1. Intended Use

Rift Valley Fever Virus (RVFV) real time RT-PCR kit is used for the detection of RVFV in serum sample by using the real time PCR systems.

2. 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 is directly proportional to the amount of the specific PCR product. Monitoring the fluorescence intensities in real-time allows the detection of the accumulating product without having to re-open the reaction tube after the amplification.

3. Product Description

Rift Valley fever (RVF) is a viral zoonosis that primarily affects animals but also has the capacity to infect humans. Infection can cause severe disease in both animals and humans, leading to high rates of disease and death. The disease also results in significant economic losses due to death and abortion among RVF-infected livestock.

4. Kit Contents

- Super Mix should be stored in the dark.
- Repeated thawing and freezing (> 3x) should be avoided, as this may reduce the sensitivity of the test.
- All reagents can be used until the expiration date indicated on the kit label.

5. 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 freezing and thawing (> 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 at −20°C.

6. Additionally Required Materials and Devices

- Biological cabinet
- Vortex mixer
- Pipets, vials and other working materials should not be stored in a laminar flow hood.
- 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.

7. Analysis sensitivity

- 5 copies/ml

8. PCR system without HEX/VIC/JOE channel may be used with [1x Molecular Grade Water instead of [ul Ref].

9. Procedure

9.1 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:

**Nucleic Acid Isolation Kit**

<table>
<thead>
<tr>
<th>Cat. Number</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>ME-0010/ME-0012</td>
<td>ZJ Biotech</td>
</tr>
</tbody>
</table>

9.2 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.

9.3 Quantification

The kit can be used for quantitative or qualitative real-time RT-PCR.

- A. The positive control contains +10 copies/ml of Molecular Grade Water into next three tubes. Do three dilutions as the following figures:

**Dilution of Standards**

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Amount</th>
<th>Fluorescence</th>
</tr>
</thead>
<tbody>
<tr>
<td>40 copies/ml</td>
<td>2 μl</td>
<td>100%</td>
</tr>
<tr>
<td>20 copies/ml</td>
<td>4 μl</td>
<td>50%</td>
</tr>
<tr>
<td>10 copies/ml</td>
<td>8 μl</td>
<td>25%</td>
</tr>
</tbody>
</table>

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

**Attention:**

- Please use ABI Prism® 7000/7500/7700/StepOne Plus; (ICycler IQ®) 4iQ-S Smart Cycler II/Bio-Rad CFX 360/Rotor Gene® 6000; Mx3000P/3005P; MJ-Option2/Chromo4; LightCycler®480 instrument.

**For use with ABI Prism®7000/7500/7300/7500/7900/Step One Plus; (ICycler IQ®) 4iQ-S Smart Cycler II/Bio-Rad CFX 360/Rotor Gene® 6000; Mx3000P/3005P; MJ-Option2/Chromo4; LightCycler®480 instrument.**

**Manufacturer**

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