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Product Catalogue Number RP-010

Corona Virus Disease 2019 (CoViD-19) Nucleic Acid Detection Kit

Instructions Manual

[Product Name] Corona Virus Disease 2019 (CoViD-19) Nucleic Acid Detection Kit (Real-Time PCR Method).

[Packing Specifications] 100 Tests/Box.

[Intended Usage]

This kit is used for the qualitative detection in vitro of suspected cases of pneumonia with novel coronavirus infection, patients with suspected aggregated cases, and other nasopharyngeal swabs, sputum, and bronchoalveolar lavage fluid samples that require diagnosis or differential diagnosis of new coronary virus infection , new coronavirus ORF1ab and N genes.

[Detection Principle]

This kit is designed for the newly released coronavirus ORF1ab and the conserved region encoding the nucleocapsid protein N gene sequence newly published on the Global Initiative on Sharing All Influenza Data (GISAID) platform. Two pairs of specific primers and Taqman probes were used to analyze quantitatively the viral nucleic acids in the sample by using one-step fluorescence PCR detection technology.

The PCR reaction system contains primers and probes of the endogenous internal standard RNaseP. The sample collection, extraction, and reaction processes are monitored by detecting the internal standard to avoid false negative results.

[Product Main Components]

The kit consists of the following components: reaction solution, primer probe mixed solution, mixed enzyme solution, RNase-free ddH2O, negative control, positive control, and instructions, as shown in Table 1.

Table 1 Kit composition

Component name	Specifications	Quantity
Reaction solution	1250μL	2 Tube
Primer probe mixture	300μL	1 Tube
Mixed enzyme solution	200μL	1 Tube
RNase-free ddH_2O	1500μL	1 Tube
Negative control	500μL	1 Tube
Positive control	500μL	1 Tube
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Required but not provided: Nucleic acid extraction or purification reagents.

Note: *components of kits of different batches cannot be used interchangeably.*

[Storage Conditions & Validity]

1. The kit should be stored frozen and protected from light at or below -15°C. The validity period is of 10 months. The foam box and blue ice bag are used for sealing and transportation, and the temperature does not exceed 8°C. The production date and validity period are shown on the outer packing box.
2. Avoid repeated freeze-thaw cycles of the kit. The number of freeze-thaw cycles should not exceed 7 times.
3. After unsealing, the kit should be stored in a refrigerated and dark place at or below -15°C, which will not affect the use within the validity period.

[Applicable Instruments]

1. This kit is suitable for ABI7500 Real-Time PCR instrument.
2. For other models not listed, this kit has not been performed relevant experiments. If the user needs to use any other instrument to carry out the detection of this reagent, please contact our Technical Department for relevant support.

[Sample Requirements]

1. Samples are from nasopharyngeal swabs, oropharyngeal swabs, etc. The specific operation method is as follows:

i) Nasopharyngeal swabs: The operator gently rotates the wet sterile swabs parallel to the angle of the upper jaw from left to right and from one nostril to the inner nasopharynx of the nasal canal. Generally, when the swab is inserted with resistance, the swab will stay for 2-3s and then slowly turn to exit.

ii) Oropharyngeal swab: The operator holds the tongue depressor against the posterior root of the patient's tongue, the other hand holds the root of the sterile swab, and then moves the left and right sides of the wet sterile swab and the swab head quickly and firmly scrape the secretions from both sides of the tonsil and the back of the throat.

iii) Put the nasopharyngeal swab and the oropharyngeal swab together in a 1.0 mL normal saline centrifuge tube for later use.

2. Alveolar lavage fluid sample: Use a sterile syringe to take the sample and place it in a centrifuge tube for inspection.
3. Cross-contamination between samples should be avoided.
4. Samples should be tested immediately after collection, or stored at -20±5°C for inspection. For long-term storage, keep at or below -70°C.

[Test Method]

1. Reagent preparation: (Reagent preparation area)

In each PCR reaction, positive and negative controls were simultaneously detected. Each sample was tested for the ORF1ab region, the N gene, and the endogenous internal standard.

