

PCLMD™ nCoV one step RT-PCR Kit



Instruction for use

This Instruction for use must be read carefully prior to use, all steps must be and followed accordingly. The assay results may not be reliable if there are any deviations from the instructions in this package insert.

- ※ For in vitro diagnostic use only
- ※ For professional use only
- ※ Refer the Lot No. and expiration date on the label of bottle and box of each component.

MD01EA-IFU-001

Intended use

The PCLMD™ nCoV one step RT-PCR Kit is a qualitative real-time reverse transcription PCR (RT-PCR) IVD medical device for the detection of SARS-CoV-2 in human nasopharyngeal specimen.

Summary and explanation of test

COVID19 is a respiratory disease caused by a new type of coronavirus (SARS-CoV-2) virus first identified in December 2019 in Wuhan, China. Common signs of infection include respiratory symptoms, fever, cough, shortness of breath, and more. In severe cases, the infection can cause pneumonia, severe acute respiratory syndrome, kidney failure and death. Coronaviruses are a group of viruses that cause symptoms from common cold to more serious illnesses such as Middle East Respiratory Syndrome (MERS-CoV) and Severe Acute Respiratory Syndrome (SARS-CoV).

Principle of the procedure

The PCLMD™ nCoV one step RT-PCR Kit is designed for one-step, qualitative RT-PCR kit for the detection of SARS-COV-2 using real-time detection instruments. This kit diagnoses coronavirus by detecting amplified products based on the fluorogenic probe-based technology, TaqMan probes. This kit uses RT PCR Pre Mix which allows efficient cDNA synthesis and PCR amplification and the products are detected and monitored in real time with probes. Three types of gene-specific primer-probe mixtures are used for tagging FAM dye to the 5'end, Primer+Probe Mixture 1 for confirmatory test, Primer+Probe Mixture 2 for screening test and IC Primer+Probe Mixture for internal control purpose.

Kit Components (50 Tests)

Master mix	1500 µl	
Primer+Probe Mixture 1 (Confirmatory)	200 µl	
Primer+Probe Mixture 2 (Screening)	200 µl	
IC Primer+Probe Mixture	200 µl	
Positive Control	160 µl	
DW	1 ml	

Required materials not included

- PCLMD™ nCoV one step RT-PCR Kit can be used with RT-PCR instruments ,
- ※ PCLMD™ nCoV one step RT-PCR Kit was tested by Applied Biosystems® 7500 Real-time PCR.
- Pipettes and pipette tips to deliver the specified volumes
- PCR tubes, cap-strips, or plates
- Racks for reagent tubes
- RNA Extraction system: QIAamp Viral RNA Mini Kit (QIAGEN)
- Disposable gloves and surgical gowns

Storage and stability



PCLMD™ nCoV one step RT-PCR Kit should be stored at -20 °C. When stored and handled as directed, the reagents are stable until the expiration date.

- Always check the expiration date prior to use. Do not use expired reagents.
- Protect fluorogenic probes from light.

Handling precautions

- Do not substitute or mix reagent from different kit lots or from other manufacturers.
- Keep reagent tubes and reactions capped as much as possible.
- Do not use the kit if pouch is damaged.
- The kit is reusable repeatedly after the first opening by designated expiration date. But please be careful of the possibility of contamination and deterioration.
- Wear disposable gloves and masks when handling specimens and reagents to prevent contamination. Change gloves between samples and whenever contamination is suspected.

Safety precautions

- Follow standard precautions. All patient specimens should be considered potentially infectious and handle carefully.
- Handle all specimens as if infectious using safe laboratory procedures according to the national biological safety regulations.
- It is recommended that this kit be used in facility with biosafety level II (or higher) for infectious pathogens.
- Use personal protective equipment such as (but not limited to) gloves, eye protection, and lab coats when handling kit reagents.
- If any of the reagents come into contact with the skin or eyes, wash the area extensively with water. Consult a doctor if there is a problem.
- All waste including unused reagent and human specimens should be disposed in accordance with the national regulations.

