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

Instructions Manual

For Emergency Use Authorization Use Only

For In Vitro Diagnostic Use

Rx Only

REF **RP-010**

100 Tests/Box

Manufactured for OaCP by:

Wuhan HealthCare Biotechnology Co., Ltd.
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[Intended Use]

This kit is used for in vitro qualitative detection of nasopharyngeal swabs, oropharyngeal swabs, and alveolar lavage fluids of suspected cases of pneumonia, suspected aggregation cases, and other patients who need to be diagnosed or differentially diagnosed for new coronavirus infection. This kit is used to detect ORF1ab and N genes.

Results are for the identification of SARS-CoV-2 RNA. SARS-CoV-2 RNA is generally detectable in nasopharyngeal and oropharyngeal swab specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA. Clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease.

The Corona Virus Disease (COVID-19) Nucleic Acid Detection Kit is intended for use by qualified and trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures. The Corona Virus Disease (COVID-19) Nucleic Acid Detection Kit is only for use under the Food and Drug Administration's Emergency Use Authorization.

[Summary and Explanation]

An outbreak of pneumonia caused by a novel coronavirus (SARS-CoV-2) in Wuhan City, Hubei Province, China was identified and reported to the WHO on December 31, 2019. The rapid spread of SARS-CoV-2 to numerous areas throughout the world necessitates preparedness and response in healthcare and lab facilities. The availability of specific and sensitive assays for the detection of the virus are essential for accurate diagnosis of cases, assessment of the extent of the outbreak, monitoring of intervention strategies, and surveillance studies. The Corona Virus Disease (COVID-19) Nucleic Acid Detection Kit is a molecular in vitro diagnostic test that aids in the detection and diagnosis of SARS-CoV-2 and is based on widely used nucleic acid amplification technology. The product contains oligonucleotide primers, hydrolysis probes (TaqMan®) and control material used in rRT-PCR for the in vitro qualitative detection of SARS-CoV-2 RNA in respiratory specimens.

[Detection Principle]

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

The PCR reaction system contains primers and probes with internal standards.

RNA isolated and purified from upper and lower respiratory specimens is reverse transcribed to cDNA and subsequently amplified in the ABI7500 quantitative fluorescence PCR instrument. In the process, the probe anneals to a specific target sequence located between the forward and reverse primers. During the extension phase of the PCR cycle, the 5' nuclease activity of Taq polymerase degrades the probe, causing the reporter dye to separate from the quencher dye, generating a fluorescent signal. With each cycle, additional reporter dye molecules are cleaved from their respective probes, increasing the fluorescence. Through the detection of internal standards, the sample collection, extraction and reaction process are monitored to avoid false negative results.

[Materials Provided]

Kit composition

Component name	Specifications	Quantity	Main components
Reaction solution	850μL	2 Tubes	Specific primers, probes, 5 × RT PCR Buffer
Mixed enzyme solution	150μL	2 Tubes	Reverse transcriptase, DNA polymerase, dNTP, Mg2 +
Negative control	400μL	1 Tube	Pseudovirus containing internal standard fragments
Positive control	400μL	1 Tube	Pseudovirus containing target fragments and internal standard fragments
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Materials required but not provided

Nucleic acid extraction or purification reagents.
Vortex
Microcentrifuge (with rotor for 1.5-2 mL tubes)
Bench top centrifuge (with rotor and adaptor for 96-well plates)
DNase/RNase free microcentrifuge tubes (1.5-2 mL)
Calibrated DNase/RNase-free pipettes of varying sizes (2 or 10 µL, 200 µL, 1000 µL)
DNase/RNase-free aerosol barrier pipette tips of varying sizes
Cold block(s) or ice
Nuclease-free water
Ethanol (96-100%)
Ethanol (70%)
10% bleach
DNAZapTM PCR DNA Degradation Solutions (Ambion, catalog # AM9890) or equivalent
RNase AWAYTM Decontamination Reagent (Invitrogen, catalog # 10328011) or equivalent
Disposable powder-free gloves

AND

7500 Fast Dx Real-Time PCR Instrument (Applied Biosystems) or equivalent
Sequence Detection System (SDS) Software, version 1.4 (Applied Biosystems) or equivalent
MicroAmp™ Fast Optical 96-Well Reaction Plate with Barcode, 0.1 mL (Applied Biosystems) or equivalent
MicroAmp™ Optical Adhesive Film (Applied Biosystems) or equivalent
MicroAmp™ Fast 8-Tube Strips, 0.1 mL (Applied Biosystems) or equivalent
MicroAmp™ Optical 8-Cap Strips (Applied Biosystems) or equivalent

[Storage Conditions & Validity]

1. The kit should be stored frozen at -20℃±5℃ and protected from light; the expiration date is 6 months; the production date and expiration date are shown in the outer packaging box.
2. Avoid repeated freezing and thawing of the kit and the number of freezing and thawing shall not exceed 7 times.
3. After opening, it should be stored at -20℃±5 ℃ and protected from light. The number of bottle openings shall not exceed 7 times.

