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Real Time PCR Kit for Novel Coronavirus 2019-nCoV (ORF1ab, N, S)

INSTRUCTION FOR USE

Product Name

Real Time PCR Kit for Novel Coronavirus 2019-nCoV (ORF1ab, N, S)

Product Description

The kit is used for in vitro qualitative detecting the ORF1ab/N/S genes of novel coronavirus 2019-nCoV in respiratory specimens including oropharyngeal swabs, nasopharyngeal swab, sputum and bronchoalveolar lavage fluid, and performing mutation typing on HV69-70 del simultaneously. As reported in GISAID database, more than 95% Omicron variant sequences reported include a deletion in the HV69-70, which can cause an S gene target failure (SGTF) in PCR assays. SGTF can be used as a proxy marker to screen for Omicron.

Primer sets and FAM labeled probe are designed for specific detection of ORF lab gene of 2019-nCoV, VIC labeled probe for N gene of 2019-nCoV, ROX labeled probe for S gene HV69-70 del mutation of 2019-nCoV. Human RNase P gene extracted concurrently with the test sample provides an internal control to validate nucleic extraction procedure and reagent integrity. Probe targeting human RNase P gene is labeled with CY5.

Size

50 tests/kit

Kit Contents

Components	50 tests/kit
RT-PCR reaction buffer	925 μL × 1 tube
RT-PCR enzyme mix	75 μL × 1 tube
Wild-type positive control	100 μL × 1 tube
Mutant positive control	100 μL × 1 tube
Negative control	100 μL × 1 tube

Precautions:
1. Components with different lot numbers cannot be used together.
2. User prepared RNA extraction Kit.

Storage and Expiration

This kit expires in 12 months when stored at -20±5℃ and in prevention of light.

Avoid repeated freeze-thaw cycles (5 times maximum).

Manufacture date and expiration date are printed on the label.

Applicable Instruments

Real-Time PCR System: Molarray MA-6000, ABI 7500, ViiA™ 7, QuantStudio 5, QuantStudio 6/7 pro, QuantStudio 6/7 flex, Agilent Mx3000P/3005P, Rotor-Gene™ 6000/Q, Bio-Rad CFX96 Touch™/iQ™ 5，Hongshi SLAN-96S/96P，AGS8830, AGS4800

Specimen Handling and Storage

1. Acceptable Specimens: oropharyngeal swab, sputum, bronchoalveolar lavage fluid and nasopharyngeal swab.
2. Collect samples in sterile tubes.
3. Contamination should be avoided during collection, storage and transportation of the samples.
4. All the samples collected should be treated as contagious, deactivate the sample at 56℃ for 30 min.
5. Specimens can be stored at 2~8℃ for up to 24 hours after collection. If a delay in extraction is expected, store specimens at -70℃ or lower. Avoid repeated freeze-thaw cycle of the sample and make sure sample is completely thawed before RNA extraction.
6. Transport samples in sealed box with dry ice or ice bag.

Protocol

Precaution: All the reagents should be thawed completely before use, then vertex and spin down at 6,000rpm.
1. Sample treatment (Sample treatment zone)
Extract RNA with viral RNA extraction kit. RNA extracted can be stored at -70℃ or lower for 6 months. Positive control and negative control can be used without extraction.
2. Preparation of amplification reagents (Mix preparation zone)
Take N (N=negative control number + RNA sample number +positive control number) PCR tubes, add 18.5 μL RT-PCR reaction buffer and 1.5 μL RT-PCR enzyme mix to each tube.

