BioSewoom CE

Real-Q 2019-nCoV Detection Kit
Instructions for Use

For in vitro Diagnostic Use Rev.3(April 10, 2020)

REF BS7nCoV

1. Introduction

1.1 Intended use

It is an in vitro diagnostic medical device that qualitatively detects the gene (E gene, RdRp gene) of the coronavirus disease (COVID-19) in samples (sputum, oropharyngeal and nasopharyngeal specimens) of patients with respiratory infections using real-time reverse transcription polymerase chain reaction. The Real-Q 2019-nCoV Detection Kit is intended for use by qualified and trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures.

1.2 Test principle

The Real-Q 2019-nCoV Detection Kit is a real-time reverse transcription polymerase chain reaction (rRT-PCR) test. The 2019-nCoV primer and probe set(s) is designed to detect RNA from the 2019-nCoV in nasopharyngeal (NP) swab, NP aspirates, oropharyngeal (throat) swab, sputum, endotracheal aspirates, endobronchial aspirates, bronchoalveolar lavage (BAL) from patients with signs and symptoms of infection who are suspected of COVID-19.

1.3 Applicable real time PCR instrument

Real-time PCR instrument	Manufacturer
CFX96 real-time PCR detection system	Bio-Rad
Applied Biosystems 7500/7500 fast Real-Time PCR Instrument System	ThermoFisher Scientific

1.4 Target gene and fluorescent probe composition

The oligonucleotide primers and probes for detection of 2019-nCoV were selected from regions of the virus RNA dependent RNA polymerase (RdRP) gene and, Envelope (E) gene. An additional primer/probe set to detect the human RNase P gene (HRP) in clinical specimens is also included in the kit. The target gene for detection and the fluorescent dye of the probe are shown in the table below. It is designed to detect TaqMan probe fluorescence signals in three different wavelength signals in a 1-tube.

FAM	HEX	Cy5
2019-nCoV, RdRP gene	Beta CoV, E gene	Human RNase P

2. Reagents

2.1 Materials provided

Component	Description	Volume (100T)
PCR MIX	2X PCR reaction mixture	1,250 µl
PROBE	nCoV probe&primer mixture	300 µl
POSITIVE CONTROL	Positive control	50 µl
ENZYME	RT-PCR enzyme	100 µl
WATER	Water, sterile, DNase/RNase free	1,000 µl
ROX DYE	ROX reference dye	10 ul

Note : In the nCoV probe & primer mixture, RdRP detection primer/probe, E gene detection primer/probe and HRP detection primer/probe are all included. The PC will yield a positive result with the RdRP gene, E gene and HRP primer/ probe sets. All components are taken out immediately before use, thawed and used for centrifugation. Immediately after use, store below -20 ℃.

2.2 Materials required but not provided

- CFX96 real-time PCR detection system
- Applied Biosystems 7500/7500 fast Real-Time PCR Instrument System
- Pipettes(adjustable)
- Powder-free gloves
- Table top centrifuge with rotor for 2 ml reaction tubes
- Viral RNA isolation Kit
- Optical 96-well reaction plate or tubes
- Optical caps or optical adhesive cover
- Aerosol barrier, RNase, DNase-free tips
- Vortex mixer
- 1.5 ml tube

