Novel Coronavirus (SARS-CoV-2) Nucleic Acid Detection Kit (Real-time Fluorescent RT-PCR) Instruction for Use

● Product Name

Novel Coronavirus (SARS-CoV-2) Nucleic Acid Detection Kit (Real-time Fluorescent RT-PCR)

● Pack Formats

50 tests/kit

● Intended Use

The kit is intended for the qualitative detection of ORF1ab gene and N gene of Novel Coronavirus (SARS-CoV-2) from oropharyngeal swabs, nasopharynx swabs and sputum specimens to identify SARS-CoV-2 infection among suspected pneumonia cases, suspected clustering cases, and other cases that require diagnosis or differential diagnosis of SARS-CoV-2 infection.

● Principle

This product is a dual-color multiplex fluorescent probe-based Taqman RT-qPCR assay system. The Taqman fluorescent probe is a specific oligonucleotide based on a reporter-quencher mechanism. For each probe, the 5'-end is labeled with a fluorophore, while the 3'-end is labeled with a quencher. When the probe is intact, the fluorescence emitted by the fluorophore is absorbed by the quencher, and no fluorescent signal is detected. However, during amplification of the template, the probe will be degraded due to the 5'-3'exonuclease activity of Taq DNA polymerase, and the fluorescent reporter and the quencher are cleaved and separated, then a fluorescent signal can be detected. The generation of each molecular amplicon is accompanied by the generation of a fluorescent signal. Real-time monitoring of the entire PCR process can be assessed by monitoring the accumulation of fluorescent signals. This product provides dual-detections of two independent genes of SARS-CoV-2 in a single tube. Specific primers and probes were designed for the detection of conserved region of ORF1ab gene and N gene of SARS-CoV-2, respectively. Detection is performed by a fluorescent PCR detector to achieve qualitative detection of RNA of SARS-CoV-2.

● Kit Components

Component	Volume (μl/Vial)	Number of Vials	Ingredient
Reaction Mix	1000	1	dNTPs、$MgCl_2$、specific primers and probes for ORF1ab and N gene
Reverse Transcriptase	20	1	Reverse Transcriptase
Taq DNA Polymerase	50	1	Taq DNA polymerase
Negative Control	200	1	5% Bovine Serum
Positive Control	200	1	Plasmid mix containing ORF1ab and N gene fragments of SARS-CoV-2

Note:

1.Don't interchange components between kits of different batches or other kits.

2.Additional instruments and reagents required: biosafety cabinet, microcentrifuge, vortex mixer, swab, sampling tube, sterile virus sampling solution, nuclease-free 1.5 mL microcentrifuge tubes.

● Storage and Stability

1.The production date and expiration date of the kit are printed on the outer box.

2.Store at -20±5°C, the validity is tentatively set for 6 months.

● Compatibility

The kit can be used with the following real-time PCR instruments: ABI Prism 7500, ABI Prism 7000, Roche LightCycler 480, Agilent Mx3000P.

● Specimens Requirements

1.Specimen type: oropharyngeal swab, nasopharyngeal swab, sputum.

2.Sampling swab, sampling tube and sampling solution:

a.Sampling swab: use swabs with a synthetic tip (such as polyester) and aluminum or plastic shafts.

b.Sampling tube: external spiral tube which is resistant to - 70°C and can hold 3 mL virus sampling solution.

c.Sterile virus sampling solution: sterilized and containing protein stabilizers, antifungal and antibiotic supplements, buffers.

3.Specimen collection, shipment and storage

a.Collection

Pharyngeal Swab Specimen: Collect swab specimen of patient for pathogen detection. Use the sampling swab to wipe the posterior wall of the pharynx and the tonsils on both sides with moderate force, and avoid touching the tongue or nasal cavity. Quickly put the swab into a 15 mL external spiral sampling tube containing 3-5 mL sampling solution (containing 5% bovine serum maintenance solution or 0.9% saline, maintenance solution is preferred) and break off shaft near the top. Tighten and seal the tube cap to prevent drying, and attach a label with a unique identification number outside.

