SARS-CoV-2 Nucleic Acid RT-PCR Test Kit (Dry Powder)

English

For professional and in vitro diagnostic use only.

[INTENDED USE]

The SARS-CoV-2 Nucleic Acid RT-PCR Test Kit is a real-time RT-PCR test intended for the qualitative detection of nucleic acids from the SARS-CoV-2 in nasal swab, nasopharyngeal swab, oropharyngeal swab, bronchoal-veolar lavage and sputum specimens from individuals suspected of COVID-19 by their healthcare provider.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in respiratory specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

The SARS-CoV-2 Nucleic Acid RT-PCR Test Kit is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures.

[SUMMARY]

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.

[PRINCIPLE]

The SARS-CoV-2 Nucleic Acid RT-PCR Test Kit for Detecting SARS-CoV-2 is a real-time reverse transcription polymerase chain reaction (rRT-PCR) test. Firstly, the RNA of SARS-CoV-2 will be reverse transcribed into cDNA by reverse transcriptase, and then PCR amplification will be performed with cDNA as template. During amplification of the template, the TaqMan probe will be degraded due to the 5'-3' polymerase activity and exonuclease activity of Taq DNA polymerase, then the separation of fluorescent reporter and quencher enables the fluorescent signal to be detected by instrument. The oligonucleotide primers and probes for specific detection of SARS-CoV-2 are selected from regions of Open Reading Frame 1ab (ORF1ab) and the nucleocapsid gene (N) of the SARS-CoV-2 genome. The ORF1ab gene of SARS-CoV-2 will be detected qualitatively by FAM channel, the N gene of SARS-CoV-2 will be detected qualitatively by ROX channel. Internal reference is used in the kit for quality control starting from sample collection.

[WARNINGS AND PRECAUTIONS]

- For professional in vitro diagnostic use only. Do not use reagents after the expiration date.
- The procedures should be performed in separated areas to aid in preventing contamination.
- Do not use reagents from different lots in the same tests.
- Prevent the introduction of RNase into samples during the extraction procedure. We recommend using only single-use pipettes and pipette tips to prevent cross-contamination of patient samples.
- The reagent dispensing process should be prevented from leaking to avoid contamination of the instrument by fluorescent substances.
- All the equipment should be used with care, calibrated regularly, and maintained following the equipment manufacture's instruction.
- Wear protective disposable gloves, laboratory coats and eye-wear when handing clinical specimens and kit reagents. Wash hand thoroughly after handing specimens and test reagents.
- Handle all contaminated material, specimens and reagents using established good laboratory working practices. Handing all specimens as if potentially infections. The sensitivity and titer of potential pathogens in the specimen material varies, the operator has to optimize pathogen inactivation by using appropriate measure according to local safety regulations.
- Discard unused reagents, contaminated material, specimens and waste in accordance with country, federal, state and local regulations.
- This procedure is for professional laboratory use only and assumes familiarity with RNA extraction methods and real-time RT-PCR assays.

[COMPOSITION]

Materials Provided

Component	Specification			
	25tests/kit	50tests/kit	100tests/kit	200tests/kit
RT-qPCR Reaction Solution (Dry Powder)	25 tests/bottle ×1	25 tests/bottle ×2	50 tests/bottle ×2	50 tests/bottle ×4
Positive Control	1 tube	1 tube	1tube	2 tube

NOTE: Before use, 100μL ultra-pure water was added into the Positive Control to dissolve and mix well. It needs to be stored at -20°C after dissolving. Negative control needs to be prepared by laboratory by using ultra-pure water.

Materials Required But Not Provided

- Vortex mixer
- Sodium hypochlorite solution for decontamination
- Pipettes with disposable tips
- Disposable gloves
- ultra-pure water

Compatible Instrument

- Applied Biosystems® 7500 Real-Time PCR System

[STORAGE AND STABILITY]

- This kit is required sealed and stored at 10-30°C. Protect from direct sunlight. The shelf life of the reagent kit is 6 months under the required storage condition.
- Repeated freezing-thawing should be avoided. When repeated freezing-thawing is no more than 1 time, the performance of this kit can be stable. The kit should be used as soon as possible after opening. If the kit is not used up after opening, it can be stored in -20°C for no more than a week.
- Please refer to the label of the kit for production date and expiry date.

