Respiratory Syncytial Virus
Real Time RT-PCR Kit

Cat. No.: RR-0098-01

For Use with LightCycler2.0/LightCycler480 (Roche)
Real Time PCR Systems

For in vitro Diagnostic use only
User Manual

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1. Intended Use

RSV real time RT-PCR kit is used for the detection of respiratory syncytial virus in sputum or nasal and pharyngeal secretions by using real time PCR systems.

2. Introduction

Respiratory syncytial virus (RSV) is a negative-sense, enveloped RNA virus. It is the most common cause of bronchiolitis and pneumonia among infants and children under 1 year of age. Illness begins most frequently with fever, runny nose, cough, and sometimes wheezing. During their first RSV infection, between 25% and 40% of infants and young children have signs or symptoms of bronchiolitis or pneumonia, and 0.5% to 2% require hospitalization. RSV also causes repeated infections throughout life, usually associated with moderate-to-severe cold-like symptoms.

3. Principle of Real-Time PCR

The principle of the real-time detection is based on the fluorogenic 5’nuclease assay. During the PCR reaction, the DNA polymerase cleaves the probe at the 5’ end and separates the reporter dye from the quencher dye only when the probe hybridizes to the target DNA. This cleavage results in the fluorescent signal generated by the cleaved reporter dye, which is monitored real-time by the PCR detection system. The PCR cycle at which an increase in the fluorescence signal is detected initially (Ct) is proportional to the amount of the specific PCR product. Monitoring the fluorescence intensities during Real Time allows the detection of the accumulating product without having to re-open the reaction tube after the amplification.

4. Product Description

RSV real time RT-PCR kit contains a specific ready-to-use system for the detection of the RSV by Reverse Transcription Polymerase Chain Reaction (RT-PCR) in the real-time PCR system. The master contains a Super Mix for the specific amplification of RSV RNA. The reaction is done in one step real time RT-PCR. The first step is a reverse transcription (RT), during which RSV RNA is transcribed into cDNA. Afterwards, a thermostable DNA polymerase is used to amplify the specific gene
fragments by means of polymerase chain reaction (PCR). Fluorescence is emitted and measured by the real time systems’ optical unit during PCR. The detection of amplified RSV DNA fragment is performed in fluorimeter channel FAM. In addition, the kit contains a system to identify possible PCR inhibition by measuring the VIC/JOE fluorescence of the internal control (IC). An external positive control (1× 10^7 copies/ml) allows the determination of the gene load. For further information, please refer to section 10.3 Quantitation.

5. Kit Contents

<table>
<thead>
<tr>
<th>Ref.</th>
<th>Type of reagent</th>
<th>Presentation</th>
<th>25rxns</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>RSV Super Mix</td>
<td>1 vial, 220 µl</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>RT-PCR Enzyme Mix</td>
<td>1 vial, 28 µl</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Molecular Grade Water</td>
<td>1 vial, 400 µl</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Internal Control (IC)</td>
<td>1 vial, 30 µl</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>RSV Positive Control(1×10^7 copies/ml)</td>
<td>1 vial, 30 µl</td>
<td></td>
</tr>
</tbody>
</table>

6. Storage

- All reagents should be stored at -20°C. Storage at +4°C is not recommended.
- All reagents can be used until the expiration date indicated on the kit label.
- Repeated thawing and freezing (> 3x) should be avoided, as this may reduce the sensitivity of the assay.
- Cool all reagents during the working steps.
- Super Mix should be stored in the dark.

7. Additionally Required Materials and Devices

- Biological cabinet
- *Real time* PCR system
- Desktop microcentrifuge for “eppendorf” type tubes (RCF max. 16,000 x g)
- Vortex mixer
- RNA extraction kit
- *Real time* PCR reaction tubes/plates
• Cryo-container
• Pipets (0.5 μl – 1000 μl)
• Sterile filter tips for micro pipets
• Sterile microtubes
• Disposable gloves, powderless
• Biohazard waste container
• Refrigerator and freezer
• Tube racks

8. Warnings and Precaution

Carefully read this instruction before starting the procedure.

