmodium* spp. specific amplification and detection systems.

The No Template Control (NTC) contains no *Plasmodium* spp. specific DNA but does contain the IC template. The NTC is used as negative control for the *Plasmodium* spp. DNA specific real-time PCR and indicates possible contamination of Master A and Master B.

## 4.2 Real-time PCR instruments

The AltoStar® Malaria PCR Kit 1.5 can be used with the following real-time PCR instruments:

- ABI Prism® 7500 SDS (Applied Biosystems)
- CFX96™ Deep Well Real-Time System (Bio-Rad)
- CFX96™ Real-Time System (Bio-Rad)
- LightCycler® 480 Instrument II (Roche)
- QuantStudio™ 5 Real-Time PCR System (Applied Biosystems)
- Rotor-Gene® Q5/6 plex Platform (QIAGEN)

### NOTE



Ensure that all instruments used have been installed, calibrated, checked and maintained according to the manufacturer's instructions and recommendations.

## 5. Material required but not provided

The following additional instruments and consumables are required for use of the AltoStar® Malaria PCR Kit 1.5 but not provided with this product:

- Appropriate real-time PCR instrument (see chapter 4.2 Real-time PCR instruments)
- Appropriate nucleic acid extraction system or kit (see chapter 6.1 Sample preparation)
- Vortex mixer
- Centrifuge (e.g. desktop centrifuge) for centrifugation of kit reagents
- Centrifuge for centrifugation of PCR plates
- Appropriate 96 well reaction plates or reaction tubes with corresponding (optical) closing material
- Pipettes (adjustable)
- Pipette tips with filters (disposable)
- Powder-free gloves (disposable)

Reagents required but not included in the AltoStar® Malaria PCR Kit 1.5:

- AltoStar® Internal Control 1.5 (Order No. IC15-06)

## 6. Procedure

### 6.1 Sample preparation

Extracted DNA is the starting material for the AltoStar® Malaria PCR Kit 1.5. The quality of the extracted DNA has a profound impact on the performance of the product.

For additional information and technical support regarding pre-treatment and sample preparation, contact Altona Diagnostics technical support (see chapter 9. Technical support).



## 6.2 Master mix setup

All reagents and samples should be thawed completely, mixed (by pipetting or gentle vortexing) and centrifuged briefly before use.

The AltoStar® Malaria PCR Kit 1.5 is configured for use with the AltoStar® Internal Control 1.5 (IC), which allows to control the sample preparation procedure (nucleic acid extraction) and the subsequent PCR.

1. Add the IC during the lysis step of the nucleic acid extraction procedure.

No matter which method/system is used for nucleic acid extraction, never add the IC directly to the sample. The IC should always be added to the sample/lysis buffer mixture. The volume of the IC which has to be added, always and only depends on the elution volume. It represents 50 % of the elution volume. For instance, if the nucleic acid is going to be eluted in 60 µl of elution buffer or water, 30 µl of IC per sample must be added into the sample/lysis buffer mixture.

2. Set up the master mix according to the following pipetting scheme:

**Table 2:** Pipetting scheme (master mix setup)

| Number of reactions (rxns) | 1            | 12            |
|----------------------------|--------------|---------------|
| Master A                   | 5 µl         | 60 µl         |
| Master B                   | 15 µl        | 180 µl        |
| <b>Volume master mix</b>   | <b>20 µl</b> | <b>240 µl</b> |

### NOTE



No matter which method/system is used for nucleic acid extraction, never add the IC directly to the specimen.

## 6.3 Reaction setup

1. Pipette 20 µl of the master mix into each required well of an appropriate optical 96-well reaction plate or an appropriate optical reaction tube.
2. Add 10 µl of the sample (eluate from the nucleic acid extraction) or 10 µl of the controls (PC or NTC).

**Table 3:** Pipetting scheme (reaction setup)

| Reaction setup      |              |
|---------------------|--------------|
| Master mix          | 20 µl        |
| Sample or control   | 10 µl        |
| <b>Total volume</b> | <b>30 µl</b> |

### NOTE



Do not add the IC to the PC reactions and the NTC.

