3. Interfering Substances Study

There is no interference of the following endogenous substances and therapeutic drugs: Whole blood, Mucins, Phenylephrine, Oxymetazoline, Sodium chloride, Beclomethasone, Dexamethasone, Flunisolide, Triamcinolone acetonide, Budesonide, Momitasone, Fluticasone, Histamine hydrochloride, Watermelon frost, alpha-interferon, Zhanamivir, Ribavirin, Oseltamivir, Peramivir, Lopinavir, Ritonavir, Abidore, Levofloxacin, Ceftriaxone, Azithromycin, Meropenem, Tobramycin, Ethanol, SDS, EDTA.

4. Precision

The weak positive sample and medium/strong positive sample were tested with three batches of SARS-CoV-2 RT-PCR Assay. Each batch of reagents is tested by 2 people per day, and each person test once in the morning and afternoon. Each sample was tested 2 times in parallel and is tested continuously for 20 days. The results met the following criteria.

4.1 Weak positive sample: the positive detection rate should be 100%, the Coefficient of variation

4.2 of Ct of FAM and HEX (VIC) channels should ≤5.0%.

Medium positive sample: the positive detection rate should be 100%, the Coefficient of variation of Ct of FAM and HEX (VIC) channels should ≤5.0%.

Precautions

1. This product is only suitable for in vitro diagnostic testing, and the experimenter should have received professional training and obtained the relevant work permit.
2. It shall be implemented strictly in accordance with the management norms of gene amplification laboratory promulgated by the relevant competent authorities. The laboratory is divided into three areas for operation (reagent preparation area, sample preparation area and amplification area), items in each area shall not be cross-used, and special instrument and equipment shall be used in each area.
3. The performance characteristics of this test kit for the sample types listed in the section "Intended use" have been identified. The performance of this test kit for other sample types has not been evaluated
4. The tip with filter and centrifuge tube used in the experiment should be autoclaved, and without DNase and RNase. After use, it is directly put in the disposal bottle containing 1% sodium hypochlorite.
5. All clinical samples should be treated as infectious substances, operation and disposal shall comply with relevant regulations, such as the General guidelines for biosafety in Pathogenic Microbiological laboratory and the Clinical Waste Management Ordinance issued by the Ministry of Health.

Symbols meaning

	Do not re-use		Manufacturer	EC REP	Authorized representative in the European Community
	Keep dry		Manufacture Date		Do not use if package is damaged
	Caution		Store at 2-8℃	IVD	In vitro diagnostic medical device
CE	CE Symbol		Keep away from sunlight	LOT	Lot number
REF	Catalogue number		Consult instructions for use		Use by date

Reference

Hui, D. S., I Azhar, E., et.al (2020). The continuing SARS-CoV-2 epidemic threat of novel coronaviruses to global health-The latest 2019 novel coronavirus outbreak in Wuhan, China. International Journal of Infectious Diseases, 91, 264–266.

Basic Information

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Date of approval and amendment of IFU： 2022.06.01
Version number： V1.03

techstar®

SARS-CoV-2 RT-PCR Assay
Instruction For Use

For in vitro diagnostic use only
For Prescription Use only

Name	SARS-CoV-2 RT-PCR Assay	
REF	SC508482	SC508962
Specification	48 reactions/kit	96 reactions/kit

Intend Use

This product is an in vitro diagnostic realtime RT-PCR assay intended for the qualitative detection of N gene and ORF1ab gene of SARS-CoV-2 in human throat (oropharyngeal) swabs and nasopharyngeal swabs collected by a healthcare provider from individuals suspected of COVID-19. Test results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable 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.

Principle

This product uses RT-PCR technology to qualitatively analyze the nucleic acid of SARS-CoV-2,First design specific primers and probes for the nucleocapsid protein N gene and ORF1ab gene of SARS-CoV-2, The specific probe of ORF1ab gene was labeled with FAM fluorescent, and the specific probe of N gene was labeled with HEX fluorescent. During the amplification process, when the reaction system does not contain SARS-CoV-2 nucleic acid,the probe is complete, because the 3 'quenched group is close to the 5' reporter group, the fluorescence emitted by the reporter group is absorbed by the quenched group, and no fluorescence signal is emitted. When the SARS-CoV-2 nucleic acid was contained in the reaction system, the fluorescent probe bound to the template during primer extension is cleaved by Taq enzyme (5'-3' exonuclease activity), and the reporter group was separated from the quenched group to emits a fluorescence signal.

The real-time amplification curve can be generated by fluorescent quantitative PCR instrument automatically according to the detected fluorescence signal, so as to achieve the purpose ofqualitative analysis of SARS-CoV-2 nucleic acid in the sample.

The assay also includes an endogenous ribonuclease P (RNaseP) as an internal control (labeled with CY5), to monitor the entire process of specimen collection, nucleic acid extraction, and PCR amplification.

