Multiplex Real time PCR Kit for SARS-CoV-2/ Influenza A/Influenza B/RSV

INSTRUCTION FOR USE

FOR PROFESSIONAL USE ONLY

【Product Name】

Multiplex Real time PCR Kit for SARS-CoV-2/Influenza A/Influenza B and RSV

【Product Description】

This kit is used for in vitro qualitative nucleic acid detection of SARS-CoV-2, Influenza A, Influenza B and respiratory syncytial virus (RSV) simultaneously in respiratory specimens including oropharyngeal swabs and sputum, bronchoalveolar lavage fluid and nasopharyngeal swab. Primer sets and FAM labeled probes are designed for specific detection of both ORFlab and N genes of SARS-CoV-2, ROX labeled probe for M gene of Influenza A, CY5 labeled probe for NS gene of Influenza B, CY5.5 labeled probe for M gene of respiratory syncytial virus (RSV). 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 VIC.

Size

48 Tests/kit, 96 Tests/kit

Kit Contents

Components	48 Tests/kit	96 Tests/kit
RT-PCR reaction buffer	888 μL × 1 tube	888 μL × 2 tube
RT-PCR enzyme mix	75 μL × 1 tube	150 μL × 1 tube
positive control	100 μL × 1 tube	200 μL × 1 tube
Negative control	100 μL × 1 tube	200 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, QuantStudio 5, QuantStudio 6/7 pro, QuantStudio 6/7 flex, Bio-Rad CFX96 Touch™

【Specimen Handling and Storage】

Acceptable Specimens: Nasopharyngeal swabs, Oropharyngeal swabs, Bronchoalveolar lavage fluid and sputum.

1. Collect samples in sterile tubes.
2. Contamination should be avoided during collection, storage and transportation of the samples.
3. All the samples collected should be treated as contagious, deactivate the sample at 56℃for 30 min.
4. 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.
5. 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 controls and negative controls 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	

Collect fluorescent signals at step 3: 60℃;

NOTE: for 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	CY5.5
Negative control	Ct＞40 or UNDET	Ct＞40 or UNDET	Ct＞40 or UNDET	Ct＞40 or UNDET	Ct＞40 or UNDET

Positive control	Ct≤35	Ct≤35	Ct≤35	Ct≤35	Ct≤35

【Result Determination】

FAM channel for both ORF1ab and N genes of SARS-CoV-2**, 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 VIC channel for the second test.
3. Negative：Ct >40 or null Ct and Ct≤35 in VIC channel.
4. Re-test：Ct >40 or null Ct and Ct >35 in VIC channel.

ROX channel for M gene of Influenza A, 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 VIC channel for the second test.
3. Negative：Ct >40 or null Ct and Ct≤35 in VIC channel.
4. Re-test：Ct >40 or null Ct and Ct>35 in VIC channel.

CY5 channel for NS gene of Influenza B, 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 VIC channel for the second test.
3. Negative：Ct >40 or null Ct and Ct≤35 in VIC channel.
4. Re-test：Ct >40 or null Ct and Ct>35 in VIC channel.

CY5.5 channel for M gene of RSV, detection result should be interpreted as below.

5. Positive： Ct ≤ 38 and amplification curve is S-shaped.
6. 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 VIC channel for the second test.
7. Negative：Ct >40 or null Ct and Ct≤35 in VIC channel.
8. Re-test：Ct >40 or null Ct and Ct>35 in VIC 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 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 SARS-CoV-2 or Influenza A or Influenza B or RSV 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 maylead 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.
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, pipettethe 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 tossedinto a waste tank containing 1% sodium hypochlorite and disposed together with other wastesafter sterilization.
10. Disinfect the working benches and other items regularly with 1% sodium hypochlorite, 75% alcohol or ultraviolet lamp.

【Company Information】

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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
LOT	Batch Code		Do Not Reuse
	Expiration Date in Year-Month-Day Format		Date of Manufacture
	Keep away from sunlight		Consult instructions for use
	caution		Temperature Limitation