CE IVD

Novel Coronavirus (2019-nCoV) Real-time RT-PCR Kit

User Manual REF RT1004

PRODUCT NAME

Novel Coronavirus (2019-nCoV) Real-time RT-PCR Kit

PRODUCT TYPE

L02	Lyophilized Type

PACKAGE SPECIFICATION

24 Tests/kit, 48 Tests/kit

SUMMARY AND DESCRIPTION

The novel coronaviruses belong to the β genus. COVID-19 is an acute respiratory infectious disease. People are generally susceptible. Currently, the patients infected by the novel coronavirus are the main source of infection; asymptomatic infected people can also be an infectious source. Based on the current epidemiological investigation, the incubation period is 1 to 14 days, mostly 3 to 7 days. The main manifestations include fever, fatigue and dry cough. Nasal congestion, runny nose, sore throat, myalgia and diarrhea are found in a few cases.

The kit can quickly identify whether a patient is infected with a novel coronavirus, thereby effectively controlling viral infection. The test results of this kit are for clinical reference only and should not be used as the only standard for clinical diagnosis. It is recommended to combine the patient's clinical manifestations and other laboratory tests for a comprehensive analysis of the condition.

INTENDED USE

Novel Coronavirus (2019-nCoV) Real-time RT-PCR Kit is a real-time RT-PCR test intended for the qualitative detection of nucleic acid from the 2019-nCoV in oropharyngeal swabs and bronchoalveolar lavage fluid collected from individuals suspected of COVID-19 by their healthcare provider.

Results from the test should not be used as the sole basis for diagnosis and exclusion of 2019-nCoV infection and patient management decisions. Positive results are indicative of the presence of 2019-nCoV RNA. Negative results do not preclude 2019-nCoV infection. Clinical observations, patient history, epidemiological information and other diagnostic information should also be necessary to determine patient infection status.

This test is only 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. This test is only for laboratory use, not for home testing.

TEST PRINCIPLE

The kit uses highly conserved sequence of ORF1ab and N gene of the novel coronavirus (2019-nCoV) as the target region, and specific primers and probes are designed for 2019-nCoV PCR detection, which is used to prepare PCR reaction solution. With the technology of real-time RT-PCR, the RNA of sample can be detected by the change of the fluorescent signal.

The PCR reaction system contains endogenous housekeeping gene internal standard primers and probes, which can monitor the sample sampling and its nucleic acid extraction and detection process by detecting whether the internal standard is normal, to avoid false negative results.

KIT COMPONENTS

No.	Contents	Volume		Qty	Main ingredients
		24 Tests/kit	48 Tests/kit		
1	2019-nCoV-PCR Master Mix (Lyophilized Powder)	520 μL/bottle	1000 μL/bottle	1	Specific primers and probes, RT-PCR Buffer, Rnasin, Hot-start Taq enzyme, RT enzyme, Protective agent for lyophilization, etc.
2	2019-nCoV Positive Control (Lyophilized Powder)	500 μL/bottle	500 μL/bottle	1	In vitro transcription RNA containing target genes (ORF1ab, N genes) and internal standard gene fragments
3	2019-nCoV Diluent	1500 μL/bottle	2000 μL/bottle	1	DEPC Water

Notes: The above components of different batch kits cannot be used interchangeably.

STORAGE AND STABILITY

1. Store the kit at -20℃ with a valid period of 6 months.
2. Protect components from light. Excessive exposure to light may affect the fluorescence probes.
3. The kit does not require cold chain transportation and can be transported at room temperature.
4. Repeated thawing and freezing of opened reagents (more than 3 times) should be avoided
5. Please refer to the label on the box for the production date and expiration date.

APPLICABLE DEVICES

The kit is suitable for real-time PCR instruments with FAM, HEX (VIC/JOE) , ROX channels from various manufacturers, such as ABI 7500 real-time PCR instrument.

SPECIMEN COLLECTION AND PREPARATION

1. Specimen Type

Oropharyngeal swab, bronchoalveolar lavage fluid.

2. Specimen Collection

1) Oropharyngeal swab

- Use the special sampling swab to wipe the posterior wall of the pharynx and the tonsils on both sides, avoid touching the tongue; Quickly put the cotton swab into a sample tube containing 3 ~ 5 mL of preservation solution (maintaining solution or normal saline, and maintenance solution is recommended);
- Break the cotton swab rod near the top, tighten the tube cap and seal to avoid drying.

2) Bronchoalveolar lavage

- Collect bronchoalveolar lavage fluid for examination.