2.3 Precautions for use

1. It is used only for in vitro diagnosis.
2. Do not use products beyond the expiration date.
3. Store the product below -20 ° C.
4. Do not mix with other product numbers.
5. Always wear laboratory gloves, lab coats, and goggles when handling the product to protect it from reagents or samples.
6. It is recommended to use aerosol barrier, RNase, DNase-free tip to prevent contamination.
7. 2X PCR reaction mixture and RT-PCR enzyme are also removed immediately before use, thawed and used by centrifugation.
8. After using the 2X PCR reaction mixture and RT-PCR enzyme, immediately store it in a freezer (below -20 ℃), and limit freezing and thawing to 5 times.
9. The composition should be used immediately after thawing to reduce the time at room temperature.
10. When dispensing sample RNA, dispense RNA into the designated well.
11. Positive control is used after vortexing and centrifugation simply after thawing, and cold and thawing is limited to 5 times.
12. Strip cap is used with vinyl gloves or rubber gloves without powder.
13. If the tube is not well closed with a strip cap, the contents may evaporate and abnormal results may occur.
14. After stripping the sample RNA, close the strip tube with a strip cap, lightly centrifuge and mount it on a real time PCR instrument.
15. Plate cover should be worn with plastic gloves or rubber gloves without powder.
16. If the plate is not well covered with a plate cover, the contents may evaporate and abnormal results may appear.
17. After completing the sample RNA loading, cover it well with a plate cover, lightly centrifuge and install it on a real time PCR instrument.
18. Check if the real time PCR conditions and fluorescence dye selection described in the inspection method are correctly set before proceeding.
19. If the positive control is not amplified, recheck.
20. If a positive amplification signal appears in the negative control, retest.
21. If a non-specific signal is seen in the positive control and sample, retest is recommended.
22. Verify the amplification curve for each sample to verify that the Ct analysis is correct.
23. Since the threshold value can be set differently for each equipment due to fluorescens variation between equipments when analyzing the results after the inspection is over, analyze the results with the threshold values set during product evaluation or setting.
24. PCR is a very sensitive method, so be careful of carry-over contamination.
25. Positive and negative controls avoid microbial and ribonuclease contamination.
26. Discard the positive control DNA in the used kit immediately.
27. Dispose of unused reagents, waste, and samples according to regulations.
28. If reagent gets into your eyes, immediately rinse with water and follow doctor's instructions.
29. If reagent comes into contact with skin, immediately rinse with water.
30. When handling samples that may cause infection, treat them safely according to CLSI Guideline M29-A.
31. Real time PCR equipment is managed periodically by the manufacturer's instructions.

2.3 Storage of kit

Store below -20℃. The shelf life is 12 months from the date of manufacture.

3. Specimen

- Upper and lower respiratory specimens such as Nasopharyngeal (NP) swab, NP aspirates, oropharyngeal (throat) swab, sputum, endotracheal aspirates, endobronchial aspirates, bronchoalveolar lavage (BAL) of patients with suspected respiratory infections
- Collecting the Specimen: Refer to Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Patients Under Investigation (PUIs) for 2019 Novel Coronavirus (2019-nCoV)
- https://www.cdc.gov/coronavirus/2019-nCoV/guidelines-clinical-specimens.html, https://www.cdc.gov/coronavirus/2019-ncov/downloads/processing-sputum-specimens.pdf

4. Viral RNA extraction

For extraction of RNA, we recommend using the extraction kit or automatic extraction instrument presented below.

- QIAGEN (QIAmp DSP Viral RNA Mini Kit, QIAamp Viral RNA Mini Kit)
- Roche MagNA Pure 96, QIAGEN QIAcube, bioMérieux EMAG automated extraction instruments

5. How to use

5.1 CFX96 real-time PCR detection system

1) Prepare a real time PCR master mixture for the reaction.

※ Total required reactions = (n sample+1 positive control+1 negative control)+1

※ Preparation of master mixture

Component	Description	Volume
PCR MIX	2X PCR reaction mixture	12.5 ul
PROBE	nCoV probe&primer mixture	3 ul
ENZYME	RT-PCR enzyme	1 ul
WATER	Water, sterile, DNase/RNase free	3.5 ul
Total		20 ul

Note **:** When mixing the master mixture, do not vortex, tap gently.

2) After mixing the master mixture well, dispense 20 μl in strip tubes or plates.

3) Dispense 5 μl of the sample RNA and positive control into each well.

4) Add 5 μl of water to the negative control well to confirm contamination of the PCR reaction.

5) After dispensing, close the tube well with a cap and centrifuge lightly. Plate is covered well with plate cover.

6) After attaching strip tube or plate to CFX96 equipment, inspect it under the following conditions.

※Real time PCR conditions

Step	Temperature	Time	Cycle	Acquisition mode
1	50 ℃	30 min	1 cycle	
2	95 ℃	15 min	1 cycle	
3	95 ℃	15 sec	40 cycles	none
	62 ℃	45 sec		Acquiring on FAM, HEX, Cy5

Note **:** The fluorescence is designated by selecting FAM, HEX, and Cy5 at 62 ° C, the last step of the cycling step.

