BioSewoom

Real-Q 2019-nCoV Detection Kit

AB 7500 (ThermoFisher)

CFX96 (Bio-Rad)

Rev.2(2020.03.25)

※ This product has obtained approval for emergency use from Korea Centers for Disease Control and Prevention(KCDC) and Ministry of Food and Drug Safety(MFDS).

1. Overview

1.1 purpose of 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 polymerase chain reaction.

1.2 Applicable real time PCR instrument

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

1.3 Target gene and fluorescent probe composition

The target gene for detection and the fluorescent dye of the probe are shown in the table below.

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

2. Type and amount of composition

2.1 Composition

Components	volume(100T)
1) 2X PCR reaction mixture (red cap ●)	1250 µl
2) nCoV probe&primer mixture (green cap ●)	300 µl
3) Positive control (blue cap ●)	50 µl
4) RT-PCR enzyme (pink cap ●)	100 µl
5) Water, sterile, DNase/RNase free	1000 µl
6) ROX reference dye (yellow cap ●)	10 ul

Note : All components are taken out immediately before use, thawed and used for centrifugation. Immediately after use, store in frozen (below -20 ℃).

2.2 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 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.
32. This product should be used by experts.

2.3 Storage

Store below -20℃.

3. Specimen

RNA extracted from specimens (sputum, oropharyngeal and nasopharyngeal specimens) of patients with suspected respiratory infections

4. How to use

4.1 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

Components	volume (ul)
2X PCR reaction mixture	12.5
nCoV probe&primer mixture	3
RT-PCR enzyme	1
Water	3.5
Total	20

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.

4.2 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)

Components	volume (ul)
2X PCR reaction mixture	12.5
ROX Reference Dye	0.07
nCoV probe&primer mixture	3
RT-PCR enzyme	1
Water	3.43
Total	20

※ ***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.

5. 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

① Be sure to check the raw data for each sample to check whether it is normal or not.

② Threshold 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.

③ Cut-off Ct is as shown in the table below.

FAM	HEX / VIC	Cy5
≤38	≤38	≤35

④ Analyze the results according to the table below.

FAM (RdRP)	HEX (E gene)	Cy5 (IC)	Result	Comment
+	+	+	2019-nCoV positive	The signal (Cy5 signal) of the IC may not be reduced or displayed due to PCR competition.
+	-	+	Inconclusive	Retest is recommended.
-	+	+	Inconclusive	Retest is recommended.
-	-	+	Negative	
-	-	-	Invalid	Perform a retest.

6. 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

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