Components	Volume per test
RT-PCR reaction buffer	18.5 μL
RT-PCR enzyme mix	1.5 μL

Centrifuge the PCR tubes at 6,000rpm for 30s and transport to sample treatment zone.
3. Sample loading (Sample treatment zone)
Add 20 μL RNA sample, negative control and positive control to the above PCR tubes respectively. Cap the tubes tightly and centrifuge at 6,000rpm for 10s and then transport to PCR amplification zone.
* Precaution: Adding samples in the following order is recommended: negative control-> RNA sample -> positive control.
4. PCR amplification (PCR amplification zone).
4.1. Put the caped PCR tubes into real-time PCR machine for amplification.
4.2. Thermal cycling setting

Steps	Temperature	Duration	Cycle
1	50℃	10min	1
2	95℃	3min	1
3	95℃	5s	45
	60℃	20s /35s	

Collect fluorescent signals at step 3:60℃; 35s for ABI 7500，while 20s for other Real-Time PCR Systems. The total volume: 40 μL.

NOTE: for ABI7500, ViiA™ 7, QuantStudio series instruments, choose 'none' as both passive reference and quencher.

4.3. Disposal after detection
Dispose the PCR tubes in a sealed bag after reaction and treat the used tubes as medical wastes.
5. Settings for result analysis
Set the baseline at a region before the exponential amplification where the fluorescent signals of all the samples are relatively stable (no significant fluctuations in all the samples); set the starting point (cycle number) away from the signal fluctuations at the starting phase of fluorescence collection; set the end point (cycle number) 1~3 cycles before the Ct of the first sample to enter exponential amplification. 4~15 cycles are recommended.

Set the threshold right above the highest point of the negative control amplification curve (irregular noise curve).
6. Quality control criteria
Prior to evaluating the specimen results, the Positive Control and Negative Control should be interpreted using the interpretation table below, and the Positive Control and Negative Control curve must be performed correctly, otherwise the sample result is invalid.

Channels Controls	Cycle threshold（Ct）value			
	FAM	VIC	ROX	CY5
Negative control	Ct > 40 or UNDET	Ct > 40 or UNDET	Ct > 40 or UNDET	Ct > 40 or UNDET
Wild-type positive	Ct ≤ 32	Ct ≤ 32	Ct ≤ 32	Ct ≤ 32

control				
Mutant positive control	Ct ≤ 32	Ct ≤ 32	Ct > 40 or UNDET	Ct ≤ 32

Result Determination

FAM channel for ORF1ab gene of 2019-nCoV, detection result should be interpreted as below.

1. Positive: Ct ≤ 38 and amplification curve is S-shaped.
2. Suspected: 38 < Ct ≤ 40 and amplification curve is S-shaped, a second test is needed. Consider positive if Ct ≤ 40 and amplification curve is S-shaped for the second test. Considered negative if Ct > 40 or null Ct and Ct ≤ 35 in CY5 channel for the second test.
3. Negative: Ct > 40 or null Ct and Ct ≤ 35 in CY5 channel.
4. Re-test: Ct > 40 or null Ct and Ct > 35 in CY5 channel.

VIC channel for N gene of 2019-nCoV, detection result should be interpreted as below.

1. Positive: Ct ≤ 38 and amplification curve is S-shaped.
2. Suspected: 38 < Ct ≤ 40 and amplification curve is S-shaped, a second test is needed. Consider positive if Ct ≤ 40 and amplification curve is S-shaped for the second test. Considered negative if Ct >40 or null Ct and Ct ≤ 35 in CY5 channel for the second test.
3. Negative: Ct > 40 or null Ct and Ct ≤ 35 in CY5 channel.
4. Re-test: Ct >40 or null Ct and Ct > 35 in CY5 channel.

ROX channel for S gene HV69-70 del mutation of 2019-nCoV, detection result should be interpreted as below.

1. Positive: Ct ≤ 38 and amplification curve is S-shaped.
2. Suspected: 38 < Ct ≤ 40 and amplification curve is S-shaped, a second test is needed. Consider positive if Ct ≤ 40 and amplification curve is S-shaped for the second test. Considered negative if Ct >40 or null Ct and Ct ≤ 35 in CY5 channel for the second test.
3. Negative: Ct >40 or null Ct and Ct ≤ 35 in CY5 channel, HV69-70 del mutation exists in the sample which causes a S gene target failure (SGTF).
4. Re-test: Ct >40 or null Ct and Ct > 35 in CY5 channel.

