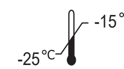
Swine flu [H1N1] Real-time PCR Kit

(For Qualitative Detection)

REF 8006



INTENDED USE

The Swine flu H1N1 Real-time PCR Kit is an *in-vitro* nucleic acid amplification kit for the detection of Swine flu HIN1 specific RNA.

KIT CONTENTS

Components	Number of vials	Volume Per vials
One step RT-PCR Master Mix	1	200µl
RT-Taq enzyme mix	1	50µl
H1N1 Primer Probe Mix [H1N1 PP mix]	1	125µl
Positive control mix	1	250µl
Water, PCR grade	1	4µl

STORAGE

1. 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.
2. Repeated thawing and freezing should be avoided, as this might affect the performance of the assay.
3. 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.

PRODUCT DESCRIPTION

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.

ANALYTICAL SENSITIVITY

The analytical sensitivity is defined as the concentration of RNA molecules (copies/µl) 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/µl in triplicates. Under optimal PCR conditions, the analytical sensitivity is 0.92 copies per micro liter. [275copies/ml-60µl eluate – 0.2ml sample]

DNA 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.

ENDOGENOUS CONTROL

Add 5µl of the internal control template to each test sample. Do not add directly to test sample. Add after adding lysis buffer to the test sample [sample/lysis buffer mix]. Complete purification according to the manufacturer's protocols.

The primer and probe present at PCR limiting concentrations which allows multiplexing with the target sequence primers. Amplification of the internal control template does not interfere with detection of the pathogen target gene even when present at low copy number. The Internal control is detected through the HEX channel and gives a CT value of 21 +/- 10.

DETECTION PROTOCOL

- 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	8µl
RT-Taq enzyme mix	2µl
H1N1 PP Mix	5µl
Volume of master mix	15µl
Purified DNA	10µl
Total volume	25µl

Negative Control setup [NTC]
Add 10µl of PCR grade water.

Qualitative Positive Control setup [H1N1 & Influenza-A]
Add 10µl of Positive control mix

PROGRAMMING THERMAL CYCLER

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

THERMAL PROFILE

Cycles	Step	Time	Temp
1	Reverse transcriptase	20min	50ºC
	Taq enzyme activation / Hold	15min	95ºC
45 cycles	Denaturation	20sec	95ºC
	Annealing/Data collection*	20sec	56ºC
	Extension	20sec	72ºC

Data collection/Acquisition	Targets
FAM	H1N1 Endogenous
HEX	Internal control
Cy5	Influenza-A

READING THE GRAPH

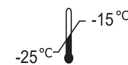
Step-1: Endogenous control validation:
Select NTC and test sample wells, select HEX channel and view the graph of endogenous [human gene] amplification. A successful amplification must be less than Ct 23 +/-7. This range indicates that test sample is collected and purified well and there is NO PCR inhibition 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.

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 OF RESULTS

Endogenous Control [Human RNaseP gene]	Universal Influenza- A	H1N1	Interpretation
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



Influenza-A	H1N1
Influenza A/ H1N1 Influenza A / H1N2 Influenza A / H2N2 Influenza A / H2N3 Influenza A / H3N1 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	H1N1-2009pdm - California/2009 H1N1-2009pdm - NY/2009 Globally reported human infected H1N1 strains in the year of 2010/11/12/13/14/15/16/17/18/19

LIMITATIONS

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.

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 Swine flu H1N1 Real-time PCR results need to be interpreted in consideration of all clinical and laboratory findings.

QUALITY CONTROL

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

Index of symbols

Consult instructions for use	LOT Batch code	Tests per kit
IVD For *in vitro* diagnostic use only	Do not re-use	Keep dry
Temperature limit - 25 °C to - 15 °C	Date of manufacture	Biologial Risks
Manufacturer	Use-by date	

Marketed by:

ATHENESE-Dx
Early diagnosis for better life

Athenese-Dx Pvt. Ltd.

PI-8006 Rev. A
Effective date: 05.08.2020
English version