Real Time PCR Kit for Novel Coronavirus 2019-nCoV and Delta variant of Novel Coronavirus 2019-nCoV

INSTRUCTION FOR USE

Product Name

Real Time PCR Kit for Novel Coronavirus 2019-nCoV and Delta variant of Novel Coronavirus 2019-nCoV

Product Description

The kit is used for in vitro qualitative detecting the ORF1ab/N genes of novel coronavirus 2019-nCoV in respiratory specimens including oropharyngeal swabs, nasopharyngeal swab, sputum and bronchoalveolar lavage fluid, and performing D950N mutation detection of Delta variant of Novel Coronavirus 2019-nCoV. As reported in GISAID database, more than 95% Delta variant of Novel Coronavirus 2019-nCoV carry D950N mutation.

Primer sets and FAM labeled probe are designed for specific detection of ORFlab gene of 2019-nCoV, VIC labeled probe for N gene of 2019-nCoV, ROX labeled probe for D950N mutation of Delta variant. 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
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, Daan 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 area.

3. Sample loading (Sample treatment area)

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 area.

* 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
	55℃	35s or 20s	

Collect fluorescent signals at step 3: 55℃; 35s for ABI 7500，while 20s for other Real-Time PCR System.

Fluorescent Channel: FAM for ORF1ab gene, VIC for N gene, ROX for D950N mutation of Delta variant, CY5 for internal control gene. The total volume: 40 μL.

NOTE: for ABI 7500, ViiATM 7, QuantStudio 5, QuantStudio 6/7 pro, QuantStudio 6/7 flex, 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
Positive control	Ct ≤ 32	Ct ≤ 32	Ct ≤ 32	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 cure is S-shaped.
2. Suspected: 38 < Ct ≤ 40 and amplification cure is S-shaped, a second test is needed. Consider positive if Ct ≤ 40 and amplification cure 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 cure is S-shaped.
2. Suspected: 38 < Ct ≤ 40 and amplification cure is S-shaped, a second test is needed. Consider positive if Ct ≤ 40 and amplification cure 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 D950N mutation of Delta variant, detection result should be interpreted as below.

1. Positive: Ct ≤ 38 and amplification cure is S-shaped.
2. Suspected: 38 < Ct ≤ 40 and amplification cure is S-shaped, a second test is needed. Consider positive if Ct ≤ 40 and amplification cure 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.

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 product, 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 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 Delta 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 Delta variant of Novel Coronavirus 2019-nCoV do not carry D950N mutation. Some other rare variants of Novel Coronavirus 2019-nCoV carry D950N mutation, such as B.1.625, B.1.621 and B.1.621.1.

Company Information

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SYMBOL	DESCRIPTION	SYMBOL	DESCRIPTION
	Manufactured By	CE	CE Mark
EC REP	Authorized Representative	REF	Catalog Number
IVD	In Vitro Diagnostic Medical Device		Potential Biological Hazards After Use
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