1) Remove the kit from the refrigerator below -15°C and put at room temperature (20-25°C) for dethawing. After complete dissolution, shake and mix well and centrifuge at low speed for 10s.

2) Calculate the number of reactions required for the experiment, and dispense the reagents into the PCR reaction tube in sequence according to the reaction system preparation method in Table 1. The PCR reaction tube is transferred to the sample preparation area, and the remaining reagents are stored at or below -15°C and kept way from light.

Table 1: Reaction system preparation method

Composition	Dosage (Volume)
Reaction solution	25μL
Primer probe mixture	3μL
Mixed enzyme solution	2μL
RNase-free ddH_2O	15μL

2. Sample preparation: (Sample preparation area)

1) RNA extraction:

It is recommended to use commercially available nucleic acid extraction kits to extract RNA sample for PCR detection. Follow the extraction kit instructions to extract.

2) Sample addition:

a. Remove the reagents prepared in the reagent preparation area and centrifuge at low speed for 10s.

b. Add the RNA to be tested, the positive control and the negative control to the PCR reaction tubes, respectively, in an amount of 5μL/well.

c. Cover the PCR reaction tubes, record the template loading sequence, and centrifuge at low speed for 10s.

d. Transfer the PCR reaction tubes to the nucleic acid amplification area for operation.

Note: *Avoid contamination during RNA sample extraction and loading. If the extracted RNA template cannot be immediately tested, it is recommended to store at or below -70°C.*

3. PCR: (Nucleic acid amplification region)

1) Boot, warm up and check the performance of the machine.

2) Take the PCR reaction tubes prepared in the sample preparation area and place them in the corresponding positions in the sample tank of the machine (Before checking the machine, check whether the reaction tubes are tightly closed to avoid aerosol pollution of the instrument and the environment due to PCR products leakage) and record the placement order.

3) Set the nucleic acid amplification relevant parameters in the machine according to Table 2 and carry out PCR amplification.

System	Set the reaction system to 50μL		
Signal acquisition	Select FAM channel to detect virus N gene; Select VIC/HEX channel to detect virus ORF-1ab region; Select the ROX channel to detect the internal standard.		
PCR reaction conditions	**Stage**	**Conditions**	**Number of cycles**
	Reverse Transcription	50℃: 30min	1
	Pre-denaturation	95℃: 3min	1
	PCR	95℃: 15s	45
		60℃: 30s (Collect fluorescence signal at the end of this step)	

Note: *ROX calibration is not selected for ABI series Real-Time PCR instrument, and none is used for quenching group.*

[Result Analysis]

For data analysis, select ΔRn VS Cycle and Linear mode; set the appropriate threshold, select Auto Baseline, view the fluorescence curve, and obtain the fluorescence quantitative curve and Ct value in the Real-Time PCR instrument.

[Positive Value Determination]

1. Test kit validity determination:

i) Positive control: Ct values of FAM, VIC/HEX channel and internal standard (ROX) channel are <37, with significant exponential increase.

ii) Negative control: FAM, VIC/HEX channel and internal standard (ROX) channel Ct values are >40 or no Ct value, the line is straight or slightly oblique, with no significant exponential growth period and plateau period.

iii) The above requirements must be met in the same experiment at the same time. Otherwise, the experiment is invalid and needs to be repeated.

2. Sample result reading:

i) Positive: The Ct values of the target gene and internal standard are <37, with significant exponential increase.

ii) Suspicious: The target gene and internal standard Ct value is between 37 and 40. The sample should be tested repeatedly.

iii) Negative: The target gene and internal standard Ct values are >40 or no Ct value.

[Test Results Interpretation]

1. Firstly, analyze whether the internal standard has an amplification curve in the ROX channel, and Ct ≤40. If there is, it indicates that the test is valid, and the subsequent analysis can be carried out:

	Detection results		Test results and interpretation
	N gene (FAM channel)	ORF1ab (VIC / HEX channel)	
a	+	-	Suspected positive, repeat test
b	-	+	Suspected positive, repeat test
c	+	+	Viral nucleic acid detected in the sample
d	-	-	No viral nucleic acids detected in the sample

Note: *A negative PCR test cannot clinically exclude the possibility of COVID-19.*

2. Samples with positive single target detection items a and b need to be repeatedly sampled. If the single target is still positive, the sample test result is positive.

