Novel Coronavirus (COVID-19) Nucleic Acid Assay Kit (RT-PCR) User Manual

Product name: Novel Coronavirus (COVID-19) Nucleic Acid Assay Kit (RT-PCR)
Catalog No.: MBS2563835
Package: 24 T/Kit, 48 T/Kit

INTENDED USE

This kit is used for the qualitative detection of nucleic acids of novel coronavirus (2019). The test results can only be used for clinical auxiliary diagnosis and cannot be used as the basis for diagnosis or exclusion of cases alone.

The Coronavirus (CoV) belongs to the order Nestoroviridae, family Coronaviridae. This family is divided into four genera: α, β, γ, and δ according to serotype and genomic characteristics. Previously there were six reported coronavirus infections in humans, including 229E and NL63 of the α genus and OC43, MERS-CoV and SARS-CoV of β genus. CoV is mainly transmitted through direct contact with secretions or through aerosols and droplets, and there is evidence that it can be transmitted through the fecaloral dissemination.

This novel coronavirus (COVID-19) belongs to β genus and has a capsule. The particles are round or oval and often multiform with a diameter of 60-140 nm. There are obvious differences between genetic characteristics of COVID-19, SARS-CoV and MERS-CoV. The present study shows that it has more than 85% homology with SARS like coronavirus originated from bat (bat-SL-CoVZC45).

The clinical symptoms of novel coronavirus pneumonia are fever, fatigue, and other systemic symptoms, accompanied by dry cough, dyspnea, and so on. It can rapidly develop into severe pneumonia, respiratory failure, acute respiratory distress syndrome, septic shock, metabolic acidosis difficult to correct and coagulation dysfunction, and multiple organ failure leading to death. At present, it is mainly being detected by virus isolation, nucleic acid detection, and serological detection.

TEST PRINCIPLE

This kit uses multiplex fluorescence RT-PCR and hydrolytic probe techniques to design specific primers and probes targeting conserved regions in ORF1ab genes and N genes of COVID-19. The qualitative detection of nucleic acid levels at two target sites is achieved in the same reaction tube. The operation is simple, the whole process does not need open the cover, which can reduce the pollution. The instrument software system automatically draws the real-time amplification curve to perceive the instant result judgment. This kit is equipped with internal standard, which is used to monitor the collection, transportation and extraction of test samples to avoid false negative test results.

MAJOR CONSTITUENT

Component Name	Specification/Size/Amount	
	24 T	48 T
COVID-19 qPCR Mix	400 μL	800 μL
Enzyme Mix	25 μL	50 μL
Positive Control	25 μL	50 μL
Negative Control	100 μL	200 μL

Note: Do not mix or use components with those from other lots. This kit does not contain the matching extraction reagents necessary for detection.

STORAGE AND EXPIRATION

Transport at 2~8℃. Store at -20±5℃ in the dark. Valid for 12 months. It is recommended to repackage the reagents and freeze them. Avoid repeated freeze-thaw cycles.

APPLICABLE INSTRUMENTS

ABI 7500/7500FAST, Roche LightCycler®480, BioRad CFX96, BigFish-BFQP16/48 fluorescence quantitative PCR.

SAMPLE REQUIREMENTS

1. Sample type: Pharynx swab, nasopharyngeal extract, sputum, bronchial lavage fluid, alveolar lavage fluid, etc.
2. Collection method: The collection was carried out according to the *Technical Guidelines for Laboratory Detection of Pneumonia with Novel Coronavirus Infection*.
3. Storage of sample: Specimens for virus isolation and nucleic acid detection should be tested as soon as possible. Specimens that can be detected within 24 hours can be stored in 4℃, otherwise specimens should be stored in -70℃ or under -70℃ for a long-term storage(if -70℃ conditions are not available, specimens should be stored temporarily in -20℃ refrigerator). Specimens after collection should be sent to the laboratory as soon as possible. For long-distance transportation of specimens, it is recommended to use dry ice or other refrigeration methods for preservation.

TEST PROCEDURE

1. **Reagent preparation (reagent preparation area)**

   Take out the kit, melt the reagents at room temperature, oscillate and mix the reagents. Centrifuge at 3000 rpm for 10 seconds. Calculate the number of reagents required. (n= sample number+2 control).

