

Primer Design Ltd

R01025

# Influenza A Virus (M1)

**Kit version: v2**

**Target region:**

M1/M2 genes

genesig<sup>®</sup> Standard Kit

150 tests

GENESIG

Kits by Primerdesign

For general laboratory and research use only

# Introduction to Influenza A Virus

Influenza, commonly known as the flu, is an infectious disease of birds and mammals caused by an RNA virus of the family Orthomyxoviridae (the influenza viruses). In people, common symptoms of influenza are fever, sore throat, muscle pains, severe headache, coughing, and weakness and fatigue. In more serious cases, influenza causes pneumonia, which can be fatal, particularly in young children and the elderly. Although the common cold is sometimes confused with influenza, it is a much less severe disease and caused by a different virus. Similarly, gastroenteritis is sometimes called "stomach flu" or "24-hour flu" but is unrelated to influenza.

Typically, influenza is transmitted from infected mammals through the air by coughs or sneezes creating aerosols containing the virus, and from infected birds through their droppings. Influenza can also be transmitted by saliva, nasal secretions, faeces, and blood. Infections either occur through direct contact with these bodily fluids, or by contact with contaminated surfaces. Flu viruses can remain infectious for over 30 days at 0°C (32°F) and about one week at human body temperature, although they are rapidly inactivated by disinfectants and detergents.

Flu spreads around the world in seasonal epidemics, killing millions of people in pandemic years and hundreds of thousands in non-pandemic years. The most recent influenza pandemic was the swine flu pandemic of 2009 with an estimated death toll between 150,000-500,000 people. Often, these pandemics result from the spread of a flu virus between animal species. Influenza virus 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"

## Specificity

The genesig® Standard Kit for Influenza A Virus (M1) FluA\_M1 is designed for the in vitro quantification of Influenza A Virus genomes. The kit is designed to have a broad detection profile. Specifically, the primers will detect over 95% of sequences available on the GISAID EpiFlu database at the time of last review.

This is the global FluA kit designed to detect all subtypes of influenza A virus. When designing this kit, sequences from a three-year period were analysed from the GISAID EpiFlu database. Due to the sequence evolution observed in the influenza A genome over time, this is considered to be an adequate time period for analysis.

The dynamics of genetic variation mean that new sequence information may become available after the most recent review. If you require further information or have a specific question about the detection profile of this kit then please send an e-mail to [techsupport@primerdesign.co.uk](mailto:techsupport@primerdesign.co.uk) and our team will answer your question.

## Kit contents

- **1x FluA\_M1\_v2.0 primer/probe mix (150 reactions, BROWN)**  
FAM labelled
- **1x FluA\_M1\_v2.0 positive control template (for Standard curve, RED)**
- **1x RNase/DNase-free water (WHITE)**  
for resuspension of primer/probe mixes
- **2x Template preparation buffer (YELLOW)**  
for resuspension of positive control template and standard curve preparation

## Reagents and equipment to be supplied by the user

### Real-time PCR Instrument

#### Extraction kit

This kit is recommended for use with genesig® Easy DNA/RNA extraction kit or exsig®Mag. However, it is designed to work well with all processes that yield high-quality nucleic acid with minimal PCR inhibitors.

#### oasig® lyophilised OneStep or PrecisionPLUS® OneStep 2X RT-qPCR Master Mix

This kit is intended for use with oasig® lyophilised OneStep or PrecisionPLUS® OneStep 2X RT-qPCR Master Mix

#### Pipettors and filter tips

#### Vortex and centrifuge

#### 1.5 ml microtubes

#### qPCR plates or reaction tubes

## Kit storage and stability

This kit is stable for shipping at ambient temperature but should be stored at -20°C upon arrival. Once the lyophilised components have been resuspended, they should not be exposed to temperatures above -20°C for longer than 30 minutes at a time and unnecessary repeated freeze/thawing should be avoided. The kit is stable for six months from the date of resuspension under these circumstances.

If a standard curve dilution series is prepared this can be stored frozen for an extended period. If you see any degradation in this serial dilution a fresh standard curve can be prepared from the positive control.

Primer Design Ltd does not recommend using the kit after the expiry date stated on the pack.

## Suitable sample material

This kit can be used with all types of samples from various origins. Please ensure that the extracted nucleic acid samples are suitable in terms of purity, concentration, and RNA integrity.

## Dynamic range of test

Under optimal PCR conditions the kit can achieve priming efficiencies between 90-110% and detect less than 100 copies of target template. If running a positive control standard curve for a quantitative result, and an efficiency of between 90% to 110% is not achieved, then the run should be repeated with a freshly prepared standard curve.

# Principles of the test

## Real-time PCR

A target specific primer/probe mix is provided, and this can be detected through the FAM channel.

The primer/probe mix provided exploits with the so-called TaqMan<sup>®</sup> principle. During PCR amplification, forward and reverse primers hybridize to the target RNA. A fluorogenic probe is included in the same reaction mixture which consists of a DNA probe labelled with a 5'-dye and a 3'-quencher. During PCR amplification, the probe is cleaved, and the reporter dye and quencher are separated. The resulting increase in fluorescence can be detected on a range of qPCR platforms.

