1. Intended Use

By using real time PCR systems, chlamydia pneumoniae real time PCR kit is used for the detection of chlamydia pneumoniae in samples like nasal and pharyngeal secretions and swabs, sputum, provoked sputum, bronchial lavage, lung biopsy) are used for DNA extraction. In addition, the kit DNA extraction buffer is available in the kit and samples (e.g. nasal and pharyngeal secretions, sputum, provoked sputum, bronchial lavage, lung biopsy etc.) can be used for PCR template.

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 fluorescence 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 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

Chlamydia pneumoniae is a species of chlamydiae bacteria that infects humans and is a major cause of pneumonia. The full genome sequence for Chlamydia pneumoniae was published in 1999. Chlamydia pneumoniae also infects and causes disease in Koalas, emerald tree boa (Corallus caninus), iguanas, chameleons, frogs, and turtles. Chlamydia pneumoniae is a common cause of pneumonia around the world. Chlamydia pneumoniae is typically acquired by otherwise healthy people and is a form of community-acquired pneumonia. Chlamydia pneumoniae real time PCR Kit contains a specific ready-to-use system for the detection of the Chlamydia Pneumoniae by polymerase chain reaction (PCR) in the real-time PCR system. The master contains reagents and enzymes for the specific amplification of Chlamydia Pneumoniae DNA. Fluorescence is emitted and measured by the real time systems’ optical unit during the PCR. The detection of amplified Chlamydia Pneumoniae DNA fragment is performed in fluorometer channel 530nm with the fluoroscope buffer BHQ1. DNA extraction buffer is available in the kit and samples (e.g. nasal and pharyngeal secretions, sputum, provoked sputum, bronchial lavage, lung biopsy etc.) can be used for PCR template.

4. Kit Contents

<table>
<thead>
<tr>
<th>Ref.</th>
<th>Type of reagent</th>
<th>Presentation 25μml</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>DNA extraction buffer</td>
<td>2 vials, 1.5mL</td>
</tr>
<tr>
<td>2</td>
<td>CP Reaction Mix</td>
<td>1 vial, 450μl</td>
</tr>
<tr>
<td>3</td>
<td>PCR Enzyme Mix</td>
<td>1 vial, 12μl</td>
</tr>
<tr>
<td>4</td>
<td>Molecular Grade Water</td>
<td>1 vial, 40μl</td>
</tr>
<tr>
<td>5</td>
<td>Internal Control (IC)</td>
<td>1 vial, 30μl</td>
</tr>
<tr>
<td>6</td>
<td>CP Positive control (1×10⁴ copies/ml)</td>
<td>1 vial, 30μl</td>
</tr>
</tbody>
</table>

Analysis sensitivity: 1×10⁴ copies/ml

Note: Analysis sensitivity depends on the sample volume, elution volume, 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.

5. Storage

- All reagents should be stored at -20°C. Storage at +4°C is not recommended.
- All reagents can be used after 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.

6. Additionally Required Materials and Devices

- Biological cabinet
- Real time PCR system
- Desktop microcentrifuge for “eppendorf” type tubes (RCP max. 16,000 x g)
- Vortex mixer
- Real time PCR reaction tubes/plates
- Cryo-container
- Pipets (0.5μl – 1000μl) 
- Sterile filter tips for micro pipets
- Sterile microtubes
- Disposable gloves, powderless
- Biobased waste container
- Refrigerator and freezer
- Tube racks

7. Usage and Preparation

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.
- Only 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.
- Avoid aerosols.

8. Sample Collection, Storage and transport

- Collect 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

9. Procedure

9.1 DNA-Extraction

DNA extraction buffer is supplied in the kit.

Attention: please thaw the buffer thoroughly and mix the buffer well before use because it contains insoluble particles. You may use your own extraction systems or commercial kits.

9.1.1 Spontum sample

1) Trypsin digestive Solution preparation

Add 10g trypsin to 200ml sterile purified water and mix thoroughly. Adjust the PH value to 8.0 with 2% NaOH solution. Add 1.25% L-CaCl₂, mix thoroughly and store at 4°C.

Please incubate at 37°C for 10 minutes before use.

2) Estimate the volume of the spumum and add parts aqueous 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 10ml normal saline. Resuspend the pellet with vortex vigorously. Centrifuge at 13000rpm for 5 minutes. Carefully remove and discard supernatant from the tube without disturbing the pellet.

4) Repeat step 3)

5) Add 10μl DNA extraction buffer, close the tube then resuspend the pellet with vortex vigorously.

Spin down briefly in a table centrifuge.

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

7) Centrifuge the tube at 13000rpm for 10 minutes. The supernatant contains the DNA extracted and can be used for PCR template.

9.1.2 Fluid samples (nasal and pharyngeal secretions, and etc.)

1) Take 1ml sample in a tube, centrifuge the tube at 13000rpm for 2min, and remove the supernatant and keep the pellet.

2) Add 10μl DNA extraction buffer to the pellet, 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.

4) Centrifuge the tube at 13000rpm for 10 minutes. The supernatant contains the DNA extracted and can be used for PCR template.

9.1.3 Tissue and swabs sample

1) Wash the sample (lung biopsy or swabs) in 0.5ml normal saline and vortex vigorously. Centrifuge the tube at 13000rpm for 2 minutes. Carefully remove and discard supernatant from the tube without disturbing the pellet.

2) Add 10μl DNA extraction buffer to the tube, close the tube then vortex for 10 seconds.

3) Incubation the tube for 10 minutes at 100°C.

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

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/μl and the result should be shown in the 560nm Channel.

9.3 PCR Protocol

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

1) Take 1ml sample in a tube, centrifuge the tube at 13000rpm for 2min, and remove the supernatant and keep the pellet.

2) Pipet 1μl Master Mix with micropipets of sterile filter tips to each Real time PCR reaction plates/tubes. Then separately add 3μl DNA sample supernatant, positive and negative controls to different plates/tubes. Immediately close the plates/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:

- 37°C for 2min 1cycle
- 95°C for 4min 1cycle
- 95°C for 5sec, 60°C for 30sec 40cycles
- Fluorescence measured at 60°C

5) Selection of fluorescence channels

<table>
<thead>
<tr>
<th>Channel</th>
<th>Selection of fluorescence channels</th>
</tr>
</thead>
<tbody>
<tr>
<td>530nm</td>
<td>Target Nuclear Acid</td>
</tr>
<tr>
<td>560nm</td>
<td>K</td>
</tr>
</tbody>
</table>

10. Threshold setting: Choose Arithmetic as back ground and noise as Noise Band method, then adjust the Noise band just above the maximum level of molecular grade water, and adjust the threshold just below the minimum of PCR cycle.

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

12. Data Analysis and Interpretation

The following sample results are possible:

<table>
<thead>
<tr>
<th>Crossing point value</th>
<th>Result Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 35</td>
<td>Blank</td>
</tr>
<tr>
<td>35&lt; ≤38</td>
<td>Below the detection limit or negative</td>
</tr>
<tr>
<td>38 ≤ 40</td>
<td>Positive</td>
</tr>
<tr>
<td>40 ≥ 45</td>
<td>Positive</td>
</tr>
<tr>
<td>≥ 45</td>
<td>Blank</td>
</tr>
<tr>
<td></td>
<td>PCR Inhibition, No diagnosis can be concluded</td>
</tr>
</tbody>
</table>

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