Materials Provided

Constituent	Component	48 reactions/kit	96 reactions/kit
Dilution Buffer	Nuclease-free water	1.0mL/vial, 1 vial	2.0mL/vial, 1 vial
AD RT-qPCR Mix	Primer、Probe、dNTPs、Enzyme	1 vial	2 vial
Positive control	Pseudovirus containing the target gene and internal control	1 vial	1 vial
Negative control	Nuclease-free water	2.0mL/vial, 1 vial	2.0mL/vial, 1 vial

Note: The components in different batch are not interchangeable.

Materials and Equipment Required But Not Provided

- Applied Biosystems™ Real-Time PCR System 7500.
  Alternatively, Roche LightCycler® 480 System, or QuantStudio 5 Real-Time PCR System.
- QIAamp Virus RNA Mini Kit (cat. #52904 or 52906).
  Alternatively, Nucleic Acid Extraction Kit (cat. SC906).
  Optionally, Nucleic Acid Extraction Kit (cat. SC902/905).
- Vortex mixer.
- Microcentrifuge.
- Micropipettes (2 or 10 μL, 200 μL and 1000 μL).
- Racks for 1.5 mL microcentrifuge tubes.
- 10% bleach (1:10 dilution of commercial 5.25-6.0% hypochlorite bleach).
- Disposable powder-free gloves and surgical gowns.
- 1.5 mL microcentrifuge tubes (DNase/RNase free).
- 96-well 0.2 mL PCR reaction plates or appropriate optical reaction tube.
- Nuclease-free water.

Storage conditions and Expiration period

Store at 2-8℃ away from light for 12 months or 20-25℃ away from light for 6 months.
Date of manufacture: see label
Expiration Date: See label

Applicable Instrument

ABI7500、ABI QuantStudio 5 and Roche LightCycler480

Specimen Collection and Preparation

1. Available sample types: throat (oropharyngeal) swabs, nasopharyngeal swabs.
2. After collection, the swab was placed in normal saline or other sample preservation solution (Hank`s solution or guanidine-based virus transport media).
3. Appropriate specimen collection, transport, storage and processing procedures are required for the optimal performance of this test. Improper collection, storage, or transport of specimens may lead to false negative results.

The specimen may be tested immediately after collection, or it may be stored at 2-8℃ for up to 24 hours before testing. If the expected detection time exceeds 24 hours, you can choose to store the samples below -70°C.

Laboratory Procedures

1. Sample processing

1.1 RNA should be collected from fresh specimen to ensure suitable RNA quality and quantity. RNA should be extracted using the QIAamp Viral RNA Mini Kit (Qiagen) or Nucleic Acid Extraction kit manually (Art.No.SC902/SC905/SC906, Wuxi Techstar Technology Co.,Ltd) according to the manufacturer`s Instruction of Use.

1.2 Add 500μL Negative control or Nuclease-free water to the Positive control. Vortex the tubes to mix then centrifuge at 6000rpm for 30s. Positive Control and Negative control should be processed simultaneously alongside the specimen.

1.3 After extraction, the RNA should be used immediately or stored at -70℃ for use later. When handling the Positive control, please take precautions to avoid contamination of the specimen sample.

⚠ The Positive Control and Negative Control only need to be extracted once, and the extracted nucleic acid can be directly used in the next experiment.

2. Reagent preparation

2.1 Take out the kit contents and thaw thoroughly at ambient temperature. Prepare 96-well plates or appropriate optical reaction tube for real-time RT-PCR based on the estimated number of reactions.

2.2 Adding 980μl Dilution Buffer to the AD RT-qPCR Mix for redissolve and mix it.

2.3 Pipette 20μL of PCR-Mix into each well.

⚠ The remaining PCR-Mix, Positive control and Negative control must be stored at under -18℃ immediately, and it should be used within 20 days, repeated freezing and thawing times should not exceed 7 times.

3. Sample Addition

Add 5μL extracted sample RNA, Negative control or Positive control, and close the 96-well reaction plate with appropriate lids or optical adhesive film, and centrifuge at 6000rpm for 30s.

⚠ Make sure that at least one Positive Control and one Negative Control is used per run.

4. Amplification（ABI7500, for example）

4.1 Double-click 7500 software or select Start>>All Programs>>Applied Biosystems>>7500 Software.

4.2 Click “New Experiment” to enter Experiment menu. In the Experiment Properties interface, enter identifying information for the experiment; you can leave other fields empty.

4.3 Select “7500 (96 Wells)", “Quantitation-Standard Curve” (for the experiment type); “TaqMan Reagents” (for reagent); and “Standard” (for ramp speed).

4.4 Go to Plate Setup > Define Targets and Samples > Define targets > Add New Target > set Target Name and Reporter as shown below:
Target 1. ORF1ab: Reporter FAM; Quencher None
Target 2. N gene: Reporter VIC; Quencher None
Target 3. IC: Reporter CY5; Quencher None

4.5 Go to Define Samples > Add New Sample > Input PC, NC and Sample (Test Specimen).

4.6 Click “Assign Target and Samples” to set targets and well positions for PC, NC and Samples to be analyzed. in the “View Plate Layout” interface, enter the name of samples and controls to include in the reaction plate in corresponding well, and select the sample/target reactions to set up. Select None for passive reference.