5.2 Applied Biosystems 7500/7500 fast Real-Time PCR Instrument System

1) Prepare a real time PCR master mixture for the reaction.

※ Total required reactions = (n sample+1 positive control+1 negative control)+1

※ Preparation of master mixture(With ROX dye)

Component	Description	Volume
PCR MIX	2X PCR reaction mixture	12.5 ul
ROX DYE	ROX reference dye	0.07 ul
PROBE	nCoV probe&primer mixture	3 ul
ENZYME	RT-PCR enzyme	1 ul
WATER	Water, sterile, DNase/RNase free	3.43 ul
Total		20 ul

Note **:** ROX dye may be added to the 2X PCR reaction mixture before using the kit. In this case, 12.57 ul of a 2X PCR reaction mixture containing ROX can be used.

Note **:** When mixing the master mixture, do not vortex, tap gently.

2) After mixing the master mixture well, dispense 20 μl in strip tubes or plates.

3) Dispense 5 μl of the prepared sample RNA and positive control into each well.

4) Add 5 μl of water to the negative control well to confirm contamination of the PCR reaction.

5) After dispensing, close the tube well with a cap and centrifuge lightly. Plate is covered well with plate cover.

6) After attaching strip tube or plate to AB 7500(or AB 7500 fast) equipment, inspect it under the following conditions.

※Real time PCR conditions

Step	Temperature	Time	Cycle	Acquisition mode
1	50 ℃	30 min	1 cycle	
2	95 ℃	15 min	1 cycle	
3	95 ℃	15 sec	40 cycles	none
	62 ℃	45 sec		Acquiring on FAM, VIC, Cy5

Note **:** When designating Detector, specify FAM, VIC, Cy5 for Reporter, and set all Quencher to None.

6. Results analysis

1) Positive control

You should check the Ct value amplified in the positive control. If the Ct value is out of the standard range, re-examination should be performed. In the case of negative control, when the signal is amplified, the entire reaction must be performed again.

	FAM Ct	HEX/VIC Ct	Cy5 Ct	Result	Comment
Positive control	28±5	28±5	28±5	Positive	Valid
Negative control	Neg	Neg	Neg	Negative	Valid

2) Interpretation of results

Assessment of clinical specimen test results should be performed after the positive and negative controls have been examined and determined to be valid and acceptable. If the controls are not valid, the patient results cannot be interpreted.

①Analyses should be performed separately for each target using a manual threshold setting.

②Thresholds should be adjusted to fall within exponential phase of the fluorescence curves and above any background noise signal. The procedure chosen for setting the threshold should be used consistently.

③Threshold setting: FAM and HEX are basically set to 300 and Cy5 is set to 200(CFX96). FAM and VIC are basically set to 0.1 and Cy5 is set to 0.05(AB 7500, with ROX). However, because the threshold value can be set differently for each device due to fluorescence variation between devices, the result is analyzed with the threshold value set by the manufacturer when evaluating or setting the product.

Note **:** If there are any inquiries in threshold settings contact to BioSewoom.

④ Cut-off Ct is as shown in the table below.

FAM(RdRP gene)	HEX / VIC (E gene)	Cy5 (HRP gene)
≤38	≤38	≤35

All clinical samples should exhibit fluorescence growth curves in the RNase P reaction that cross the threshold line within 35 cycles (≤35 Ct)

⑤ Analyze the results according to the table below.

FAM (RdRP)	HEX (E gene)	Cy5 (HRP)	Result	Comment
+	+	+/-	2019-nCoV positive	
+	-	+/-	Inconclusive	Retest is recommended.
-	+	+/-	Inconclusive	Retest is recommended.
-	-	+	Negative	
-	-	-	Invalid	Perform a retest.

7. Performance evaluation

7.1 Analytical sensitivity (Limit of Detection, LOD)

To determine the LoD, RdRP in-vitro transcribed RNA and E gene in-vitro transcribed RNA was serially diluted in simulated nasal matrix. The LoD was confirmed by testing 5 panel members with target concentrations at 30, 15, 7.5, 3.75 and 0.5 copies/uL tested on AB 7500 and CFX96 instrument in 20 repetitions. Then, the 95% LOD concentration was determined through probit analysis. The results are summarized in below.

