MiCo BioMed

Coronavirus disease 2019(COVID-19)

Detection Kit

nCoV-OS

Cat. No. 7K106

CE IVD

Contents

1. Intended Use

This Real-time PCR kit is qualitatively detecting coronavirus disease 2019 (COVID-19) by using the extracted RNA from sputum, nasopharyngeal swab or oropharyngeal swab of suspected patients. This Kit is designed as a professional use In Vitro diagnostic medical device with experience in molecular diagnosis experiments. This test can help diagnose infections of indivisual suspected of coronavirus disease 2019. Consultation with a medical specialist is required for final diagnosis. Calibration of the system is traceable to SARS-CoC-2 RNA NCCP 43326

2. Principle of the Procedure

Coronavirus disease 2019 detection kit is based on TaqMan detection method. TaqMan® chemistry is the key feature of detection system. TaqMan® probe contains a reporter fluorescent dye on the 5'-end and a quencher dye. The probe is designed to bind specific target sequence between forward and reverse primers. In every cycle, reporter dye is cleaved by binding to specific target and fluorescent intensity increased as a result. The intensity of fluorescence represents the amount of target genome in certain specimen.

3. Material Provided

3.1. Kit Contents & Volume

Cap Color	Components name	Model name	Volume	Quantity 50 test/kit	Description
Purple	2X One-Step RT-PCR Master mix	nCoV-MMGR06	500 ㎕	2	Polymerase, reverse transcriptase, buffer and stabilizer
Brown	Primer/Probe Mixture1	nCoV-PPM1	50 ㎕	1	Specific primer & probe mixture
Brown	Primer/Probe Mixture2	nCoV-PPM2	50 ㎕	1	Specific primer & probe mixture
Red	Positive Control DNA	nCoV-PC	200 ㎕	1	Positive Control DNA
Yellow	Internal Positive Control DNA	nCoV-IPC	100 ㎕	1	Internal Positive Control DNA
Green	Nuclease Free Water	nCoV-DW	300 ㎕	1	Ultra-pure water

※ Please avoid light when storing or using the Primer & Probe Mixture.

※ This kit provides sufficient volume for 50 reactions when using 20 ㎕ per reaction

3.2. Material Required but Not Provided

- 0.2 ㎖ or 1.5 ㎖ tube
- Micro pipette and sterilized pipette tips
- Table top centrifuge
- Powder-free gloves
- Heating block and Vortex mixer
- Clean bench and Bio Safety Cabinet (BSC)
- Ethanol and Isopropyl alcohol
- VERI-Q PREP M16 / Machine (Cat. No. 9S101, MiCo BioMed. Co., Ltd. Korea)
- VERI-Q PREP M16 - 16TU-CV19(Cat. No. 7A133, MiCo BioMed. Co., Ltd. Korea)
- VERI-Q ManuPrep Viral DNA/RNA kit(Cat. No. 7A231, MiCo BioMed. Co., Ltd. Korea)

3.2.1. Compatible methods for RNA extraction

Product	Description	Catalog No.
VERI-Q PREP M16 16TU-CV19	Manufacturer : MiCo BioMed Instrument : VERI-Q PREP M16(Cat No. 9S101)	7A133
VERI-Q ManuPrep Viral DNA/RNA kit	Manufacturer : MiCo BioMed Manual method	7A231
QIAamp MinElute Virus Spin Kit	Manufacturer : QIAGEN Manual method This kit can be on the QIAcube(Cat No. 9001293)	57704

3.2.2. Compatible instruments for Real-time PCR

Product	Description	Catalog No.
CFX96 Real-time PCR Detection system	-Manufacturer: Bio-rad -96-well format, five fluorescence detection	1845096
Applied Biosystems® 7500 Real-time PCR system	-Manufacturer: Thermofisher -96-well format, five fluorescence detection	4345241

4. Warning and Precaution

Please read the instruction for use thoroughly before using the kit and check integrity of all components in the kit before use.

1) Use for in vitro diagnostic only.

2) This kit is optimized to use with recommended system and it couldn't guaranteed performance excepting the system.

