Liferiver

Novel Coronavirus (2019-nCoV) Real Time Multiplex RT-PCR Kit (Detection for 3 Genes)

Instructions for Use

NON STERILE

REF RR-0479-02 RR-0479-02-50 RR-0479-02-200

Σ25 Σ50 Σ200

CE

-25℃ -15℃

IVD

For use with ABI Prism®7500; Bio-Rad CFX96; SLAN; MIC POC Dx48 Instrument

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1. Intended Use

Novel Coronavirus (2019-nCoV) Real Time Multiplex RT-PCR Kit (Detection for 3 Genes) is used for the in vitro qualitative detection of 2019 novel coronavirus (2019-nCoV) RNA in nasopharyngeal swab, oropharyngeal swab and sputum specimens by real time PCR systems. It is considered as an aid in the diagnosis of the 2019-nCoV infection.

2. Principle of Real-Time RT-PCR

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 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 the amplification.

Real time reverse-transcription polymerase chain reaction (real-time RT-PCR) is used when the starting material is RNA. In this method, RNA is first transcribed into the complementary DNA (cDNA) by reverse transcriptase from total RNA. The cDNA is then used as a template for the real time PCR.

3. Product Description

Coronaviruses are a large family of viruses, some causing illness in human and others circulating among animals such as camels, cats and bats. 2019-nCoV is a novel coronavirus. The kit contains a specific ready-to-use system for the detection of Novel Coronavirus (2019-nCoV) RNA by the real-time RT-PCR. The reaction is done in a one-step real time RT-PCR assay in a single tube. It includes a reverse transcription (RT) for the transcription of virus RNA into cDNA and real time PCR for the amplification and detection of the cDNA. Fluorescence is emitted and measured by the real time systems´ optical unit during PCR. The detection of amplified virus DNA fragment is performed in fluorimeter **channel FAM, HEX/VIC** and **Cal Fluor Red610/TEXAS RED**.

4. Kit Contents

Ref.	Type of Reagent	Presentation 25rxns	Presentation 50rxns	Presentation 200rxns
1	Novel CoV (2019-nCoV) Super Mix	1 vial, 513µL	1 vial, 988µL	3 vial, 1300µL
2	RT-PCR Enzyme Mix	1 vial, 27µL	1 vial, 52µL	1 vial, 210µL
3	Novel CoV (2019-nCoV) Internal Control	1 vial, 30µL	1 vial, 60µL	1 vial, 250µL
4	Novel CoV (2019-nCoV) Negative Control	1 vial, 400µL	1 vial, 400µL	1 vial, 1000µL
5	Novel CoV (2019-nCoV) Positive Control	1 vial, 400µL	1 vial, 400µL	1 vial, 1000µL

Limit of detection (LoD): 200 copies/mL;

Note: LoD depends on the sample volume, elution volume, nucleic acid extraction method and other factors. If you use the RNA extraction kits recommended, the LoD is the same as it declares. However, if you use other extraction method, additional verification should be performed to ensure the analytical performance of the assay.

5. Storage

- All reagents should be stored at -20±5°C. The validity period is 12 months.
- The assay should be used within 1 month after opening.
- Repeated thawing and freezing (> 3x) should be avoided as this may reduce the sensitivity of the assay.
- Cool all reagents during the working steps.
- Super Mix should be stored away from light.

6. Additionally Required Materials and Devices

- Biological cabinet
- Vortex mixer
- Cryo-container
- Sterile filter tips for micro pipets
- Disposable gloves, powderless
- Refrigerator and freezer
- Desktop microcentrifuge for "eppendorf" type tubes (RCF max. 16,000 x g)
- Real time PCR system
- Real time PCR reaction tubes/plates
- Pipets (0.5µL – 1000µL)
- Sterile microtubes
- Biohazard waste container
- Tube racks

7. ⚠Warnings and Precautions

Carefully read this instructions for use before starting the procedure.

- This assay needs to be carried out by skilled personnel.
- Clinical samples should be regarded as potentially infectious materials and be prepared in a laminar flow hood.
- This assay needs to be run according to Good Laboratory Practice.
- Do not use the kit after its expiration date.
- Avoid repeated thawing and freezing of reagents as this may reduce the sensitivity of the test.
- Once the reagents have been thawed, vortex and centrifuge briefly the tubes before use.
- Prepare quickly the reaction mix on ice or in the cooling block.
- Set up separate working areas for: 1) Reaction setup, 2) Isolation of the RNA and 3) Amplification/detection of amplification products.
- Pipets, vials and other working materials should not circulate among working units.
- Use always sterile pipette tips with filters.
- Wear separate coats and gloves in each area.
- Discard sample and assay waste according to your local safety regulations.
- Do not pipette by mouth. Do not eat, drink or smoke in laboratory.
- Avoid aerosols

8. Sample Collection, Storage and Transport

- Collect samples in sterile tubes;
- Specimens can be extracted immediately or stored at 2°C~8°C within 24 hours or frozen at -70°C for long-term.
- Transportation of clinical specimens must comply with local regulations for the transport of etiologic agents

9. Procedure

9.1 RNA-Extraction

Different brand RNA extraction kits are available. You may use your own extraction systems or the commercial kits based on the yield. For the RNA extraction, please follow the manufacturer's instructions. The recommended extraction kits are as follows:

