

Primerdesign™ Ltd

Influenza A virus subtype H7N9 (avian influenza)

Hemagglutinin H7
&
Neuramidase N9

genesig® Standard Kit

150 tests

GENESIG

Kits by Primerdesign

For general laboratory and research use only

Introduction to Influenza A virus subtype H7N9 (avian influenza)

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, feces 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. Three influenza pandemics occurred in the 20th century—each following a major genetic change in the virus—and killed tens of millions of 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 Primerdesign genesig Kit for Influenza A virus subtype H7N9 (avian influenza) (H7N9) genomes is designed for the in vitro quantification of H7N9 genomes. The kit is designed to have a broad detection profile. Specifically, the primers represent 100% homology with over 95% of the NCBI database reference sequences available at the time of design.

The dynamics of genetic variation means that new sequence information may become available after the initial design. Primerdesign periodically reviews the detection profiles of our kits and when required releases new versions.

The primers have 100% homology with over 95% of avian H7 and N9 isolates globally that have been entered into the influenza sequence database in the NCBI database. The primers have low sequence homology to other influenza subtypes. The quantification of both subtyping genes ensures accurate determination of the H7N9 genotype in a single experiment.

If you require further information, or have a specific question about the detection profile of this kit then please send an e.mail to enquiry@primerdesign.co.uk and our bioinformatics team will answer your question.

Kit contents

- **Hemagglutinin H7 primer/probe mix (150 reactions BROWN)**
FAM labelled
- **Neuramidase N9 primer/probe mix (150 reactions BROWN)**
FAM labelled
- **Hemagglutinin H7 positive control template (for Standard curve RED)**
- **Neuramidase N9 positive control template (for Standard curve RED)**
- **RNase/DNase free water (WHITE)**
for resuspension of primer/probe mixes
- **Template preparation buffer (YELLOW)**
for resuspension of positive control templates 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. However, it is designed to work well with all processes that yield high quality RNA and DNA with minimal PCR inhibitors.

oasig™ lyophilised OneStep or Precision® PLUS OneStep 2X RT-qPCR Master Mix

Contains complete OneStep RT-qPCR master mix

Pipettors and Tips

Vortex and centrifuge

Thin walled 1.5 ml PCR reaction tubes

Kit storage and stability

This kit is stable at room temperature but should be stored at -20°C on 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.

Primerdesign does not recommend using the kit after the expiry date stated on the pack.

Suitable sample material

All kinds of sample material suited for PCR amplification can be used. Please ensure the samples are suitable in terms of purity, concentration, and RNA/DNA integrity. Always run at least one negative control with the samples. To prepare a negative-control, replace the template RNA sample with RNase/DNase free water.

Dynamic range of test

Under optimal PCR conditions genesig H7N9 detection kits have very high priming efficiencies of >95% and can detect less than 100 copies of target template.

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 Primerdesign genesig detection kits allow precise and reproducible data recovery combined with excellent sensitivity. For data obtained by violation to the general GLP guidelines and the manufacturer's recommendations the right to claim under guarantee is expired. PCR is a proprietary technology covered by 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 practice 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 U.S. 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

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Principles of the test

The kit contains two primer and probe sets. The H7 primer and probe set are designed to detect all influenza H7 sequences from the avian H7N9 lineage. The N9 primer and probe set detect all N9 sequences regardless of the species of origin. Results from both primer sets can be used to make a positive determination of the H7N9 genotype. Samples that test negative for H7 and positive for N9 represent alternative circulating subtypes that contain the N9 gene.

Real-time PCR

A H7N9 specific primer and probe mix is provided and this can be detected through the FAM channel.

The primer and probe mix provided exploits the so-called TaqMan® principle. During PCR amplification, forward and reverse primers hybridize to the H7N9 DNA/cDNA. A fluorogenic probe is included in the same reaction mixture which consists of a DNA probe labeled 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 H7N9 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 and probes for detecting the target H7N9 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 the RNase/DNase free water should be used instead of 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 DNA, we recommend that all pipetting be 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 lyophilised primer and probe mix is in the base of the tube and is not spilt 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
Pre-PCR pack	
H7 primer/probe mix (BROWN)	165 µl
N9 primer/probe mix (BROWN)	165 µl

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

To ensure complete resuspension, vortex each tube thoroughly.

