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LiCovid™

2019-nCoV RT-qPCR Detection Kit
100 Reactions

DKB_61/0

Introduction

LiCovid RT-qPCR Detection Kit is designed to detect 2019 novel corona virus (2019-nCoV) in human respiratory specimens. This kit employs two Real-time PCR assays to detect 2019-nCoV and specifically targeting the E and RdRp genes as recommended by the WHO reference method. We offer the complete solution kit including one-step RT-qPCR mix, specific primer and probe and positive control as well.

The Dual-probe system is based on the standard hydrolysis probe system known as TaqMan Technology. The COVID-19 specific probes are labelled with the FAM fluorophore for diagnose E and RdRp genes. Also the internal control probe is labelled with the HEX fluorophore.

Biosafety Precautions

- Personnel must be familiar with the protocol and instruments used.
- Wear appropriate personal protective equipment (e.g. gowns, gloves, eye protection) when working with clinical specimens.
- Specimen processing should be performed in a certified class II biological safety cabinet following biosafety level 2 or higher guidelines.
- Positive controls and All patient specimens should be considered potentially infectious and applied accordingly.
- Do not drink, eat, smoke, apply cosmetics or handle contact lenses in areas where reagents and human specimens are handled.
- Attention should be paid to expiration dates printed on the kit box. Do not use expired product.
- Ensure that all instruments used have been installed, calibrated and maintained according to the manufacturer's instruction and recommendations.
- Work quickly on ice or in the Cooling Block and always use disposable powder-free gloves.
- It is essential to transfer the kit to the appropriate storage condition immediately after use.

Acceptable Specimens

Respiratory specimens including: nasopharyngeal or oropharyngeal aspirates or washes, nasopharyngeal or oropharyngeal swabs, broncheoalveolar lavage, tracheal aspirates, and sputum.

Specimen Handling and Storage

- Specimens can be stored at 4°C for up to 72 hours after collection.
- If a delay in extraction is expected, store specimens at -80°C .
- Extracted nucleic acids should be stored at -80°C .

Equipment Required (not included)

- PCR Work Station [UV lamp; Laminar flow (Class 100 HEPA filtered)]
- Vortex mixer
- Microcentrifuge
- Micropipettes (2 or 10 µl, 200 µl and 1000 µl) with related tips
- cold blocks
- -20 °C and -80 °C freezers; 4 °C refrigerator
- Real-time PCR Apparatus
- Nucleic acid extraction system or Kit
- PCR reaction tubes for the specific Real-time PCR device
- Sterile (DNase and RNase free) microcentrifuge tubes

Shipping and Storage Condition

Kit is shipped on dry ice and should be stored immediately upon receipt at –20 ± 5°C in a constant temperature freezer.

Nucleic Acid Extraction

Performance of RT-PCR amplification-based assays depends on the amount and quality of sample template RNA. RNA extraction procedures should be qualified and validated for recovery and purity before testing specimens.

Commercially available extraction procedures that have been shown to generate highly purified RNA when following manufacturer's recommended procedures for sample extraction ®include: bioMérieuxNucliSens® systems, QIAamp® Viral RNA Mini Kit, QIAamp MinElute Virus Spin Kit or RNeasy® Mini Kit (QIAGEN), EZ1 DSP Virus Kit (QIAGEN), Roche MagNA Pure Compact RNA Isolation Kit, Roche MagNA Pure Compact Nucleic Acid Isolation Kit, and Roche MagNA Pure 96 DNA and Viral NA Small Volume Kit, and Invitrogen ChargeSwitch® Total RNA Cell Kit.

Retain residual specimen and nucleic extract and store immediately at -80°C .

Only thaw the number of specimen extracts that will be tested in a single day. Do not freeze/thaw specimen and nucleic extract more than once before test.

Kit components

Component Part	Volume (µl)
One-Step RT qPCR Mix	500
Probe/Primer Mix 1 (E & IC Genes)	500
Probe/Primer Mix 2 (RdRp Gene)	500
Positive Control 1 (E Gene)	100
Positive Control 2 (RdRp Gene)	100
RNase Inhibitor	100
Nuclease-free Water	500

Applied Instruments

This kit is compatible with Real-time PCR instruments with FAM and HEX channels.

RT qPCR Detection Protocol

According to the WHO protocol, we recommended the E gene assay as the first-line screening followed by confirmatory testing with RdRp gene assay.

1- E & Internal Control Genes Assay

For each sample set up 1 reaction for E target gene and Internal control gene, according to the table below:

E & Internal Control Genes

Component	Volume (µl)
One-Step RT qPCR Mix	5
RNase Inhibitor	1
Probe/Primer Mix 1	5
Sample	4
Nuclease-free Water	Up to 20

2- RdRp Gene Assay

For each sample set up 1 reaction for RdRp target gene, according to the table below:

RdRp Gene

Component	Volume (µl)
One-Step RT qPCR Mix	5
RNase Inhibitor	1
Probe/Primer Mix 2	5
Sample	4
Nuclease-free Water	Up to 20

3- Positive Control

-Add 4 µl of Positive Control 1 instead of sample RNA for E and Internal Control Genes Assay.

