

# HELINI

## Swine flu [H1N1]

### Real-time PCR Kit

(Single tube assay)

Instructions for use

**For use with:** Agilent, Bio-Rad, Roche Lightcycler-96, Roche-Z480/Cobas-480, Applied Bio systems [ABI], Thermo-Piko-Real, Rotor gene 5/6plex, Alta-96, Cepheid Real time PCR machines.

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HELINI Biomolecules, Chennai, INDIA



## Intended Use

The HELINI Swine flu H1N1 Real-time PCR Kit is an in vitro nucleic acid amplification kit for the detection of Swine flu H1N1 specific RNA.

## Kit components

<b>Components</b>	<b>Volume Per reaction</b>	<b>Number of vials</b>	<b>Volume Per vials</b>
One step RT-PCR Master Mix	8 $\mu$ l	1	200 $\mu$ l
RT-Taq enzyme mix	2 $\mu$ l	1	50 $\mu$ l
H1N1 Primer Probe Mix [H1N1 PP mix]	5 $\mu$ l	1	125 $\mu$ l
Positive control mix	10 $\mu$ l	1	150 $\mu$ l
Water, PCR grade		1	4ml

## **Storage**

- The kit is shipped on gel ice. Upon arrival, all components should be stored in -20°C. They are stable until the expiration date stated on the label.
- Repeated thawing and freezing should be avoided, as this might affect the performance of the assay.
- If the reagents are to be used only intermittently, they should be frozen in aliquots. Storage at 2 to 8°C should not exceed a period of 5 hours.

## **Material and instruments required**

- Real-time PCR instrument having FAM, HEX & Cy5 channels
- Automatic Nucleic acid extraction system or spin column based purification kit for the purification of nucleic acids
- Desktop centrifuge having 13000rpm or above with a rotor for 1.5/2 ml reaction tubes
- Centrifuge with a rotor for PCR strips/tubes and 96 well plates
- Optical cap qPCR tubes or strips or 96 wells
- Micro Pipettes (variables)
- Micro Pipette tips with filters (disposable)
- Powder-free gloves (disposable)

*[Please ensure that all instruments used have been installed, calibrated, checked and maintained according to the manufacturer's instructions and recommendations.]*

## **Product Use Limitations**

- All reagents may exclusively be used in molecular diagnosis.
- The product is to be used by personnel specially instructed and trained in Molecular diagnosis.
- Strict compliance with the user manual is required for optimal PCR results.
- Attention should be paid to expiration dates printed on the box and labels of all components. Do not use expired components.
- Wear protective disposable powder-free gloves, a laboratory coat and eye protection when handling specimens and kit components.
- Avoid microbial and nuclease (RNase/RNase) contamination of the specimens and the components of the kit.
- Always use RNase/RNase-free disposable pipette tips with aerosol barriers.
- Use separated and segregated working areas for sample preparation, reaction setup and amplification/detection activities.
- The workflow in the laboratory should proceed in unidirectional manner. Always wear disposable gloves in each area and change them before entering a different area.
- Store positive and/or potentially positive material separated from all other components of the kit.
- Do not open the reaction tubes/plates post amplification, to avoid contamination with amplicons.

- Additional controls may be tested according to guidelines or requirements of local, state and/or federal regulations or accrediting organizations.
- Do not autoclave reaction tubes after the PCR, since this will not degrade the amplified nucleic acid and will bear the risk to contaminate the laboratory area.
- Discard sample and assay waste according to your local safety regulations.

### **Technical Assistance**

For technical assistance and more information, please contact;

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## **Product description**

HELINI Swine flu H1N1 Real-time PCR Kit constitutes a ready-to-use system for the detection of Influenza-A and H1N1 specific RNA using polymerase chain reaction (PCR). It contains reagents and enzymes for the specific amplification of the conserved region of the Swine flu H1N1 genome, and for the direct detection of the specific amplicon in FAM and Cy5 channel. In addition, it contains an endogenous control amplification system to identify possible PCR inhibition and RNA purification efficiency. External positive control is supplied to assist the run.

## **Specificity**

Swine flu H1N1 primer and probe have been designed for the specific and exclusive *in vitro* detection of PAN-Influenza-A and Swine flu H1N1. The target sequence is highly conserved and has previously been shown to be a good genetic marker for Swine flu H1N1. The primers and probe sequences in this kit have 100% homology with a broad range of clinically relevant reference sequences based on a comprehensive bioinformatics analysis.

