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**Influenza Virus A Real Time RT-PCR Kit**

Cat. No.: RR-0051-02

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For use with ABI Prism<sup>®</sup> 7000/7300/7500/7900; Smart CyclerII;  
iCycler iQ<sup>™</sup>4/iQ<sup>™</sup>5; Rotor Gene<sup>™</sup>2000/3000; Mx3000P/3005P;  
MJ-Option2/Chromo4 real time PCR systems

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User Manual

For in vitro Diagnostic use only

## 1. Intended Use

Influenza virus A real time RT-PCR kit is used for the detection of Influenza virus A in nasal and pharyngeal secretions by using real time PCR systems.

## 2. Introduction

Influenzavirus A is a genus of the Orthomyxoviridae family of viruses. Influenzavirus A includes only one species: *Influenza A virus* which causes influenza in birds and some mammals. Strains of all subtypes of influenza A virus have been isolated from wild birds, although disease is uncommon. Some isolates of influenza A virus cause severe disease both in domestic poultry and, rarely, in humans. Occasionally viruses are transmitted from wild aquatic birds to domestic poultry and this may cause an outbreak or give rise to human influenza pandemics.

Virions consist of an envelope, a matrix protein, a nucleoprotein complex, a nucleocapsid, and a polymerase complex. Virus capsid is enveloped. Virions are spherical to pleomorphic; filamentous forms occur (sometimes). Virions measure 80-120 nm in diameter; 200-300nm in length. The genome is segmented and consists of eight segments of linear negative-sense, single-stranded RNA. The complete genome is 13600 nucleotides long.

Firm diagnosis is by means of virus isolation and serology. The virus can be isolated from the nose or a throat swab. This is used to infect cells in culture (or eggs). Hemadsorption may be used to detect infected cells. Polymerase chain reaction (PCR) test are being developed to detect viral RNA.

## 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

The Influenza virus A real time RT-PCR kit contains a specific ready-to-use system for the detection of the Influenza virus A 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 Influenza virus A 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 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 systems' optical unit during the PCR. The detection of amplified Influenza virus A DNA fragment is performed in fluorimeter **channel FAM** with the fluorescent quencher BHQ1. In addition, the kit contains a system to identify possible PCR inhibition by measuring the VIC/JOE fluorescence of the internal control (IC). An external positive control defined as  $1 \times 10^7$  copies/ml is supplied which allow the determination of the gene load. For further information, please refer to section 10.3 Quantitation.

## 5. Kit Contents

Ref.	Type of reagent	Presentation 25rxns
1	Influenza Virus A Super Mix	1 vial, 480µl
2	RT-PCR Enzyme Mix	1 vial, 28µl
3	Molecular Grade Water	1 vial, 400µl
4	Internal Control (IC)	1 vial, 30µl
5	Influenza Virus A Positive Control ( $1 \times 10^7$ copies/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 assay sensitivity.
- 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.
- Quickly prepare 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

Different brand RNA extraction kits are available. 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 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.

Add the internal control (IC) 1µl/rxn and the result will be shown in the VIC/JOE channel.

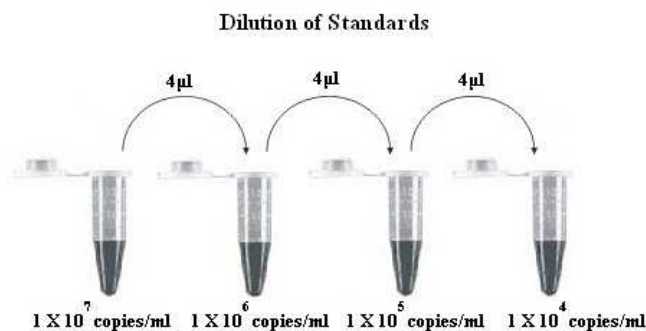
#### 10.3 Quantitation

The kit can be used for **quantitative** or **qualitative** real-time RT-PCR. A positive control defined as  $1 \times 10^7$  copies/ml is supplied in the kit.

**For performance of quantitative real-time PCR, Standard dilutions must prepare first as follows. Molecular Grade Water is used for dilution.**

**The step of dilution is not needed for performance of qualitative real-time PCR.**

Take positive control ( $1 \times 10^7$  copies/ml) as the starting high standard in the first tube. Respectively pipette **36µl** 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.

