Primer Design Ltd

R01290

Avian Influenza A Virus Subtype H9N1

Kit version: 3

H9 - Hemagglutinin (HA) gene
N1 - Neuramidase (N) gene

genesig® Standard Kit

150 tests

GENESIG
Kits by Primerdesign

For general laboratory and research use only

Product Description

This genesig® Standard qPCR detection kit targets the hemagglutinin (HA) gene and neuraminidase (N) gene from avian influenza A virus subtype H9N1 which is commonly known as H9N1. H9N1 is an RNA virus which primarily infects poultry but may also infect wild birds and in rare cases, humans. Infection can lead to avian influenza. Influenza A is divided into subtypes based on two surface proteins: hemagglutinin (H) and neuraminidase (N). There are 18 different hemagglutinin subtypes and 11 neuraminidase subtypes, of which only H9N1 subtype will be detected by this qPCR kit.

Specificity

The kit is designed for the in vitro quantification of avian influenza A virus subtype H9N1 genomes and 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.

Sequences from the prior five-year period and ten-year period were used for the H9 and N1 assay reviews respectively. Due to the sequence evolution observed in the Influenza genome over time, this is considered to be an adequate time period for analysis. Due to the limited sequence information for N1, a greater time period was used.

The H9 primer and probe set is designed to detect H9 sequences and therefore may detect H9 sequences from non-H9N1 subtypes.

The N1 primer and probe set is designed to detect N1 sequences from the H9N1 lineage but may detect N1 sequences from other subtypes.

A positive result for both H9 and N1, indicates the presence of H9N1 specifically

The dynamics of genetic variation means that new sequence information may become available after the initial design. 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 and our team will answer your question.

Kit contents

Quantity	Component	Tube	Cap Colour
1	**H9_v2.0 primer/probe mix (150 reactions)** FAM labelled		**BROWN**
1	**N1(H7N1&H9N1) primer/probe mix (150 reactions)** FAM labelled		**BROWN**
1	**H9_v2.0 positive control template**		**RED** (in silver foil wrapper)
1	**N1(H7N1&H9N1) positive control template**		**RED** (in silver foil wrapper)
2	**Template preparation buffer** for resuspension of positive control template and standard curve preparation		**YELLOW**
1	**RNase/DNase free water** for resuspension of primer/probe mixes		**WHITE**

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 nucleic acid with minimal PCR inhibitors.

oasig®PLUS OneStep Lyophilised qPCR Master Mix
This kit is intended for use with oasig®PLUS OneStep Lyophilised 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 at room temperature but should be stored at -20ºC 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.

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

Target specific primer/probe mixes are provided, and these can be detected through the FAM channel.

The primer/probe mixes provided exploit the so-called TaqMan® principle. During PCR amplification, forward and reverse primers hybridise to the target 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 positive control templates. This can be used to generate standard curves of the target copy number/Cq value. Alternatively, the positive controls 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 genes 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 controls do 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 positive controls to the run. This can be avoided by sealing all other samples and negative controls before pipetting the positive controls into the positive control wells.

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 minimise the risk of contamination with foreign RNA/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 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 primer/probe mixes 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	
H9_v2.0 primer/probe mix (BROWN)	165 µl
N1(H7N1&H9N1) primer/probe mix (BROWN)	165 µl

3. **Resuspend the positive control template 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	
H9_v2.0 Positive Control Template (RED) *	500 µl
N1(H7N1&H9N1) 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 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®PLUS OneStep Lyophilised qPCR Master Mix	10 µl
H9_v2.0 or N1(H7N1&H9N1) primer/probe mix (BROWN)	1 µl
RNase/DNase free water (WHITE)	4 µl
Final Volume	**15 µl**

2. **Pipette 15µl of these mixes into each well according to your qPCR experimental plate set up.**

3. **Pipette 5µl of RNA template into each well, according to your experimental plate set up.**
   For negative control wells use 5µl of RNase/DNase free water. The final volume in each well is 20µl.

4. **If a standard curve is included for quantitative analysis prepare a reaction mix according to the table below:**

Component	Volume
oasig®PLUS OneStep Lyophilised qPCR Master Mix	10 µl
H9_v2.0 or N1(H7N1&H9N1) primer/probe mix (BROWN)	1 µl
RNase/DNase free water (WHITE)	4 µl
Final Volume	**15 µl**

5. **Preparation of standard curve dilution series.**
   a) **Pipette 90µl of template preparation buffer into 5 tubes and label 2-6**
   b) **Pipette 10µl of Positive Control Template (RED) into tube 2**
   c) **Vortex thoroughly**
   d) **Change pipette tip and pipette 10µl from tube 2 into tube 3**
   e) **Vortex thoroughly**
   Repeat steps d and e 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®PLUS OneStep Lyophilised 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.

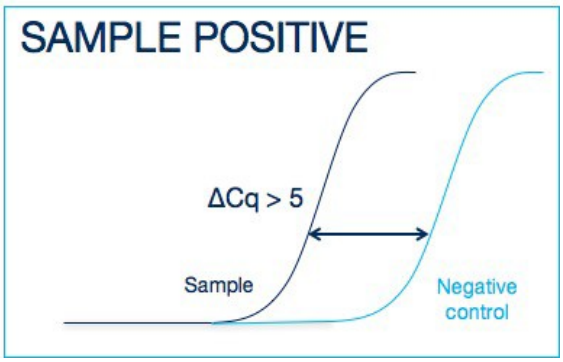
Interpretation of results

H9_v2.0 (FAM)	N1 (H7N1&H9N1) (FAM)	*Positive control	Negative control	**Interpretation
+	-	+	-	**POSITIVE RESULT FOR H9**
-	+	+	-	**POSITIVE RESULT FOR N1**
+	+	+	-	**POSITIVE RESULT FOR H9N1**
-	-	+	-	**NEGATIVE RESULT**
+ / -	+ / -	+	≤ 35	**EXPERIMENT FAILED** due to test contamination
+ / -	+ / -	+	> 35	***
+ / -	+ / -	-	+ / -	**EXPERIMENT FAILED**

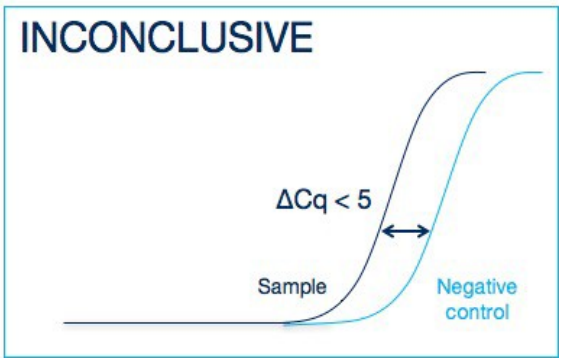
*Positive control template (**RED**) is expected to amplify between Cq 16 and 23. Failure to satisfy this quality control criterion is a strong indication that the experiment has been compromised.

**If a sample is positive for H9 but negative for N1, this could be due to the presence of one of the other H9 subtypes. Conversely if a sample is positive for N1 but negative for H9, this could be due to the presence of one of the other N1 subtypes. A sample is confirmed as H9N1 if both targets (FAM) are positive.

***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 in violation of the general GLP guidelines and the manufacturer's recommendations, the right to claim under guarantee is expired.

Trademarks

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.