## **Dynamic linear range**

The linear range was evaluated by analyzing a logarithmic dilution series of RNA concentrations ranging from 1.00E+09 to 1.00E+00 copies/ $\mu$ l. At least six replicates per dilution were analyzed. The linear range is 1.00E+09 to 1.00E+00 copies/ $\mu$ l.

### **Analytical Sensitivity**

The analytical sensitivity is defined as the concentration of RNA molecules (copies/ $\mu$ l) that can be detected with a positivity rate of 95%. The analytical sensitivity was determined by analysis of dilution series of quantified Influenza-A and H1N1 specific RNA from 0.001copies to 10copies/ $\mu$ l in triplicates. Under optimal PCR conditions, the analytical sensitivity is 0.42 copies per micro liter.

### **Note:**

#### **RNA Purification**

Purified RNA is the starting material for the Real-time PCR assay. The quality of the purified RNA has a profound impact on the performance of the entire test system. It has to be ensured that the purification system used for RNA purification is compatible with real-time PCR technology.

If you are using a spin column based sample preparation procedure having washing buffers containing ethanol, it is highly recommended to perform an additional centrifugation step for 3min at approximately 17000 x g (~ 13000 rpm), using a new collection tube, prior to the elution of the RNA.



### **Endogenous control**

Human gene is given as endogenous control. It amplifies a single copy human gene from the test samples. A successful amplification indicates that test samples are properly collected and has its biological property.

The primer and probe present at PCR limiting concentrations which allows multiplexing with the target sequence primers. Amplification of the endogenous control template does not interfere with detection of the mutation even when present at low copy number. The endogenous control is detected through the HEX channel and gives a CT value of 23 +/-10.

## Detection Protocol

### Things to do before starting

- Before use, all kit components need to be thawed completely, mixed by gently inverting and centrifuged briefly.
- Make sure that Positive and Negative control is included in every run.
- Include 0.5 reaction volume for pipetting error while calculating the volume for total number of reactions.

Components	Volume per reaction
One step RT-PCR Master Mix	8 $\mu$ l
RT-Taq enzyme mix	2 $\mu$ l
H1N1 PP Mix	5 $\mu$ l
	<b>15<math>\mu</math>l</b>
<b>Purified RNA</b>	<b>10<math>\mu</math>l</b>
Final reaction volume	25 $\mu$ l

### Negative Control setup [NTC]

Add 10 $\mu$ l of PCR grade water.

### Qualitative Positive Control setup [H1N1 & Influenza-A]

Add 10 $\mu$ l of Positive control mix

Centrifuge PCR vials briefly before placing into thermal cycler.

*[Note: There should not be any bubbles in the reaction mix. Bubbles interfere with fluorescence detection.]*

## Programming Thermal cycler

<b>Sample volume</b>	25µl
<b>Fluorescence Dyes</b>	FAM & HEX & Cy5
<b>Passive reference</b>	None
<b>Ramping rate</b>	Default

## Thermal Profile

	<b>Step</b>	<b>Time</b>	<b>Temp</b>
	Reverse transcriptase	20min	50°C
	Taq enzyme activation / Hold	15min	95°C
<b>45 cycles</b>	Denaturation	20sec	95°C
	Annealing/Data collection*	20sec**	56°C
	Extension	20sec	72°C

<b>Data collection/Acquisition</b>	<b>Targets</b>
FAM	H1N1
HEX	Endogenous control
Cy5	Influenza-A

\*\* Some qPCR machines may require minimum 30sec for data collection; in that case, set to 30sec, this will not affect the performance.

## **Reading the graph:**

### **Step-1: Endogenous control validation:**

Select NTC and test sample wells, select HEX channel and view the graph of endogenous [human gene] amplification. A successful amplification must be less than Ct 23 +/-10. This range indicates that test sample is collected and purified well and there is NO PCR inhibition in the reaction. Any sample value goes beyond Ct value 34 indicates that either sample has some issues in the purification or inhibiting PCR reaction. Please note that Endogenous control will not amplify in both NTC and Positive control.