3) This assay needs to be carried out by skilled personnel.

4) It couldn't guaranteed performance when used modified protocol.

5) All specimens should be handled as potentially high-harm factor and also it have to trash or disinfection after using of that.

***** The handling of high risk infectious materials should be accordant with the law of the relevant country.

6) Wear protective disposable glove, laboratory coat and eye protection goggle when handling specimens and kit reagent.

7) Do not eat, drink or smoke in laboratory areas.

8) Do not use the kit after its expiration date, stated on the label.

9) Do not mix reagents different lot or different tube of the same lot.

10) Avoid repeated thawing and freezing of the reagents because of this may reduce the sensitivity of the test.

11) All reagents have to be sufficiently thawed, mix well and centrifuge briefly before use.

12) Use always sterilized filter tip and recommend use of separating the pipette.

***** It should be handled carefully for preventing of contamination by positive control DNA.

13) Use always calibrated equipment.

14) It should be disinfected table and the around after experiment.

15) The long-time light exposure of Primer & Probe Mixture should be protected because it can cause the damage of fluorescence property of probes.

16) In order to get the reasonable results, always use Positive control and Negative control.

17) After testing, all wastes should be processed with fulfillment of regulation of each country/region.

18) Do not expose the product to heat and keep it at the specified temperature, as there is a risk of performance degradation.

19) Laboratory Biosafety: Non-propagative diagnostic laboratory work (e.g. sequencing, NAAT) should be conducted at facilities and procedures equivalent to BSL-2

20) If any damage is found at Kit during shipment or before using, please contact the manufacturer or dealer.

21) Contact the manufacturer if the performance of the kit has changed.

5. Reagents Storage, Shelf life and Handling

5.1. Storage

The Kit should be stored at -20±5℃.

5.2. Shelf life

12 months after manufacturing / 20 days after opening.

5.3. Handling

All reagents should be handled on ice during preparation of mixture. Do not repeatedly freeze and thaw more than 5 times and avoid light when store or using the kit.

6. Procedure

6.1. RNA Extraction

1) This kit is not included for Nucleic Acid (NA) extraction reagent.
2) The quality of the extracted NA is important on the performance of the test.
3) If you confirmed the suitability of the NA extraction, alternative NA extraction systems and kits might also be available.

6.1.1. Recommendation for RNA extraction with VERI-Q PREP M16-16TU-CV19

1) The extraction of the NA using the Kit has to be performed following the manufacturer's instructions using at least 500 ㎕ of specimen. For elution of the extracted NA, 50 ㎕ elution buffer should be used.
2) Please refer to IFU of PREP M16 instrument and 16TU-CV19 reagent.

6.1.2. Recommendation for RNA extraction with VERI-Q ManuPrep Viral DNA/RNA kit

1) The extraction of the NA using the Kit has to be performed following the manufacturer's instructions using at least 200 ㎕ of specimen. For elution of the extracted NA, 50 ㎕ elution buffer should be used.
2) Please refer to IFU of VERI-Q ManuPrep Viral DNA/RNA kit.

6.1.3. Recommendation for RNA extraction with QIAamp MinElute Virus Spin Kit

1) The extraction of the NA using the Kit has to be performed following the manufacturer's instructions using 200 ㎕ of specimen. For elution of the extracted NA, 50 ㎕ elution buffer should be used.
2) Please refer to IFU of QIAamp MinElute Virus Spin Kit

6.2. Sample preparation for Real-time PCR

⚠ The preparation described in this part should be performed within 20 min.

⚠ Filter tips and gloves must be used to prevent splashing and potential cross-contamination of specimen. Use extreme care to ensure selective amplification.

⚠ Completely thaw the reagent on ice.

⚠ Briefly centrifuge the reagent tubes to remove drops from the inside of cap.

⚠ Completely protect the reagent from light.

⚠ Use RNA extracted from fresh samples.