CFX96 real-time PCR detection system	Applied Biosystems 7500 Real-Time PCR Instrument System
RdRP gene, 6.87 copies/ul	RdRP gene, 6.51 copies/ul
E gene, 7.99 copies/ul	E gene, 7.60 copies/ul

7.2 Inclusivity

Inclusivity was demonstrated by comparing the Real-Q 2019-nCoV Detection Kit primers and probes to an alignment of all SARS-CoV-2 sequences available in Genbank (https://www.ncbi.nlm.nih.gov/nucleotide). Multiple sequence alignment by The MUSCLE alignment software was performed all SARS-CoV-2 sequences. The inclusivity of the 2019-nCoV detection was confirmed by in silico analyzing the position of the detection probe and primer used in the Real-Q 2019-nCoV Detection Kit in the alignment data through the GeneDoC MSA program. As a result of GeneDoC multiple sequence alignment on primer and probe sequences, all of the primers and probes in the Real-Q 2019-nCoV Detection Kit test had 100% homology to all of the available circulating SARS-CoV-2 sequences.

7.3 Cross reactivity

Cross reactivity performance of Real-Q 2019-nCoV Detection Kit test was evaluated by testing 64 virus and bacterial standard strains representative samples listed in below. The cross-reactivity test was repeated twice. No cross-reactivity of the Real-Q 2019-nCoV Detection Kit test duplicate was observed at the concentrations tested. The results are summarized in below.

NO.	Strains	Concentration	Result
1	Adenovirus	$1x10^6$copies/ul	Negative
2	Parainfluenza virus 4	$1x10^6$copies/ul	Negative
3	Enterovirus	$1x10^6$copies/ul	Negative
4	Influenza A virus	$1x10^6$copies/ul	Negative
5	Influenza B virus	$1x10^6$copies/ul	Negative

