SARS-CoV-2 RT-qPCR Detection Kit (Triple Gene RdRp/N/E)

LS-K1080-25 (25 Tests) • **See Storage Conditions Below**
(DB Biotech, a.s. Catalog DB 2019)

LSBio
LifeSpan BioSciences, Inc.

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

For use with ABI Prism®7500/7900;Bio-Rad CFX 96;Rotor Gene™6000; Bio-Rad CFX96; SLAN; MIC POC Dx48 Instrument

Introduction

SARS-CoV-2 Nucleic Acid Triple Gene Detection Kit is used for the qualitative detection of a novel coronavirus that was identified in 2019 in Wuhan, Hubei Province, China. It was found in upper respiratory tract specimens (nasopharyngeal extracts, deep cough sputum, etc.) and lower respiratory tract specimens (bronchoalveolar lavage fluid, etc.) by real-time PCR systems.

The primer and probe design for this kit are based on the nucleotide sequences for newly characterized strain, 2019-nCoV (GeneBank accession: MN908947) and covers six 2019-nCoV strain sequences (EPI_ISL_402119, EPI_ISL_402120, EPI_ISL_402121, EPI_ISL_402122, EPI_ISL_402123, and EPI_ISL_402124). This kit contains a specific ready-to-use system for the detection of novel coronavirus by reverse transcription polymerase chain reaction (RT-PCR) in a real-time PCR system.

Assay Principle

The principle of the real-time detection is based on the fluorogenic 5'nuclease assay. During the PCR reaction, the DNA polymerase cleaves the probe at the 5' end and separates the reporter dye from the quencher dye only when the probe hybridizes to the target DNA. This cleavage results in the fluorescent signal generated by the cleaved reporter dye, which is monitored in real time by the PCR detection system. The PCR cycle at which an increase in the fluorescence signal is detected initially (Ct) is proportional to the amount of the specific PCR product. Monitoring the fluorescence intensities in real time allows the detection of the accumulating product without having to re-open the reaction tube after amplification. The reaction is done as a one-step, real-time RT-PCR. The first step is a reverse transcription, during which the virus RNA is transcribed into cDNA. Next, a thermostable DNA polymerase is used to amplify the specific gene fragments by PCR. Fluorescence is emitted and measured by the real-time system's optical unit during PCR. The detection of amplified DNA from the virus is performed in fluorimeter channels FAM, HEX/VIC/JOE, and Cal Red 610/ROX/TEXAS Red with the fluorescent quencher BHQ1.

Components

Component	LS-K1080-25 25 Tests
Novel CoV (2019-nCoV) Super Mix	513 µl
RT-qPCR Enzyme Mix	27 µl
Novel CoV (2019-nCoV) Internal Control	30 µl
Novel CoV (2019-nCoV) Negative Control	400 µl
Novel CoV (2019-nCoV) Positive Control	30 µl

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

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Storage Conditions

All reagents should be stored at -20°C. Avoid repeated freeze-thaw cycles. SARS-CoV-2 Super Mix should be stored in the dark.

Verified for Use on the following Instruments

- ABI Prism 7500/7900
- Bio-Rad CFX 96
- Rotor Gene 6000
- SLAN
- MIC POC Dx48 Instrument

Additional Materials Required

- Biological safety cabinet
- Vortex mixer
- Cryo-container
- Sterile filter tips for micropipettes
- Powder-free disposable gloves
- Refrigerator and freezer
- Real-Time PCR system
- Optical grade, Real-Time RT-PCR reaction tubes and plates
- Pipettes (0.5 - 1000 µl)
- Sterile microtubes
- Biohazardous waste container
- Tube racks
- Desktop microcentrifuge for Eppendorf type tubes (RCF max. 16,000 x *g*)

Sample Storage

Specimens for virus isolation and nucleic acid detection should be tested as soon as possible. Specimens to be tested within 24 hours of collection can be stored at 4°C. For longer storage, specimens should be stored at -70°C.

Sample Collection, Storage, and Transport

1. Collect samples in sterile tubes.
2. Specimens can be extracted immediately or frozen at -20°C to -80°C.
3. Transportation of clinical specimens must comply with local regulations for the transport of infectious agents.

Precautions

1. Please read instructions carefully before use. This assay should be carried out by laboratory professionals while practicing Good Laboratory Practice guidelines.
2. Clinical samples should be regarded as potentially infectious materials and should be prepared in a laminar flow hood.
3. Do not use the kit after its expiration date.
4. Avoid repeated freeze-thaw cycles of reagents as this may reduce the sensitivity of the test.
5. Once the reagents have been thawed, vortex and centrifuge briefly before use.
6. Quickly prepare the Reaction Mix on ice or in a cooling block.
7. Set up two separate working areas, one for the isolation of RNA/DNA and another for the amplification/detection of the amplified products.

