Quality approved by SD BIOSENSOR / For *in vitro* Diagnostics

REF M-NCOV-01

STANDARD M

nCoV Real-Time Detection kit

STANDARD M nCoV Real-Time Detection kit
2019 New Coronavirus (2019-nCoV) RT-PCR Kit

SD BIOSENSOR

NOTE

- Please read instructions carefully before you perform the test.
- Refer to the instrument's manual for the instructions of the instrument.

EXPLANATION AND SUMMARY

[Introduction]

Coronavirus is a single-stranded positive-sense RNA virus with an envelope of about 80 to 120 nm in diameter. Its genetic material is the largest of all RNA viruses and is an important pathogen of many domestic animals, pets, and human diseases. It can cause a variety of acute and chronic diseases. Common signs of a person infected with a coronavirus include respiratory symptoms, fever, cough, shortness of breath, and dyspnea. In more severe cases, infection can cause pneumonia, severe acute respiratory syndrome, kidney failure, and even death. The 2019 new coronavirus, or "2019-nCoV", was discovered because of Wuhan Viral Pneumonia cases in 2019, and was named by the World Health Organization on January 12, 2020, confirming that it can cause colds and the Middle East Respiratory Syndrome (MERS) and more serious diseases such as acute respiratory syndrome (SARS). This kit is helpful for the auxiliary diagnosis of coronavirus infection. The test results are for clinical reference only and cannot be used as a basis for confirming or excluding cases alone.

[Intended Use]

STANDARD M nCoV Real-Time Detection kit is used for identification and detection of novel coronavirus (2019-nCoV) ORF1ab (RdRp) gene and E gene in human nasopharyngeal swab, oropharyngeal swab, and sputum specimens using reverse transcription (RT) real-time PCR. This kit is helpful for the auxiliary diagnosis of 2019 novel coronavirus disease (COVID-19). The test results are for clinical reference only and cannot be used as a basis for confirming or excluding cases alone.

[Principle of the Procedure]

STANDARD M nCoV Real-Time Detection kit is designed according to "WHO interim guidance for laboratory testing for 2019 novel coronavirus (2019-nCoV) in humans". This kit is based on TaqMan probe real-time fluorescent PCR technology. Coronavirus RNA was first transcribed into cDNA by reverse transcriptase, and then cDNA was used as a template for PCR amplification. During the PCR reaction, the 5'→ 3' polymerase activity of Taq DNA polymerase and exo-nuclease were simultaneously used. Dicer activity, which causes the degradation of the TaqMan probe, and the separation of the fluorophore and quencher makes the fluorescence signal detected by the instrument: FAM channel qualitative detection of the new coronavirus (2019-nCoV) ORF1ab (RdRp) gene, JOE (VIC or HEX) channel qualitative detection of the coronavirus E gene, and CY5 channel detection internal reference. The kit uses dUTP and UNG enzymes to prevent contamination of amplification products.

Target	Channel
ORF1ab (RdRp) gene	FAM
E gene	JOE (VIC or HEX)
Internal control (IC)	Cy5

KIT CONTENTS

	Contents	Quantity	Dosage in each reaction
1	2019-nCoV Reaction Solution	750㎕/vial x 2	14㎕
2	RTase Mix	630㎕/vial x 1	6㎕
3	2019-nCoV Positive control	600㎕/vial x 1	-
4	Negative control	600㎕/vial x 1	-
5	Internal control A	525㎕/vial x 1	5㎕ (add with specimen) 0.5㎕ (amplification directly)
6	ROX	55㎕/vial x 1	0.5㎕
7	Instructions for use	1	-

[Instruments for the Kit]

LightCycler 480 (Roche), CFX96™ Dx System (Bio-Rad), Applied Biosystems 7500 Real-Time PCR Instrument System (Thermo Fisher Scientific)

[Materials Required but not Provided]

① Micropipette and tip ② Blood collection tube ③ PPE (Personal Protective Equipment) ④ Biohazard container ⑤ Viral RNA extraction kit ⑥ PCR reaction tube ⑦ Vortex mixer ⑧ Centrifuge

[Kit Storage and Stability]

The kit should be stored in a sealed place at −25°C to −15°C. Kit materials are stable until expiration date printed on the outer packaging. Freeze-thaw times no more than 5 times.