Sputum Specimen: collect sputum specimen of patient for pathogen detection. Sputum is taken from the lower respiratory tract and stored in a sterile sampling tube. Tighten and seal the tube cap to prevent drying, and attach a label with a unique identification number outside.

b.Storage and Shipment

After the specimens are transported to the laboratory, they should be processed and aliquoted immediately to avoid repeated freezing-thawing. Pharyngeal swab specimens should be stored at 4-8 °C (no more than 5 days), sputum specimens should be stored at 4-8 °C (no more than 48 hours) or - 70°C or below, and should not be stored at - 20 °C. Fresh specimens should be transported to the laboratory within 48 hours at 4-8 °C, while frozen specimens should be transported to the laboratory with dry ice. SARS-CoV-2 is a pathogen of infectious diseases. Transport of specimens should comply with applicable national regulations, and they musts be packed and transported in class A level. The packing box should be filled with water-absorbing material, and the specimen collection tube should be kept upright during transportation and cannot be tilted.

● Protocol

1.Nucleic Acid Extraction

Use 200 μL specimen to perform nucleic acid extraction. Following the instruction of the kit when commercial nucleic acid extraction kit is used to extract SARS-CoV-2 RNA. The negative and positive controls in the kit are involved in extraction.

2.RT-PCR Amplification

2.1 Assay Design

2.1.1 Specimen test: Each specimen is tested with the reaction mixture and the result is used to make the final interpretation of the specimen.

2.1.2 Control test: It is recommended to set both negative and positive controls for each test.

2.2 Reaction Mix Preparation

2.2.1 Thaw the reaction mix, mix well by shaking, and centrifuge at 3000 rpm for 10 seconds.

2.2.2 Take a 1.5 mL nuclease-free centrifuge tube, calculate the numbers of specimens to be tested (n) and take 20 μL × (n+2) reaction mixture, 1.0 μL× (n+2) Taq DNA polymerase and 0.35 μL× (n+2) Reverse Transcriptase. Mix well and centrifuge at 3000 rpm for 10 seconds.

Note: n+2=n specimens +l positive control +1 negative control

2.3 Reaction Mix Aliquoting

Aliquot 20 μL reaction mix into the corresponding PCR amplification tube.

2.4 Template Addition

Add 5 μL of extracted specimen RNA/negative control/positive control to different PCR reaction tubes which contain 20 μL of PCR mixture.

2.5 Amplification and Test

The specimen-loaded PCR tube is transferred to the full-automatic fluorescent quantitative PCR detector for amplification test. The settings of different instruments are as follows:

2.5.1 For ABI Prism 7500 and ABI Prism 7000 automatic fluorescent quantitative PCR detector, the fluorescent signal is set to: Reporter Dye 1: FAM, Quencher Dye 1: NONE; Reporter Dye 2: HEX, Quencher Dye 2: NONE; Passive Reference: NONE. Set up and run the following cycling procedure (Table 1). The total volume of reaction is 25 μL.

2.5.2 For other automatic fluorescent quantitative PCR detector, the fluorescent channels are set as FAM and HEX, and the amplification procedure is the same as Table 1.

Table. 1 Fluorescent PCR Amplification Procedure

Step	Temperature	Time	Fluorescent Collection	Cycles
Reverse Transcription and Denaturation	50 °C	30 minutes	No	1
	95 °C	3 minutes	No	
Preamplification	95 °C	15 seconds	No	5
	50 °C	30 seconds	No	
	72 °C	30 seconds	No	
Amplification and Fluorescence Collection	95 °C	10 seconds	No	40
	55 °C	40 seconds	Yes	

3. Data Analysis

3.1 ORF1ab gene of SARS-CoV-2 is detected in the FAM channel and N gene of SARS-CoV-2 is detected in the HEX channel. FAM and HEX channels should be selected to analyze results.

