RealStar®

Chagas PCR Kit 1.0

For research use only!

(RUO)
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1. Application

The RealStar® Chagas PCR Kit 1.0 is a reagent system, based on real-time PCR technology, for the qualitative detection of Trypanosoma cruzi specific DNA.

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

2. Kit Components

<table>
<thead>
<tr>
<th>Lid Color</th>
<th>Component</th>
<th>Number of Vials</th>
<th>Volume [µl/Vial]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blue</td>
<td>Master A</td>
<td>8</td>
<td>60</td>
</tr>
<tr>
<td>Purple</td>
<td>Master B</td>
<td>8</td>
<td>180</td>
</tr>
<tr>
<td>Green</td>
<td>Internal Control</td>
<td>1</td>
<td>1000</td>
</tr>
<tr>
<td>Red</td>
<td>Positive Control</td>
<td>1</td>
<td>250</td>
</tr>
<tr>
<td>White</td>
<td>Water (PCR grade)</td>
<td>1</td>
<td>500</td>
</tr>
</tbody>
</table>

3. Storage

- The RealStar® Chagas PCR Kit 1.0 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 between -25°C and -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. The reagents should be frozen in aliquots, if they are to be used intermittently.
- Storage between +2°C and +8°C should not exceed a period of two hours.
- Protect Master A and Master B from light.
4. Product Description

The RealStar® Chagas PCR Kit 1.0 is a reagent system, based on real-time PCR technology, for the qualitative detection of Trypanosoma cruzi specific DNA. The assay includes a heterologous amplification system (Internal Control) to identify possible PCR inhibition and to confirm the integrity of the reagents of the kit.

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 labelled with fluorescent reporter and quencher dyes.

Probes specific for T. cruzi DNA are labelled with the fluorophore FAM™. The probe specific for the Internal Control (IC) is labelled with the fluorophore JOE™. Using probes linked to distinguishable dyes enables the parallel detection of T. cruzi specific DNA and the Internal Control in corresponding detector channels of the real-time PCR instrument.

The test consists of two processes in a single tube assay:

- PCR amplification of target DNA and Internal Control
- Simultaneous detection of PCR amplicons by fluorescent dye labelled probes

The RealStar® Chagas PCR Kit 1.0 consists of:

- Two Master reagents (Master A and Master B)
- Internal Control (IC)
- Positive Control
- PCR grade water

Master A and Master B contain all components (PCR buffer, DNA polymerase, magnesium salt, primers and probes) to allow PCR mediated amplification and target detection of T. cruzi specific DNA and Internal Control in one reaction setup.

4.1 Real-Time PCR Instruments

The RealStar® Chagas PCR Kit 1.0 can be used with the following real-time PCR instruments:

- Mx 3005P™ QPCR System (Stratagene)
- VERSANT® kPCR Molecular System AD (Siemens Healthcare)
- ABI Prism® 7500 SDS (Applied Biosystems)
- ABI Prism® 7500 Fast SDS (Applied Biosystems)
- Rotor-Gene® 6000 (Corbett Research)
- Rotor-Gene® Q5/6 plex Platform (QIAGEN)
- CFX96™ Real-Time PCR Detection System (Bio-Rad)
- CFX96™ Deep Well Real-Time PCR Detection System (Bio-Rad)
- LightCycler® 480 Instrument II (Roche)

NOTE

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

5. Procedure

5.1 Sample Preparation

The quality of the extracted DNA has a profound impact on the performance of the entire test system. It has to be ensured that the system used for nucleic acid extraction is compatible with real-time PCR technology. The following kits and systems are suitable for nucleic acid extraction:

- QIAamp® DNA Mini Kit (QIAGEN)
- QIAasympnomy® (QIAGEN)
5.2 Master Mix Setup

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

The RealStar® Chagas PCR Kit 1.0 contains a heterologous Internal Control (IC), which can either be used as a PCR inhibition control or as a control of the sample preparation procedure (nucleic acid extraction) and as a PCR inhibition control.

- If the IC is used as a PCR inhibition control, but not as a control for the sample preparation procedure, set up the Master Mix according to the following pipetting scheme:

<table>
<thead>
<tr>
<th>Number of Reactions (rxns)</th>
<th>1</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Master A</td>
<td>5 µl</td>
<td>60 µl</td>
</tr>
<tr>
<td>Master B</td>
<td>15 µl</td>
<td>180 µl</td>
</tr>
<tr>
<td>Internal Control</td>
<td>1 µl</td>
<td>12 µl</td>
</tr>
<tr>
<td>Volume Master Mix</td>
<td>21 µl</td>
<td>252 µl</td>
</tr>
</tbody>
</table>

- If the IC is used as a control for the sample preparation procedure and as a PCR inhibition control, add the IC during the nucleic acid extraction procedure. No matter which method/system is used for nucleic acid extraction, the IC must not be added 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 10% of the elution volume. For instance, if the nucleic acid is going to be eluted in 60 µl of elution buffer or water, 6 µl of IC per sample must be added into the sample/lysis buffer mixture.