3. Specimen Storage

1) The Specimens collected should be tested immediately or stored at 2~8℃ temporarily and tested in 24 hours.
2) If the testing is delayed, it can be stored at -70 ℃ for 6 months.
3) Avoid multiple freeze-thaw cycles.

TEST PROCEDURE

1. Reconstitution of reagent components (in the reagent preparation area, sample processing area)

1) Reconstitution of 2019-nCoV-PCR master mix lyophilized powder (in the reagent preparation area)

a. 24 Tests/kit:

Please add ***520 μL*** of 2019-nCoV diluent to dissolve 2019-nCoV-PCR master mix lyophilized powder, 450 μL of 2019-nCoV diluent can be used as 2019-nCoV negative control. After dissolution, store all the reagents at -20 ℃ for using.

b. 48 Tests/kit:

Please add ***1000 μL*** of 2019-nCoV diluent to dissolve 2019-nCoV-PCR master mix lyophilized powder, 450 μL of 2019-nCoV diluent can be used as 2019-nCoV negative control. After dissolution, store all the reagents at -20 ℃ for using.

2) Reconstitution of 2019-nCoV-positive control lyophilized powder (in the sample processing area)

a. 24 Tests/kit:

Please add ***500 μL*** of 2019-nCoV diluent to dissolve 2019-nCoV-positive control lyophilized powder. After dissolution, store all the reagents at -20 ℃ for using.

b. 48 Tests/kit:

Please add ***500 μL*** of 2019-nCoV diluent to dissolve 2019-nCoV-positive control lyophilized powder. After dissolution, store all the reagents at -20 ℃ for using.

2. Master Mix Preparation (in the reagent preparation area)

Notes: All reagents should be thawed completely, mixed (by pipetting or gentle vortexing) before using.

1) According to the number of specimens to be tested, the number of positive controls and negative controls, take 20 μL of 2019-nCoV-PCR master mix into the PCR reaction tube.
2) Transfer the prepared reagents to the sample processing area for using.

3. Sample Preparation and Nucleic acid Extraction (in the sample processing area)

QIAGEN-- QIAamp Viral RNA Mini Kit (Product No.: 52904) is recommended for nucleic acid extraction. Nucleic acid extraction should be performed simultaneously for the specimens and the positive and negative controls in the kit for monitoring of environment and quality control of PCR detection reagents. The extraction should be performed according to the manufacturer's instructions.

4. Sample Addition (in the sample processing area)

Respectively add 5 μL of the extracted sample nucleic acid, positive control nucleic acid, and negative control nucleic acid to the PCR reaction tube containing master mix, mix thoroughly after centrifugation, and transfer it to the PCR detection area.

5. PCR Amplification (in the PCR detection area)

1) Place the PCR reaction tube in the sample tank of the instrument. Set the positive control, negative control, and specimens to be tested in the corresponding order, and set the sample name.
2) Define the fluorescence detect channel

- Select FAM (ORF1ab) and ROX (N gene) channels to detect 2019-nCoV nucleic acid;

- Select the HEX (VIC/JOE) channel to detect the internal standard.
- For ABI 7500 real-time PCR instrument, please select “passive reference” and “quencher” as “none”.

3) PCR Program setting

Steps	Temperature	Time	Cycles
Reverse Transcription	48℃	10 min	1
cDNA pre-denaturation	95℃	2 min	1
Denaturation	95℃	10 s	45
Annealing, extension, fluorescence acquisition	55℃	30 s	

4) Result analysis (Please set the machine according to the PCR machine user manual)

a. After the reaction, save the results and analyze the amplification curves of target gene and the internal standard separately.

b. According to the analysis of amplification curve, user can adjust the Start value, End value, and Threshold value of the baseline, the Start value can be 3 to 15, and the End value can be 5 to 20. Make the negative control amplification curve straight or below the threshold line.

c. Click “Analysis”, make the parameters meet the requirements of “Quality Control" below.

d. Record the qualitative results in the Plate window.

5) Quality control

- For the positive control
  FAM, ROX and HEX (internal standard) channels: Ct ≤ 40;
- For the Negative control
  FAM, ROX, HEX (internal standard) channel: without Ct value or Ct > 40.

Notes: The above requirements must be met at the same time in one experiment, otherwise, this experiment is considered as invalid and needs to be tested again.

POSITIVE JUDGMENT VALUE

According the study of the reference value, the Ct reference value of the target gene detected by this kit is 40, and the reference value of the internal standard Ct is 40.