## Positive control

For copy number determination and as a positive control for the PCR set-up, the kit contains a positive control template. This can be used to generate a standard curve of the target copy number/Cq value. Alternatively, the positive control can be used at a single dilution where full quantitative analysis of the samples is not required. Each time the kit is used, at least one positive control reaction must be included in the run. A positive result indicates that the primers/probes for detecting the target gene worked properly in that particular experimental scenario. If a negative result is obtained the test results are invalid and must be repeated. Care should be taken to ensure that the positive control does not contaminate any other kit component which would lead to false-positive results. This can be achieved by handling this component in a Post PCR environment. Care should also be taken to avoid cross-contamination of other samples when adding the positive control to the run. This can be avoided by sealing all other samples and negative controls before pipetting the positive control into the positive control well.

## Negative control

To validate any positive findings a negative control reaction should be included every time the kit is used. For this reaction, RNase/DNase-free water should be used instead of the template. A negative result indicates that the reagents have not become contaminated while setting up the run.

# Resuspension Protocol

To minimize the risk of contamination with foreign RNA/DNA, we recommend that all pipetting is performed in a PCR clean environment. Ideally, this would be a designated PCR lab or PCR cabinet. Filter tips are recommended for all pipetting steps.

**1. Pulse-spin each tube in a centrifuge before opening.**

This will ensure that the lyophilised primer/probe mix or template is in the base of the tube and is not lost upon opening the tube.

**2. Resuspend the kit components in the RNase/DNase-free water supplied, according to the table below.**

To ensure complete resuspension, vortex each tube thoroughly.

Component - resuspend in water	Volume
<b>Pre-PCR pack</b>	
FluA_M1_v2.0 primer/probe mix ( <b>BROWN</b> )	165 µl

**3. Resuspend the positive control template in the template preparation buffer supplied, according to the table below:**

To ensure complete resuspension, vortex the tube thoroughly.

Component - resuspend in template preparation buffer	Volume
<b>Post-PCR heat-sealed foil</b>	
FluA_M1_v2.0 Positive Control Template ( <b>RED</b> )	500 µl

\* This component contains a high copy number template and is a VERY significant contamination risk. It must be opened and handled in a separate laboratory environment, away from the other components.

# OneStep RT-qPCR detection protocol

## For optimum performance and sensitivity.

All pipetting steps and experimental plate set up should be performed on ice. After the plate is prepared proceed immediately to the OneStep amplification protocol. Prolonged incubation of reaction mixes at room temperature can lead to PCR artifacts that reduce the sensitivity of detection.

1. For each RNA sample prepare a reaction mix according to the table below:  
Include sufficient reactions for positive and negative controls.

Component	Volume
oasig <sup>®</sup> lyophilised OneStep or PrecisionPLUS <sup>®</sup> OneStep 2X RT-qPCR Master Mix	10 µl
FluA_M1_v2.0 primer/probe mix ( <b>BROWN</b> )	1 µl
RNase/DNase-free water ( <b>WHITE</b> )	4 µl
<b>Final Volume</b>	<b>15 µl</b>

2. Pipette 15 µl of this mix into each well according to your qPCR experimental plate set-up.
3. Pipette 5 µl of RNA template into each well, according to your experimental plate set up.  
For negative control wells use 5 µl of RNase/DNase free water (**WHITE**). For positive control wells use 5 µl of the positive control template (**RED**). The final volume in each well is 20 µl.



#### 4. (Optional) Standard curve preparation for quantitative analysis.

For quantitative analysis of the samples, a standard curve dilution series can be prepared using the positive control template (**RED**). This is not required for qualitative analysis.

##### 4.1 Reaction mix preparation for the standard curve.

Include sufficient reactions for each dilution of the standard curve.

Component	Volume
oasig® lyophilised OneStep or PrecisionPLUS® OneStep 2X RT-qPCR Master Mix	10 µl
FluA_M1_v2.0 primer/probe mix ( <b>BROWN</b> )	1 µl
RNase/DNase-free water ( <b>WHITE</b> )	4 µl
<b>Final Volume</b>	<b>15 µl</b>

##### 4.2 Preparation of a 10-fold standard curve dilution series.

- pipette 90 µl of template preparation buffer (**YELLOW**) into 5 tubes and label them 2-6. The neat positive control tube (**RED**) is considered tube 1.
- Pipette 10 µl of positive control template (**RED**) into tube 2.
- Vortex thoroughly.
- Change pipette tip and pipette 10 µl from tube 2 into tube 3.
- Vortex thoroughly.

Repeat steps **d** and **e** across the tubes to complete the dilution series.

Standard Curve	Copy Number
Tube 1 Positive control ( <b>RED</b> )	$2 \times 10^5$ per µl
Tube 2	$2 \times 10^4$ per µl
Tube 3	$2 \times 10^3$ per µl
Tube 4	$2 \times 10^2$ per µl
Tube 5	20 per µl
Tube 6	2 per µl

##### 4.3 Pipette 15 µl of reaction mix and 5 µl of the respective standard into each well for the standard curve according to your plate set up.

