1. Intended Use
Zika Virus (ZIKV) Real Time RT-PCR Kit is used for the detection of Zika virus in serum or plasma by using real time RT-PCR. The principle of real-time PCR is based on the fluorogenic 5' nuclelease 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 by the real time systems’ optical unit during the PCR. The PCR cycle at which an increase in the fluorescence signal is detected initially is proportional to the amount of the specific PCR product.

2. Principle of Real-Time PCR
The principle of the real-time detection is based on the fluorogenic 5’ nuclelease 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 by the real time systems’ optical unit during the PCR. The PCR cycle at which an increase in the fluorescence signal is detected initially is 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
Zika virus is enveloped andicosahedral with a non-segmented, single-stranded, positive sense RNA genome. It is most closely related to the Spondweni virus, which is one of the two viruses in the Spondweni virus clade. The virus was first isolated in 1947 from a rhesus monkey in the Zika Forest of Uganda, Africa and was isolated for the first time from human in 1968 in Nigeria. Common symptoms of infection with the virus include mild headaches, pruritis, arthralgia, and conjunctivitis, and arthralgia. In 2009, it was proved that Zika virus can be sexually transmitted between humans.

4. Kit Contents
- ZIKV Super Mix
- RT-PCR Enzyme Mix
- Molecular Grade Water
- Internal Control (IC)
- ZIKV Positive Control (>10 copies/ml)

Analysis sensitivity: 5x10^4 copies/ml LOQ: 1x10^4~1x10^6 copies/ml
Note: Analysis sensitivity depends on the sample volume, elution volume, nucleic acid extraction methods and other factors. If you use the RNA extraction kits recommended, the analysis sensitivity in the same as it declares. However, when the sample volume is dozens or even hundreds of times greater than elution volume by some concentrating method, it can be much higher.

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 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.

6. Additionally Required Materials and Devices
- Biological safety cabinet
- Real time PCR system
- Desktop microcentrifuge
- Pipettors (0.5 μl – 1000 μl)
- Sterile filter tips for micro pipettes
- Sterile microtubes
- Disposable gloves, powderless
- Biohazard waste container
- Pipettor and filter
tube racks

7. 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.
- This assay repeated thawing and freezing of the reagents, this may reduce the sensitivity of the test.
- Once the reagents have 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 2) Amplification detection of amplification products.
- Pipets, vials and other working materials should not circulate among working units.
- Use always sterile pipette tips with filter.
- Wear separate coats and gloves in each area.

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

A. Use thoroughly before next transfer.
B. The positive control contains high concentration of the target DNA. Therefore, be careful during the dilution in order to avoid contamination.

9.4 RT-PCR Protocol

The Master Mix volume for each reaction should be pipetted as follows:

<table>
<thead>
<tr>
<th>No</th>
<th>PCR System</th>
<th>Volume (μl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>1 μl</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>1 μl</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>1 μl</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>1 μl</td>
</tr>
</tbody>
</table>

10. Threshold setting: Choose Arithmetic as back ground and none as Noise Band method, then adjust the Noise band just above the maximum level of molecular grade water, and adjust the threshold just under the minimum of the positive control.

11. Calibration for quantitative detection: Input each concentration of standard controls at the end of run, and a standard curve will be automatically formed.

12. Quality control: Negative control, positive control, internal control and QS curve must be performed correctly, otherwise the sample results is invalid.

13. Data Analysis and Interpretation
The following results are possible:

<table>
<thead>
<tr>
<th>Crossing point value</th>
<th>Result Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;25</td>
<td>Positive (qualitative assay)</td>
</tr>
<tr>
<td>25~35</td>
<td>Negative (qualitative assay)</td>
</tr>
<tr>
<td>&gt;35</td>
<td>Negative (qualitative assay)</td>
</tr>
<tr>
<td>PCR inhibition; no diagnosis can be concluded.</td>
<td></td>
</tr>
</tbody>
</table>

For further details or technical support, please contact trade@liferiver.com.cn