• For in vitro diagnostic use only.
• This assay needs to be carried out by skilled personnel.
• Clinical samples should be regarded as potentially infectious materials and should be prepared in a laminar flow hood.
• This assay needs to be run according to Good Laboratory Practice.
• Do not use the kit after its expiration date.
• Avoid repeated thawing and freezing of the reagents, this may reduce the sensitivity of the test.
• Once the reagents have been thawed, vortex and centrifuge briefly the tubes before use.
• Prepare quickly the Reaction mix on ice or in the cooling block.
• Set up two separate working areas: 1) Isolation of the RNA/ DNA and 2) Amplification/ detection of amplification products.
• Pipets, vials and other working materials should not circulate among working units.
• Use always sterile pipette tips with filters.
• Wear separate coats and gloves in each area.
• Do not pipette by mouth. Do not eat, drink, smoke in laboratory.
• Avoid aerosols
9. Sample Collection, Storage and transport

• Collected samples in sterile tubes;
• Specimens can be extracted immediately or frozen at -20°C to -80°C.
• Transportation of clinical specimens must comply with local regulations for the transport of etiologic agents

10. Procedure

10.1 RNA-Extraction

RNA extraction kits are available from various manufacturers. You may use your own extraction systems or the commercial kit based on the yield. For the RNA extraction, please comply with the manufacturer’s instructions. The recommended extraction kit is as follows:

<table>
<thead>
<tr>
<th>Nucleic Acid Isolation Kit</th>
<th>Cat. Number</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>RNA Isolation Kit</td>
<td>ME-0001</td>
<td>ZJ Biotech</td>
</tr>
<tr>
<td>QIAamp Viral RNA Mini extraction Kit (50)</td>
<td>52904</td>
<td>QIAGEN</td>
</tr>
</tbody>
</table>

10.2 Internal Control

It is necessary to add internal control (IC) in the reaction mix. Inhibition Control (IC) allows the user to determine and control the possibility of PCR inhibition. Add the internal control (IC) 1 μl/rxn and the result will be shown in the VIC/JOE channel.

10.3 Quantitation

The kit can be used for quantitative or qualitative real-time RT-PCR. A positive control ($1 \times 10^7$ copies/ml) is supplied in the kit.

For performance of quantitative real-time PCR, standard dilutions must be prepared first as follows. Molecular Grade Water is used for the diluent.

Dilution is not need for performance of qualitative real-time PCR.

Take positive control ($1 \times 10^7$ copies/ml) as the starting high standard in the first tube.
Respectively pipette 36ul of Molecular Grade Water into next three tubes. Do three dilutions as the following figures:

To generate a standard curve on the real-time system, all four dilution standards should be used and defined as standard with specification of the corresponding concentrations.

**Attention:**

A. Mix thoroughly before next transfer.

B. The positive control (1×10⁷ copies/ml) contains high concentration of the target DNA. Therefore, be careful during the dilution in order to avoid contamination.

**10.4 RT-PCR Protocol**

The Master Mix volume for each reaction should be pipetted as follows:
1) Depending upon the number of sample(n) the following pipetting scheme can be followed. (For reasons of unprecise pipetting, always add an extra virtual sample.)

<table>
<thead>
<tr>
<th>Reaction Volume</th>
<th>Master Mix Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>13μl Super Mix</td>
<td>13μl × (n+1)</td>
</tr>
<tr>
<td>1μl Enzyme Mix</td>
<td>1μl × (n+1)</td>
</tr>
<tr>
<td>1μl internal control (IC)</td>
<td>1μl × (n+1)</td>
</tr>
</tbody>
</table>

Mix completely then spin down briefly in a centrifuge.

2) Pipet 15 μl Master Mix with micropipets of sterile filter tips to each Real time PCR reaction plate/tubes. Separately add 5μl RNA sample template, positive and negative controls to different plate/tubes. Immediately close the plate/tubes to avoid contamination.

3) Spin down briefly in order to collect the Master Mix in the bottom of the reaction tubes.

4) Perform the following protocol in the instrument:
45°C for 10 min, 1 cycle;

95°C for 15 min, 1 cycle;

95°C for 5 sec, 60°C for 30 sec, 50 cycles.

Fluorescence is measured at 60°C

11. Data Analysis and Interpretation

The following results are possible:

1) A signal is detected in channel FAM. **The result is positive: The sample contains RSV RNA.**

In this case, the detection of a signal in channel VIC/JOE (Internal control) is dispensable, as high initial concentrations of RSV cDNA can lead to a reduced or absent fluorescence signal of the internal control (competition).

2) In channel FAM no signal is detected, at the same time, a VIC/JOE signal from the Internal Control appears. **The sample does not contain any RSV RNA. It can be considered negative.**

In the case of a negative RSV RT-PCR the detected signal of the internal control rules out the possibility of PCR inhibition.

3) Neither in channel FAM nor in channel VIC/JOE is a signal detected. **A diagnostic statement can not be made.** Inhibition of the RT-PCR reaction.

For further questions or problems, please contact our technical support at trade@liferiver.com.cn