3. Make sure that at least 1 PC and 1 NTC is used per run.
4. Thoroughly mix the samples and controls with the master mix by pipetting up and down.
5. Close the 96-well reaction plate with appropriate lids or optical adhesive film and the reaction tubes with appropriate lids.
6. Centrifuge the 96-well reaction plate in a centrifuge with a microtiter plate rotor for 30 seconds at approximately 1,000 x g (~ 3,000 rpm).

## 7. Programming the real-time PCR instrument

For basic information regarding the setup and programming of the different real-time PCR instruments, refer to the instructions for use of the respective instrument.

For detailed programming instructions regarding the use of the AltoStar® Malaria PCR Kit 1.5 on specific real-time PCR instruments, contact altona Diagnostics technical support (see chapter 9. Technical support).

## 7.1 Settings

Define the following basic settings:

**Table 4:** Run settings

| Settings           |         |
|--------------------|---------|
| Reaction volume    | 30 µl   |
| Ramp rate          | Default |
| Passive reference* | None    |

\* If applicable

## 7.2 Fluorescence detectors (dyes)

Define the following fluorescence detectors (dyes):

**Table 5:** Fluorescence detectors

| Target                              | Detector name    | Reporter | Quencher |
|-------------------------------------|------------------|----------|----------|
| <i>Plasmodium</i> spp. specific DNA | Plasmodium spp.  | FAM™     | (None)   |
| IC                                  | Internal Control | JOE™     | (None)   |

## 7.3 Temperature profile and dye acquisition

Define the following temperature profile and dye acquisition:

**Table 6:** Temperature profile and dye acquisition

|               | Stage   | Cycle repeats | Acquisition | Temperature [°C] | Time [min:s] |
|---------------|---------|---------------|-------------|------------------|--------------|
| Denaturation  | Hold    | 1             | -           | 95               | 02:00        |
| Amplification | Cycling | 45            | -           | 95               | 00:15        |
|               |         |               | Yes         | 58               | 00:45        |
|               |         |               | -           | 72               | 00:15        |

## 8. Data analysis

For basic information regarding data analysis on specific real-time PCR instruments, refer to the instructions for use of the respective instrument.

For detailed instructions regarding the analysis of the data generated with the AltoStar® Malaria PCR Kit 1.5 on different real-time PCR instruments, contact altona Diagnostics technical support (see chapter 9. Technical support).

### 8.1 Interpretation of results

#### 8.1.1 Qualitative analysis

**Table 7:** Qualitative analysis

| Detection channel                    |           | Result interpretation   |
|--------------------------------------|-----------|---|
| FAM™<br>( <i>Plasmodium</i><br>spp.) | JOE™ (IC) |   |
| +                                    | +/-*      | <i>Plasmodium</i> spp. specific DNA detected.   |
| -                                    | +         | No <i>Plasmodium</i> spp. specific DNA detected. Sample does not contain detectable amounts of <i>Plasmodium</i> spp. specific DNA. |
| -                                    | -         | PCR inhibition or reagent failure. Repeat testing from original sample or collect and test a new sample.                            |

\* Detection of the IC in the JOE™ detection channel is not required for positive results in the FAM™ detection channel. A high *Plasmodium* spp. DNA load in the sample can lead to a reduced or absent IC signal.

## 9. Technical support

For customer support, contact altona Diagnostics technical support:

**e-mail:** [support@altona-diagnostics.com](mailto:support@altona-diagnostics.com)

**phone:** +49-(0)40-5480676-0

## 10. Trademarks and disclaimers














AltoStar® (altona Diagnostics); ABI Prism®, QuantStudio™ (Applied Biosystems); CFX96™ (Bio-Rad); Rotor-Gene® (QIAGEN); LightCycler® (Roche); FAM™, JOE™, VIC™ (Thermo Fisher Scientific).



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## 11. Symbols

| Symbol  | Explanation  |
|---|--|
|    | Research use only                                  |
|    | Batch code   |
|    | Content  |
|    | Cap color  |
|    | Catalogue number                                   |
|    | Number   |
|    | Component  |
|    | Consult instructions for use                       |
|  | Contains sufficient for "n" tests/reactions (rxns) |
|  | Temperature limit                                  |
|  | Use-by date  |
|  | Manufacturer                                       |
|  | Material number                                    |

| Symbol  | Explanation  |
|---|--|
|  | Version  |
|  | Note: Information is given to the user that is useful but not essential to the task at hand. |

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**always a drop ahead.**

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