Specimen collection

- Nasopharyngeal specimen collected in accordance with the normal laboratory practice can be used for the sample (other specimens have not been tested with this kit)
- Training in specimen collection is recommended due to the importance of specimen quality.

Preparation for use

RNA sample preparation

- Extract RNA from the specimens using QIAamp Viral RNA Mini Kit (QIAGEN)
- RNA should be maintained on cold block or on ice during preparation and use to ensure stability.

Reagent and instrument preparation.

- Prepare an instrument according to the instrument operation manual.
- Thaw the kit reagents by tapping and inverting except RT PCR Enzyme Mix.
- Reagent should only be handled in a clean area and stored at appropriate temperatures.

Assay procedure

- Prepare the RQ-PCR master mixture by mixing reagents to get the required volume according to the below table.
- Three master mixtures should be prepared with each Primer+Probe Mix for each sample.

Components	Volume
Master mix	5 µl X N
Primer+Probe Mixture (PPM1,2,IC)	2 µl X N
DW	8 µl X N
Total	15 µl X N

$N = \text{sample \# (n)} + \text{NC} + \text{PC} = n + 2$

- ⊗ Determine the number of reactions (N) to set up per assay. It is necessary to make excess reaction mix for NC and PC and for pipetting error.
- ⊗ Prepare the three types of master mixture to make one sample be tested by Primer+Probe Mixture 1 (confirmatory), Primer+Probe Mixture 2 (screening) and IC Primer+Probe Mixture (internal control).

- Dispense the 15 µl of each master mix into the appropriate PCR tubes respectively and add 5 µl of RNA sample to the tube.

⊗ Make three tubes for testing one sample.

	Master mixture			Sample	Total
	PPM 1	PPM 2	IC PPM		
Tube 1	15 µl	-	-	5 µl	20 µl
Tube 2	-	15 µl	-	5 µl	20 µl
Tube 3	-	-	15 µl	5 µl	20 µl

- To achieve accurate assay results, test both Positive Control and Negative Control (DW).

⊗ DW included in this product may be used as No Template Control (NTC) to check for contamination

during RNA preparation

- Briefly centrifuge reaction tube for 10 seconds
- Set the instrument according to the reaction conditions in the table below.

Standard cycling mode

Step	Temp.	Time	Cycle	Acquisition mode
Hold	50°C	30min	1	
	95°C	10min	1	
Cycle	95°C	15sec	40	Data detection
	55°C	1min		

Fast cycling mode

Step	Temp.	Time	Cycle	Acquisition mode
Hold	50°C	5min	1	
	95°C	2min	1	
Cycle	95°C	5 sec	40	Data detection
	55°C	30 sec		

*Fast cycling mode can be used with ABI RT-PCR instruments : Applied Biosystems® QuantStudio and 7500 Fast

Interpretation of Results

Amplification curve must be verified after the reaction is complete.

PPM1 Ct	PPM2 Ct	IC PPM Ct	Result
< 35	< 35	< 35	COVID-19 Positive
> 35	< 35	< 35	COVID-19 Negative *
> 35	> 35	< 35	COVID-19 Negative
> 35	> 35	>35	Invalid **

* It is recommended to test other respiratory diseases.

** It is recommended to re-extract RNA.

Quality control

Test Negative Control and Positive Control and check the Primer+Probe Mixture (FAM) Ct values for quality control. Ct values of Positive Control and Negative Control should be as indicated in the table below.

	PPM1 Ct	PPM2 Ct	IC PPM Ct
PC	< 35	< 35	< 35
NC	> 35	> 35	>35

Bibliography

- WHO Clinical management of severe acute respiratory infection when Novel coronavirus (2019-nCoV) infection is suspected: Interim Guidance
- Detection of 2019 novel coronavirus (2019-nCoV) by real time RT PCR

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