[SPECIMEN]

- Only the following validated specimens should be used for testing: Upper respiratory specimens such as nasal swab, nasopharyngeal swab and oropharyngeal swab, and lower respiratory specimens such as bronchoalveolar lavage and sputum.
- Please refer to https://www.cdc.gov/coronavirus/2019-nCoV/lab/guidelines-clinical-specimens.html for information on collection of appropriate s pecimens to test for SARS-CoV-2.
- Store specimens at 2-8°C for up to 72 hours after collection. If a delay in testing or shipping is expected, store specimens at -70°C or below.
- Specimen packaging and transportation should follow https://www.cdc.gov/coronavirus/2019-nCoV/lab/lab-biosafety-guidelines.html#specimen.

[DIRECTIONS FOR USE]

1 Specimen Preparation (Sample Preparation Area)

- Users can use our company's nucleic acid extraction or purification reagent，or commercialized universal virus RNA extraction kit.
- The extracted RNA should be used for further detection in time. If not, it should be stored at -20±5°C for no more than 1 week or stored at -70°C for a long time.

2 Reagent preparation(Reagent Preparation Area)

- Take out testing sample and kit components from storage condition, thawing at room temperature.
- Add 250μL of ultra-pure water to each bottle of dry powder (25 tests) or add 500μL of ultra-pure water to each bottle of dry powder (50 tests); shake the bottle completely. The unused reagents should be sealed at -20°C as soon as possible after dissolution, and the freezing and thawing should not exceed 1 time.
- Add 100μL ultra-pure water into the bottle of Positive Control to dissolve and mix well. It needs to be stored at -20°C after dissolving. Take ultra-pure water as Negative Control.
- Add 10μL RT-qPCR reaction solution to each PCR tube.

3 Sample Adding (Sample Preparation Area)

- Add 10μL Testing sample RNA to PCR tube.

Components of reaction buffer	Volume (μL)
RT-qPCR reaction solution	10
Testing sample RNA	10
Total Volume	20

NOTE: Add 10μL Positive Control solution into PCR tube with 10μL RT-qPCR reaction solution inside. Add 10μL Negative Control solution separately into another PCR tube with 10μL RT-qPCR reaction solution inside.

- Tightly close the PCR reaction tube and transfer them to the Detection Area.

4 PCR Amplification and Detection (Detection Area)

- Checking whether the reaction tube tight before started.
- Load the PCR reaction vessels in the Real-Time PCR System.
- The reaction system was 20.0μL. The reported group is FAM (for the ORF1ab), ROX (for the N) and VIC (for the internal control) fluorescence, the quenched group is none, the calibration channel is none, and the fluorescence data is collected at 55°C, 45sec.
- Edit and run the program as below:

Steps	Cycle(s)	Temperature	Time
1	1	50°C	15min
2	1	95°C	5min
3	5	95°C	10sec
		55°C	30sec
4	40	95°C	10sec
		55°C	45sec

[VALIDATION REQUIREMENTS AND QUALITY CONTROL]

1 Principle for threshold value setup

- Starting analysis according to the relevant equipment software after the end of the experiment.
- Choose the FAM, ROX and VIC channels, set the base line cycler at 3- 15 cycles. Baseline of threshold should be just over the peak of the amplification cure of the Negative Control.
- The recording instrument automatically analyzes the calculated specimen Ct value.

2 Detailed requirements for valid results

- The Ct value in any fluorescent detection channel of negative control should be ≥35 or Undet.
- The Ct value in any fluorescent detection channel of positive control should be < 30 and present a typical S-type amplification curve.
- Generally, the internal control gene should show a typical amplification curve, and the VIC Ct value should be <32. If the internal control is negative and one or two genes are positive, the results are still reliable and can be determined as positive under the above quality control requirements.If the internal control is negative and all the target genes are negative, the result is not reliable and the test should be repeated.

The requirements above should be met at the same time, if not, the results are considered invalid. Please check if there is something failed with your instruments, reagents or amplification conditions et al.