4.7 Click “Run Method”. On the Run Method interface, select either the “Graphical View” tab (default) or the “Tabular View” to edit the run method. Make sure the thermal profile displays the holding and cycling stages shown below. Set “Reaction volume Per Well” to 25 μL.

Step	Temperature	Time	Fluorescence measured	Cycle
1	50℃	20min	No	1
2	95℃	3min	No	1
3	95℃	15sec	No	40
4	58℃	30sec	Yes	

4.8 Click “Run”. In the Run interface, save the experiment. Click “START”.

4.9 After the run completes, take out the sample andproceed to data analysis.

5. Analysis of result

The results are automatically saved after the reaction, and the Baseline Starting Cycle, Baseline End Cycle and Threshold of the Baseline are adjusted according to the analyzed images. Generally, the Baseline Starting point is set to 3--15. The baseline endpoint should be set at 5-20, and the threshold should be in the exponential phase of the amplification curve, and the curve for negative quality control should be flat or below the threshold line. Click Analysis to automatically analyze the results.

Interpretation of result

1. Quality control standards

1.1 Negative control:No typical S-type amplification curve or no Ct for FAM, HEX (VIC) and CY5 channels.

1.2 Positive control: FAM, HEX (VIC) and CY5 channels showed a typical S-type a amplification curve.

If both meet the requirements of 1.1 and 1.2, the experiment is effective, otherwise it is invalid.

2. Result interpretation

When the above quality control conditions are met, analysis as follows:

2.1 **Positive:** The FAM and HEX (VIC) channels has aS-type amplification curve, and Ct,< 38, it can be determined as SARS-CoV-2 nucleic acid positive;

2.2 **Negative:** The FAM and HEX (VIC) channels to be tested have no Ct value, and the CY5 channel showed a typical S-type amplification curve, which was judged to be SARS-CoV-2 nucleic acid negative.

2.3 **Suspected:** If only one of FAM and HEX(VIC) channels to be tested has S-type amplification curve, and Ct>38, and the other channel have no Ct value, the sample needs to be extracted and tested again. If the retest results are consistent, it can be judged as SARS-CoV-2 nucleic acid positive; if the retest results are negative, it can be judged as SARS-CoV-2 nucleic acid negative.

2.4 If there is no typical S-type amplification curve in the FAM, HEX(VIC) and CY5 channels, it indicates that the system is inhibited or mis operated, and the test is invalid. Therefore, the samples should be retested.

Limitations

1. Negative result cannot completely exclude pathogen infection, and the concentration of target genes in sample below the detection limit or the mutation of target sequences to be tested can also result in negative result.
2. False negative or false positive result may be caused by improper sample collection, transport and handling, as well as improper experimental operation and experimental environment.
3. The samples collected from the patients who used drug therapy may result in false negative results.
4. Diseases caused by other bacterial or viral pathogens cannot be ruled out.
5. The testing result of this product are only for clinical reference and should not be used as the sole basis for clinical diagnosis and treatment, and clinical management of patients should be considered in combination with their symptoms/signs, medical history, other laboratory tests and treatment responses.
6. The nucleic acid may remain in the body for a long period of time, which is not related to virus activity. A positive test result does not necessarily mean that the virus is infectious, or that the virus is a pathogen that causes clinical symptoms.

Performance Characteristics

1. Limit of Detection

The LoD of the SARS-CoV-2 RT-PCR Assay was estimated by testing the standardized dilutions of virus (n = 20 each). The lowest target level at which ≥19/20 produced positive results was 500 Copies/mL. This value was then confirmed by testing 20 replicates.

2. Analysis specificity

No cross-reactivity were observed with Coronavirus -OC43, Coronavirus -229E, Coronavirus -NL63, Coronavirus -HKU1, SARS Coronavirus, MERS Coronavirus, New Influenza A Virus（H1N1 2009）, Influenza A virus (seasonal H1N1), H3N2, H7N9, H5N1, Influenza B Yamagata, Influenza B Victoria, RSV Type A, RSV Type B, Parainfluenza virus Type Ⅰ, Parainfluenza virus Type Ⅱ, Parainfluenza virus Type Ⅲ, Rhinovirus A, Rhinovirus B (HRV-70), Rhinovirus C, Adenovirus type 1, Adenovirus type 2, Adenovirus type 3, Adenovirus type 4, Adenovirus type 5, Adenovirus type 7, Adenovirus type 55, Enterovirus A（EV-A71), Enterovirus B（CoxsackievirusB1）, Enterovirus C, Enterovirus D, Human Metapneumovirus, EB Virus, Measles virus, Human Cytomegalovirus, Rotavirus, Norovirus, Mumps virus, Chickenpox-shingles virus, Mycoplasma pneumoniae, Chlamydia pneumoniae, Haemophilus influenzae, Staphylococcus aureus, Streptococcus pneumoniae, Klebsiella pneumoniae, Mycobacterium tuberculosis, Candida albicans.