1) Centrifuge the Kit components at 3,000 rpm for 5 sec.
   *At this time, centrifuge the other components first and then centrifuge the positive control to prevent contamination between positive control and others.
2) Vortex for 3 sec and then centrifuge at 3,000 rpm for 2 sec.
   * Positive control should be centrifuged separately to prevent contamination.
3) Prepare the PCR mixture by placing each reagent No.1 to No.4 in a 1.5 ml tube. (Refer to the table ‘PCR Mixture’.)

[PCR Mixture]

<Total number of reaction = n sample + 1 positive control +1 negative control +1=n+3>

No.	Components name	Model name	PPM1	PPM2	Ex) 9 reaction
1	2X One-Step RT-PCR Master mix	nCoV-MMGR06	10 ㎕	10 ㎕	90 ㎕
2	Primer/Probe Mixture1	nCoV-PPM1	1 ㎕	-	9 ㎕
3	Primer/Probe Mixture2	nCoV-PPM2	-	1 ㎕	
4	Internal Positive Control DNA	nCoV-IPC	1 ㎕	1 ㎕	9 ㎕
5	Template		8 ㎕	8 ㎕	-
	Total		20 ㎕	20 ㎕	

※ For the preparation method of template, see ‘6.1 step.’

4) Vortex for 3 sec and centrifuge at 3,000 rpm for 2 sec.
5) Aliquot 12 ㎕ of reaction mixture in each well. (Not provided)
6) Add 8 ㎕ to each well in the order negative control, template, and positive control.
   * Be careful contamination.
7) Mix the PCR mixture and centrifuge at 1,000 rpm for 30 sec.
8) Set up the time and temperature of instrument as shown in the table ‘Real-time PCR condition’.

[Real-time PCR condition]

Step	Temperature	Time	Cycle
1	50℃	10 min	1
2	95℃	3 min	1
3	95℃	9 sec	45
4	58℃	30 sec	

7. Results Analysis

All the results are based on Ct values that automatically calculated by software.

7.1. Fluorophore and cut-off value

	Target	Fluorophore	Cut-off of Ct value
nCoV-PPM1	*ORF3a*	FAM	< 40
	IPC	Cy5	< 40
nCoV-PPM2	*N*	Cy5	< 40
	IPC	HEX	< 40

7.2. Interpretation of sample results

Sample	nCoV-PPM1		nCoV-PPM2		Result
	ORF3a	IPC	N	IPC	
	FAM	Cy5	Cy5	HEX	
Negative Control	-	+	-	+	**Valid**
	+/-	-	+/-	-	**Invalid, re-test**
Positive Control	+	+	+	+	**Valid**
	+/-	-	+/-	-	**Invalid, re-test**
Case 1	-	+	-	+	**Negative**
Case 2	+	+/- [b]	+	+/- [b]	**COVID-19**
Case 3	+	+/- [b]	-	+	**Potential COVID-19 [a]**
Case 4	-	+	+	+/- [b]	**Potential COVID-19 [a]**
Case 5	+	+/-	-	-	**Invalid, re-test**
Case 6	-	-	+	+/-	**Invalid, re-test**
Case 7	-	-	-	-	**Invalid, re-test**

* Cut off: < 40 Ct

** Quality control is performed using PC (Positive Control) and IPC (Internal Positive Control).

[a] If one of two reaction is positive, the test should be recommended re-test and second result is same or ‘case2’, the case should be assessed to ‘potential COVID-19’. In this case, the infection may be low in concentration, so be careful about judgement.

[b] Due to the high amplification of the sample, the amplification of IPC could decrease or not be detected.

8. Trouble shooting

Problems	Probable cause	Recommendation
Cannot see any signal in all channel including positive control	Wrong operation of instrument	Please check Real-time PCR condition and run the assay under correct setting.
	Incorrect preparation of mixture	Please check all components and repeat assay.
	Not available storage condition	Repeat the assay using fresh reagents.
False positive at the negative control	Carry-over contamination	Discard all the components of assay. Repeat the assay using new components.
Not acceptable positive control	Degradation of positive control	Aliquot when thaw positive control. Avoid repeated freezing and thawing.
	Incorrect preparation	Please confirm the protocol and repeat assay.
No appearance or high Ct value of IPC	High concentration of sample	Retest after diluting the DNA using nuclease free water.

