1. Intended Use
Respiratory adenovirus Real time PCR kit is used for the detection of adenovirus in sputum, gargle, nasopharyngeal swab or blood samples by real time PCR. This clearance statement is based on the fluorescent signal generated by the cleared reactor dye, which is monitored in real-time by the PCR detection system. PCR cycle at which an increase in the fluorescence signal is detected (initial Cq) 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.

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 PCR. This cleavage results in the fluorescent signal generated by the cleared reporter dye, which is monitored in real-time by the PCR detection system. The PCR cycle at which an increase in the fluorescence signal is detected (initial Cq) 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.

3. Product Description
Human adenoviruses are classified into 47 serotypes and six subgroups (A-F) with different tropism. Among the 47 serotypes, the clinically important Ad species are those associated with febrile respiratory disease (AdB1, AdC, and AdE) since these can cause widespread outbreaks with severe clinical symptoms, including viral pneumonia and death.

Respiratory adenovirus real time PCR kit contains a specific ready-to-use system for the detection of adenovirus by polymerase chain reaction (PCR) in the real-time PCR system. The master contains the conventional assay and enhancers for the specific amplification of the adenovirus DNA includes types 1.2 and 5. Fluorescence is emitted and measured by the real time systems’ optical unit during PCR. The detection of amplified Adenovirus DNA fragment is performed in fluorimeter channel FAM with the fluorescence quencher BHQ1. DNA extraction buffer is available in the kit and stored at -20°C. Nasopharyngeal swab or blood samples are used for DNA extraction.

4. Kit Contents

- 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.
- Reaction mix should be stored in the dark.

5. Additional Required Materials and Devices

- *B* DNA Extraction Buffer
- *P* R-ADV Reaction Mix
- *E* PCR Enzyme Mix
- *G* Molecular Grade Water
- *I* Internal Control (IC)
- *C* R-ADV Positive Control(10×copies/ml)

**Analysis sensitivity:** 5×10² copies/ml

**LOQ:** 1×10⁻¹⁸ to 1×10⁻⁰ copies/ml

Note: Analysis sensitivity depends on the sample volume, elution nucleic acid extraction methods and other factors. If you use the DNA extraction buffer in the kit, the analysis sensitivity is 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.

6. Storage

- All reagents should be stored at -20°C. Storage at 4°C is not recommended.
- Repeated thawing and freezing (≥3x) should be avoided, as this may reduce the sensitivity of the assay.
- Cool all reagents during the working steps.
- Reaction mix should be stored in the dark.

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 handled 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, which may reduce the sensitivity of the test.
- Once the reagents have thawed, vortex and centrifuge briefly the tubes before use.
- To reduce reproducibility errors, use the same batch of reagents and controls.
- Set up separate working areas: 1) Isolation of the DNA/ RNA and 2) Amplification/ detection of amplification products.
- Pipets, vials and other working materials should not circulate among working units.
- Use separate sterile pipets tips with the same identification code.
- Use separate working stations.
- Wear separate coats and gloves in each area.

8. Sample Collection, Storage and Transportation

- Collect samples in sterile tubes.
- The 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 diagnostic agents.

9. Procedure

9.1 DNA Extraction

DNA extraction buffer is supplied in the kit, please thaw the buffer thoroughly and spin down briefly in the centrifuge before use. It is better to use commercial kits for nucleic acid extraction.

9.1.1 Sample preparation

1) Trypsin Digestion Solution Preparation

Add 10g trypsin to 200ml sterilized purified water and mix thoroughly. Adjust the pH value to 8.0 with 25%NaOH solution. Add 2.5mlmL/CaCl₂, mix thoroughly and store at 4°C. Please incubate at 37°C for 10 minutes before use.

2) Estimate the volume of the spumnum and add parts aeques of the Trypsin Digestive Solution then vortex vigorously. Set at room temperature for 30 minutes. Transfer 0.5ml mixture to a new tube. Centrifuge the tube at 13000rpm for 5 minutes, carefully remove and discard supernatant from the tube without disturbing the pellet.

3) Add 1.0ml normal saline. Resuspend the pellet with vortex vigorously, Centrifuge the tube at 1300rpm for 5 minutes. Carefully remove and discard supernatant from the tube without disturbing the pellet.

4) Repeat step 3.

5) Add 50µl DNA extraction buffer, close the tube then resuspend the pellet with vortex vigorously.

