SARS-CoV-2-RT-qPCR Detection Kit (Dual Gene RdRP/N)

LS-K1081-100 (100 Tests) • **See Storage Conditions Below**
(Bio-Helix Catalog No QP019-0100)

For research use only. Intended for use by laboratory professionals.

For use with ABI 7500 Fast Series; Agilent Mx3005p; Bio-Rad CFX96; Roche Applied Science Light Cycler Series; Qiagen Rotor-Gene 3000; QuantStudio 7 Flex Instrument

Introduction

SARS-CoV-2-RT-qPCR Detection Kit for analysis of human respiratory tract specimens. This kit shows high specificity for the RdRP and N target markers as recommended by the World Health Organization (WHO) and the US Centers for Disease Control and Prevention (CDC), yields data in less than 2 hours, and is compatible with standard RT-qPCR machines.

Components

Component	LS-K1081-100
	100 Tests
2X RT-qPCR Master Mix	1.25 mL
RT-qPCR Enzyme Mix	40 µl
COVID-19 Primers/Probes	200 µl
Negative Extraction Control	1.0 mL
Positive Control	100 µl
Nuclease-Free Water	1.0 mL

Note: Do not mix or use components from different lots.

Storage Conditions

Store at -20°C. The kit is valid for 12 months from manufacture date. Please refer to box for date of manufacture.

LifeSpan BioSciences, Inc. • 2401 Fourth Avenue, Suite 900, Seattle, WA 98121
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Assay Procedure

1. Thaw and combine the following components in a 0.2-mL PCR tube on ice: COVID-19 Primers, COVID-19 Probes, 2X RT-qPCR Master Mix, and RT-qPCR Enzyme Mix. Caution: Do not add more than one RNA sample per 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 Master Mix	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. Cap tubes and place in the thermal cycler.

3. Process in the thermal cycler as follows:

	Phase	Condition	Cycle Number
PCR Reaction Conditions	cDNA Synthesis	42°C for 15 minutes	1
	Pre-denaturation	95°C for 10 seconds	1
	Denaturation	95°C for 15 seconds	40
	Annealing	60°C for 60 seconds	
	Melting Curve	Refer to specific guidelines for instrument used	

Note: Optimal conditions for amplification will vary depending on the qPCR system used.

Detection: As three channels (FAM, ROX, HEX) are used in this single-tube qPCR, we recommend that you perform a channel calibration as specified by the manufacturer. Choose the FAM, ROX, and HEX channels for each sample to be tested. Select “None” for ROX passive reference on any qPCR machine requiring ROX as the reference dye.

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Control Performance

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, improper 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	SARS-CoV-2 detected.
+	-	+/-	Inconclusive results	Repeat RT-qPCR of samples or repeat from extraction step. If result is still inconclusive, we recommend collecting new specimens from the subject.
-	+			
-	-	+	Negative	SARS-CoV-2 not detected.
-	-	-	Invalid result	Repeat from extraction step. If the repeated result remains invalid, we recommend collecting new specimens from the subject.

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Troubleshooting

Issue	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. Exercise care with the nucleic acid extraction steps to minimize carryover of PCR inhibitors.
	Degraded template	1. Do not store diluted template in water or at low concentrations. 2. Check the integrity of template material by gel electrophoresis.
	Sub-optimal thermal-cycling conditions	1. Try using a minimum extension time of 15 seconds for cDNA.
Signal in negative control	Contamination of reaction components	1. To minimize the possibility of contamination of PCR components, 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 precipitate DNA in your work area, whereas 10% bleach solution will hydrolyze and 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 at different annealing temperatures.
Low or high reaction efficiency	Primer dimer formation	1. Reduce the primer concentration.

		2. Perform melt-curve analysis to determine if primer dimers are present. 3. Please contact us for replacements if the above suggestions do not work.
	Insufficient optimization	1. Use a thermal gradient to identify the optimal thermal cycling conditions for a specific primer set.

Cautions

1. Before use, shake reagents gently. Avoid foaming.
2. Reduce primers/probes exposure time to light as much as possible.
3. During operation, always wear a lab coat, disposable gloves, and eye protection.

Manufacture

1. Manufacturer Name: Bio-Helix
2. Address: Room MAF 312, No.2, Beining Rd., Keelung City Taiwan (202)
3. Telephone: +886-2-24624956
4. Email: info@bio-helix.com

LifeSpan BioSciences, Inc. is an authorized distributor of this product.

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