6	Coronavirus 229E	1x10^{6}copies/ul	Negative
7	Coronavirus OC43	1x10^{6}copies/ul	Negative
8	Coronavirus NL63	1x10^{6}copies/ul	Negative
9	Rhinovirus	1x10^{6}copies/ul	Negative
10	Parainfluenza virus 3	1x10^{6}copies/ul	Negative
11	Parainfluenza virus 2	1x10^{6}copies/ul	Negative
12	Parainfluenza virus 1	1x10^{6}copies/ul	Negative
13	Respiratory syncytial virus A	1x10^{6}copies/ul	Negative
14	Respiratory syncytial virus B	1x10^{6}copies/ul	Negative
15	Metapneumovirus	1x10^{6}copies/ul	Negative
16	Bocavirus	1x10^{6}copies/ul	Negative
17	Mycoplasma pneumoniae	1x10^{6}copies/ul	Negative
18	Chlamydophila pneumoniae	1x10^{6}copies/ul	Negative
19	Streptococcus pneumoniae	1x10^{6}copies/ul	Negative
20	Haemophilus influenzae	1x10^{6}copies/ul	Negative
21	Legionella pneumophila	1x10^{6}copies/ul	Negative
22	Bordetella pertussis	1x10^{6}copies/ul	Negative
23	Klebsiella pneumoniae	1x10^{6}copies/ul	Negative
24	Haemophilus haemolyticus	1x10^{6}copies/ul	Negative
25	Bordetella parapertussis	1x10^{6}copies/ul	Negative
26	Streptococcus mitis	1x10^{6}copies/ul	Negative
27	Haemophilus parainfluenza	1x10^{6}copies/ul	Negative
28	Streptococcus Pseudopneumonia	1x10^{6}copies/ul	Negative
29	Mycobacterium abscessus	1x10^{6}copies/ul	Negative
30	Mycobacterium avium	1x10^{6}copies/ul	Negative
31	Mycobacterium bolletii	1x10^{6}copies/ul	Negative
32	Mycobacterium celatum	1x10^{6}copies/ul	Negative
33	Mycobacterium chelone	1x10^{6}copies/ul	Negative
34	Mycobacterium conceptionense	1x10^{6}copies/ul	Negative
35	Mycobacterium fortuitum	1x10^{6}copies/ul	Negative
36	Mycobacterium gordonae	1x10^{6}copies/ul	Negative
37	Mycobacterium intracellulare	1x10^{6}copies/ul	Negative
38	Mycobacterium kansasii	1x10^{6}copies/ul	Negative
39	Mycobacterium marinum	1x10^{6}copies/ul	Negative
40	Mycobacterium massiliense	1x10^{6}copies/ul	Negative
41	Mycobacterium scrofulaceum	1x10^{6}copies/ul	Negative
42	Mycobacterium szulgai	1x10^{6}copies/ul	Negative
43	Mycobacterium terrae	1x10^{6}copies/ul	Negative
44	Mycobacterium xenopi	1x10^{6}copies/ul	Negative
45	Chlamydia Trachomatis	1x10^{6}copies/ul	Negative
46	Ureaplasma parvum	1x10^{6}copies/ul	Negative
47	Mycoplasma genitalium	1x10^{6}copies/ul	Negative
48	Trichomonas vaginalis	1x10^{6}copies/ul	Negative
49	Neisseria Gonorrhoeae	1x10^{6}copies/ul	Negative
50	Ureaplasma urealyticum	1x10^{6}copies/ul	Negative
51	Mycoplasma hominis	1x10^{6}copies/ul	Negative
52	Herpes simplex virus I	1x10^{6}copies/ul	Negative
53	Herpes simplex virus II	1x10^{6}copies/ul	Negative
54	BK virus	1x10^{6}copies/ul	Negative
55	Cytomegalovirus	1x10^{6}copies/ul	Negative
56	Epstein–Barr virus	1x10^{6}copies/ul	Negative
57	Salmonella bongori	1x10^{6}copies/ul	Negative
58	Campylobacter coli	1x10^{6}copies/ul	Negative
59	Shigella sonnei	1x10^{6}copies/ul	Negative
60	Clostridium perfringens	1x10^{6}copies/ul	Negative
61	Yersinia enterocolitica	1x10^{6}copies/ul	Negative
62	GroupA Rotavirus	1x10^{6}copies/ul	Negative
63	Astrovirus	1x10^{6}copies/ul	Negative
64	Aeromonas sobria	1x10^{6}copies/ul	Negative

7.4 Clinical evaluation

A clinical evaluation study was performed to evaluate the performance of the Real-Q 2019-nCoV Detection Kit test using nasopharyngeal swab specimens. A total of 20 contrived positive specimens at approximately 2X LOD and 10 contrived positive specimens at approximately 20x to 100x LOD were tested. Samples were contrived by spiking known concentrations of SARS-CoV-2 genomic RNA*(NCCP #43326) into negative patient specimens. In addition to the contrived positive specimens, 30 negative specimens were tested.

There were 30 total samples tested twice at the 2X to 100X LOD level with all results valid and included in the analysis. There were 30 total samples tested for the negative level with all results valid and included in the analysis

SARS-CoV-2 Concentration	Number Tested	Positive Detected	% Detection
2X LOD	40	40	100
20x to 100x LOD	20	20	100
Negative	30	0	0

• SARS-CoV-2 genomic RNA*: This is RNA sample from the National Culture Collection for Pathogen (NCCP) in Republic of Korea.

8. References

1) Zhang Y-Z. Novel 2019 coronavirus genome. Virological. [Accessed 21 Jan 2020]. Available from: http://virological.org/t/novel-2019-coronavirus-genome/319
2) Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR Euro Surveill. 2020 Jan;25(3).
3) Genomic characterization of the 2019 novel human-pathogenic coronavirus isolated from a patient with atypical pneumonia after visiting Wuhan Emerg Microbes Infect. 2020 Dec;9(1):221-236

9. Explanation of symbols

Symbol	Explanation
IVD	In vitro diagnostic medical device
LOT	Batch code
REF	Catalogue number
	Use by
	Temperature limitation
Σ	Contains sufficient for <n> tests
	Caution, consult accompanying documents
	Manufacturer
EC REP	Authorized Representative in the European Community
CE	CE mark

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