



Streptococcus Pneumoniae Real Time PCR Kit
Cat. No.: RD-0146-01

For Use with LightCycler 1.0/LightCycler2.0 Real Time PCR Systems

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, provoked sputum, bronchial lavage, lung biopsy and etc.

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 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 (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 genus Streptococcus. A significant human pathogenic bacterium, S. pneumoniae was recognized as a major cause of pneumonia in the late 19th century and is the subject of many humoral immunity studies. The organism causes many types of pneumococcal infection other than pneumonia, including acute sinusitis, otitis media, meningitis, bacteremia, sepsis, osteomyelitis, septic arthritis, endocarditis, peritonitis, 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 master 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 fluorimeter channel FAM with the fluorescent quencher BHQ1. DNA extraction buffer is available in the kit and samples (e.g. nasal and pharyngeal secretions, sputum, provoked sputum, bronchial lavage, lung biopsy) are used for DNA extraction. In addition, the kit contains a system to identify possible PCR inhibition by measuring the HEX/VIC/JOE fluorescence of the internal control (IC). An external positive control (1×10^8 copies/ml) allows the determination of the gene load. For further information, please refer to section 9.3 Quantitation.

4. Kit Contents

Ref.	Type of Reagent	Presentation 25rxns
1	DNA Extraction Buffer	2 vials, 1.5ml
2	S.pneumoniae Reaction Mix	1 vial, 450 μ l
3	PCR Enzyme Mix	1 vial, 12 μ l
4	Molecular Grade Water	1 vial, 400 μ l
5	Internal Control (IC)	1 vial, 30 μ l
6	S.pneumoniae Positive Control (1×10^8 copies/ml)	1 vial, 30 μ l

Analysis sensitivity: 1×10^3 copies/ml; LOQ: $2 \times 10^3 \sim 1 \times 10^8$ copies/ml.

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
- Cryo-container
- Sterile filter tips for micro pipets
- Disposable gloves, powderless
- Refrigerator and freezer
- Desktop microcentrifuge for "eppendorf" type tubes (RCF max. 16,000 x g)
- Real time PCR system
- Real time PCR reaction tubes/plates
- Pipets (0.5 μ l – 1000 μ l)
- Sterile microtubes
- Biohazard waste container
- 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.
- 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.
- 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 etiologic 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.

9.1.1 Sputum 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 2ml 25mmol/L CaCl₂, mix thoroughly and store at 4°C. **Please incubate at 37°C for 10 minutes before use.**

- Estimate the volume of the sputum and add partes aequales 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.
- Add 1.0ml 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.
- Repeat step 3)
- Add 50 μ l DNA extraction buffer, closed the tube then resuspend the pellet with vortex vigorously. Spin down briefly in a table centrifuge.
- Incubate the tube for 10 minutes at 100°C.
- 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 (Pleural effusion , and etc.)

- Take 400 μ l (3ml for water sample) sample in a tube, centrifuge the tube at 13000rpm for 2min, and remove the supernatant and keep the pellet. 2) Add 100 μ l 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 sample

- Wash the sample (lung biopsy) in 1ml normal saline and vortex vigorously. Centrifuge at 13000rpm for 2 minutes. Carefully remove and discard supernatant from the tube without disturbing the pellet. 2) Add 100 μ l DNA extraction buffer to the tube, closed 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.

Attention:

- 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.
- The extraction sample should be used in 3 hours or stored at -20°C for one month.
- 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.

9.2 Internal Control and Positive 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) 1 μ l/rxn and the result will be shown in the HEX/VIC/JOE.

Attention: It is necessary to dilute the internal control and positive control supplied in the kit by 10 times with molecular grade water before detection, and close the tube immediately then vortex for 10 seconds.

Because of transportation with carbon dioxide ice, there may be white precipitate in tubes of internal control and positive control, but it will disappear in a few minutes when it is incubated at room temperature. Besides, the white precipitate have no effect on the detection result.

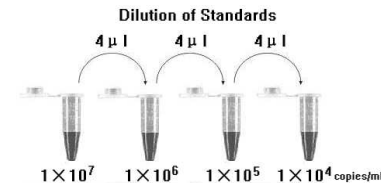
9.3 Quantitation

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

For performance of quantitative real-time PCR, standard dilutions must be prepared first as follows. Molecular Grade Water is used for dilution.

Dilution is not needed for performance of qualitative real-time PCR detection.

Take positive control (1×10^7 copies/ml) as the starting high standard in the first tube. Respectively pipette 36 μ l of Molecular Grade Water into next three tubes. Do three dilutions as the following figures:



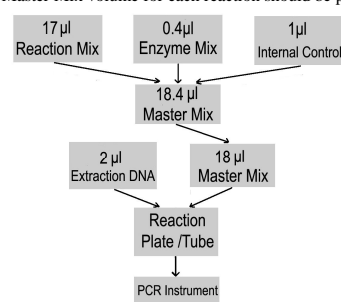
To generate a standard curve on the real-time system, all four dilution standards should be used and defined as standard with specification of the corresponding concentrations.

Attention:

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

9.4 PCR Protocol

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



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

- The volumes of Reaction Mix and Enzyme Mix per reaction multiply with the number of samples, which includes the number of 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. Mix the master mix completely then spin down briefly in a centrifuge.
- Pipet 18 μ l Master Mix with micropipets of sterile filter tips to

each Real time PCR reaction plate/tubes. Then separately add 2 μ l DNA sample supernatant, positive and negative controls to different 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:
37°C for 2 min, 1 cycle; 94°C for 2 min, 1 cycle; 93°C for 30 sec, 40 cycles.

Fluorescence is measured at 60°C; FAM and HEX/VIC/JOE channels should be chosen.

10. Baseline setting: just above the maximum level of molecular grade water.

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: The crossing point value of molecular grade water and positive control in FAM channel shows blank and ≤ 35 respectively; The CT value of internal control in HEX/VIC/JOE channel shows 25-33; Correlation coefficient of standard curve should be ≤ -0.98 , otherwise the result is invalid.

13. Data Analysis and Interpretation

The following results are possible:

- The crossing point value in channel FAM shows ≤ 38 . **The result is positive: The sample contains S. pneumoniae DNA.**
- The crossing point value in channel FAM shows 38-40, please repeat again. **If the result still shows 38-40, it can be considered negative.**
- In channel FAM no signal is detected, at the same time, a HEX/VIC/JOE signal from the Internal Control appears. **The sample does not contain any S. pneumoniae DNA. It can be considered negative.**
- Neither in channel FAM nor in channel HEX/VIC/JOE signal is detected. **A diagnostic statement can not be made.** Inhibition of the PCR reaction.

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