

Primerdesign

R01384

## **Influenza A virus subtype H5N1 (Avian influenza)**

**Kit version: 3**

**Target region:**

Haemagglutinin gene

Neuraminidase gene

**genesig® Standard RNA Kit**

150 tests

**G E N E S I G**

Kits by Primerdesign

For general laboratory and research use only

GENESIG

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# Product Description

This genesig® Standard qPCR detection kit comprises a two-target approach for the detection of avian influenza A virus subtype H5N1. The H5 assay targets the haemagglutinin (HA) gene (segment 4), and the N1 assay targets the neuraminidase (NA) gene (segment 6) from H5N1. H5N1 is a highly pathogenic RNA virus that primarily infects birds but can be transmitted to humans through close contact. In birds, infection can cause respiratory distress, diarrhoea and decreased egg production, and has high mortality rates. Human infection is rare, causing flu-like symptoms that may progress to pneumonia.

## Specificity

The kit is designed for the in vitro quantification of avian influenza A virus subtype H5N1 genomes and to have a broad detection profile. Specifically, the primers will detect over 95% of sequences from avian origin available on the GISAID EpiFlu database in the previous three years. Due to the sequence evolution observed in genomes of RNA viruses, three years is considered an adequate time period for analysis.

The H5 assay is designed to detect H5 sequences from the H5N1 lineage but may cross-react with H5 sequences from other subtypes.

The N1 assay is designed to detect N1 sequences from the H5N1 lineage but may cross-react with N1 sequences from other subtypes.

A positive result for both H5 and N1 indicates the presence of H5N1 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, please send an email to [techsupport@primerdesign.co.uk](mailto:techsupport@primerdesign.co.uk) and our team will answer your question.

# Kit contents

Quantity	Component	Tube	Cap Colour
1	<b>H5 (H5N1) primer/probe mix</b> FAM labelled		<b>BROWN</b>
1	<b>N1 (H5N1) v2.0 primer/probe mix</b> FAM labelled		<b>BROWN</b>
1	<b>H5 (H5N1) positive control template</b>		<b>RED</b> (in foil wrapper)
1	<b>N1 (H5N1) v2.0 positive control template</b>		<b>RED</b> (in foil wrapper)
1	<b>RNase/DNase free water</b> for resuspension of the primer/probe mixes		<b>WHITE</b>
2	<b>Template preparation buffer</b> for resuspension of positive control templates and standard curve preparation		<b>YELLOW</b>

# 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®PLUS OneStep 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 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® principle. During PCR amplification, forward and reverse primers hybridize to the target cDNA. 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 minimise 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 allow primer/probe mix to rehydrate for 10 minutes at room temperature. Vortex each tube thoroughly, followed by pipetting up and down 10 times. Failure to mix well can produce poor kit performance.

Component - resuspend in water	Volume
<b>Pre-PCR pack</b>	
H5 (H5N1) primer/probe mix (BROWN)	165 µl
N1 (H5N1) v2.0 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 the tube thoroughly.

Component - resuspend in template preparation buffer	Volume
<b>Post-PCR heat-sealed foil</b>	
H5 (H5N1) Positive Control Template (RED) *	500 µl
N1 (H5N1) v2.0 Positive Control Template (RED) *	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 RT-qPCR 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 qPCR Master Mix ( <b>GOLD</b> )	10 µl
H5 (H5N1) <b>or</b> N1 (H5N1) 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®PLUS OneStep Master Mix ( <b>GOLD</b> )	10 µl
H5 (H5N1) <b>or</b> N1 (H5N1) 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.

- a. 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.
- b. Pipette 10 µl of positive control template (**RED**) into tube 2.
- c. Vortex thoroughly.
- d. Change pipette tip and pipette 10 µl from tube 2 into tube 3.
- e. Vortex thoroughly.

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

Standard Curve	Copy Number
Tube 1 H5 (H5N1) <b>or</b> N1 (H5N1) v2.0 Positive control ( <b>RED</b> )	$2 \times 10^5$ per $\mu\text{l}$
Tube 2	$2 \times 10^4$ per $\mu\text{l}$
Tube 3	$2 \times 10^3$ per $\mu\text{l}$
Tube 4	$2 \times 10^2$ per $\mu\text{l}$
Tube 5	20 per $\mu\text{l}$
Tube 6	2 per $\mu\text{l}$

**4.3 Pipette 15  $\mu\text{l}$  of reaction mix and 5  $\mu\text{l}$  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  $\mu\text{l}$ .

# OneStep RT-qPCR Amplification Protocol

Amplification conditions using oasig®PLUS OneStep Master Mix.

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

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

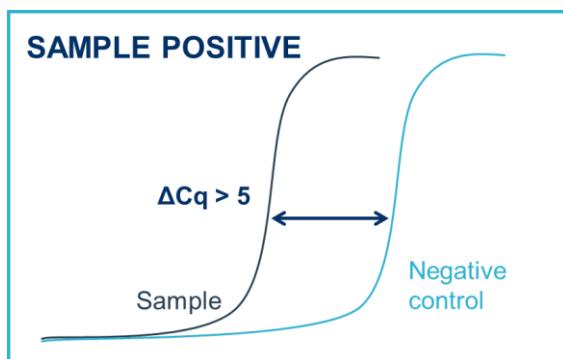
# Interpretation of results

H5 (H5N1) (FAM)	N1 (H5N1) v2.0 (FAM)	Positive control	Negative control	Interpretation
+	-	+	-	<b>POSITIVE RESULT FOR H5*</b>
-	+	+	-	<b>POSITIVE RESULT FOR N1*</b>
+	+	+	-	<b>POSITIVE RESULT FOR H5N1*</b>
-	-	+	-	<b>NEGATIVE RESULT</b>
+/-	+/-	+	$\leq 35$	<b>EXPERIMENT FAILED</b> Due to test contamination
+/-	+/-	+	> 35	**
-	-	+	-	<b>SAMPLE PREPARATION FAILED</b>
+/-	+/-	-	+/-	<b>EXPERIMENT FAILED</b>

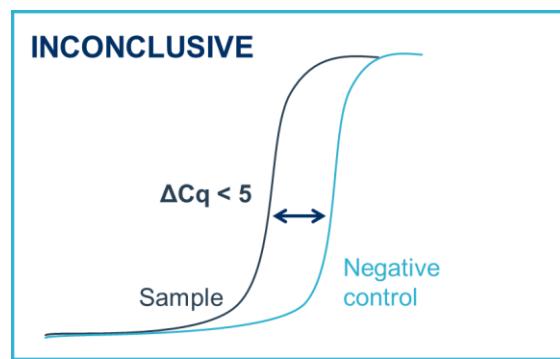
The positive control template (RED) is expected to amplify between Cq 16 and 23. Failure to satisfy this quality control criterion is a strong indication that the experiment has been compromised.

\*If a sample is positive for H5 but negative for N1, this could be due to the presence another H5 subtype. Conversely, if a sample is positive for N1 but negative for H5, this could be due to the presence of another N1 subtype. A sample is confirmed as H5N1 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 by violation of the general GLP guidelines and the manufacturer's recommendations the right to claim under guarantee is expired.

# Trademarks

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