The final volume in each well is 20 µl.

# OneStep RT-qPCR Amplification Protocol

Amplification conditions using oasisig<sup>®</sup> lyophilised OneStep or PrecisionPLUS<sup>®</sup> OneStep 2X RT-qPCR Master Mix.

	<b>Step</b>	<b>Time</b>	<b>Temp</b>
	Reverse Transcription	10 min	55 °C
	Enzyme activation	2 min	95 °C
Cycling x50	Denaturation	10 s	95 °C
	<b>DATA COLLECTION *</b>	60 s	60 °C

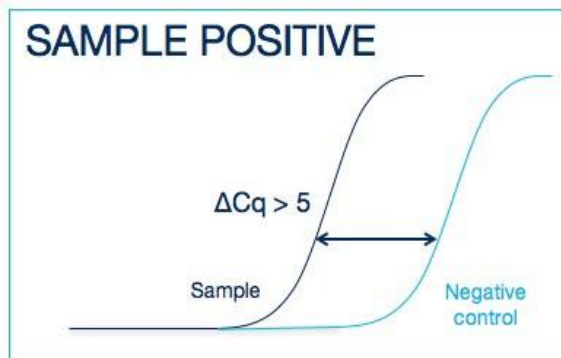
\* Fluorogenic data should be collected during this step through the FAM channels

# Interpretation of results

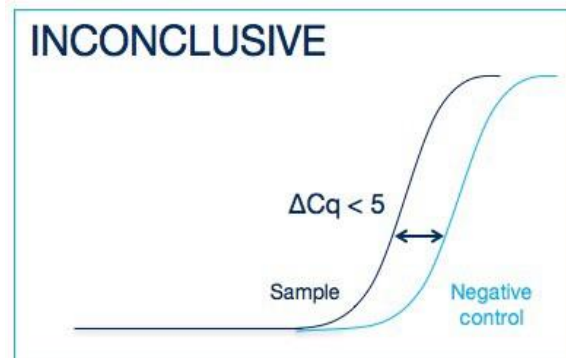
Target	Positive control	Negative control	Interpretation
+	+	-	<b>POSITIVE QUANTITATIVE RESULT</b> calculate copy number
-	+	-	<b>NEGATIVE RESULT</b>
+ / -	+	$\leq 35$	<b>EXPERIMENT FAILED</b> due to test contamination
+ / -	+	$> 35$	*
+ / -	-	+ / -	<b>EXPERIMENT FAILED</b>

A positive control template is expected to amplify between Cq 16 and 23. Failure to satisfy this quality control criterion is a strong indication that the experiment has been compromised.

\*Where the test sample is positive and the negative control is positive with a Cq > 35, the sample must be reinterpreted based on the relative signal strength of the two results:



If the sample amplifies > 5 Cq earlier than the negative control, then the sample should be reinterpreted (via the table above) with the negative control verified as negative.



If the sample amplifies < 5 Cq earlier than the negative control, then the positive sample result is invalidated, and the result should be determined inconclusive due to test contamination. The test for this sample should be repeated.

## Notices and disclaimers

This product is developed, designed and sold for research purposes only. It is not intended for human diagnostic or drug purposes or to be administered to humans unless clearly expressed for that purpose by the Food and Drug Administration in the USA or the appropriate regulatory authorities in the country of use. During the warranty period, Primer Design Ltd genesig® detection kits allow precise and reproducible data recovery combined with excellent sensitivity. For data obtained by violation of the general GLP guidelines and the manufacturer's recommendations the right to claim under guarantee is expired. PCR is a proprietary technology covered by the several US and foreign patents. These patents are owned by Roche Molecular Systems Inc. and have been sub-licensed by PE Corporation in certain fields. Depending on your specific application you may need a license from Roche or PE to practice PCR. Additional information on purchasing licenses to practise the PCR process may be obtained by contacting the Director of Licensing at Roche Molecular Systems, 1145 Atlantic Avenue, Alameda, CA 94501 or Applied Biosystems business group of the Applied Biosystems Corporation, 850 Lincoln Centre Drive, Foster City, CA 94404. In addition, the 5' nuclease assay and other homogeneous amplification methods used in connection with the PCR process may be covered by US Patents 5,210,015 and 5,487,972, owned by Roche Molecular Systems, Inc, and by U.S. Patent 5,538,848, owned by The Perkin-Elmer Corporation.

## Trademarks

PrecisionPLUS® is a trademark of Primer Design Ltd.

genesig® is a registered trademark of Primer Design Ltd.

oasig® is a trademark of Primer Design Ltd.

exsig®Mag is a trademark of Primer Design Ltd.

The PCR process is covered by US Patents 4,683,195, and 4,683,202 and foreign equivalents owned by Hoffmann-La Roche AG. TaqMan® is a registered trademark of Roche Molecular Systems, Inc., The purchase of the Primer Design Ltd reagents cannot be construed as an authorization or implicit license to practice PCR under any patents held by Hoffmann-La Roche Inc.