1. Application

The RealStar® hMPV RT-PCR Kit 2.0 is a reagent system, based on real-time PCR technology, for the qualitative detection of human metapneumovirus (hMPV) specific RNA. Furthermore, the test allows the differentiation between hMPV subtype A (hMPV A) and hMPV subtype B (hMPV B) specific RNA.

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 hMPV A</td>
<td>1</td>
<td>250</td>
</tr>
<tr>
<td>Orange</td>
<td>Positive Control hMPV B</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® hMPV RT-PCR Kit 2.0 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® hMPV RT-PCR Kit 2.0 is a reagent system, based on real-time PCR technology, for the detection and differentiation of Human metapneumovirus A (hMPV A) and Human metapneumovirus B (hMPV B) specific RNA. The assay includes a heterologous amplification system (Internal Control) to identify possible RT-PCR inhibition and to confirm the integrity of the reagents of the kit.

Real-time RT-PCR technology utilizes reverse-transcriptase (RT) reaction to convert RNA into complementary DNA (cDNA), 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 hMPV A RNA are labelled with a fluorophore showing similar characteristics to Cy5™ whereas the probes specific for hMPV B RNA are labelled with the fluorophore FAM™. The probe specific for Internal Control (IC) is labelled with the fluorophore JOE™.

Using probes linked to distinguishable dyes enables the parallel detection of hMPV A and hMPV B specific RNA as well as the detection of the Internal Control in corresponding detector channels of the real-time PCR instrument.

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

- Reverse transcription of target and Internal Control RNA to cDNA
- PCR amplification of target and Internal Control cDNA
- Simultaneous detection of PCR amplicons by fluorescent dye labelled probes

The RealStar® hMPV RT-PCR Kit 2.0 consists of:

- Two Master reagents (Master A and Master B)
- Internal Control (IC)

4.1 Real-Time PCR Instruments

The RealStar® hMPV RT-PCR Kit 2.0 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

Extracted RNA is the starting material for the RealStar® hMPV RT-PCR Kit 2.0.

The quality of the extracted RNA 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® Viral RNA Mini Kit (QIAGEN)
- QIAasympohony® (QIAGEN)
- NuclisENS® easyMag® (bioMérieux)
- Magna Pure 96 System (Roche)
- m2000sp (Abbott)
- Maxwell® 16 IVI Instrument (Promega)
- VERSANT® kPCR Molecular System SP (Siemens Healthcare)

Alternative nucleic acid extraction systems and kits might also be appropriate.

If using a spin column based sample preparation procedure including washing buffers containing ethanol, it is highly recommended to perform an additional centrifugation step for 10 min at approximately 17000 x g (~13000 rpm), using a new collection tube, prior to the elution of the nucleic acid.

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® hMPV RT-PCR Kit 2.0 contains a heterologous Internal Control (IC), which can either be used as a RT-PCR inhibition control or as a control of the sample preparation procedure (nucleic acid extraction) and as a RT-PCR inhibition control.

► If the IC is used as a RT-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 RT-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><strong>Volume Master Mix</strong></td>
<td>20 µl</td>
<td>240 µl</td>
</tr>
</tbody>
</table>

**CAUTION**

If the IC (Internal Control) was added during the sample preparation procedure, at least the negative control must include the IC.

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

### 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 control (Positive or Negative Control).

<table>
<thead>
<tr>
<th>Reaction Setup</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Master Mix</strong></td>
</tr>
<tr>
<td><strong>Sample or Control</strong></td>
</tr>
<tr>
<td><strong>Total Volume</strong></td>
</tr>
</tbody>
</table>

- Make sure that each Positive Control and at least 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® hMPV RT-PCR Kit 2.0 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>Value</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>ROX™</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>hMPV A specific RNA</td>
<td>hMPV A</td>
<td>Cy5</td>
<td>(None)</td>
</tr>
<tr>
<td>hMPV B specific RNA</td>
<td>hMPV B</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>Stage</th>
<th>Cycle Repeats</th>
<th>Acquisition</th>
<th>Temperature [°C]</th>
<th>Time [min:sec]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reverse Transcription</td>
<td>Hold</td>
<td>1</td>
<td>-</td>
<td>55</td>
</tr>
<tr>
<td>Denaturation</td>
<td>Hold</td>
<td>1</td>
<td>-</td>
<td>95</td>
</tr>
<tr>
<td>Amplification</td>
<td>Cycling</td>
<td>45</td>
<td>-</td>
<td>95</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>yes</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-</td>
<td>72</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® hMPV RT-PCR Kit 2.0 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>Cy®5</td>
<td>+</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 either in the Cy®5 detection channel or in the FAM™ detection channel. High hMPV A and/or hMPV B RNA load/s in the sample can lead to reduced or absent Internal Control signals.

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®, QIAasyphony® (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
- **版**: Version
- **册**: Consult instructions for use
- **件**: Contains sufficient for “n” tests/reactions (rxns)
- **度**: Temperature limit
- **日**: Use-by date
- **生**: Manufacturer
- **警**: Caution
- **注**: Note
always a drop ahead.

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