Measles Virus Real Time RT-PCR Kit
Cat No: RR-0054-02

For use with ABI Prism®7000/7300/7500/7900; Smart CyclerII;
iCycler iQTM4/iQTM5; Rotor Gene®2000/3000; Mx3000P/3005P;
MJ-Option2/Chromo4 real time PCR systems.

For in vitro Diagnostic use only
User Manual

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1. Intended Use
Measles virus real time RT-PCR Kit is used for the detection of measles virus in nasal and pharyngeal secretion samples by real time PCR systems.

2. Introduction
Measles is one of the most contagious of all human viruses, with about forty million infections worldwide each year, and one to two million deaths. Measles outbreaks are common in underdeveloped countries where there is lower socioeconomic status, crowding, and low access to health care. In the third world, there may be up to 900,000 measles related deaths per year. Therefore, there is a lot of pressure on health in different countries in controlling the disease through vaccination. Measles causes rash, cough, and fever, and can lead to ear infection, pneumonia, conjunctivitis, diarrhea, seizures, brain damage, and death.

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
Measles virus real time RT-PCR kit contains a specific ready-to-use system for the detection of the measles virus 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 measles virus RNA. The reaction is done in one step real time RT-PCR. The first step is a reverse transcription (RT), during which the measles virus 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 the PCR. The detection of amplified measles virus DNA fragment is performed in fluorimeter channel FAM with the fluorescent quencher BHQ1. 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) contained, 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 25rxns</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Measles virus Super Mix</td>
<td>1 vial, 480µl</td>
</tr>
<tr>
<td>2</td>
<td>RT-PCR Enzyme Mix</td>
<td>1 vial, 28µl</td>
</tr>
<tr>
<td>3</td>
<td>Molecular Grade Water</td>
<td>1 vial, 400µl</td>
</tr>
<tr>
<td>4</td>
<td>Internal Control (IC)</td>
<td>1 vial, 30µl</td>
</tr>
<tr>
<td>5</td>
<td>Measles virus Positive Control(1×10(^{7}) copies/ml)</td>
<td>1 vial, 30µl</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.
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:
10.2 Internal Control
It is necessary to add internal control (IC) in the reaction mix. Internal Control (IC) allows the user to determine and control the possibility of PCR inhibition. Add the internal control (IC) 1µl/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 \((1 \times 10^7 \text{ copies/ml})\) is supplied in the kit.

For **performance of quantitative real-time PCR**, Standard dilutions must prepare first as follows. **Molecular Grade Water** is used for dilution.

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

Take positive control \((1 \times 10^7 \text{ copies/ml})\) 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 \((1 \times 10^7 \text{ copies/ml})\) 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 samples \( n \) 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>18( \mu )l Super Mix</td>
<td>18( \mu )l ( \times ) ( n+1 )</td>
</tr>
<tr>
<td>1( \mu )l Enzyme Mix</td>
<td>1( \mu )l ( \times ) ( n+1 )</td>
</tr>
<tr>
<td>1( \mu )l Internal Control (IC)</td>
<td>1( \mu )l ( \times ) ( n+1 )</td>
</tr>
</tbody>
</table>

Mix completely then spin down briefly in a centrifuge.

2) Pipet 20\( \mu \)l Master Mix with micropipets of sterile filter tips to each Real time PCR reaction plate/tubes. Separately add 5\( \mu \)l RNA sample, positive and negative controls to each reaction 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 15 sec, 60°C for 60sec, 40 cycles.

Fluorescence is measured at 60°C

5) If you use ABI Prism® system, please choose “none” as passive reference and quencher.

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 measles virus RNA.

In this case, the detection of a signal in channel VIC/JOE (Internal control) is dispensable, as high initial
concentrations of measles virus 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 measles virus RNA. It can be considered negative.**

   In the case of a negative measles virus 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**