

# HELINI

## Human metapneumovirus

### Real-time PCR Kit

#### 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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8289



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HELINI Biomolecules, Chennai, INDIA

**Intended Use**

The HELINI Human metapneumovirus Real-time PCR Kit is an in vitro nucleic acid amplification kit for the detection and quantification of Human metapneumovirus 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
Human metapneumovirus & Endogenous control Primer Probe Mix [HMPV/Endo PP mix]	5µl	1	125µl
Positive control [QS1]	10µl	1	250µl
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 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 Human metapneumovirus Real-time PCR Kit constitutes a ready-to-use system for the detection of Human metapneumovirus specific RNA using polymerase chain reaction (PCR). It contains reagents and enzymes for the specific amplification of the conserved region of the Human metapneumovirus genome, and for the direct detection of the specific amplicon in 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, which can be used as both qualitative and quantitative to determination the amount of viral load.

### Specificity

Human metapneumovirus primer and probe have been designed for the specific and exclusive *in vitro* quantification of Human metapneumovirus and its linkage genotypes A & B (A1/A2 & B1/B2). It detects all genotypes and will not discriminate them. The target sequence is highly conserved and 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 Human metapneumovirus specific pDNA from 0.001copies to 10copies/ $\mu$ l in triplicates. Under optimal PCR conditions, the analytical sensitivity is 0.25 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.

**Preparation of standard curve dilution series:**

1. Pipette 90µl of PCR grade water into three 1.5ml micro centrifuge tubes and label as QS2 to QS4.
2. Pipette 10µl of Positive control-QS1 into tube QS2.
3. Vortex thoroughly and spin down briefly.
4. Change pipette tip and pipette 10µl from tube QS2 into tube QS3.
5. Vortex thoroughly and spin down briefly.
6. Repeat steps 4 and 5 to complete the dilution series.
7. Use 10µl per reaction.
8. Prepare every time fresh and use.

Standards	Copies per µl
QS-1	1000000
QS-2	100000
QS-3	10000
QS-4	1000

## 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.
- Make sure that internal control template is added during RNA purification. If not, pipette 2.5µl of the internal control template directly into the purified RNA.
- 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
HMPV/Endo PP Mix	5µl
<b>Purified RNA</b>	<b>10µl</b>
Final reaction volume	25µl

### Negative Control setup [NTC]

Add 10µl of PCR grade water.

### Qualitative Positive Control setup

Add 10µl of any one of the Positive controls [From QS1 to QS4]

### Quantitative Positive controls setup

10µl of all Positive controls prepared from QS1 to QS4.

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>	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>40 cycles</b>	Denaturation	20sec	95°C
	Annealing/Data collection*	20sec**	60°C
	Extension	20sec	72°C

<b>Data collection/Acquisition</b>	<b>Targets</b>
HEX	Human metapneumovirus
Cy5	Endogenous control

\*\* 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 Cy5 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 – HEX - Negative and Positive control validation**

Select the NTC and Positive control [Qualitative] or Standards wells [Quantitative], select HEX 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 or Standards must be amplified as per their copy numbers.

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 –HEX - Test Sample status**

In HEX channel, select test sample well one by one, analyze the graph/amplification.

**Qualitative interpretation of results:**

Test Sample	Negative control	Positive control	Internal Control	Interpretation
Positive	Negative	Positive	Positive	Human metapneumovirus specific RNA detected
Negative	Negative	Positive	Positive	No HMPV specific RNA Detected. Sample does not contain detectable amounts of HMPV specific RNA.
Negative	Negative	Negative	Negative	Experiment fail
Positive	Positive	Positive	Positive	Experiment fail

**Qualitative**

Observation		Interpretation
HEX-HMPV	Cy5-Endogenous Control	
<37	<31	Human metapneumovirus Specific DNA detected

**Recommendation:**

In HEX [Human metapneumovirus] 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.,



### Standard Curve analysis

- Interpret the values for unknown samples, only if the  $R > 0.98$
- Slope of calibrators is between -3.0 to -3.7
- PCR efficiency is between 85% and no amplification in HEX channel of negative control.

### Calculating copies per ml

Input the machine indicated copy number into the following formula

$$\text{Result (copies/ml)} = \frac{\text{Result (copies/}\mu\text{l)} \times \text{Elution Volume (}\mu\text{l)}}{\text{Sample Volume (ml)}}$$

#### Note:

Elution volume: must be typed in micro liter format, example 30 $\mu$ l, 60 $\mu$ l or 100 $\mu$ l. Sample volume: must be typed in milliliter format, example 0.2ml or 0.5ml

Observation	Interpretation
<75 copies/ml	Human metapneumovirus detected, but below limit of detection
75 copies to 3 x 10 <sup>9</sup> copies/mL	Human metapneumovirus detected and quantified
<3 x 10 <sup>6</sup> copies/mL	Human metapneumovirus detected, but upper limit of quantitation

### 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 similar 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 Human metapneumovirus 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 Human metapneumovirus 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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