HELINI
Swine flu [H1N1]
Real-time PCR Kit

(Single tube assay –RNaseP/InfA/InfB/H1N1)

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, Cepheid Real time PCR machines.

CE IVD

REF 8006

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Intended Use

The HELINI Swine flu H1N1 Real-time PCR Kit is an in vitro nucleic acid amplification kit for the detection of Influenza A & B, Swine flu HIN1 specific RNA.

Kit components

Components	Volume Per reaction	Number of vials	Volume Per vials
One step RT-PCR Master Mix	8µl	1	200µl
RT-Taq enzyme mix	2µl	1	50µl
H1N1 Primer Probe Mix [H1N1 PP mix]	5µl	1	125µl
Positive control mix	10µl	1	150µl
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, ROX & 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 & B and H1N1 specific RNA using polymerase chain reaction (PCR). It contains reagents and enzymes for the specific amplification of the conserved region of the Influenza A & B genome, and for the direct detection of the specific amplicon in FAM, HEX, ROX 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. PAN-Influenza-B 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/µl. At least six replicates per dilution were analyzed. The linear range is 1.00E+09 to 1.00E+00 copies/µl.

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, Influenza-B and H1N1 specific RNA from 0.001copies to 10copies/µl in triplicates. Under optimal PCR conditions, the analytical sensitivity is 0.32 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	8µl
RT-Taq enzyme mix	2µl
H1N1 PP Mix	5µl
	15µl
Purified RNA	**10µl**
Final reaction volume	25µl

Negative Control setup [NTC]

Add 10µl of PCR grade water.

Positive Control setup

Add 10µl 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

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

Thermal Profile

	Step	**Time**	**Temp**
	Reverse transcriptase	20min	50ºC
	Taq enzyme activation / Hold	15min	95ºC
40 cycles	Denaturation	20sec	95ºC
	Annealing/Data collection*	20sec	60ºC
	Extension	20sec	72ºC

Data collection/Acquisition	**Targets**
FAM	H1N1
HEX	Endogenous control
ROX	Influenza-B
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. Make sure that ROX passive reference option to none.

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.

Step-4 – Universal-Influenza-B

Select the NTC and Positive control wells, select ROX 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

Endo-genous Control [Human RNaseP gene]	PAN Inf-A	H1N1	PAN Inf-B	Interpretation
Positive	Positive	Positive	Negative	H1N1 RNA detected
Positive	Positive	Negative	Negative	Influenza-A RNA detected
Positive	Negative	Negative	Negative	InfA/HIN1 RNA Not Detected
Positive	Negative	Negative	Positive	Influenza-B RNA Detected
Negative	Negative	Negative	Negative	Sample not suitable for qPCR or repeat with fresh sample

FAM-H1N1	HEX-Endo	Cy5-InfA
<37	<33	<37

Influenza-B	HEX-Endo
<37	<33

Recommendation:

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], Influenza-B [ROX] & 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

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
Influenza B	PAN – All subtypes

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.

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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

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