   Reaction system per person prepared as follows:

Preparation of reaction solution (per person)	
Reaction solution	volume/person
COVID-19 qPCR Mix	14 μL
Enzyme Mix	1 μL

   Notes: When preparing the reaction system, the number of reactions to be prepared per PCR run maybe calculated by *n (samples to be tested) +2(positive control, negative control) × 4*. Calculate the amount of each of the above reagents and add to a proper volume centrifuge tube. After full mixing, pack the reagents 15μL into each PCR reaction tubes.

2. **Sample preparation (sample preparation area)**

   2.1 Nucleic acid extraction: Extract the nucleic acid of tested samples according to the instructions of nucleic acid extraction kit.

   2.2 Sample loading: Add 5 μL of nucleic acid, positive control and negative control of the tested samples to the PCR reaction tubes. The final volume should be 20 μL/ tube. Plugin the tube lid tightly, then centrifuge immediately at low speed.

3 **RT-PCR Amplification detection (Nucleic acid amplification region)**

   3.1 Place the reaction tubes into the fluorescence PCR instrument for amplification.

3.2 Example of ABI 7500 operating instructions

3.2.1 Cycle parameter setting

Step		Cycle	Temperature (℃)	Reaction time
1	Reverse transcription reaction	**1**	**55**	**15 min**
2	Prior denaturalization	**1**	**95**	**30 sec**
3	Denaturalization	**40**	**95**	**10 sec**
	Annealing, extension and fluorescence detection		**58**	**30 sec**
For fluorescent detection at 60℃ in step 3, detection channel: FAM, HEX.				

Note: ABI 7500 real-time fluorescence PCR instrument should be set without ROX correction, and the quenching groups should be selected "None".

3.2.2 Analysis of result

ABI 7500: After the reaction is over, the results are automatically saved. Adjust the Start value, End value and Threshold value of the BaseLine according to the analyzed image (These parameters can be adjusted according to the actual situation, the Start value can be set at 3~15, the value can be set at 5 and 20, and the amplification curve of the blank control is straight or below the threshold line). Click on the Analysis to automatically obtain the results and read the results at the Report interface.

POSITIVE RESULT JUDJEMENTS

Positive: CT≤37, the curve is S type and has obvious exponential growth period, which is judged to be COVID-19 positive.

Negative: CT>40 or not detected, judged as COVID-19 negative.

INTERPRETATION OF RESULTS

Quality control

Control	CT	
	FAM channel	HEX channel
Negative Control	Ct≥37 or UNDET	Ct≥37or UNDET
Positive Control	ct≤35	ct≤35

Result judgement

The results were analyzed when the instrument was normal and both positive and negative controls were normal: the FAM channel was the Novel Coronavirus (2019) ORF1ab gene and the HEX channel was the Novel Coronavirus (2019) N gene. Experimental validity judgment: if the result satisfy the following two points at the same time, the experiment is effective, otherwise invalid.

- **Positive control:** Typical S amplification curve and ct≤30.
- **Negative control:** The target gene had no typical S amplification curve and the Ct>40.

Experimental validity judgment: if the result satisfy the following two points at the same time, the experiment is effective, otherwise invalid.

1) **Positive control:** Typical S amplification curve and ct≤35.
2) **Negative control:** The target gene had no typical S amplification curve and the Ct>37.

COVID -19 result judgment		FAM (ORF1ab gene)	
		CT≤37	No CT value or CT>37
BHQ/ROX (N gene)	CT≤37	COVID-19 positive	Retest. If the results are consistent, the judgment is COVID-19 positive. If inconsistent, the judgment is suspicious.
	No CT value or CT>37	Retest. If the results are consistent, the judgment is COVID-19 positive. If inconsistent, the judgment is suspicious.	COVID-19 negative

LIMITATIONS OF DETECTION METHOD

1. This kit is used for qualitative detection of nucleic acids in samples. Clinical diagnosis and treatment of patients should be combined with their symptoms/ signs, medical history, other laboratory examination and treatment response, etc.
2. Possibility analysis of false positive results
   2.1 If cross contamination occurs during transportation and processing, false positive results may result.
   2.2 Aerosol contamination such as PCR products in the experimental environment may lead to false positive results.
   2.3 Contamination of consumables and equipment used in the experiment may lead to false positive results.
3. Possibility analysis of false negative results
   3.1 Incorrect sample collection, transport and treatment, and low pathogen content in the sample may lead to false negative results.
   3.2 Variations in the target sequence to be tested or other changes in the sequence of the pathogen may result in false negative results.
   3.3 Additional unvalidated interference or PCR inhibitors may lead to false negative results.