- If the IC was added during the sample preparation procedure, set up the Master Mix according to the following pipetting scheme:

<table>
<thead>
<tr>
<th>Number of Reactions (rxns)</th>
<th>1</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Master A</td>
<td>5 µl</td>
<td>60 µl</td>
</tr>
<tr>
<td>Master B</td>
<td>15 µl</td>
<td>180 µl</td>
</tr>
<tr>
<td>Volume Master Mix</td>
<td>20 µl</td>
<td>240 µl</td>
</tr>
</tbody>
</table>
5.3 Reaction Setup

► 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.

► Add 10 µl of the sample (eluate from the nucleic acid extraction) or 10 µl of the controls (Positive or Negative Control).

<table>
<thead>
<tr>
<th>Reaction Setup</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Master Mix</td>
<td>20 µl</td>
</tr>
<tr>
<td>Sample or Control</td>
<td>10 µl</td>
</tr>
<tr>
<td>Total Volume</td>
<td>30 µl</td>
</tr>
</tbody>
</table>

► Make sure that at least one Positive and one Negative Control is used per run.

► Thoroughly mix the samples and controls with the Master Mix by pipetting up and down.

► Close the 96-well reaction plate with appropriate lids or optical adhesive film and the reaction tubes with appropriate lids.

► Centrifuge the 96-well reaction plate in a centrifuge with a microtiter plate rotor for 30 seconds at approximately 1000 x g (~ 3000 rpm).

6. Programming the Real-Time PCR Instrument

For basic information regarding the setup and programming of the different real-time PCR instruments, please refer to the user manual of the respective instrument. For detailed programming instructions regarding the use of the RealStar® Chagas PCR Kit 1.0 on specific real-time PCR instruments please contact our Technical Support (see chapter 8. Technical Assistance).

6.1 Settings

► Define the following settings:

<table>
<thead>
<tr>
<th>Settings</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Reaction Volume</td>
<td>30 µl</td>
</tr>
<tr>
<td>Ramp Rate</td>
<td>Default</td>
</tr>
<tr>
<td>Passive Reference</td>
<td>None</td>
</tr>
</tbody>
</table>

6.2 Fluorescence Detectors (Dyes)

► Define the fluorescence detectors (dyes):

<table>
<thead>
<tr>
<th>Target</th>
<th>Detector Name</th>
<th>Reporter</th>
<th>Quencher</th>
</tr>
</thead>
<tbody>
<tr>
<td>T. cruzi specific DNA</td>
<td>T. cruzi</td>
<td>FAM™</td>
<td>(None)</td>
</tr>
<tr>
<td>Internal Control</td>
<td>IC</td>
<td>JOE™</td>
<td>(None)</td>
</tr>
</tbody>
</table>
### 6.3 Temperature Profile and Dye Acquisition

Define the temperature profile and dye acquisition:

<table>
<thead>
<tr>
<th>Analysis Mode</th>
<th>Cycle Repeats</th>
<th>Acquisition</th>
<th>Temperature [°C]</th>
<th>Time [min:sec]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Denaturation</td>
<td>Hold</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td></td>
<td>95</td>
<td>02:00</td>
</tr>
<tr>
<td>Amplification</td>
<td>Cycling</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>45</td>
<td></td>
<td>95</td>
<td>00:15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>yes</td>
<td>58</td>
<td>00:45</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>72</td>
<td>00:15</td>
</tr>
</tbody>
</table>

### 7. Data Analysis

For basic information regarding data analysis on specific real-time PCR instruments, please refer to the user manual of the respective instrument.

For detailed instructions regarding the analysis of the data generated with the RealStar® Chagas PCR Kit 1.0 on different real-time PCR instruments please contact our Technical Support (see chapter 8. Technical Assistance).

#### 7.1 Interpretation of Results

##### 7.1.1 Qualitative Analysis

<table>
<thead>
<tr>
<th>Detection Channel</th>
<th>Result Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>FAM™</td>
<td>JOE™</td>
</tr>
<tr>
<td>+</td>
<td>+*</td>
</tr>
<tr>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* Detection of the Internal Control in the JOE™ detection channel is not required for positive results in the FAM™ detection channel. A high T. cruzi DNA load in the sample can lead to a reduced or absent Internal Control signal.

† Trypanosoma rangeli is a non-human pathogenic Trypanosoma species, having the same prevalence and transmission route as Trypanosoma cruzi. Due to the assay design Trypanosoma rangeli positive samples generate a positive signal in the FAM™ channel.
8. Technical Assistance
For technical advice, please contact our Technical Support:

e-mail: support@altona-diagnostics.com
phone: +49-(0)40-5480676-0

9. Trademarks and Disclaimers

RealStar® (altona Diagnostics); ABI Prism® (Applied Biosystems); ATCC® (American Type Culture Collection); CFX96™ (Bio-Rad); Cy® (GE Healthcare); FAM™, JOE™, ROX™ (Life Technologies); LightCycler® (Roche); Maxwell® (Promega); Mx 3005P™ (Stratagene); NucliSENS®, easyMag® (bioMérieux); Rotor-Gene®, QIAamp®, QIAsymphony® (QIAGEN); VERSANT® (Siemens Healthcare).

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10. Explanation of Symbols

RUO For research use only
LOT Batch code
CAP Cap color
REF Product number
CONT Content
NUM Number
COMP Component
VER Version
CONS Consult instructions for use
RF Contains sufficient for “n” tests/reactions (rxns)
TEMP Temperature limit
UD Use-by date
MAN Manufacturer
CAT Caution
REM Note
always a drop ahead.

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