Component - resuspend in template preparation buffer	Volume
Post-PCR heat-sealed foil	
H7 Positive Control Template (RED) *	500 µl
N9 Positive Control Template (RED) *	500 µl

* This component contains 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 poured 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 OneStep or PrecisionPLUS OneStep 2X RT-qPCR Master Mix	10 μ l
H7 or N9 primer/probe mix (BROWN)	1 μ l
RNase/DNase free water (WHITE)	4 μ l
Final Volume	15 μl

- 2. Pipette 15 μ l of this mix into each well according to your qPCR experimental plate set up.**
- 3. Pipette 5 μ l of RNA template into each well, according to your experimental plate set up.**
For negative control wells use 5 μ l of RNase/DNase free water. The final volume in each well is 20 μ l.
- 4. If a standard curve is included for quantitative analysis prepare a reaction mix according to the table below:**

Component	Volume
oasig OneStep or PrecisionPLUS OneStep 2X RT-qPCR Master Mix	10 μ l
H7 or N9 primer/probe mix (BROWN)	1 μ l
RNase/DNase free water (WHITE)	4 μ l
Final Volume	15 μl

5. Preparation of standard curve dilution series

- 1) Pipette 90µl of template preparation buffer into 5 tubes and label 2-6
- 2) Pipette 10µl of Positive Control Template (**RED**) into tube 2
- 3) Vortex thoroughly
- 4) Change pipette tip and pipette 10µl from tube 2 into tube 3
- 5) Vortex thoroughly

Repeat steps 4 and 5 to complete the dilution series

Standard Curve	Copy Number
Tube 1 Positive control (RED)	2 x 10 ⁵ per µl
Tube 2	2 x 10 ⁴ per µl
Tube 3	2 x 10 ³ per µl
Tube 4	2 x 10 ² per µl
Tube 5	20 per µl
Tube 6	2 per µl

6. Pipette 5µl of standard template into each well for the standard curve according to your plate set up

The final volume in each well is 20µl.

OneStep RT-qPCR amplification protocol

Amplification conditions using oasig OneStep or PrecisionPLUS OneStep 2X RT-qPCR Master Mix.

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

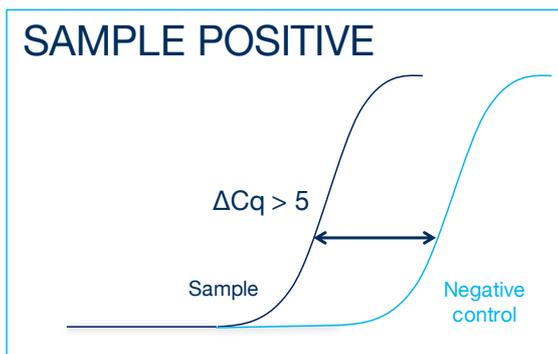
* Fluorogenic data should be collected during this step through the FAM channel

Interpretation of results

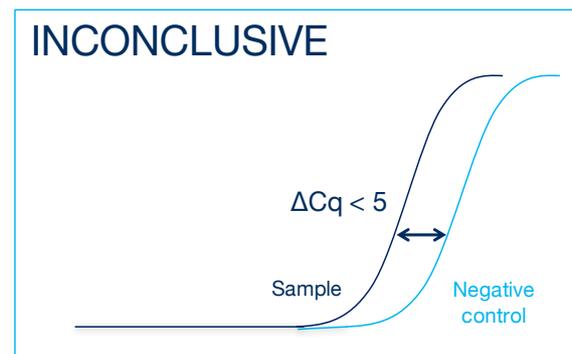
Target	Positive control	Negative control	Interpretation
+	+	-	POSITIVE QUANTITATIVE RESULT calculate copy number
-	+	-	NEGATIVE RESULT
+ / -	+	≤ 35	EXPERIMENT FAILED due to test contamination
+ / -	+	> 35	*
+ / -	-	+ / -	EXPERIMENT FAILED

Positive control template (**RED**) 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.