8. Pipettes, vials and other working materials should not circulate among working units.
9. Always use sterile pipette tips with filters.
10. Wear separate coats and gloves in each area.
11. Do not pipette by mouth. Do not eat, drink or smoke in laboratory.
12. Avoid aerosols.

Assay Procedure

1. RNA-Extraction
Extract sample RNA according to the instructions for the extraction kit (kit not provided).

2. Internal Control
One microliter (1 µl) of internal control should be added to each extraction mixture to monitor the process.

3. Reagent Preparation
Calculate the amounts of reagents required. Reaction volume per sample prepared as follows:

Preparation of Master Mix (Per Sample)	
Reaction Solution	**Volume per sample**
Novel CoV (2019-nCoV) Super Mix	19 µl
RT-qPCR Enzyme Mix	1 µl

Note: The volumes of Novel CoV (2019-nCoV) Super Mix and RT-qPCR Enzyme Mix per reaction multiply with the number of samples, which includes the number of controls, standards, and samples prepared.

4. RT-PCR Protocol

1. Pipette 20 µl of Master Mix into each Real-Time PCR reaction tube or plate well.
2. Add 1 µl of internal control to each reaction well.
3. For experimental specimens, add 5 µl of extracted nucleic acid sample per reaction.
4. For the positive control, add 1 µl of the provided positive control to the positive control well.
5. For the negative extraction control, add 5 µl of nucleic acid from the negative control extraction to the appropriate well.
6. For the negative control, add 5 µl of nuclease-free water to the negative control well.
7. Immediately close the plates/tubes to avoid contamination.
8. Centrifuge briefly to collect the Master Mix in the bottom of the reaction tubes.
9. For the listed systems, use the following reaction conditions.
    a) ABI Prism 7500/7900
    b) Bio-Rad CFX 96
    c) Rotor Gene 6000
    d) SLAN

	Phase	Condition	Cycle Number
PCR Reaction Conditions	Reverse transcription	45°C for 10 minutes	1
	Pre-denaturation	95°C for 3 minutes	1
	PCR	95°C for 15 seconds	45
		58°C for 30 seconds (Fluorescence measured at 58°C)	

Selection of Fluorescence Channels	
FAM	Gene RdRP
HEX/VIC/JOE	Gene N
Cal Red 610/ROX/TEXAS RED	Gene E
Cy5	IC

Note: For the ABI Prism system, choose "none" as Passive Reference and Quencher

1. For the listed system, use the following reaction conditions:
   a. MIC POC Dx48

	Phase	Condition	Cycle Number
PCR Reaction Conditions	Reverse transcription	45°C for 10 minutes	1
	Pre-denaturation	95°C for 90 seconds	1
	PCR	95°C for 15 seconds	45
		58°C for 20 seconds (Fluorescence measured at 58°C)	

Reference Range

Quality Control

Control	Ct			
	FAM Gene RdRP	**HEX/VIC/Joe** Gene N	**Cal Red 610** Gene E	**Cy5**
Negative Control	Ct≥37 or Not detected	Ct≥37 or Not detected	Ct≥37 or Not detected	Ct 25-40
Positive Control	Ct ≤ 35	Ct ≤ 35	Ct ≤ 35	Ct ≤ 35

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Analysis of Results

Result	Ct Value			
	FAM	**HEX/VIC/JOE**	**Cal Red 610**	**Cy5**
Below the detection limit	Undetected	Undetected	Undetected	Ct 25-43
SARS CoV-2 Positive	Ct ≤ 40	Undetected	Ct ≤ 40	-
	Ct ≤ 40	Ct ≤ 40	Undetected	-
	Ct ≤ 40	Ct ≤ 40	Ct ≤ 40	-
Retest; If FAM channel is still Ct≤40 it is considered positive	Ct ≤ 40	Undetected	Undetected	-
Retest; If either HEX/VIC/JOE or Cal Red 610 channels are Ct ≤ 40, the specimen might be positive for a different type of coronavirus	Undetected	Ct ≤ 40	Undetected	-
	Undetected	Undetected	Ct ≤ 40	-
PCR Inhibition; inconclusive	Undetected	Undetected	Undetected	Undetected

Limitations

1. The test results of this kit are for **RESEARCH USE ONLY** and not for diagnostic/clinical use.
2. Cross contamination during transportation and processing, aerosol contamination (such as PCR products) in the experimental environment, and contamination of any consumables and/or equipment could all lead to false-positive results.
3. Improper sample collection, transportation and treatment, low pathogen content, variations in the target sequence to be tested, or additional interfering factors or PCR inhibitors may lead to false-negative results.

Product Performance

1. Minimum detection limit: ≤1x10^3 copies/ml

Manufacture

1. Manufacturer Name: DB Biotech, a.s.
2. Address: Popradska 80, 040 11 Kosice, Slovakia, EU
3. Telephone: +421 55 789 0010
4. Email: dbbiotech@dbbiotech.com

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

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