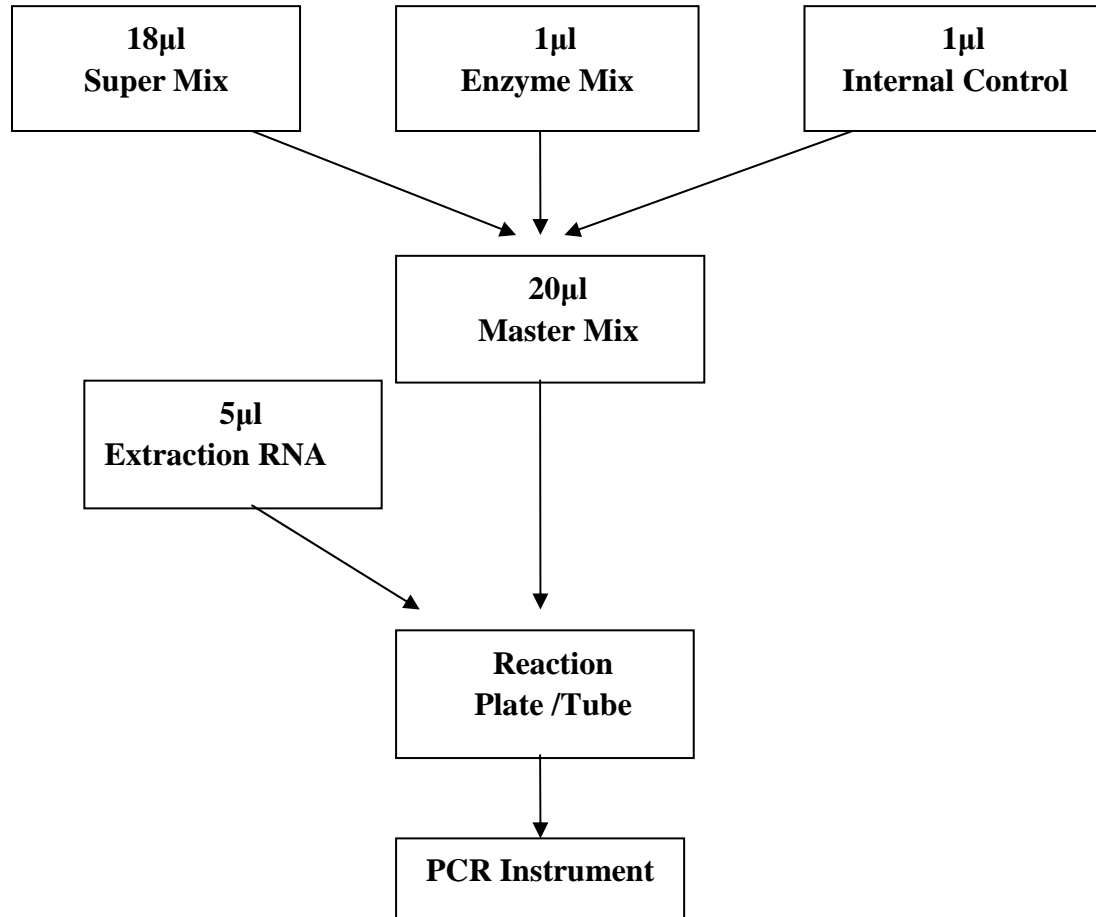
**Attention:**

A. Mix thoroughly before next transfer.

B. The positive control ( $1 \times 10^7$  copies/ml) contains high concentration of the target DNA. Therefore, be careful of 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) The volumes of Super Mix and Internal Control 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. (**n: the number of reaction** ).

Reaction Volume	Master Mix Volume
18µl Super Mix	$18\mu\text{l} \times (n+1)$
1µl Enzyme Mix	$1\mu\text{l} \times (n+1)$
1µl Internal control (IC)	$1\mu\text{l} \times (n+1)$

Mix completely then spin down briefly in a centrifuge.

- 2) Pipet **20 µl** 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;  
↓  
95°C for 15 min, 1 cycle;  
↓  
95°C for 15 sec, 60°C for 60sec, 40 cycles.  
Fluorescence is measured at 60°C

5) If you use ABI Prism<sup>®</sup> 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.**

In this case, the detection of a signal in channel VIC/JOE (Internal control) is dispensable, as high initial concentrations of Influenza virus A cDNA can lead to a reduced or absent fluorescence signal of the internal control (competition).

2) In channel FAM no signal is detected, at the same time, a VIC/JOE signal from the Internal Control appears. **The sample does not contain any Influenza virus A RNA. It can be considered negative.**

In the case of a negative Influenza virus A RT-PCR the detected signal of the internal control rules out the possibility of PCR inhibition.

3) Neither in channel FAM nor in channel VIC/JOE is a signal detected. **A diagnostic statement can not be made.** Inhibition of the RT-PCR reaction.

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