— PROTOCOL —

LifeDireX COVID-19 RT-qPCR Detection Kit

Cat No. QP019-0100

Size: 100 Reactions

Sample: 5pg ~ 1μg RNA / 20μl 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 RdRP 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	Reactions/Kit
QP019-0100-1	2X RT-qPCR MasterMix	1.25 ml	100
QP019-0100-2	RT-qPCR Enzyme Mix	40 μl	100
QP019-0100-3	COVID-19 Primers/Probes	200 μl	100
QP019-0100-4	Positive Control	100 μl	20
QP019-0100-5	Negative Extraction Control	1.0 ml	20
QP019-0100-6	Nuclease-Free Water	1.0 ml	100

Required Materials

》Real-Time PCR tubes 》Real-Time PCR instrument

Real-Time PCR Instrument

ABI: 7500 Fast Series

Bio-Rad: CFX96

Roche: LightCycler Series

Agilent: Mx3005p

Qiagen: RotorGene 3000

Application

》Gene Expression (mRNA) Analysis

》Copy Number Analysis

》SNP Genotype Analysis

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 µl Patient Sample	20 µl Positive Extraction Control	20 µl Negative Extraction Control	Negative Control
RNA Sample	5 µl	0 µl	0 µl	0 µl
COVID-19 Primers/Probes	2 µl	2 µl	2 µl	2 µl
2X RT-qPCR MasterMix	10 µl	10 µl	10 µl	10 µl
RT-qPCR Enzyme Mix	0.4 µl	0.4 µl	0.4 µl	0.4 µl
Positive Extraction Control	0 µl	5 µl	0 µl	0 µl
Negative Extraction Control	0 µl	0 µl	5 µl	0 µl
Nuclease – Free H_2O	2.6 µl	2.6 µl	2.6 µl	7.6 µl

2. Use the Nuclease-free H_2O for the Negative Control while using Positive Control 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	40
Annealing	60 seconds at 60°C	
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 three channels (FAM, ROX, HEX) are used in this one tube qPCR assay, we recommend to perform the channel calibration as requested by its manufacturer. Please refer to the instrument's user manual to perform this calibration. Choose the FAM, ROX, and HEX channels for each sample to be tested with the LifeDireX COVID-19 RT-qPCR Detection Kit. Select "None" for ROX passive reference on any qPCR machine requiring ROX as the reference dye.

Expected Performance of Controls

Control Type	Used to Monitor	Expected Results and Ct Values		
		N (FAM)	RP (HEX)	RdRP (ROX)
Positive	Flawed assay setup and reagent failure, including degraded primer and probe	Positive Ct < 40.0	Negative Ct ND	Positive Ct < 40.0
Positive Extraction Control ("RP")	Poor specimen lysis, undesirable specimen collection, improper assay setup, extraction failure, or PCR inhibition	Negative Ct ND	Positive Ct < 40.0	Negative Ct ND
Negative ("NTC")	Assay or extraction reagent contamination	Negative Ct ND	Negative Ct ND	Negative Ct ND
Negative Extraction Control	Cross-contamination	Negative Ct ND	Positive Ct < 40.0	Negative Ct ND

ND = Not Detected.

Results are considered invalid if any control does not perform as specified above.

Interpretation of Results

SARS-CoV-2			Interpretation	Action
N	RdRP	RP		
+	+	+/-	Positive	Report result to sender health authority.
If only one of the two targets is positive		+/-	Inconclusive Result	Repeat RT-qPCR of samples or repeat from extraction step. If result is still inconclusive, recommend collection of new specimen(s) from the patient.
-	-	+	Negative	SARS-CoV-2 not detected. Report result to sender health authority
-	-	-	Invalid Result	Repeat from extraction step. If the repeated result remains invalid, recommend collection of a new specimen(s) from the patient.

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.