6) Spin down briefly in a table centrifuge.

7) Centrifuge the tube at 13000rpm for 5 minutes. The supernatant contains DNA extracted and can be used for PCR template.

9.1.2 Blood sample

1) Take 2ml anticoagulant, and transfer the plasma layer and buffy-coat layer to another tube after it is naturally stratified.

2) Add 50µl DNA extraction buffer into the tube, and close the tube then vortex for 10 seconds. Spin down briefly in a table centrifuge.

3) Incubate the tube for 10 minutes at 100°C. Centrifuge the tube at 13000rpm for 5 minutes. The supernatant contains DNA extracted and can be used for PCR template.

9.1.3 Gargle and nasopharyngeal swab sample

1) Take 1ml sample in a tube, centrifuge the tube at 13000rpm for 2min, and remove the supernatant and keep the pellet. Add 50µl DNA extraction buffer into the tube, close the tube then vortex for 10 seconds. Spin down briefly in a table centrifuge.

2) Incubate the tube for 10 minutes at 100°C.

3) Centrifuge the tube at 13000rpm for 5 minutes. The supernatant contains DNA extracted and can be used for PCR template.

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.

- Add the internal control (IC) 1µl/xen and the result will be shown in the HEX/VIC/JOE channel.

9.3 Quantitation

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

For performance of quantitative real-time PCR, Standard dilutions must prepare first as follows.

**Molar Grade Water** is used for dilution.

The step of dilution is not needed for performance of qualitative real-time PCR.

- Take positive control (1×10⁵/copies/ml) as the starting high standard in the first tube. Respectively pipette 36µl of Molecular Grade Water into next three tubes. Do three dilutions as the following graph.

**Dilution of Standards**

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Copies/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/10⁵</td>
<td>1.0×10⁵</td>
</tr>
<tr>
<td>1/10⁴</td>
<td>1.0×10⁴</td>
</tr>
<tr>
<td>1/10³</td>
<td>1.0×10³</td>
</tr>
<tr>
<td>1/10²</td>
<td>1.0×10²</td>
</tr>
<tr>
<td>1/10¹</td>
<td>1.0×10¹</td>
</tr>
<tr>
<td>1/10⁰</td>
<td>1.0×10⁰</td>
</tr>
</tbody>
</table>

**PCR system without HEX/VIC/JOE channel** may be treated with 1µl Molecular Grade Water instead of 1µl IC.

- The volumes of Reaction Mix and Enzyme Mix per reaction multiply with the number of samples, which includes the number of the controls. standards and sample prepared. Molecular Grade Water is used as the negative control. For reasons of unprecise pipetting, always add an extra virtual sample, (the number of reaction ) Mix completely then spin down briefly in a centrifuge.

- Take 5µl 50µl (2:52 for SmartCycler II) Master stock and mix gently, then add the sample tips to each Real Time PCR reaction plate/ tube. Then separately add 4µl (2.5µl for SmartCycler II) DNA sample, positive and negative controls to different reaction plates/tubes. Immediately close the plate/tubes to avoid contamination.

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

- Perform the following protocol in the instrument:

  1. 75°C for 2min
  2. 94°C for 2min
  3. 94°C for 15sec, 60°C for 1min
  4. 1cycle

  **Threshold setting:** just above the maximum level of molecular grade water.

- Calibration for quantitative detection: Input each concentration of standard controls at the end of each cycle. The standard curve will be automatically formed.

- 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 sample results are possible:**

- **Cq value**
- **Result Analysis**

<table>
<thead>
<tr>
<th><strong>Cq</strong></th>
<th><strong>Cq value</strong></th>
<th><strong>Result Analysis</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>UNDET</td>
<td>Below the detection limit or negative</td>
</tr>
<tr>
<td>29</td>
<td>≤3.98</td>
<td>Positive, and the software displays the quantitative value.</td>
</tr>
<tr>
<td>38</td>
<td>3.98–40</td>
<td>Quantitative Detection. Correlation coefficient of QS curve = 0.98</td>
</tr>
<tr>
<td>40</td>
<td>UNDET</td>
<td>Positive Control(qualitative assay) ≤3.98</td>
</tr>
<tr>
<td>41</td>
<td>UNDET</td>
<td>Quality Control (Quantitative detection) ≤3.98</td>
</tr>
</tbody>
</table>

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