HELINI
Coronavirus
Real-time PCR Kit

[RdRp & ORF gene – Dual target - Single tube assay]

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.

Cat.No:8052 – 25/50/100tests

Instruction manual

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Intended Use

The HELINI Coronavirus Real-time PCR Kit is an in vitro nucleic acid amplification kit for the detection of novel Coronavirus-2019 specific RNA.

Kit components

Components	Volume Per reaction	25tests	50tests	100tests
One step RT-PCR Master Mix	8µl	200µl	400µl	800µl
RT-Taq enzyme mix	2µl	50µl	100µl	200µl
CoV Primer Probe Mix [CoV PP mix]	5µl	125µl	250µl	500µl
Positive control	10µl	100µl	100µl	100µl
Water, PCR grade		1ml	1ml	1ml

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 channel
- 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 Coronavirus Real-time PCR kit constitutes a ready-to-use system for the detection of Novel 2019 Coronavirus specific RNA using polymerase chain reaction (PCR). It contains reagents and enzymes for the specific amplification of the conserved region of the Novel Coronavirus genome, and for the direct detection of the specific amplicon in FAM & Cy5 channel. In addition, it contains an endogenous control amplification system to identify the sample collection, possible PCR inhibition and RNA purification efficiency.

Specificity

Novel Coronavirus primer and probe have been designed for the specific and exclusive *in vitro* detection of Novel Coronavirus 2019. The target gene [RdRp and ORF gene] 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 cDNA 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 Coronavirus specific pDNA from 0.001copies to 10copies/µl in triplicates. Under optimal PCR conditions, the analytical sensitivity is 0.65 copy 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 10 min at approximately 17000 x g (~ 13000 rpm), using a new collection tube, prior to the elution of the RNA.

Endogenous control

Human gene [RNaseP] is given as endogenous control. It amplifies a single copy human gene from the test samples. A successful amplification indicates that test sample is properly collected and has its biological property with required number of human cells for PCR.

The Endogenous control 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 pathogen even when present at low copy number. The endogenous control is detected through the HEX channel and gives a CT value of 25 +/-9.

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**
One step RT-PCR Master Mix	8µl
RT-Taq enzyme mix	2µl
CoV PP Mix	5µl
	15µl
Thoroughly mix the sample RNA by pipetting up and down.	
Purified RNA	**10µl**
Final reaction volume	25µl

Negative Control setup [NTC]

Add 10µl of PCR grade water.

Qualitative Positive Control setup

Add 10µl of the Positive control

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 & **Cy5**
Passive reference	None
Ramping rate	Default

Thermal Profile

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

*Some model of Real-time PCR machines requires minimum 30seconds for annealing and data acquisition. You can increase the duration to 30secconds and it will not affect the performance of the reaction. [for example - ABI-7500]

*Data collection/Acquisition	Targets
FAM	RdRp-Novel Coronavirus-2019
Cy5	ORF-Novel Coronavirus-2019
HEX	Endogenous control

Reading the graph:

Step-1 – Endogenous control Validation

Select **ONLY test samples** for the endogenous control analysis. Select HEX dye and view the graph of endogenous control amplification. A successful amplification Ct value must be within Ct 25 +/- 10.

This range indicates that test sample has enough cells to perform PCR reaction and NO PCR inhibition in the reaction. Any sample value goes beyond Ct 35 indicates that either sample does not have enough cells OR issues in the purification OR inhibition in the PCR reaction.

Endogenous control will not get amplified in the negative control and Positive control. Ignore a late noise HEX amplification graph in the NTC & PC. In case, any late HEX amplification in the test sample, analyze the graph in log scale mode and accept if there is a minimum 2 Ct difference between the NTC/PC noise and test sample. Log scale analysis indicates test sample starting fluorescence cycle.

Step-2

FAM-RdRp: 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.

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Cy5-ORF: 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.

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: Test samples

Select each sample well and analyze with one dye at a time. First FAM and then Cy5. There will be a difference of Ct/Cq value between each gene targets which is normal.

Gene	Dye	Cut off
RdRp	FAM	**<36**
ORF	Cy5	**<36**
RNaseP	HEX	**<36**

Note:

NO Endogenous control amplification is acceptable when both viral targets RDRP & ORF amplified and show Ct value difference within 2 Ct value.

Qualitative interpretation of results:

Negative Control	Positive Control	Endo Control	RdRp gene	ORF gene	Interpretation
Negative	Positive	Positive/ Negative*	Positive	Positive	2019 Novel Coronavirus RNA detected
Negative	Positive	Positive/ Negative*	Negative	Negative	Not Detected
Negative	Negative	Negative	Negative	Negative	Experiment failed – repeat
Positive	Positive	Positive	Positive	Positive	Experiment failed – Check the reagent or environment contamination

*NO Endogenous control amplification is acceptable When both viral targets RDRP & ORF amplified and show Ct value difference within 2 Ct value.

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 research, the HELINI Coronavirus [COVID-19] 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 Coronavirus [COVID-19] Real-time PCR kit is tested against predetermined specifications to ensure consistent product quality.

Manufactured by

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