WARNINGS AND PRECAUTIONS

1. This kit is only for *in vitro* diagnosis.
2. Please read the product manual carefully before testing.
3. All instruments used in the experiment should be sterilized.
4. Improper specimen collection, transfer, storage, and processing may cause erroneous test results.
5. Nucleic acid extraction should be performed as soon as possible after specimen collection to avoid viral nucleic acid degradation; if it cannot be performed as soon as possible, it should be stored in accordance with SPECIMEN COLLECTION AND PREPARATION.
6. After the operation of the nucleic acid extraction instrument, the used consumables should be sealed. After the instrument is cleaned, turn on the UV lamp for 30 minutes.
7. As this test involves extraction of viral RNA and PCR amplification, care should be taken to avoid contamination of the amplification reaction mixture of the kit. Regular monitoring of laboratory contamination is recommended.
8. Clinical laboratories should be equipped with equipment and operators in strict accordance with the "Code of Practice for Clinical Gene Amplification Laboratories."
9. When using this kit, it should be operated strictly in accordance with the instructions; the specimen processing and specimen addition steps must be performed in a biological safety cabinet or other basic protective facilities, and follow the technical requirements of the clinical gene amplification laboratory.

SPECIMEN COLLECTION AND PREPARATION

[Nasopharyngeal swab]

1. Hold the nasopharyngeal swab close to the nasal septum slowly and deeply to the back of the nasopharynx.
2. Rotate it several times to obtain secretions.
3. Quickly dip the swab into the specimen collection tube, and discard the tail.
4. Tighten the tube cap to seal in case of drying.
5. The swab specimens to be tested can be stored for 1 day at room temperature, 4 days at 2-8°C, and long-term storage below −20°C.

[Oropharyngeal swab]

1. Use moderate swab to wipe the posterior wall of the pharynx and the tonsils on both sides avoiding touching the tongue.
2. Quickly dip the swab into the specimen collection tube, and discard the tail.
3. Tighten the tube cap to seal in case of drying.
4. The swab specimens to be tested can be stored for 1 day at room temperature, 4 days at 2-8°C, and long-term storage below −20°C.

[Sputum]

1. Collect sputum specimen by inducing a cough into a sterile container.
2. Specimens should be taken carefully to avoid contamination and completely sealed to prevent leakage during transportation (Triple packaging).
3. The sputum specimens to be tested can be stored for 1 day at room temperature, 4 days at 2-8°C, and long-term storage below −20°C.

ASSAY PROTOCOL

[Nucleic Acid Extraction]

It is recommended to use QIAamp Viral RNA mini kit (QIAGEN, Cat. No. 52904) for nucleic acid extraction of specimens and reference materials.

1. The specimen volume required for nucleic acid extraction is 200㎕, (5㎕ of internal control A is added to each specimen to be extracted (including positive and negative controls))
2. After the nucleic acid extraction is completed, the nucleic acid extraction solution should be added to the reaction tube within 10 minutes. Transfer the nucleic acid extract to a centrifuge tube and store at −25°C to −15°C for long-term storage.

[Reagent Preparation]

1. LightCycler 480 or CFX96™ Dx System

Prepare the PCR mixture according to the table below and dispense 20㎕ into each PCR reaction tube. (If the nucleic acid extracted by other methods is directly detected, dispense 20.5㎕ into each PCR reaction tube including Internal control A.)

	Reagent	Volume/reaction
1	2019-nCoV Reaction Solution	N x 14㎕
2	RTase Mix	N x 6㎕
3	Internal control A (amplification directly)	N x 0.5㎕
Total volume/well		20㎕ (IC from NA extraction step) 20.5㎕ (IC from amplification step)

PCR reaction mixture can be stored below 8°C for 3 hours.

2. Applied Biosystems 7500 Real-Time PCR Instrument System

Prepare the PCR mixture according to the table below and dispense 20.5㎕ into each PCR reaction tube. (If the nucleic acid extracted by other methods is directly detected, dispense 21㎕ into each PCR reaction tube including Internal control A.)

	Reagent	Volume/reaction
1	2019-nCoV Reaction Solution	N x 14㎕
2	RTase Mix	N x 6㎕
3	ROX*	N x 0.5㎕
4	Internal control A (amplification directly)	N x 0.5㎕
Total volume/well		20.5㎕ (IC from NA extraction step) 21㎕ (IC from amplification step)

* ROX is used as a Reference dye

PCR reaction mixture can be stored below 8°C for 3 hours.

