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Chlamydia Pneumoniae Real Time PCR Kit User Manual

For In Vitro Diagnostic Use Only

REF RD-0099-01

For use with LightCycler1.0/2.0 Instrument



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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, pleural effusion 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

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 fluorimeter channel 530nm 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 560nm fluorescence of the internal control (IC). An external positive control (1×10⁷ copies/ml) allows the determination of the gene load.

4. Kit Contents

Ref.	Type of reagent	Presentation 25rxns
1	DNA extraction buffer	2 vials, 1.5ml
2	CP Reaction Mix	1 vial, 450µl
3	PCR Enzyme Mix	1 vial, 12µl
4	Molecular Grade Water	1 vial, 400µl
5	Internal Control (IC)	1 vial, 30µl
6	CP Positive control (1×10 ⁷ copies/ml)	1 vial, 30µl

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 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
- Real time PCR system
- Desktop microcentrifuge for "eppendorf" type tubes (RCF 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
- Biohazard waste container
- Refrigerator and freezer
- 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.

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 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.

2) 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.

3) 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.

4) Repeat step 3)

5) Add 100µl 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 10 minutes. The supernatant contains the DNA extracted and can be used for PCR template.

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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 100µl 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.

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