Performance Index

1. Sensitivity: 200 copies/mL.

2. Specificity: No cross reaction with SARS-CoV, MERS-CoV, CoV-HKU1, CoV-OC43, CoV-229E, CoV-NL63 and H1N1, H3N2, H5N1, H7N9, Influenza B, Parainfluenza Virus（1,2,3）, Rhinovirus (A,B,C), Adenovirus (1,2,3,4,5,7,55), Human interstitial pneumovirus, Human metapneumovirus, EBv, Measles virus, Human cytomegalic virus, Rota virus, Norovirus, Mumps virus, Varicella Zoster Virus, Mycoplasma pneumonia, Chlamydia pneumonia, Legionella, Bordetella pertussis, Haemophilus influenza, Staphylococcus Aureus, Streptococcus Pneumonia, Streptococcus pyogenes, Klebsiella pneumonia, Tuberculous bacillus, Aspergillus fumigatus, Candida albicans, Candida glabrata, Cryptococcus neoformans.

3. Precision: CV ≤ 5%.

Limitations

1. Negative results do not preclude infection with Novel Coronavirus (2019-nCoV) and should not be the sole basis of a patient treatment decision.
2. Reliable results are dependent on the adequate specimen collection, proper transportation storage and processing procedures.
3. Inhibitors present in the sample and/or errors in the following assay procedure may lead to false negative results.
4. A trained health care professional should interpret assay results in conjunction with the patient's medical history, clinical signs and symptoms, and the results of other diagnostic tests.
5. Potential mutations within the target regions of the virus genome covered by the test primers and/or probes may result in failure to detect the presence of the pathogens.
6. There is a risk of false positive values resulting from cross-contamination by target organisms, their nucleic acids or amplified products, or from non-specific signals in the assay.

Precautions

1. Management of the lab should comply with national regulations on gene amplification testing labs.
2. This kit is used for *in vitro* diagnosis only. It is for medical professional use only.
3. To prevent contamination, the same item should not be used cross different zones. Clean the bench immediately after conducting experiments.
4. Reagents of the kit should be thoroughly thawed and centrifuge briefly before use.
5. Reaction tubes containing reaction aliquots should be capped or placed in sealed bags before being delivered to the sample zone.
6. When loading sample, pipette the sample directly into the reaction solution without contacting the tube wall. Cap the tube immediately after loading the sample.
7. After the amplification is completed, the reaction tubes should be taken out immediately, placed in designated sealed bags and disposed at designated locations.
8. Avoid generating bubbles when aliquoting reaction mixture. Ensure the reaction tubes are capped before loading them into the PCR machine to avoid contamination.
9. Pipette tips used in the experiments should be directly tossed into a waste tank containing 1% sodium hypochlorite and disposed together with other wastes after sterilization.
10. Disinfect the working benches and other items regularly with 1% sodium hypochlorite, 75% alcohol or ultraviolet lamp.
11. The detection result of Omicron variant maybe incorrect for samples with very low copies (such as samples with Ct >35 in FAM or VIC channel) due to instability of amplification. Very little Omicron variant of Novel Coronavirus 2019-nCoV do not carry HV69-70 del mutation. Some other variants of Novel Coronavirus 2019-nCoV carry HV69-70 del mutation, such as Alpha and Eta.
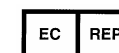
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SYMBOL	DESCRIPTION	SYMBOL	DESCRIPTION
⌂	Manufactured By	CE	CE Mark
EC REP	Authorized Representative	REF	Catalog Number
IVD	InVitro Diagnostic Medical Device	☣	Potential Biological Hazards After Use

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LOT	Batch Code	⊗	Do Not Reuse
⧖	Expiration Date in Year-Month-Day Format	~	Date of Manufacture
🌡	Temperature Limitation	📖	Consult instructions for use
⚠	caution	☀	Keep away from sunlight