[RT-PCR Amplification]

1. Take 10㎕ of each of the negative control, positive control, and nucleic acid extract of the specimen to be tested, add them to the PCR mixture dispensed reaction tube.
2. Centrifuge at low speed for a few seconds, and place them on the real-time fluorescence quantitative PCR instrument.
3. Set the cycle condition below on the PCR instrument for the NA amplification.

Reaction	Temp. (℃)	Time	Cycle
Reverse transcription	50℃	15 minutes	1
Initial denaturation	95℃	3 minutes	1
Pre-amplification	95℃	5 seconds	5
	60℃	40 seconds	
Amplification	95℃	5 seconds	40
	60℃	40 seconds	
	Collect the signals (FAM/JOE*/Cy5)		

* JOE/VIC/HEX

NOTE

- In the software operation interface of the Applied Biosystems 7500 real-time PCR instrument, select "ROX" from the Passive Reference pull-down menu.

[Interpretation of Results]

Open the experiment data with the analysis software and perform the Ct analysis according to the instrument manual. See the table below for the Ct cut-off for each fluorescent channel.

Target	Ct Value	Interpretation
ORF1ab gene (FAM)	Ct≤36	2019-nCov ORF1ab (RdRp) gene positive
E gene (JOE/VIC/HEX)	Ct≤36	Coronavirus E gene positive
E gene (JOE/VIC/HEX)	Ct≤32	Internal control positive

Refer to the table below for the validity and the interpretation of each specimen result according to the results of each channel.

ORF1ab (RdRp) (FAM)	E gene (JOE/VIC/HEX)	IC (Cy5)	Interpretation
Positive	Positive	Positive	2019-nCoV positive
Positive	Negative	Positive	2019-nCoV positive
Negative	Positive	Positive	Near-source Coronavirus positive
Negative	Negative	Positive	2019-nCoV negative
Negative	Negative	Negative	Invalid / Re-test

NOTE

- **In case FAM/JOE* signal is strong, Cy5 (IC) could be undetermined. If the retest result is still the same, interpret the result except for the Cy5 (IC) result.**

* JOE/VIC/HEX

QUALITY CONTROL

A negative control and a positive control should be set for each test. The test results should meet the requirements of the following table, otherwise the test is invalid. Check the instrument, reagents and amplification conditions for errors and repeat the experiment.

Control	QC requirements		
	ORF1ab gene (FAM)	E gene (JOE/VIC/HEX)	IC (Cy5)
2019-nCoV Positive control	Ct≤26	Ct≤26	No requirements for Ct
Negative control	Undetermined	Undetermined	Ct≤26

LIMITATIONS OF THE KIT PROTOCOLS

1. Qualitative detection of positive results in this kit does not indicate the presence of live virus. It is recommended to use other methods for confirmation at the same time.
2. This kit only classifies and identifies the new coronavirus (2019-nCoV). The test results are for clinical reference only. The clinical diagnosis and treatment of patients should be combined with their symptoms / signs, medical history, other laboratory tests and treatment responses considering.
3. Although the kit was designed to select relatively conservative fragments for amplification and detection, in theory, it is still not possible to completely avoid Missed detection of coronavirus types that may have rare mutations in the conserved regions.

REFERENCES

1. Clinical management of severe acute respiratory infection when novel coronavirus(nCoV) infection is suspected. Interim guidance. WHO.2020
2. Diagnostic detection of Wuhan coronavirus 2019 by real-time RT-PCR.2020
3. Diagnosis and treatment of pneumonia caused by new coronavirus (trial version 4) National Health Commission. 2020

SYMBOL

REF	Reference number		Consult Instructions for use
LOT	Batch code	IVD	*In vitro* Diagnostics
	Manufacturer		Date of manufacture
	Contains Sufficient for 〈n〉 Tests		To indicate the temperature limitations in which the transport package has to be kept and handled
	Use by date		Note

IVD

Manufactured by SD BIOSENSOR, Inc.
Head office : C-4th&5th, 16, Deogyeong-daero 1556beon-gil, Yeongtong-gu, Suwon-si, Gyeonggi-do, 16690, REPUBLIC OF KOREA
Manufacturing site : 74, Osongsaengmyeong 4-ro, Osong-eup, Heungdeok-gu, Cheongju-si, Chungcheongbuk-do, 28161, REPUBLIC OF KOREA

Any inquiries regarding instructions provided should be addressed to : sales@sdbiosensor.com
or you can also contact us through www.sdbiosensor.com

L28MCOV1ENR0
Issued date : 2020.02