1. Intended Use

Pneumocystis jiroveci real time PCR kit is designed for the detection of Pneumocystis jiroveci in bronchial lavage sample or lung section sample from human by using real time PCR systems.

2. Principle of Real-Time PCR

DNA extraction is based on the fluorogenic 5’ nucleic acid 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 by the PCR detection system. The PCR cycle at which an increase in the fluorescence signal is detected initially is proportional to the amount of the specific PCR product. Monitoring the fluorescence intensities in real-time allows the detection of the accumulating product without having to re-open the reaction tube after the amplification.

3. Product Description

Pneumocystis jiroveci is a yeast-like fungus of the genus Pneumocystis. It is an important human pathogen among immunocompromised hosts. At first, the name Pneumocystis carinii was applied to the organisms found in both rats and humans, as it was not yet known that the parasite was host-specific. By 1976 the name Pneumocystis jiroveci (or jirovecii) was proposed to distinguish the organism found in humans from variants of Pneumocystis in other animals. It is now considered a common opportunistic infection in persons with HIV infection. P. jiroveci is now of several organisms known to cause life-threatening opportunistic infections in patients with advanced HIV infection worldwide.

Pneumocystis jiroveci real time PCR kit contains a specific ready-to-use system for the detection of the Pneumocystis jiroveci by a reverse transcriptase in the real-time PCR system. The master contains reagents and enzymes for the specific amplification of the Pneumocystis jiroveci DNA. Fluorescence is emitted and measured by the real time systems’ optical unit. The detection of amplified Pneumocystis jiroveci DNA fragment is performed in the fluorometer Hidex Quatro. A fluorescent quencher BHQ1. DNA extraction buffer is available in the kit and bronchial lavage sample or lung section sample is used for the extraction of the DNA. In the kit, a system is provided to identify possible PCR inhibition by measuring the 560 nm fluorescence of the internal control (IC). An external positive control (1×10⁴ copies/ml) contained, allows the determination of the gene load.

4. Kit Contents

- DNA Extraction Buffer 2 vials, 1.5ml
- P. jiroveci Mix 1 vial, 450µl
- P. jiroveci Enzyme Mix 1 vial, 12µl
- Molecular Grade Water 1 vial, 400µl
- Internal Control (IC) 1 vial, 30µl
- P. jiroveci Positive Control (1×10⁴ copies/ml) 1 vial, 30µl

Analysis sensitivity: 1×10⁴ copies/ml LOQ: 2×10⁻¹ ~ 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 only 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.
- Super Mix should be stored at -20°C.

6. Additionally Required Materials and Devices

- Biocatalyst
- Vortex mixer
- Cryo-container
- Sterile filter tips for micro pipets
- Disposable gloves, powder-free
- Biohazard waste container
- Refrigerator and Freezer
- Tryptic digestive solution
- Desktop microcentrifuge for “eppendorf” type tubes (RCF max. 16,000 x g)

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, as 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.
- Carefully close and open the centrifuge.
- Do not pipette by mouth. Do not eat, drink, and smoke in laboratory.
- Avoid aerosols.

8. Sample Collection, Storage and Transport

- Collected 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. Detection

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 Bronchial lavage sample

1) Take 400µl sample in a tube, and centrifuge the tube at 13000rpm for 2min. Remove the supernatant, and keep the sediment for processing.
2) Add 100µl DNA extraction buffer in the tube (sediment), 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.

9.1.2 Lung section sample

1) Wash the lung tissue with sterile saline for several times.
2) Take 50mg sample in a tube, add 1ml sterile saline, and grind the tissue into homogenate.
3) Transfer the homogenate to a 1.5ml tube, and centrifuge the tube at 13000rpm for 5min. Remove the supernatant, and keep the sediment for processing.
4) Add 100µl DNA extraction buffer in the tube (sediment), close the tube then vortex for 10 seconds. Spin down briefly in a table centrifuge.
5) Incubate the tube for 10 minutes at 100°C.
6) Centrifuge the tube at 13000rpm for 5 minutes. The supernatant contains the 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.

A. Add internal control (IC) 1µl/50µl and the result will be shown in the 560nm.

9.3 Quantitation

The kit can be used for qualitative or quantitative real-time PCR. For performance of quantitative real-time PCR, standard dilutions must be prepared firstly as follows. Molecular Grade Water is used as the dilution.

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

Take positive control (1×10⁴ copies/ml) as the starting high standard in the first tube. Respectively pipette 36µl Molecular Grade Water into next three tubes. Do three dilutions as the following figures:

- Dilution of Standard:

  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.
  
  A. Mix thoroughly before next transfer.
  B. 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 follow:

Ref. Type of Reagent Presentation 25µl
1 DNA Extraction Buffer 2 vials, 1.5ml
2 P. jiroveci Mix 1 vial, 450µl
3 P. jiroveci Enzyme Mix 1 vial, 12µl
4 Molecular Grade Water 1 vial, 400µl
5 Internal Control (IC) 1 vial, 30µl
6 P. jiroveci Positive Control (1×10⁴ copies/ml) 1 vial, 30µl

10. Threshold setting: Choose Arithmetic as back ground and none as Noise Band method, then adjust the Noise band just above the maximum level of molecular grade water, and adjust the threshold just under the minimum of the positive control.

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: 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 results are possible:

- Crossing point value
- Result Analysis

- Molecular Grade Water
- Positive Control
- Quantitative detection
- Correlation coefficient of QS curve

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