3. If the internal standard does not detect Ct or Ct >40 in the ROX channel, it means that the concentration of the test sample is too low or there is an interference substance inhibiting the reaction. It is necessary to prepare the experiment again.

4. For positive samples and virus cultures, the internal standard test results are not required. For negative samples, the internal standard test should be positive. If the internal standard test is negative, the test result of the sample is invalid, and the cause should be found and eliminated. Resample and repeat the experiment (if the test results of the repeated experiment are still invalid, please contact our Technical Department).

5. Determination of gray area results: If the fluorescence signal of a sample has a significant increase in the FAM, VIC/HEX channel, but the Ct value is >40, the sample is in the gray area and needs re-examination. If the retest result is still in the gray area, it is judged as positive.

[Product Performance Index]

1. **Specificity**: The primer probes used in this kit are COVID-19 based.

The conserved sequence is designed to have a high detection rate for the target gene fragment. This kit has no cross-reactivity with coronavirus (HKU1, OC43, NL63, and 229E), SARS coronavirus, MERS coronavirus, influenza virus, parainfluenza virus, respiratory syncytial virus, and adenovirus positive samples. The negative and positive reference products of the testing enterprises were 100%.

2. **Minimum detection limit**: The minimum detection limit of this kit is 10^3 copies/mL.

[Test Method 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. The improper operation of the sample during the collection, transportation, storage and nucleic acid extraction process can easily cause RNA degradation and produce false negative results.
3. When the detected nucleic acids concentration in the sample is less than the minimum detection limit, false negative results may occur.
4. If cross-contamination occurs during sample collection and preparation, it is easy to lead to false positive results.
5. Some infected people have a large number of dead virus in their samples due to the taking of antiviral drugs. At this time, there may be strong positive results detected by this kit and negative results detected by the culture method. In case of such results, the recent medication situation of the tested person shall be inquired.
6. Variations of the targeted sequence of the virus to be tested or other reasons leading to sequence changes may conduct to false negative results.
7. For the new virus, the most suitable sample type and the best sampling time after infection may not be confirmed. Therefore, the possibility of false negative results will be reduced if the samples are collected in the same patient in different times and multiple parts.

[Precautions]

1. Laboratory management shall be strictly implemented in accordance with the management specifications of the "Administrative Measures for Clinical Gene Amplification Laboratory of Medical Institutions" promulgated by the General Office of the Ministry of Health.
2. Laboratory personnel must have professional training and have certain experience.
3. The experimental process should be carried out in zones (reagent preparation zone, sample processing zone, nucleic acid amplification zone). Special instruments and equipment should be used at each stage of the experiment operation, and supplies should not be used in each stage. There should be strict requirements to minimize cross-contamination.
4. Experimental consumables (such as centrifuge tubes, suction heads, etc.) should have reasonable cleaning and quality inspection procedures to prevent contamination from causing false positive results or amplification reaction inhibitors to cause false negative results.
5. The instrument and its supporting power supply system should be checked before use to ensure the normal operation of the reagent after the reagent is out on the machine.
6. The suction heads used in the experiment shall be directly put into the waste tank containing 10% sodium hypochlorite and discarded together with other waste products.
7. The workbench and various experimental items are often disinfected with 10% sodium hypochlorite, 75% alcohol and UV lamps.
8. The Real-Time PCR machine requires frequent calibration and sample plate wells (holes) cleaning.
9. To prevent fluorescence interference, avoid direct contact with the octaplex PCR reaction tubes and tubes covered by hands.
10. The positive control in this kit is not contagious and will not cause harm to the human body. However, it is recommended to treat it as a potentially infectious substance when using it.
11. The test samples involved in this kit should be considered as infectious substances, and their handling must meet the relevant requirements of the General Guidelines for Biosafety of Microbiology and Biomedical Laboratories of the Ministry of Health and the Medical Waste Management Regulations.

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Manufacturer & Registrant Information

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