8. 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.

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:

<table>
<thead>
<tr>
<th>Nucleic Acid Isolation Kit</th>
<th>Cat. Number</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>QIAamp Viral RNA Mini extraction Kit</td>
<td>ME-0010/ME-0012</td>
<td>ZJ Biotech</td>
</tr>
</tbody>
</table>

9.2 Real-Time PCR
PCR systems or the commercial kit based on the yield.

For performance of quantitative real-time PCR, standard dilution must be prepared as first as possible. 

For use with ABI Prism™7000/7300/7500/7900/Step One Plus; /Cycler IQ™48™; 5
Smart Cycler II;Bio-Rad CFX 96;Rotor Gene™6000; Mx3000P/3005P;MJ-Option2/Chromo4; LightCycler™480 instrument

1. Intended Use
CSFV real time RT-PCR kit is used for the detection of CSFV in serum, plasma or animal tissue samples by 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 if 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 is proportionate 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
CSFV (previously called hog cholera virus) is a member of the family Flaviviridae, genus Pestivirus. The virions are spherical, 50 nm in diameter, and consist of a tightly adherent lipid envelope covered with indistinct peplomers surrounding a spherical nucleocapsid with probable icosahedral symmetry. The single-stranded RNA (ssRNA) of the virus is infective and is about 12.5 kb long. Although CSFV can replicate in non-primate cells, porcine kidney cells are used most frequently for virus growth. Classical swine fever (CSF) is an economically important contagious disease of swine worldwide. It was first recognized in Ohio in 1933. The disease occurs in much of Asia, Central and South America, countries of Europe and Africa. Many countries are free of the disease among which are Australia, Canada, Ireland, New Zealand, Scandinavian countries, Switzerland, and the United States.

4. Kit Contents

5. Storage
All reagents should be stored at -20°C. Storage at 4°C is not recommended.

6. Additional Required Materials and Devices
- Biological cabinet
- Trypsin digestion Solution
- Real time PCR reaction tubes/plates
- Pipets (0.5 µl - 1000 µl)
- Sterile microtubes
- Biobased waste container
- Barcode scanner
- Rack and 96-well microplates

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 data.
- Avoid repeated thawing and freezing of the reagents, which 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.

8. Dilution Standards

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

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

11. Data Analysis and Interpretation
The following sample results are possible:

<table>
<thead>
<tr>
<th>Cq value</th>
<th>Result Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>FAM</td>
<td>HEX/VIC/IOE</td>
</tr>
<tr>
<td>UNDET</td>
<td>25-35</td>
</tr>
</tbody>
</table>

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