PRODUCT PERFORMANCES

1. **Product compliance rate of positive reference:** The positive compliance rate is 100% when testing with standardized positive reference.
2. **Product compliance rate of negative reference:** The negative compliance rate is 100% when testing with standardized negative reference.
3. **Minimum detection limit:** ≤ 1×10^3 copies/mL.
4. **Precision:** Test with standardized precision reference, each test is repeated 10 times. The coefficient of variation (CV, %) of the CT value ≤ 5%.

5. **Specificity:** This product does not have cross reaction with total nucleic acids from other common pathogens with the same site of infection or similar symptoms (Coronavirus 229E, Coronavirus OC43, Coronavirus HKU1, Coronavirus NL63, SARS Coronavirus, MERS Coronavirus, Influenza A virus, Influenza B virus, Parainfluenza virus, Adenovirus, Chimpanzee coryza agent, Partial pulmonary virus, Boca virus, Rhinovirus, Mycoplasma pneumonia, Chlamydia pneumoniae, Streptococcus pneumoniae, Klebsiella pneumoniae, Legionnella, Staphylococcus aureus, Mycobacterium tuberculosis, Haemophilus influenzae, Baumanii) and human leukocytes.

CAUTION

1. This kit is *in vitro* detection reagent. Operators should be professionally trained and have some experience. Please read this instruction carefully before use.
2. To ensure the accuracy and reliability of the experimental results, please use the calibrated pipette, select qualified disposable PCR reaction tube, centrifuge tube, pipette tips and other consumables for sample processing and reagent preparation operations. All appliances should be free of DNA and RNA enzymes.
3. The experiment should be operated strictly zonal. The articles and work clothes in each district are all special and must not be cross-used to avoid pollution. Clean the workbench immediately after the experiment.
4. This product should be fully melted at room temperature before use, proper mixing and instantaneous low speed centrifugation is necessary.
5. Sample handling shall be performed in a biosafety cabinet to ensure operator safety and prevent contamination of the environment.
6. Negative control and positive control should be set in each experiment. Do not mix reagents with different batches. Use the kit within the validity period.
7. The tested samples should be as fresh as possible, and the extraction process should strictly prevent RNA degradation caused by RNA enzyme contamination and improper operation.
8. Samples stored at -70℃should be fully melted at room temperature, mixed and centrifuged at low speed before assay.
9. Tubes with reaction solutions should be capped or packed in compact bags and then transferred to sample treatment areas.
10. When adding the sample, it should be completely added to the reaction solution, and avoid the adhesion to the pipe wall. After pipetting, the lid of the tube should be tightly closed as soon as possible.
11. Try to avoid bubbles in reaction liquid repackaging step. Check whether the reaction tube is tightly covered before operating the machine to avoid contaminating the instrument due to leakage.
12. Remove the reaction tube after amplification. Seal the tube in a special plastic bag and discarded at a designated place.
13. The pipette tips used in the experiment should be injected directly into the waste tank containing 10% sodium hypochlorite and discarded with other waste materials.
14. Operation table and various experimental items should be sterilized with 10% sodium hypochlorite, 75% alcohol and ultraviolet lamp frequently.
15. The Real-time Fluorescence PCR instrument needs to be regularly corrected and the sample plate holes should be regularly cleaned.
16. The samples to be tested in this kit shall be considered as infectious substances and shall be operated and handled in accordance with the relevant requirements of the *Ministry of Health's General Guidelines for Biosecurity in Microbiomedical Laboratories* and the *Medical Waste Management Regulations*.

REFERENCES

1. *Technical Guidelines for Laboratory Detection of Pneumonia with Novel Coronavirus Infection.*
2. *SN/T 5098-2019 Real-time fluorescence RT-PCR Detection Method for Human Coronavirus NL63 and HKU1 at border ports.*
3. *Interim Measures for the Administration of Clinical Gene Amplification Laboratory [2002]10.*

Version

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