Instruction for Use of Novel Coronavirus (COVID-19) Nucleic Acid Detection Kit (PCR-fluorescent Probe)

【Product Name】

Novel Coronavirus (COVID-19) Nucleic Acid Detection Kit
(PCR-fluorescent Probe)

【Intended Use】

This kit is an in vitro diagnostic test used for the detection of a new type of coronavirus (COVID-19). Samples can be obtained from throat swab or sputum.

【Packaging Specification】 24 tests/kit, 48 tests/kit, 96 tests/kit

【Kit Components】

Component	Volume (μl/Vial) 24T Kit	Volume (μl/Vial) 48T Kit	Volume (μl/Vial) 96T Kit
Reaction Mix	400 μL	800 μL	1600 μL
RT Enzyme Mix	11.5 μL	23 μL	46 μL
Positive control	40μL	40μL	40μL
Nuclease-free water	1.0mL	1.0mL	1.0mL

NOTIFICATION:

1. Reaction Mix is basic components of this detection kit. The reaction mixes in this kit contain specific primers and probes for N gene and ORF1ab of COVID-19, and RNase P gene as sample internal control (IC), as well as all components needed for efficient RT-PCR reactions.

	Specific Genes	Fluorescent Dyes
Gene 1	ORF1ab (RdRp)	FAM
Gene 2	N Gene	VIC
Internal control	RNase P	CY5

2. The positive control is the mix of single-stranded RNA of specific regions of novel coronavirus, required to be dispensed into 200 μL PCR tubes with 5μL per reaction according to the required number of reactions. Please avoid repeated frozen-thawed cycles to avoid the degradation of RNA templates.
3. Components from different batch number kits is not recommended to be used interchangeably.

【Storage】

1. All reagents should be stored at -20℃ until the expiration date listed on the outer kit box.
2. Protect Reaction Mix from light during storage.
3. Repeated freezing and thawing (more than five times) of reagents should be avoided.
4. Expiration date: 9 months.

【Materials and Devices Required but Not Provided】

1. Biological cabinet.
2. Appropriate real time PCR instrument: ABI7500、ABI QuantStudio 6/7/12K; Roche Lightcycler®480/1536/Nano; Agilent Mx3000P/3005P; Qiagen Rotor-Gene 6000 / Q; Bio-Rad CFX384/CFX96, Bio-Rad Touch / iQ5; Cepheid Smartcycler/Smartcycler II; Eppendorf Mastercycler.
3. Appropriate nucleic acid extraction system or kit.
4. Desktop centrifuge (suitable for 96-well plate or 8-strip tube).
5. Centrifuge with a rotor for 0.2ml reaction tubes or plate.
6. Vortex mixer.
7. Adjustable pipettes (with maximum capacity of 2μL, 10μL, 50μL, 100μL, and 200μL, respectively).
8. Disposable pipette tips with filters.
9. Disposable powder-free gloves.
10. RNase free 1.5mL centrifuge tube.
11. PCR reaction tube / PCR reaction plate.

NOTE: Please ensure that instruments have been installed, calibrated, checked, and maintained according to the manufacturer's instructions and recommendations.

【Background Information】

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.

COVID-19 positive nucleic acid can be detected via real-time fluorescence RT-PCR on the specimen such as throat swab based on the conditions that complied with the criteria for suspected case. Specific detections of N gene, and RdRp/ORF1ab gene regions in novel coronavirus (COVID-19) are detected by this kit. Gene N detected by RT-PCR is used for the screening of COVID-19 virus, and the detection of gene region ORF1ab (RdRp) will provide a further verification of the virus infection by COVID-19.

The design of this kit is based on the sequence characters of 2019 novel coronavirus and SARS-like coronavirus, with Reaction Mix containing specific primers and probes for ORF1ab、N gene and internal control (RNase P gene). The RdRp (RNA Dependent RNA Polymerase) of ORF1ab region specifically differentiate COVID-19 from SARS-CoV, therefore it will be verified that the coronavirus carried by the specimen is COVID-19 or not; N gene is designed to detect gene N (Envelope) region in coronavirus, which encodes envelope protein of coronavirus. Screening of COVID-19 is performed via detecting N gene. RNase P gene detecting human cells is used as internal control for sample collection, processing, and detection.

【Detection Principle】

The kit is based on real-time fluorescent probe Quantitative RT-PCR technology. RT-PCR （Reverse Transcription-Polymerase Chain Reaction）is a method in which RNA reverse transcription (RT) is combined with polymerase chain reaction of cDNA. First, via reverse transcriptase, RNA fragment in COVID-19 will be synthesized to cDNA, then with cDNA as the template, target fragment will be synthesized via amplification by DNA Polymerase.

