BioCore

2019-nCoV Real Time PCR Kit

Catalogue Number: BC01-0099

[Introduction]

Coronavirus can infect humans and various animals. It is an RNA virus with a size of 27 to 32 kb. Among them, six types of coronavirus are known to have an infectious to human; the types 229E, OC43, NL63 and HKU1 cause the cold, and the types SARS-CoV and MERS-CoV can cause severe pneumonia. In 2019, the newly identified coronavirus (Corona 19) is showing symptoms of pneumonia and accompanied by fever, respiratory symptoms (cough, shortness of breath), etc. It is seem to be transmitted through respiratory secretions, and infection is spreading worldwide through secondary infections among family members and medical institutions. BioCore 2019-nCoV Real Time PCR test is a product that identifies 2019-nCoV (COVID-19) using reverse transcriptase polymerase chain reaction and Taqman probe method.

[Intended Use]

This product qualitatively confirms the presence or absence of a new coronavirus (2019-nCoV, COVID-19) using reverse-transcription Real Time Polymerase Chain Reaction in sputum, oropharyngeal and nasopharyngeal specimens. It is an in vitro diagnostic medical device.

[2019-nCoV Real Time PCR Kit : 100 Test]

Reagents	Volume
2019-nCoV RT PCR Reaction Mixture	1,100 ㎕
2019-nCoV RT PCR Primer/Probe Mixture	550 ㎕
2019-nCoV RT PCR Positive control	50 ㎕
2019-nCoV RT PCR Negative control	50 ㎕

※ Recommend lightly vortex and spin-down before use.

[Storage and Stability]

1. All reagents must be stored at -20℃.
2. The expiry date is 12 months from the date of manufacture.

[Available Sample Types]

1. Sputum
2. Oropharyngeal and nasopharyngeal swabs
3. RNA extract

[Available Real-time PCR machine]

1. SLAN-96P (Shanghai Hongshi Medical Technology Co.,Ltd)
2. CFX96™ Dx System (Bio-Rad Inc.)
3. Applied Biosystems 7500 Real-Time PCR Instrument System (Thermo Fisher Scientific Inc.)

[Test Procedure]

1. RNA extraction from samples

(1) Recommended Commercial Viral RNA Extraction Reagents
ex1) AdvanSure™ Nucleic Acid R Kit (LG Chem, Korea)
ex2) AdvanSure™ Nucleic Acid R tube Kit (LG Chem, Korea)
ex3) QIAamp® Viral RNA Mini Kit
(cat no. 52904, Qiagen, Germany)

2. Preparation of 2019-nCoV RT PCR Master Mixture

(1) Add 5μl of 2019-nCoV RT PCR Primer/Probe Mixture into 10μl of 2019-nCoV RT PCR Reaction Mixture for one sample (Table 1).

Table 1. 2019-nCoV RT PCR Master Mixture

Solution \ Number of Samples	1	3
2019-nCoV RT PCR Reaction Mixture	10	30
2019-nCoV RT PCR Primer/Probe Mixture	5	15
Total (ul)	15	45

(2) Add 5μl of extracted RNA into 15μl of 2019-nCoV RT PCR Master Mixture and mix well by pipetting. Prepare the Positive control and Negative control with same procedure as samples.

(3) Place the tube in a Real Time PCR machine and start the machine using following condition (Table 2).

3. Setting the 2019-nCoV Real Time PCR condition

Set the program under the following conditions (Table 2).

Table 2. 2019-nCoV Real Time PCR condition

Temperatures / Times	Cycles
50℃ / 30 min	1 cycle
95℃ / 15 min	
95℃ / 15 sec 60℃ / 30 sec -> Fluorescence acquiring	45 cycles
N gene → FAM RDRP gene → CalRed 610 (or Texas Red) IC → Cy5	

4. Data analysis

(1) Threshold Set up

a. SLAN-96P

Channel	Item	Manual Threshold	Analysis Type
1	N gene	0.1	Qualitative
3	RDRP gene	0.1	Qualitative
4	IC	0.1	Qualitative

b. CFX96™ Dx System

Channel	Target	Threshold
1	N gene	200
3	RDRP gene	200
4	IC	200

c. Applied Biosystems 7500 Real-Time PCR Instrument System

Channel	Target	Threshold
1	N gene	10,000
3	RDRP gene	10,000
4	IC	10,000

Note 1) When setting the experimental program named "Setup", set the "Select the dye to use as the passive reference" to "None" in "Assign Targets and Samples" of "Plate Setup"
Note 2) Deselect "Auto Baseline" of "Amplification Plot" when analyzing results in "Analysis" section

(2) Result interpretation

	Ct value			Result
	N gene (FAM)	RDRP gene (CalRed610)	IC (Cy5)	
1	≤ 40	≤ 40	≤ 40	2019-nCoV Positive
2	≤ 40	≤ 40	No Ct	2019-nCoV Positive[a]
3	No Ct	No Ct	≤ 40	Negative
4	No Ct	No Ct	No Ct	Re-test[b]
5	≤ 40	No Ct	≤ 40 or No Ct	Re-test[c]
6	No Ct	≤ 40	≤ 40 or No Ct	Re-test[c]
PC	≤ 40	≤ 40	No Ct	Positive Control
NC	No Ct	No Ct	≤ 40	Negative Control

a: If the concentration of 2019-nCoV is high, the amplification of the IC may not occur.

b: Dilute the sample nucleic acid 2 to 10 times and retest. If IC amplification does not occur even after re-examination, extract RNA again and experiment.

c: Re-extract or enrich the nucleic acid from sample. Due to the low concentration of nucleic acid, the amplification reaction is not available.

[Warnings and Precautions]

1. This product is for in vitro diagnostic use only.
2. Samples cannot identify unknown microorganisms or HIV infection, so care must be taken with handling.
3. Disposable gloves and anti-contamination test tools are used throughout the diagnostic test to prevent contamination that may affect the diagnostic results.
4. Be careful not to contaminate the microorganisms when opening the lid or removing the contents of the reagent tube.
5. Reagents should be stored under the relevant storage conditions (-20℃) before and after use.
6. Waste used for inspection is disposed of in accordance with infectious waste disposal regulations.
7. Do not mix different lot reagents.
8. If the internal control (IC) is also not amplified in a COVID-19 negative sample, there is a possibility that RNA has not been extracted or a PCR inhibitor is mixed. Dilute the extracted RNA 2 to 10 times and retest. If the IC does not amplify even with diluted RNA, re-extract the RNA and test.
9. Positive control and negative control may cause contamination and must be handled with care.
10. Positive control and negative control do not repeat freezing and thawing.

[References]

1. Laboratory testing for 2019 novel coronavirus (2019-nCoV) in suspected human cases. Interim guidance. 17 January 2020
2. Real-Time RT-PCR Panel for Detection 2019-Novel Coronavirus Centers for Disease Control and Prevention, Respiratory Viruses Branch, Division of Viral Diseases. Instructions for Use
3. Diagnostic detection of Wuhan coronavirus 2019 by real-time RT-PCR. -Protocol and preliminary evaluation as of Jan 13, 2020
4. Description of the First Strain of 2019-nCoV, C-Tan-nCoV Wuhan Strain — National Pathogen Resource Center, China, 2020

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