Human Parainfluenza Virus Type 3 Real time RT-PCR kit

User Manual

For In Vitro Diagnostic Use Only

REF: RR-0158-02

For use with A/Bi Prism™7000/7500/7700/Step One Plus; Cycler™ IQ™; 4/iQ™; Smart Cycler™II/Bio-Rad Cycler; Rotorgene™6000; Mx3000P/3005P; MJ-OpenView2/ChromoMax; LightCycler™480 Instrument

1. Intended Use

Human Parainfluenza Virus Type 3 real time RT-PCR kit is used for the detection of Human Parainfluenza Virus Type 3 in nasopharyngeal secretions by using real time PCR systems.

2. Principle of Real-time RT-PCR

The principle of the real-time detection is based on the fluorescence 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 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 fluorescent signal is detected initially (Ct) is proportional to the amount of the PCR product. Multiplication of the fluorescent 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

Human parainfluenza viruses are second to respiratory syncytial virus (RSV) as a common cause of lower respiratory tract disease in young children. Similar to RSV, HPVs can cause repeated infections throughout life, usually manifested by an upper respiratory tract illness (e.g., a cold and/or sore throat). HPVs are negative-sense, single-stranded RNA viruses that possess fusion and hemagglutinin neuraminidase glycoprotein “spikes” on their surface. There are four serotypes of HPV (1 through 4) and two subtypes (4a and 4b). Each of the four HPVs has different clinical and epidemiologic features. HPV-3 is more often associated with bronchiolitis and pneumonia. HPV-1 peak activity occurs during the spring and early summer months each year, but the virus can be isolated throughout the year.

The Human Parainfluenza Virus Type 3 real time RT-PCR kit contains a specific ready-to-use system for the detection of the Human Parainfluenza Virus Type 3 using RT-PCR (Reverse Transcription Polymerase Chain Reaction) in the real-time PCR system. The master contains a Super Mix for the specific amplification of the Human Parainfluenza Virus Type 3 RNA. The reaction is carried out in one step real time RT-PCR. The first step is a reverse transcription (RT) during which the Human Parainfluenza Virus Type 3 RNA is transcribed into cDNA. Afterwards, a thermostable DNA polymerase is used to amplify the specific gene fragments by means of PCR (polymerase chain reaction). Fluorescence is emitted and measured by the real time system’s optical unit during the PCR. The detection of amplified Human Parainfluenza Virus Type 3 DNA fragment is performed in fluorimeter channel FAM with the fluorescent quencher BHQ2. 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 defined as 1×10⁴ copies/ml is supplied which allow the determination of the gene load. For further information, please refer to section 9.5 Quantitation.

4. Kit Contents

- N/A

5. Analysis Sensitivity

- N/A

6. Storage

- N/A

7. Warnings and Precautions

- N/A

8. Sample Collection, Storage and transport

- N/A

9. Procedure

- N/A

10. Internal Control

- N/A

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. Interpretation of the result:

- N/A

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