LifeDireX COVID-19 RT-qPCR Detection Kit

Cat No. QP019-0100

Size: 100 Reactions

Sample: 5pg ~ 1ug RNA / 20ul Reaction

Storage: Stable for up to 1 year at -20°C

Description

In view of the joint global efforts of advancing collaborative research in diagnostics, therapeutics, and vaccination in the fight against the COVID-19(SARS-CoV-2) pandemic, Bio-Helix has specifically developed the LifeDireX COVID-19 RT-qPCR Detection Kit for human respiratory tract specimens. The kit is characterized by: (1) High specificity for the ORF1ab and N target markers as recommended by WHO and US CDC; (2) Data obtained in less than 2 hours; and (3) Compatible with standard RT-qPCR machines (ABI 7500, Bio-Rad CFX96, QuantStudio’ s 7 Flex).

Kit Contents

Part No.	Component	Volume
QP019-0100-1	2X RT-qPCR MasterMix	1.25ml
QP019-0100-2	COVID-19 Primers	100 ul
QP019-0100-3	COVID-19 Probes	100 ul
QP019-0100-4	Positive Control Template	10 ul
QP019-0100-5	RT-qPCR Enzyme Mix	40 ul

Required Materials

Real-time PCR tubes Real-time PCR instrument Nuclease–Free H_2O

Real-Time PCR Instrument

ABI 7500, Bio-Rad CFX96, QuantStudio’ s 7 Flex

Application

Gene Expression (mRNA) Analysis

Copy Number Analysis

SNP Genotype Analysis

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Protocol

1. PCR Reaction: Thaw and assemble the following components in a 0.2 ml PCR tube on ice just prior to use: COVID-19 Primers, COVID-19 Probes, 2X RT-qPCR MasterMix, and RT-qPCR Enzyme Mix. Caution: Do not add more than one RNA sample into a single qPCR tube. Mix gently. If necessary, centrifuge briefly.

Component	20 ul Sample	20 ul Positive Control	20 ul Negative Control
RNA Sample	5pg ~ 1ug RNA / 20ul Reaction	-	-
COVID-19 Primers	1 ul	1 ul	1 ul
COVID-19 Probes	1 ul	1 ul	1 ul
2X RT-qPCR MasterMix	10 ul	10 ul	10 ul
RT-qPCR Enzyme Mix	0.4 ul	0.4 ul	0.4 ul
Nuclease – Free H_2O	Up to 20 ul	Up to 20 ul	Up to 20 ul

2. Use the Nuclease-free H_2O for the Negative Control while using Positive Control Template for the Positive Control setup. Cap tubes and place in the thermal cycler.

3. Process in the thermal cycler for 42 cycles as follows:

Steps	Temperature/Time	Cycle
cDNA Synthesis	15 minutes at 42°C	1
Pre-Denaturation	10 minutes at 95°C	1
Denaturation	15 seconds at 95°C	
Annealing	60 seconds at 60°C	40
Melting curve	Refer to specific guidelines for instrument used	

Note:

Optimal conditions for amplification will vary depending on the primers and thermal cycler used. It may be necessary to optimize the system for individual primers, template, and thermal cycler.

4. Detection: As four channels (FAM, HEX, ROX, & Cy5) are used in this one tube qPCR assay, we recommend to perform the color (channel) calibration as requested by the instrument' s manufacturer. Please refer to the instrument' s user manual to perform this calibration. Choose the FAM, HEX, ROX, and Cy5 channels for each sample to be tested with the LifeDireX COVID-19 RT-qPCR Detection Kit. Select "None" for ROX passive reference on any Applied Biosystem' s qPCR machine.

Troubleshooting

Refer to the table below to troubleshoot problems that you may encounter when quantify of nucleic acid targets with the kit.

Trouble	Cause	Solution
Poor Signal or No Signal	Inhibitor Present	1. Perform a dilution series of the PCR template to determine whether the effect of the inhibitory agent can be reduced. 2. Take extra care with the nucleic acid extraction steps to minimize carryover of PCR inhibitors.
	Degraded Template Material	1. Do not store diluted template in water or at low concentrations. 2. Check the integrity of template material by automated or manual gel electrophoresis.
	Inadequate Thermal Cycling Conditions	1. Try using a minimum extension time of 30 sec for genomic DNA and 15 sec for cDNA.
Signal in Negative Control	Contamination of Reaction Components with Target Sequence	1. To minimize the possibility of contamination of PCR components by PCR product or other template, designate a work area exclusively for PCR assay setup. 2. Use a solution of 10% bleach instead of ethanol to prepare the workstation area for PCR assay setup. Ethanol will only induce precipitation of DNA in your work area, while the 10% bleach solution will hydrolyze, as well as dissolve, any residual DNA.
Poor Reproducibility Across Replicate Samples	Inhibitor Present	1. Perform a dilution series of the PCR template to determine whether the effect of the inhibitory agent can be reduced. 2. Take extra care with the nucleic acid extraction steps to minimize carryover of PCR inhibitors.
	Primer Design	1. Verify primers design at different annealing temperatures.
Low or High Reaction Efficiency	Primer- Dimer	1. Reduce primer concentration. 2. Evaluate primer sequences for complementarity and secondary structure. Redesign primers if necessary. 3. Perform melt-curve analysis to determine if primer- dimers are present.
	Insufficient Optimization	1. Use a thermal gradient to identify the optimal thermal cycling conditions for a specific primer set.

Caution

1. Shake gently before use to avoid foaming and low-speed centrifugation.
2. Reduce the exposure time.
3. During operation, always wear a lab coat, disposable gloves, and protective equipment.