9. Limitation

- It must be kept at the storage temperature until expiry date.
  (Storage temperature -20±5℃, expiry date 12 month after manufacturing, 20 days after opening)
- It should be kept away from light.
- Use on ice during the test.
- Use of this product is limited to personnel specially instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures.
- Good laboratory practice is essential for proper performance of this assay. Extreme care should be taken to preserve the purity of the components of the kit and reaction setups. All reagents should be closely monitored for impurity and contamination. Any suspicious reagents should be discarded.
- Appropriate specimen collection, transport, storage and processing procedures are required for the optimal performance of this test.
- This assay is not to be used on the specimen directly. Appropriate nucleic acid extraction methods have to be conducted prior to using this assay.
- The presence of PCR inhibitors may cause false negative or invalid results.
- As with any diagnostic test, results of the nCoV-OS should be interpreted in consideration of all clinical and laboratory findings.

10. Performance Characteristics

- **Analytical Sensitivity (LoD)**

  Analytical sensitivity (Limit of Detection;LoD) of nCoV-OS defines each target gene as 95% detectable concentration (copies/μl). This tests were replicated 24 times for each concentration using two instruments. As a result of analyzing the analytical sensitivity is shown in the table below.

	ORF3a gene	N gene
ABI7500	1.752 copies/ul (14.016 copies/rxn)	3.629 copies/ul (29.032 copies/ rxn)
Bio-Rad, CFX96	1.549 copies/ul (12.392 copies/ rxn)	1.793 copies/ul (14.344 copies/ rxn)

- **Analytical Specificity (Cross-reactivity)**

  - The analytical specificity of the nCoV-OS was tested against 42 organisms including bacteria and virus that can be isolated from the reference material DNA or RNA and cultured medium samples.
  - Each isolate d sample was tested at a concentration at least 5 $x10^5$ copies/reaction.
  - It was confirmed that nCoV-OS was specifically detected in positive control.

- **Interfering substances**

  - The PCR inhibition reaction of the nCoV-OS was tested against 4 interfering substances.
  - As a result, the difference Ct value was ± 2, between the control and test group at each concentration.
  - The PCR reaction was not inhibited with these substances.

11. Reference

- Centers for Disease Control and Prevention (CDC), DEPARTMENT OF HEALTH & HUMAN SERVICES, Division of Viral Diseases '2019-Novel Coronavirus (2019-nCoV) Real-time rRT-PCR Panel Primers and Probes'
- World Health Organization (WHO), Laboratory testing for 2019 novel coronavirus (2019-nCoV) in suspected human cases Interim guidance_ updated 14 January 2020
- Laboratory biorisk management for laboratories handling human specimens suspected or confirmed to contain novel coronavirus: Interim recommendations. Geneva: World Health Organization; 2013.
- WHO laboratory biosafety manual, third edition. Geneva: World Health Organization; 2004.
- Guideline for the collection of clinical specimens during field investigation of outbreaks WHO/CDS/CSR/EDC/200.4

12. Manufacture

12.1. Factory address

MiCo BioMed Co.,Ltd.

3rd and 4th Floor , 54 Changeop-ro, Sujeong-gu, Seongnam-si, Gyeonggi-do, Republic of Korea 13449

www.micobiomed.com

12.2. Contact

If there is any issue when you use this kit, please contact to MiCo BioMed Co.,Ltd.

Tel. +82-70-5227-6000

Fax. +82-70-5227-6001~2

E-mail salesbio@micobiomed.com

Date of preparation: Mar. 2020

MiCo BioMed Co.,Ltd

3rd and 4th Floor , 54 Changeop-ro, Sujeong-gu, Seongnam-si, Gyeonggi-do, Republic of Korea 13449
E-mail. salesbio@micobiomed.com
TEL. +82-70-5227-6000
FAX. +82-70-5227-6001~2

EC REP

OBELIS S.A

Bd. Général Wahis, 53, 1030 Brussels, Belgium
E-mail. mail@obelis.net
TEL. +32-2-732-59-54
FAX. +32-2-732-60-03