-Add 4 µl of Positive Control 2 instead of sample RNA for RdRp Gene Assay.

4- NTC (Non-Template Control)

Add 9 µl of Nuclease-free Water insted of sample RNA in to NTC reactions.

5- qPCR Amplification Protocol

- Place the tubes in Real-time PCR Device.
- Select the channels for acquisition:

No	Name of channel	Source wavelength (nm)	Detection wavelength (nm)	Gene/s
1	FAM	470	510	E & RdRp
2	HEX	530	555	IC

- Create a temperature profile on your instrument as follows the table below:

Temperature (°C)	time	Cycles
50	20 min	1
94	5 min	1
94	30 sec	40
55	45 sec	

Result Analysis

1- Positive control

Firstly, check the positive control performance. The first point of your standard curve should amplify in a Ct range of approximately 20±3. Amplification outside of this range suggests a failure and the test should be repeated.

2- NTC

In ideal circumstances, the negative control well should deliver a flat line – negative result. Therefore:

- NTC Curve observation at Ct > 35 is not valid and don't repeat the test.
- NTC Curve observation at Ct ≤ 35 is cause to repeat the test.

3- Internal control

The Ct value obtained with the Internal control will confirm successful extraction of nucleic acid and quality of the biological material. Detection of the Internal control is through the HEX channel. For acceptance of correct results, the Internal control must be positive in all of tests.

Test Sample Evaluation

- Samples that are positive for the target genes of interest will deliver defined “sigmoidal” amplification plots.
- The signal is considered to be positive, if the corresponding fluorescence accumulation curves cross threshold.
- If the test sample has obvious amplification curve or Ct value ≤35, it can be judged that the sample is positive for 2019-nCoV.
- If the test sample has amplification curve or Ct value >35, the results need to be re-tested. If the re-test results are consistent, the sample can be judged to be positive for 2019-nCoV.
- If the test sample only has the Ct value ≤35 for E or RdRp genes, and there is no amplification curve for the other gene, the results need to be re-tested. If the re-test results are consistent, the sample can be judged to be positive for 2019-nCoV.

Interpretation of results

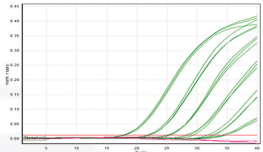
qPCR Signal								
E Target Gene	+	+	-	-	+	+	+	-
RdRp Target Gene	+	-	+	-	+	+	-	+
Positive control	+	+	+	+	+	+	+	+
Internal control	+	+	+	+	+	-	-	-
NTC	-	-	-	-	Ct<35	-/+	-/+	-/+
Result	positive	Re-test	Re-test	Negative	Not valid	Test Repeat/ Extraction of RNA	Test Repeat/ Extraction of RNA	Test Repeat/ Extraction of RNA

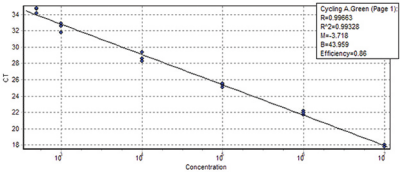
Validation Results for RdRp Gene:

Slope: -3.7 & Efficiency: 0.86

Limit of Quantitation (LOQ): 354 Copies/Reaction

Limit of Detection (LOD): 62 Copies/Reaction



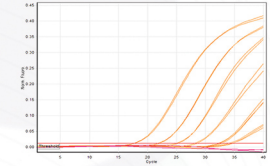


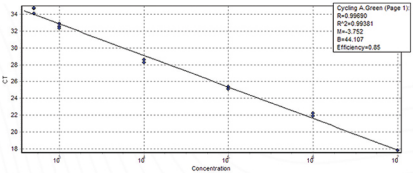
Validation Results for E Gene:

Slope: -3.7 & Efficiency: 0.85

Limit of Quantitation (LOQ): 377 Copies/Reaction

Limit of Detection (LOD): 71 Copies/Reaction





Refrences

1-2019-nCoV WHO qPCR kit (Quick User Guide). Version 1.1

2-Real-Time RT-PCR Panel for Detection 2019-Novel Coronavirus (Centers for Disease Control and Prevention, Respiratory Viruses Branch, Division of Viral Diseases) (Feb 2020)

3-Instruction for use of detection kit for 2019 Novel Coronavirus (2019-nCov) RNA (PCR-Fluorescence Probing). DAAN Gene co. Jan 2020

Symbols				
	Temperature Limitation		IVD	In Vitro Diagnostic Medical Device
	Use by MM-YYYY			Keep Dry
	REF Catalogue number/Reference number			Biological Risk
	LOT Lot Number/ Batch Code/Batch Number			Consult Instruction For Use
	Data of Manufacture			Place for opening of The Seal

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