LifeDireX COVID-19 RT-qPCR Detection Kit

05 MAR 2021

Catalog Number	Size	Concentration
QP019-0100	100 Reactions	2X

Storage Conditions

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 (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 Content(s)

2X RT-qPCR MasterMix	1.25 ml
RT-qPCR Enzyme Mix	40 µl
COVID-19 Primers/Probes	200 µl
Positive Control	100 µl
Negative Extraction Control	1.0 ml
Nuclease-Free Water	1.0 ml

Required materials but not provided

- A compatible real-time PCR instrument
- Vortex or equivalent
- Microcentrifuge
- Plates and seals for your instruments

Instrument Compatibility

ABI: 7500 Fast Series; Bio-Rad: CFX96; Roche: LightCycler Series; Agilent: Mx3005p
Qiagen: RotorGene 3000

Reaction Setup

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.

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Component	20 µl Patient Sample	20 µl Positive 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 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
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 Dtected.

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

Interpretation of Results

SARS-CoV-2			Interpretation
N	RdRP	RP	
+	+	+/-	Positive
If only one of the two targets is positive		+/-	Inconclusive Result
	-	+	Negative
-	-	-	Invalid Result

Note: The results from this kit should be interpreted in combination with all relevant laboratory findings.

Important notes

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.

Troubleshooting

Refer to the table below to troubleshoot problems that you may encounter when quantifying 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.

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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.