**Streptococcus Pneumoniae Real Time PCR Kit User Manual**

**For In vitro Diagnostic Use Only**

**EC REP**

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### 1. Intended Use

By using real time PCR systems, Streptococcus pneumoniae real time PCR kit is used for the detection of Streptococcus pneumoniae in samples like nasal and pharyngeal secretions, sputum, pleural fluid, bronchial lavage, tracheal aspirate and biopsy etc.

**2. Principle of Real-Time PCR**

The principle of the 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 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 initially (Ct) 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**

Streptococcus pneumoniae, or pneumococcus, is gram-positive, alpha-hemolytic, bile soluble, aerotolerant anaerobe and a member of the Streptococcus genus. The pneumococcus, or pneumococcus, is a member of the Streptococcus pneumoniae, or pneumococcus, is a member of the Streptococcus pneumoniae genus. The organism causes many types of pneumococcal infection other than pneumonia, including acute sinusitis, otitis media, menigitis, bacteremia, sepsis, osteomyelitis, septic arthritis, endocarditis, perinitis, pericarditis, cellulitis, and brain abscess. It is the most common cause of bacterial meningitis in adults and children.

Streptococcus pneumoniae real time PCR Kit contains a specific ready-to-use system for the detection of the Streptococcus pneumoniae by polymerase chain reaction (PCR) in the real-time PCR system. The PCR contains reagents and enzymes for the specific amplification of the Streptococcus pneumoniae DNA. Fluorescence is emitted and measured by the real time systems’ optical unit during the PCR. The detection of amplified Streptococcus pneumoniae DNA fragment is performed in fluorescence channel FAM™ with the fluorescence quencher BHQ1. DNA extraction buffer is available in the kit. An external positive control (1x10⁰ copies/ml) allows the determination of the gene load. For further information, please refer to section 2.9 Quantity.

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### 4. Kit Contents

<table>
<thead>
<tr>
<th>Ref.</th>
<th>Type of Reagent</th>
<th>Presentation</th>
<th>25 rxns</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>DNA Extraction Buffet</td>
<td>2 vials, 1.5mL</td>
<td>25 x 1 x 1.5mL</td>
</tr>
<tr>
<td>2</td>
<td>S. pneumoniae Enzyme Mix</td>
<td>1 vial, 959µl</td>
<td>25 x 1 x 959µl</td>
</tr>
<tr>
<td>3</td>
<td>PCR Enzyme Mix</td>
<td>1 vial, 12µl</td>
<td>25 x 1 x 12µl</td>
</tr>
<tr>
<td>4</td>
<td>Molecular Water</td>
<td>1 vial, 40µl</td>
<td>25 x 1 x 40µl</td>
</tr>
<tr>
<td>5</td>
<td>S. pneumoniae Positive Control(1x10⁰ copies/ml)</td>
<td>1 vial, 30µl</td>
<td>25 x 1 x 30µl</td>
</tr>
</tbody>
</table>

**Analysis sensitivity**: 5x10⁰ copies/ml

**LOQ**: 1x10⁰ ~ 1x10¹ 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.

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### 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.
- Reaction Mix should be stored in the dark.

**6. Additionally Required Materials and Devices**

- Biological cabinet
- Vortex mixer
- Cyro-container
- Sterile filter tips for micro pipets
- Disposable gloves, non-latex
- Refrigerator and freezer
- Desktop microcentrifuge for “eppendorf” type tubes (e.g., Max 16,000 x g)

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### 7. Instructions and Precautions

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.
- Once the reagents have been thawed, vortex and centrifuge briefly the tubes before use.
- Reuses of the same ice or ice cubes should be avoided.
- Use set up two separate working areas: 1) Isolation of the RNA/ DNA and 2) Amplification/ detection of amplification products.
- Use always sterile pipette tips with filters.
- Wear separate coats and gloves in each area.
- Avoid aerosols.

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### 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 biological agents.

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### 9. Procedure

**DNA Extraction**

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

**1.1 Sputum sample**

1. Trypsin digestive dilution preparation

Add 10g trypsin to 200ml sterile purified water and mix thoroughly. Adjust the pH value to 8.0 with 5%NaOH solution. Add 2mL 25mMol/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 supernatant and add paraffin oil of the trypsin digestive solution then vortex vigorously. Set at room temperature for 30 minutes. Transfer 0.5mlmixture 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 0.1ml 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 50µl DNA extraction buffer, closed 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 5 minutes. The supernatant contains the DNA extracted and can be used as PCR template.

**1.2 Fluid samples (Pleural effusion, and etc.)**

Take 40µl (1ml for water sample) in a tube, centrifuge the tube at 13000rpm for 2min, and remove the supernatant and keep the pellet.

3) Add 100µl DNA extraction buffer to the tube, closed the tube then vortex for 10 seconds.

4) Incubate the tube for 100°C for 5 minutes. The supernatant contains the extracted DNA and can be used for the template of the PCR.

**Attention:**

A. During the incubation, make sure the tube is not open. Since the vapor will volatilize into the air and may cause contamination if the sample is positive.

B. For determination of the extracted template, at least 3 hours after centrifugation at -20°C for one month.

C. DNA extraction kits are available from various manufacturers. You may use your own extraction systems or the commercial kit based on the yield. For the DNA extraction, please comply with the manufacturer’s instructions.

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### 2.0 Quantitation

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

**For performance of quantitative real-time PCR, standard dilutions must be prepared first as follows.**

1. Molecular Grade Water is used for dilution.

2. The extraction sample should be diluted in 3 steps.

3. DNA extraction buffer is available in the kit, the DNA extraction should be prepared.

4. The volumes of Reaction Mix and Enzyme should be measured by the template of the gene.

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