Influenza Virus A&B Real Time RT-PCR Kit

Cat. No.: RR-0097-02

For use with ABI Prism®7000/7300/7500/7900; Smart CyclerII;

iCycler iQ™4/iQ™5; Rotor Gene™2000/3000; Mx3000P/3005P;

MJ-Option2/Chromo4 real time PCR systems

For in vitro Diagnostic use only

User Manual

1. Intended Use

Influenza virus A&B Real Time RT-PCR Kit is used for the detection of Influenza virus A&B virus in nasal and pharyngeal secretions by real time PCR systems.

2. Introduction

Influenza is a viral infection of the lungs characterized by fever, cough, and severe muscle aches. In the elderly and infirm, it is a major cause of disability and death (often as a result of secondary infection of the lungs by bacteria). Major outbreaks of influenza are associated with influenza virus type A or B. Infection with type B influenza is usually milder than type A. Type C virus is associated with minor symptoms.

3. 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.

4. Product Description

Influenza virus A&B real time RT-PCR kit contains a specific ready-to-use system for the detection of the Influenza virus A&B virus by Reverse Transcription Polymerase Chain Reaction(RT-PCR) in the real-time PCR system. The master contains a Super Mix for the specific amplification of Influenza virus A&B virus RNA. The reaction is done in one step real time RT-PCR. The first step is a reverse transcription (RT), during which the Influenza virus A&B virus RNA is transcribed into cDNA. Afterwards, a thermostable DNA polymerase is used to amplify the specific gene fragments by means of polymerase chain reaction(PCR). Fluorescence is emitted and measured by the real time systems´ optical unit during PCR. The detection of amplified Influenza virus A&B virus DNA fragment is performed in fluorimeter **channel FAM and HEX** with the fluorescent quencher BHQ1. An external positive control(1×10^7 copies/ml) contained, allows the determination of the gene load. For further information, please refer to section 10.2 Quantitation.

5. Kit Contents

Ref.	Type of reagent	Presentation 25rxns
1	Influenza virus A&B Super Mix	1 vial, 480µl
2	RT-PCR Enzyme Mix	1 vial, 28µl
3	Molecular Grade Water	1 vial, 400µl
4	Inluenza virus A&B Positive Control(1×10^7copies/ml)	1 vial, 30µl

6. 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.
- Super Mix should be stored in the dark.

7. 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
- RNA extraction kit

- *Real time* PCR reaction tubes/plates
- Cryo-container
- Pipets (0.5 µl – 1000 µl)
- Sterile filter tips for micro pipets
- Sterile microtubes
- Disposable gloves, powderless
- Biohazard waste container
- Refrigerator and freezer
- Tube racks

8. 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.
- Do not pipette by mouth. Do not eat, drink, smoke in laboratory.
- Avoid aerosols

9. 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

10. Procedure

10.1 RNA-Extraction

RNA extraction kits are available from various manufacturers. You may use your own extraction systems or the commercial kit based on the yield. For the RNA extraction, please comply with the manufacturer's instructions. The recommended extraction kit is as follows:

Nucleic Acid Isolation Kit	**Cat. Number**	**Manufacturer**
RNA Isolation Kit	ME-0001	ZJ Biotech
QIAamp Viral RNA Mini Extraction Kit (50)	52904	QIAGEN

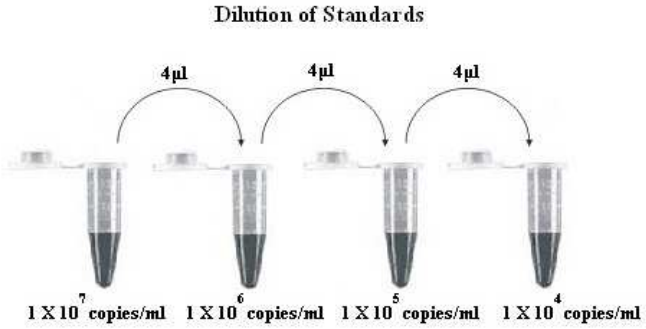
10.2 Quantitation

The kit can be used for **quantitative** or **qualitative** real-time RT-PCR. Positive control(1×10^7 copies/ml) is supplied in the kit.

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 **36ul** 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.