Nucleic Acid Isolation Kit	Cat. Number	Manufacturer
RNA Isolation Kit (Paramagnetic Beads Column)	ME-0010	Shanghai ZJ Bio-Tech
Viral DNA/RNA Isolation Kit (Centrifuge Column)	ME-0078	Shanghai ZJ Bio-Tech
Viral RNA Isolation Kit (for Auto-Extraction)	ME-0089	Shanghai ZJ Bio-Tech
Viral RNA Extraction Kit (for Auto-Extraction)	ME-0092	Shanghai ZJ Bio-Tech
Viral RNA Isolation Kit (Preloaded for Auto-Extraction)	ME-0044	Shanghai ZJ Bio-Tech
QIAamp Viral RNA Mini extraction Kit	52904/52906	QIAGEN

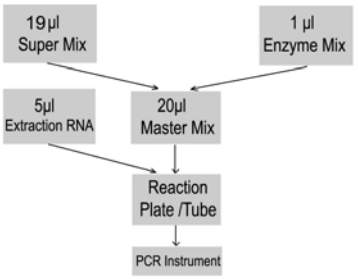
It is noted that the Negative Control and Positive Control in this kit should be extracted with the same protocol as for specimens.

9.2 Internal Control

The internal control (IC) in this kit should be added into the extraction mixture with 1µL/test to monitor the whole process.

9.3 RT-PCR Protocol

The Master Mix volume for each reaction should be pipetted as follows:



1) The volumes of Super Mix and Enzyme Mix per reaction multiply with the number of samples, which includes the number of controls and samples prepared. For reasons of imprecise pipetting, always add an extra virtual sample. Mix completely and then spin down briefly with a centrifuge.
2) Pipet **20µL** Master Mix with micropipets of sterile filter tips to each of the *Real Time* PCR reaction plate/tubes. Separately add **5µL** template (nucleic acid extracted from negative control, positive control and specimens) to different reaction plates/tubes. Immediately close the plates/tubes to avoid contamination.
3) Spin down briefly in order to collect the Master Mix and template in the bottom of the reaction tubes.
4) Perform the following protocol in the instrument of ***ABI Prism®7500; Bio-Rad CFX96; SLAN:***

45°C for 10min	1cycle
95°C for 3min	1cycle
95°C for 15sec, 58°C for 30sec (Fluorescence measured at 58°C)	45cycles

Selection of Fluorescence Channels	
FAM	ORF1ab
HEX/VIC	Gene N
Cal Fluor Red 610/TEXAS RED	Gene E
Cy5	IC

⚠: Perform the following protocol in the instrument *of* ***MIC POC Dx48:***

45°C for 10min	1cycle
95°C for 90sec	1cycle
95°C for 3sec, 58°C for 20sec (Fluorescence measured at 58°C)	45cycles

Selection of Fluorescence Channels	
FAM	ORF1ab
HEX/VIC	Gene N
Cal Fluor Red 610/TEXAS RED	Gene E
Cy5	IC

5) ⚠If you use ABI Prism® system, please choose **"none"** as **passive reference** and **quencher.**

10. Threshold Setting: Just above the maximum level of Negative Control.

11. Quality Control: Negative Control and Positive Control must be performed correctly; otherwise the sample results are invalid.

Channel / Control	Ct Value			
	FAM (ORF1ab)	HEX/VIC (Gene N)	Cal Fluor Red610/TEXAS RED (Gene E)	Cy5 (IC)
Negative Control	UNDET	UNDET	UNDET	≤35 with S-type curve
Positive Control	≤30 with S-type curve			No requirement

12. Data Analysis and Interpretation

The table below lists the expected results for the Novel Coronavirus (2019-nCoV) Real Time Multiplex RT-PCR Kit (Detection for 3 Genes). If results obtained do not follow these guidelines, contact Liferiver for consultation.

Ct value				Result interpretation[a]
ORF1ab	N	E	IC	
+	+	+	/	2019-nCoV detected
+	—	+	/	
+	+	—	/	
—	+	+	/	
—	—	—	*	2019-nCoV not detected[b]
—	—	—	—	Invalid; Re-sample and repeat testing.
+	—	—	/	Repeat testing. If at least one of ORF1ab, N and E genes is positive, the sample is 2019-nCoV positive. If none of the three genes is positive, the sample is 2019-nCoV negative.
—	+	—	/	
—	—	+	/	Re-sampling and repeat testing[c]

"+"represents a positive detection signal, which is defined as Ct≤43;
"*"represents a positive detection signal, which is defined as Ct≤35;
"-" represents a negative detection signal, which is defined as Ct>43;
"/" represents no requirement. Detection of Internal Control is not required if result positive in any of the other three detection channels.

Note:

[a] Laboratories should report their diagnostic result as appropriate and in compliance with their specific reporting system.

[b] Optimum specimen types and timing for peak viral levels during infections caused by 2019-nCoV have not been determined. Collection of multiple specimens from the same patient may be necessary to detect the virus.

[c] If the results are positive for at least two of ORF1ab, N and E genes, the sample is 2019-nCoV positive; If none of the three genes is positive, the sample is 2019-nCoV negative; If the result is positive for ORF1ab or N gene, the sample is 2019-nCoV positive; If the result is still positive for only E gene, the sample is 2019-nCoV positive or other near-source coronavirus positive (Once SARS virus and non-human samples are excluded, it can be reported as 2019-nCoV positive).

For further questions or problems，please contact our technical support at **info@liferiverbiotech.com**