In PCR-Fluorescent Probe method, the probe with specific binding to target sequence is added based on the forward primer and the reverse primer, Specific primers and probes are designed based on specific gene areas of Novel Coronavirus (COVID-19). Probes consist of a reporter fluorophore at 5' and quenching fluorophore at 3'. The fluorescent signals emitted from reporter fluorophores are absorbed by the quenchers, so it doesn't emit signals. During amplification, probes bonded to templates are cut off by Taq enzyme (5'-3' exonuclease activity), separating reporter dye from the quencher, generating fluorescent signals, the PCR instrument will then automatically draw a real-time amplification curve based on the signal change, finally realizing the qualitative detection of Novel Coronavirus (COVID-19) at the nucleic acid level.

【Warnings and Precautions】

1. For in vitro diagnostic use only.
2. Carefully read this instruction **before starting the procedure.** Components from different batch number kit cannot be used interchangeably.
3. Once each component within the kit is thawed, it is suggested to use them up within one operation based on examination demand, and the component remained should be restored at -20℃. Repeated freezing and thawing (more than three times) of reagents should be avoided.
4. Viral RNA and RT-PCR premix are sensitive to temperature. Once Sample RNA and RT-PCR pre-mix are taken out of -20±5℃ freezer, prepare the Master Mix on ice or in the cooling block.
5. Avoid microbial and nuclease (DNase/RNase) contamination of the specimen and the components of the kit.
6. Always use DNase/RNase-free disposable aerosol-blocking pipette tips.
7. Use of this product is limited to personnel specifically instructed and trained in the Wear protective disposable powder-free gloves, laboratory coat and eye protection when handling specimens and kit reagents.
8. Use separated and segregated working areas for (i) reaction set-up, (ii) specimen preparation and (iii) amplification/detection activities. Workflow in the laboratory should proceed in unidirectional manner. Wear separate coats and gloves in each area.
9. All biological samples and materials with the contact with the product should be treated as infectious biohazard, and related local regulations shall be followed for the disposal. Prevent the exposure to skin and mucosa.
10. The detection test with this kit should be conducted by medical staff and technician with professional technical training.
11. Store positive and/or potentially positive material separated from all other components of the kit.
12. Do not open the reaction tubes/plate post amplification to avoid contamination with amplicons.
13. Additional controls may be tested according to guidelines or requirements of local, state and/or federal regulations or accrediting organizations.
14. Discard sample and assay waste according to your local safety regulations.
15. Do not eat, drink, or smoke in the laboratory working area.
16. Do not use components of the kit after expiration date.

【Recommendation on Specimen according to the WHO Guideline】

1. Applicable sample type: throat swab, sputum.
2. Requirements on sample collection: the sample collection shall be conducted with polyester swab or polyester flocked swab.
   （1）Nasopharyngeal swab: extend the sterile nasopharyngeal swab (metal swab with one curved end) from the oral cavity to nasopharynx, swab the posterior wall of the pharynx to get the sample.
   （2）Oropharyngeal swab: The sampling technician shall fix the tongue with a spatula, and use the polyester swab or calcium alginate swab to cross the root of the tongue to reach the positions such as pharynx posterior wall and tonsillar crypts, side wall, swab 3 to 5 times to collect mucosa cells; take out the swab gently to avoid the contact with the tongue, horst, oral mucosa or saliva, and insert the swab back into the sampling apparatus.
3. Requirements on sample transportation and storage: It is required to ship the sample at 4℃ to the lab, and store at 4℃ if the storage time is less than 5 days, while it is required to store at -70 °C if the storage time is longer than 5 days.
4. Precautions:
   （1）Nasopharyngeal swab and Oropharyngeal swab shall be placed into one tube in order to increase the viral load;
   （2）For the transportation of virus detection sample, WHO guideline suggested to use VTM (Viral Transport Medium) with antifungal and antibiotic supplement.

5. **SPUTUM SPECIMEN**: Recommend stored in sterile container; Required to ship the sample at 4℃ to the lab, and store at 4℃ if the storage time is less than 48 hours, while it is required to store at -70 °C if the storage time is longer than 48 hours. PLEASE **ENSURE THE MATERIAL**

IS FROM THE LOWER RESPIRATORY TRACT.