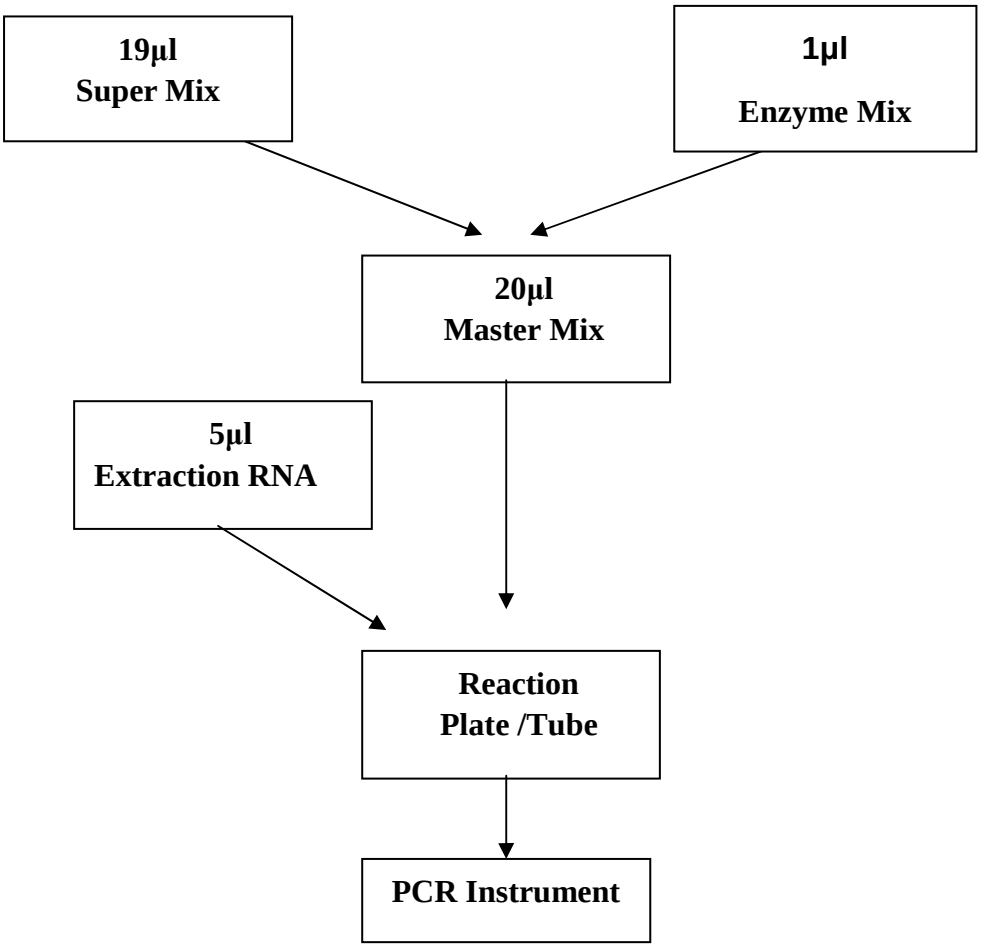
Attention:

A. Mix thoroughly before next transfer.

B. The positive control (1×10^7 copies/ml) contains high concentration of the target DNA. Therefore, be careful during the dilution in order to avoid contamination.

10.4 RT-PCR Protocol

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



1) Depending upon the number of samples(n) the following pipetting scheme can be followed. For the reason of unprecise pipetting, always add an extra virtual sample.

Reaction Volume	**Master Mix Volume**
19µl Super Mix	19µl × (n+1)
1µl Enzyme Mix	1µl × (n+1)

Mix completely then spin down briefly in a centrifuge.

2) Pipet **20µl** Master Mix with micropipets of sterile filter tips to each *Real time* PCR reaction plate/tubes. Separately add **5µl** RNA sample template, positive and negative controls to different plate/tubes. Immediately close the plate/tubes to avoid contamination.
3) Spin down briefly in order to collect the Master Mix in the bottom of the reaction tubes.
4) Perform the following protocol in the instrument:

45°C for **10 min, 1 cycle;**

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95°C for **15 min, 1 cycle;**

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95°C for **15 sec, 60°C** for **60sec, 40 cycles.**

Fluorescence is measured at 60°C

5) If you use ABI Prism® system, please choose **"none"** as **passive reference** and **quencher.**

11. Data Analysis and Interpretation

The following results are possible:

1) A signal is detected in channel FAM. **The result is positive: The sample contains Influenza virus A RNA.**

2) A signal is detected in channel HEX. **The result is positive: The sample contains Influenza virus B RNA.**

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