### **Step-2 – Universal-Influenza-A**

Select the NTC and Positive control wells, select Cy5 channel, and view the graph of amplification. The NTC must be flat with no Ct value. If required adjust the threshold value just above the NTC. The PC must be amplified. Select test samples wells one by one and view the amplification. NTC justifies NO contamination in the reagent as well as fine pipetting and its environment. PC justifies the reagents storage conditions and reaction parameters are as prescribed.

### Step-3-H1N1

Select the NTC and Positive control wells, select FAM channel, and view the graph of amplification. The NTC must be flat with no Ct value. If required adjust the threshold value just above the NTC. The PC must be amplified. Select test samples wells one by one and view the amplification. NTC justifies NO contamination in the reagent as well as fine pipetting and its environment. PC justifies the reagents storage conditions and reaction parameters are as prescribed.

### Qualitative interpretation

<b>Endogenous Control [Human RNaseP gene]</b>	<b>Universal Influenza-A</b>	<b>H1N1</b>	<b>Interpretation</b>
Positive	Positive	Positive	H1N1 RNA detected
Positive	Positive	Negative	Influenza-A RNA detected
Positive	Negative	Negative	InfA/HIN1 RNA Not Detected
Negative	Negative	Negative	Sample not suitable for qPCR or repeat with fresh sample

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Observation		Interpretation	
FAM-H1N1	HEX-Endo	Cy5-InfA	
<37	<33	<37	H1N1 Detected

**Recommendation:**

In FAM [H1N1] & Cy5 [InfA] channel, the Ct value beyond 35 is required careful analysis. The analysis may include that the status of NTC amplification curve, threshold adjustment, linear/log scale view assessment, etc.,

**Note:**

High positive amplification of Influenza-A [Cy5] & H1N1 [FAM] may suppress the amplification of endogenous control. It can be reported as “positive” after a careful verification of Negative [No amplification] and positive control [Amplification] amplification status.

**Strains detected**

<b>Influenza-A</b>	<b>H1N1</b>
Influenza A/ H1N1	H1N1-2009pdm - California/2009
Influenza A / H1N2	H1N1-2009pdm - NY/2009
Influenza A / H2N2	
Influenza A / H2N3	Globally reported human infected H1N1 strains in
Influenza A / H3N1	the year of 2010/11/12/13/14/15/16/17/18/19
Influenza A / H3N2	
Influenza A / H3N8	
Influenza A / H5N1	
Influenza A / H5N2	
Influenza A / H5N3	
Influenza A / H5N6	
Influenza A / H5N8	
Influenza A / H5N9	
Influenza A / H6N1	
Influenza A / H6N2	
Influenza A / H7N1	
Influenza A / H7N2	
Influenza A / H7N3	
Influenza A / H7N4	
Influenza A / H7N7	
Influenza A / H7N9	
Influenza A / H9N2	
Influenza A / H10N7	
Influenza A / H11N2	
Influenza A / H11N3	
Influenza A / H11N9	
Influenza A / H12N5	
Influenza A / H13N6	

## **Limitations**

Good laboratory practice is essential for proper performance of this assay. Strict compliance with the instructions for use is required for optimal results.

Analysts should be trained and familiar with testing procedures and interpretation of results prior to performing the assay.

A false negative result may occur if inadequate numbers of organisms are present in the sample due to improper collection, transport or handling. Appropriate specimen collection, transport, storage and processing procedures are required for the optimal performance of this test.

Extreme care should be taken to preserve the purity of the components of the kit and reaction setups. All reagents should be closely monitored for impurity and contamination. Any suspicious reagents should be discarded.

The presence of PCR inhibitors may cause under quantification, false negative or invalid results.

Potential mutations within the target regions of the pathogen's genome covered by the primers and/or probes used in the kit may result in under quantification and/or failure to detect.






As with any diagnostic test, the HELINI Swine flu H1N1 Real-time PCR results need to be interpreted in consideration of all clinical and laboratory findings.



## Quality Control

In accordance with the HELINI Biomolecules in house Quality Management System, each lot of HELINI Swine flu H1N1 Real-time PCR kit is tested against predetermined specifications to ensure consistent product quality.

## Explanations of symbols

	In vitro diagnostic medical device
	
	Catalogue number
	Pack size – number of tests
	Manufacturer

Manufactured by

**HELINI *Biomolecules***,

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