3.2 Set the baseline as instrument required to correct the background fluorescence interference. Generally, 3-4 cycles prior to presence of the amplification signal from the strongest specimen is set as END.

3.3 The threshold value is used to determine whether the specimen is amplified. The amplification curve of the negative control should be straight or below the threshold line or click “Analysis” to obtain the analysis result automatically.

4. Quality Control

Assay controls should be run concurrently with all test specimens. The result is valid if all the following criteria are met. Otherwise, the result is invalid.

4.1 Negative Control: No Ct or Ct=0.

4.2 Positive Control: Ct<30.0.

● Reference Interval

Positive: Ct≤37.0; Negative: No Ct or Ct=0; Gray Zone: 37.0<Ct<40.0

● Interpreting Test Results

1. If the criteria of quality control are met, analyze the data as follows:

	Type 1	Type 2	Type 3	Type 4	Type 5
FAM (ORF1ab)	No Ct	Ct≤37.0	No Ct	Ct≤37.0	37.0<Ct<40.0
HEX (N)	No Ct	Ct≤37.0	Ct≤37.0	No Ct	
Results	SARS-CoV-2 Negative	SARS-CoV-2 Positive	SARS-CoV-2 Suspicious	SARS-CoV-2 Suspicious	Gray Zone

2. Specimens with result in the gray zone need to be tested repeatedly: take 400 μL specimen to re-extract RNA and test. The specimen with Ct<40.0 is positive, otherwise it is negative.

3. Suspicious specimens can be detected by other technical methods or verified by genetic sequencing.

● Assay Limitations

The detected target sequences of this kit are the conservative region of SARS-CoV-2 ORF1ab gene and N gene. However, target sequence variations may lead to false negative result.

● Performance Characteristics

1. Detection limitation: 100 copies/mL.

2. Precision: use precision reference with strong positive level and critical positive level for within-batch and between-batch detection, repeat 10 times, the coefficient of variation (CV) of their Ct values is ≤ 5.0%.

3. Specificity: non-specific interference of Influenza A Virus, Influenza B Virus etc.

● Precautions

1.Please read this instructions carefully before use and strictly follow the operation procedures.

2.Laboratory management and test operations should be performed in accordance with the related regulations: strictly divide the whole detection area into: PCR reaction mix preparation area, specimen processing and loading area. Deal with specimens in the biosafety cabinet to protect the operators safety and prevent environmental pollution. The instruments, equipment, consumables and work clothes should be used independently and exclusively in each area.

3.Don't interchange reagents between kits of different lots and use the kit within the validity period.

4.This kit is for *in vitro* diagnostic use only. The detection result is only for clinical reference, and it should not be used as the only evidence for clinical diagnosis and treatment.

● References

1.Laboratory biosafety guidance related to coronavirus disease 2019 (COVID-19). Interim guidance. World Health Organization.

2.Laboratory testing for coronavirus disease 2019 (COVID-19) in suspected human cases. Interim guidance. World Health Organization.

3.Guidance for laboratories shipping specimens to WHO reference laboratories that provide confirmatory testing for COVID-19 virus. Interim guidance. World Health Organization.

4.Regulations on the transport management of highly pathogenic microbial bacteria (viruses) species or specimens capable of infecting humans. (No. 45 of the Ministry of Health).

5.Administrative measures for clinical gene amplification laboratory of medical institutions (No. 194 issued by the Department of Medical Administration, Ministry of Health in 2010).

6.Regulations on the management of instructions and labels of medical devices. (Provision No.6 2014 CFDA Requirements).

7.Guidelines for preparation of *in vitro* diagnostic reagent instructions. (Circular No. 17, 2014 of the State Food and Drug Administration)

● Symbols

Symbols	Description
	*In vitro* Diagnostic Medical Device
	Temperature Limitation (Storage Temp. -20±5°C)
	Consult Instruction for Use (IFU)
	Production Date
	Batch Code (Lot Number)
	Expiration Date

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