【Procedure】

1. Collect clinic sample RNA with QIAamp viral RNA mini kit or other RNA isolation kit (see the instructions).
2. Formulation of RT-PCR One-step Mix.
   2.1 Determine the amounts of samples to be tested first; RNase-free reaction tubes shall be provided with each sample.
   2.2 Thaw Reaction Mix, positive control, and RNA samples on the ice, and shake with inching on the shaker, followed by short spin on the centrifuge.
   2.3 Reaction mixes contain RT-PCR primers, probes and reaction reagents (except enzymes). Primers and probes for Internal control (IC) gene have been included in the reaction mix. Set up following ingredients in order:

Table, Detection of each assay (example with reactions for ORF1ab, N gene and IC gene).Add the components as the order below

Reagents	Individual test (μL)
Reaction Mix (ORF1ab/N gene/IC)	14.6
RT Enzyme Mix	0.4
RNA sample	5
Total volume	20

2.4 Add the 14.6 μL Reaction Mix and 0.4 μL RT Enzyme Mix together. Calculate the volume (μL) needed: number of tests multiply Reaction Mix or RT Enzyme Mix amount; prepare 10% more.
2.5 Aliquot 15 μL of final Reaction Mix into each sample tube.
2.6 The total RT-PCR reaction volume is 20 μL:
（1）RNA sample: Add 5μL of RNA sample to the corresponding reaction well in Reaction Mix.
（2）Positive control: Add 5μL of positive control to the reaction well in Reaction Mix as positive control well.
（3）Negative control: Add 5μL of Nuclease-free water to the reaction well in Reaction Mix as negative control well.
2.7 Seal the tube cap and shake with inching on the shaker several times, followed by short spin on the centrifuge.

3. RT-PCR protocol as below:

Temp (℃)	Time	Cycles
50	5 min	1
95	20 sec	1
95	5 sec	45
60	30 sec	

Probe label:
ORF1ab: FAM-BHQ1; N Gene: VIC-BHQ1; IC (RNase P) tube: CY5- BHQ3.

4. Result Interpretation
   4.1 Quality Check for the Test Results
   The following requirements on value Ct of positive control well and negative control well on the reaction plate within the same reaction plate/batch:

	Quality control requirement
Positive Control reaction well	Ct≤37
Negative Control reaction well	Ct>37 or Undet

4.2 The experiment is invalidated, and repeat is required if the positive control and/or negative control does not meet the criteria set above.
4.3 The analysis of the Ct value of the ORF1ab, N, and IC wells in each swab or sputum specimen as follows:

ORF1ab	N gene	IC	Interpretation
Ct≤37	Ct≤37	Ct≤40	Positive
Ct>40 or Undet	Ct>40 or Undet	Ct≤40	Negative
40>Ct>37 (Amplifiable)	40>Ct>37 (Amplifiable)	Ct≤40	Weak positive; Retest to confirm
Ct>40 or Undet	Ct≤37	Ct≤40	Coronaviral infection
Ct>37 or Undet	Ct>37 or Undet	Ct>40 or undet	Resampling for test or other confirmation
Ct≤37	Ct>37 or Undet		

【Performance Evaluation】

1. Limit of Detection (LOD): 400 copies/mL.
2. Interference reaction : The five potential reference(Dexamethasone, Azithromycin, Tobramycln, Levofloxacin, Ceftriaxone) will not interfere with the detection results of the kit.
3. Cross-reactivity: No cross reaction with 16 viruses(Human BK polyomavirus, Human adenovirus C serotype 5, Human adenovirus A/B1/C/D/E, Human herpesvirus 1/2/3/4/5/7, Human parvovirus B19, Human JC polyomavirus, Simian vacuolating virus 40) and human genome DNA
4. Internal precision: repeatability : cv% < 10%, between-run precision : cv% < 10%, between-day precision : cv% < 10%, total precision: cv% < 10%..
5. External precision: repeatability : cv% < 10%, between-run precision : cv% < 10%, total precision: cv% < 10%.

【Performance Limitation】

1. Test results only serve as clinical reference and comprehensive judgments based on clinical symptoms and other laboratory tests method should be considered by clinicians.
2. Negative results cannot completely rule out the existence of novel coronavirus. Improper sample collection, improper transportation, improper processing and insufficient initiation VL(viral load) may influences the experimental results.
3. Other unverified interferences or PCR inhibitors may cause false negative results.

【Explanation of Marks】

Diagram and symbol used on kit label	remarks
	Manufacturer
	Authorized representative in the European Community
	Consult instructions for use
	In vitro diagnosis reagent
	Contains sufficient for <n> tests
	Date of manufacture
	Use-by date
	Do Not Reuse
	Batch code
	Biological risks
	Storage temperature
	Keep dry
	Keep away from sunlight
	Fragile, handle with care
	Recoverable PAP material
	Recoverable PP material
	Recycled recyclable

【Manufacturing Date and Expiration Date】

See details on packaging label.

【Basic Information】

GUANGDONG ARDENT BIOMED Co.,Ltd.
4th floor of C1 Building,No.11Kaiyuan Road Science City
High-tech Industrial Development District,Guangzhou City
Guangdong 510530,China
Tel: 86-020-82207223
Web site:www.ardentbiomed.com.cn

EC REP Caretechion GmbH
Niederrheinstr 71,40474 Duesseldorf,Germany
Tel: +49 211 300 366 18
Email: jian.wang@caretechion.de

【User Manual Information】

Issue date: Mar., 2020
Rev.TCF-308 A0

CE IVD