ACCEGEN
BIOTECHNOLOGY

AceSeq™ Novel Coronavirus (SARS-CoV-2) Nucleic Acid Detection Kit (Real Time RT-PCR Method)

Instructions for Use

Package 96 Tests / Kit

Intended Use

The AceSeq™ Novel Coronavirus (SARS-CoV-2) Nucleic Acid Detection Kit is a real-time multiplex RT-PCR test intended for the qualitative detection of SARS-CoV-2 viral RNA. It is suitable for upper respiratory specimens, such as nasopharyngeal, oropharyngeal swab, and bronchoalveolar lavage specimens. In a single RT-PCR reaction, it is able to detect ORF1ab and N RNA sequences, and in addition, human RNase P as an internal control.

Principles

This kit uses the real-time fluorescent RT-PCR technology, with prime and probe sets designed targeting the ORF1ab and N gene sequences. Each probe is labeled at the 5 ' end a fluorescence reporter group and 3' end a quenching group. The 3' primers enable the reverse transcriptase to generate cDNA from viral RNA, and subsequently Taq polymerase performs multiple rounds of amplification of the target DNA. During primer extension steps, the 5 '→ 3' exonuclease activity of the Taq enzyme hydrolyzes the probes, and the reporter fluorescence group is free from the quencher, and therefore a fluorescent signal is emitted. The target detection can be achieved from the amplification curve and the Ct value.

This kit contains an endogenous internal control (IC), which targets human RNase P gene sequences. The IC is used to monitor the collection, transportation, extraction, and amplification of the test specimen to avoid false negativity.

Materials Provided

Item	Contents	Amount
RT-PCR Mix, lyophilized microsphere	Primers, probes, reaction buffer, Mg2+, dNTPs, M-MLV Reverse Transcriptase, Taq DNA Polymerase	8-tube strip x 12
Positive Control (PC), lyophilized microsphere	Synthetic RNA	Tube × 1
Negative control (NC)	Nuclease free Water	Tube x 1

Reagent Storage and Handling

1. The kit is shipped at ambient temperature and stored between 2- 30℃ for 12 months.
2. After dissolving the positive control material, use immediately or store at under - 20℃, and avoid repeat freeze-thaw cycles.

Materials Required but Not Supplied

1. ABI7500, ABI 7500 Fast or other Real-time fluorescence PCR instrument
2. Vortex mixer
3. Microcentrifuge
4. pipettor and pipette tips.
5. PCR tube or strip
6. Swap specimen kit
7. Viral RNA extraction kit

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Specimen Handling, Storage

1. The recommended specimen type is an upper respiratory specimen, such as nasopharyngeal, oropharyngeal swab or Bronchoalveolar lavage fluid in UTM or VTM.
2. Specimens can be stored at 4°C for up to 72 hours, or at -20 ° C for three months.

Procedure

1. Nucleic acid extraction

Commercial viral nucleic acids kits have been found to be able to extract high quality RNA when manufacturer's recommended procedures are followed.

2. Positive control reconstitution

Add 20 uL nuclease free water (NC) to the Positive Control tube, and mix well before use.

3. RT-PCR Reaction Setup

a. Take specimen number N+2 tubes of RT-PCR Mix 8-tube strip, add 15 uL nuclease free water to each tube, and then add 5 uL extracted test sample RNA, extracted positive control RNA, and nuclease free water (NC) respectively.

b. Close caps， mix thoroughly, and spin briefly.

c. Place the reaction tubes into the sample rack of the real-time PCR instrument.

4. RT-PCR protocols:

Recommended Settings

	Step	Temperature（℃）	Time	Cycle
1	Reverse Transcription	50	5mins	1
2	Pre-denaturation	95	5mins	1
3	Denaturation	95	5secs	45
4	Annealing / extension	60	40secs	

*Fluorescence signal is detected at 60 °C in step 4 and the fluorescence channels are: FAM, VIC, and Cy5; quenching group: BHQ.

Interpretation of Results

1. Quality Control

Positive and negative controls should have been examined and determined to be valid and acceptable before clinical specimen test results can be assessed.

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Acceptable Ct values for Positive and Negative Control

	FAM channel (Orf1ab)	VIC channel (N)	Cy5 channel (Rnase P)
Positive control	Ct≤35	Ct≤35	Ct≤35
Negative control	Undetected	Undetected	Undetected

If the requirements above are not met, the test result is invalid.

2. Examination and determination of result in each channel

FAM channel is for the ORF1ab target: if amplification curve was typical "S" shape, and Ct value≤36, it is determined positive.

VIC channel is for the N target: if amplification curve was typical "S" shape, and Ct value≤36, it is determined positive.

Cy5 channel is for the Rnase P target: if amplification curve was typical "S" shape, and Ct value≤35, it is determined positive.

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3. Interpretation of test results

Detection Results (ORF1ab、N)			Result
FAM	HEX	Cy5	
+	+	±	SARS-CoV-2 Positive
-	-	+	SARS-CoV-2 Negative
+	-	±	The result is not valid, repeat nucleic acids extraction and RT-PCR
-	+	±	The result is not valid, repeat nucleic acids extraction and RT-PCR
-	-	-	The result is not valid, repeat specimen collection

【Limitations】

（1）The test results of this kit are for clinical reference only. The clinical diagnosis and treatment of patients should be considered in combination with their symptoms / signs, medical history, other laboratory tests, and treatment response.

（2）Failed sample collection, transportation, storage and processing may cause erroneous test results.

（3）The negative test result only indicates that the virus titer in the sample is lower than the minimum detection limit of this kit, but cannot exclude the possibility of 2019-nCoV infection.

（4）Cross-contamination may occur if the sample processing process is not controlled, and false-positive results may occur.

Performance Characteristics

（1）Lower limit of detection: 100 copies/mL.

（2）Cross-reactivity: The test kit has been tested not to cross-react with coronary virus HKU1, OC43, NL63, 229E, H1N1 (2009), H3N2, Victoria influenza virus B, Yamagata influenza virus B, Respiratory Syncytial Virus A, and Respiratory Syncytial Virus B.

（3）Interfering substances: Mucin concentration in the sample under 0.05mg / mL, or the volume proportion of blood and nasal secretions in the sample under 10% does not interfere with the test results.

（4）Repeatability CV≤5%；Reproducibility CV≤5%.

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