RESULT INTERPRETATION

ORF1ab	N	Internal Control	Results
+	+	+/-	2019-nCoV Positive
+	+		
-	-	+	2019-nCoV Negative
+	-	+/-	2019-nCoV Inconclusive. Repeat test, if the repeat results are consistent with those previously reported as inconclusive
-	+		
-	-	-	Invalid

Result of (+) : Ct≤40

Result of (-) : Ct＞40 or No amplification curve (No Ct)

LIMITATION

1. The test results of the kit are for clinical reference only. The clinical diagnosis and treatment of suspected cases should be considered in combination with their symptoms/signs, medical history and treatment response.
2. Analysis of the possibility of false results.
   1) Unreasonable sample collection, processing, transportation, storage and low RNA content in the sample may lead to false negative results.
   2) Variations in the region of the target sequence to be tested or sequence changes caused by other reasons may lead to false negative results.
   3) mproper reagent storage or unverified inhibitors of nucleic acid reactions may lead to false negative results.
   4) Cross-contamination during sample processing may cause false positive results.
3. Use of this product is limited to personnel specially instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures.

PRODUCT PERFORMANCE

1. Accuracy: The enterprise positive reference products are tested, and the results are all positive. The enterprise negative control products are tested, and the results were all negative.
2. Limit of detection: 300 copies / mL.
3. Precision: The coefficient of variation (CV%) of the precision within-run is no more than 5%.
4. Cross-reactivity: No cross reaction with the following pathogens.

- Human coronavirus HKU1
- Human coronavirus OC43
- Human coronavirus NL63
- Human coronavirus 229E
- SARS coronavirus
- MERS coronavirus
- Influenza A
- Influenza B
- Adenovirus type 1
- Adenovirus type 2
- Adenovirus type 3
- Adenovirus type 4
- Adenovirus type 5
- Adenovirus type 7
- Adenovirus type 55
- Respiratory syncytial virus A
- Respiratory syncytial virus B
- Parainfluenza virus 1
- Parainfluenza virus 2
- Parainfluenza virus 3
- Rhinovirus A
- Rhinovirus B
- Rhinovirus C
- Enterovirus A
- Enterovirus B
- Enterovirus C
- Enterovirus D
- Human Metapneumovirus
- Epstein-Barr virus
- Measles virus
- Cytomegalovirus
- Rotavirus
- Norovirus
- Mumps virus
- Varicella zoster virus
- *Mycoplasma pneumonia*
- *Chlamydia pneumonia*
- Legionella
- Pertussis
- *Haemophilus influenza*
- *Staphylococcus aureus*
- *Streptococcus pneumonia*
- *Streptococcus pyogenes*
- *Klebsiella pneumonia*
- *Aspergillus fumigatus*
- *Candida albicans*
- *Candida glabrata*
- *Cryptococcus neoformans*
- Mycobacterium tuberculosis attenuated strains
- Human genome

PRECAUTION

1. This product is only used for in vitro testing. Please read this manual carefully before use.
2. The laboratory management should be strictly in accordance with the management regulations of PCR gene amplification laboratories issued by the industry administrative department. The laboratory personnel should undergo professional training, and the experimental process should be strictly divided into sections. The consumables used are sterilized for one-time use. Instruments, consumables, and lab clothes should not be used crosswise. Clean and disinfect the workbench immediately after the experiment.
3. All test samples should be regarded as infectious substances. Wear working clothes, wear disposable gloves and frequently replace gloves during the experiment to avoid cross-contamination between samples; sample handling and waste disposal must meet relevant regulatory requirements: Ministry of Health "General Guidelines for Biosafety in Microbial Biomedical Laboratories" and "Regulations on the Management of Medical Waste".
4. Tips: Improper reagent storage, transportation, and improper operation of sample collection, processing, and testing may affect the test results. Please strictly follow the instructions. Due to the characteristics of the sample collection process such as swabs and the virus infection process itself, there may be false negative results due to insufficient sample volume collected, etc. It should be comprehensively judged in conjunction with other clinical diagnosis and treatment information and retested if necessary.

DESCRIPTION OF SYMBOLS USED

The following graphical symbols used in or found on Novel Coronavirus (2019-nCoV) Real-time RT-PCR Kit are the most common ones appearing on medical devices and their packaging. They are explained in more details in the European Standard EN 980:2008 and International Standard ISO 15223-1:2016.

Key to symbols used					
	Manufacturer		Expiration date		Temperature limitation
	Do not reuse		Date of manufacture		Sufficient for
	Consult instructions for use	LOT	Batch code	CE	CE mark
REF	Catalogue number	IVD	*In vitro* diagnostic medical device	EC REP	Authorized representative in the European Community
	Do not use if package is damaged				

Thank you for purchasing Novel Coronavirus (2019-nCoV) Real-time RT-PCR Kit. Please read this user manual carefully before operating to ensure proper use.