[INTERPRETATION OF RESULTS]

SARS-CoV-2 ORF1ab gene was detected by FAM, N gene was detected by ROX.

- When both ORF1ab and N genes are negative (Ct≥35.0 or no Ct), the result should be regarded as negative.
- When both ORF1ab and N genes are positive (Ct < 32.0), or ether one of them is positive (Ct < 32.0), the result should be regarded as positive.
- When 32.0≤Ct value <35.0 in either FAM or ROX detection channel, suggest repeating the test once again. If the Ct value is ≥35.0 in either FAM or ROX detection channel, the sample should be considered as negative.If the Ct value is <35.0 in either FAM or ROX detection channel, the sample should be considered as positive.

SARS-CoV-2 ORF1ab（FAM）	SARS-CoV-2 N（ROX）	Result Interpretation	Report
-	-	SARS-CoV-2 RNA not detected	Negative
+	+	SARS-CoV-2 RNA detected	Positive
+	-	SARS-CoV-2 RNA detected	
-	+		

[LIMITATIONS]

- The kit should exclusively be used for in vitro diagnostics, and the results is an aid for diagnostics.
- As with all diagnostic tests, all results must be interpreted together with other clinical information available to the physician.
- The test results of samples are related to the collection, processing, transportation, preservation and nucleic acid extraction of samples. Erroneous result may be caused by irrational specimen collection, transfer, storage, and processing.
- The gene mutation of the virus in the process of epidemic may lead to false negative results.

[PERFORMANCE CHARACTERISTICS]

Limit of Detection (LoD)

The LoD studies determined the lowest detectable concentration of SARS-CoV-2 at which approximately 95% of all (true positive) replicates test positive. The LoD was determined by limiting dilution studies using characterized samples. 20 individuals at 500 copies/mL of SARS-CoV-2 RNA were added to sputum samples tested positive results, It indicates that the sensitivity of the SARS-CoV-2 Nucleic Acid RT-PCR Test Kit is 500 copies/mL.

Cross Reactivity and Interference

Cross-Reactivity: Other common causative agents of infectious diseases include Human Coronavirus OC43, Human Coronavirus 229E, Human Coronavirus HKU1, Human Coronavirus NL63, SARS Coronavirus, MERS-CoV, Influenza A(H1N1), Influenza A(H3N2), Influenza A(H5N1), Influenza B, Respiratory syncytial virus A,B, Parainfluenza virus 1,2,3, Rhinovirus A, B, Adenovirus 3, 5, Enterovirus, Human metapneumovirus, Epstein-Barr virus, Mycoplasma pneumoniae, Chlamydia pneumoniae, Candida albicans, Pseudomonas aeruginosa, Staphylococcus epidermis, Streptococcus salivarius, Pseudomonas aeruginosa had been evaluated with the SARS-CoV-2 Nucleic Acid Detection Kit. The result showed there was no cross-reactivity.

Interferences: The Whole Blood, common antiviral drugs Oseltamivir, Amantadine, Ribavirin, Antibiotic azithromycin were verified, the test results of the kit were not affected.

Clinical Evaluation

In total 202 clinical specimens were involved in this clinical study, including 65 SARS-CoV-2 positive and 137 SARS-CoV-2 negative, as determined using a comparator molecular diagnostic assay for the detection of SARS-CoV-2 RNA. The specimen types tested included sputum and nasopharyngeal swabs. Compared to the result of commercial RT-PCR kit bearing the CE marking, the CLUNGENE SARS-CoV-2 Nucleic Acid RT-PCR Test Kit exhibited positive and negative percent agreement of 96.92% (95%CI: 89.46%~99.15%) and 99.27% (95%CI: 95.98%~99.87%), respectively, with Kappa value 0.9658.

All Specimen Types Combined:

SARS-CoV-2		Comparator kit		Total
		Positive	Negative	
CLUNGENE®	Positive	63	1	64
	Negative	2	136	138
Total		65	137	202

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Index of Symbol

Do not reuse	IVD For in vitro diagnostic use only
Temperature Limit	Consult instructions for use
Caution	LOT Lot number
Use by	Contains sufficient for <n> tests
Manufacturer	Keep dry

EC REP Authorized representative in the European Community

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