[Applicable Instruments]

1. This kit is suitable for ABI7500 quantitative fluorescence PCR instrument.
2. For other models not listed, relevant tests have not been conducted with this kit. If the user needs to apply any other instrument to carry out the detection of this reagent, please contact our Technical Department for relevant support.

NB: *The other devices can include QuantStudio (Thermo Fisher), Applied Biosystems, Bio-Rad (CFX96 Touch 1000, iQ5 iCycler), Agilent (MX-3000, Aria-Dx), MGITech, LighCycler 480II & Z480 (Roche), M2000RT Abbott, Pentabase Gentier 48E, SLAN-96S, HIMEDIA INSTA Q96 LA1012, HIMEDIA INSTA Q48 LA1023, Rotor-Gene (6000, 3000, Q6Plex), LineGene 9600, DT-Lite, DT-96, DT-Prime thermocycler series and other quantitative fluorescence PCR platforms with FAM, VIC/HEX and Cy5 channels.*

[Sample Requirements]

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

a. 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 it to exit.

b. Oropharyngeal swab: The operator holds the tongue with the tongue depressor in one hand and the root of the sterile swab in the other. Scrape the secretions from both sides of the tonsils and the pharyngeal wall posterior separately.

c. Place the nasopharyngeal swab or the oropharyngeal swab into the centrifuge tube containing 1.0mL physiological saline solution for later use.

i) Alveolar lavage fluid sample: Use a sterile syringe to take the sample and place it in a centrifuge tube for examination.

ii) Cross-contamination between samples should be avoided.

iii) The samples should be tested in time after collection, or stored at -20±5℃ for testing, and kept below -70℃ for long-term storage.

[Testing Method]

1. Reagent preparation: (Reagent preparation area)

In each PCR reaction, a positive control and a negative control are tested simultaneously. Each sample needs to be tested for the ORF1ab region, N gene and internal standard.

1) Remove the kit from the refrigerator, equilibrate at room temperature, fully dethaw each component, vortex to mix and then centrifuge instantly.

2) Calculate the number of reactions required for the current test n (n = number of samples + negative control + positive control), and mix the reagents according to the preparation method of the reaction system in Table 1 and then divide into 20μL/well PCR reaction tube. Transfer the PCR reaction tube to the sample preparation area and put the remaining reagents back to -20℃±5℃ and keep away from light.

Table 1: Reaction system preparation method

Composition	Volume
Reaction solution	17μL
Mixed enzyme solution	3μL
Total volume	20μL

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. Extract according to the operation steps of the Extraction Kit instructions.
- The positive control and negative control of this kit are part of the extraction.

2) Loading/Sample addition:

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

b. The RNA of the sample to be tested, the positive control and the negative control after treatment are respectively added into the PCR reaction tubes at the volume of 5μL/well.

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

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^{O}C.*

3. PCR: (Nucleic acid amplification area)

1) Boot, preheat and check the performance of the instrument.

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 (be careful to check whether the reaction tubes are tightly closed before starting the machine 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.

Table 2: **Instrument nucleic acid amplification related parameters**

System		**Set the reaction system to 25μL**	
Signal acquisition	Select FAM and VIC channels to detect viral ORF1ab and N genes. Select Cy5 channel to detect internal standard.		
PCR reaction conditions	**Stage**	**Conditions**	**Number of cycles**
	Reverse Transcription	50℃: 15min	1
	Pre-denaturation	95℃: 15min	1
	PCR	94℃: 15s	45
		55℃: 45s (Collect fluorescence signal at the end of this step)	

Note: *ABI series fluorescence quantitative PCR instrument does not choose ROX calibration, and select <None> for quenching group.*

4. Reading of test results

For data analysis, select ΔRn VS Cycle and Linear mode; select Auto Threshold and Auto Baseline, view the fluorescence curve, and obtain the fluorescence quantitative curve and Ct value in the fluorescence quantitative PCR instrument.

5. Quality control procedures

Positive control: FAM and VIC signals have obvious amplification curves at the same time, Ct value ≤32, Cy5 signal has or does not have amplification curve.

2. Negative control FAM and VIC signals have no rising curve; Cy5 signal has a significant amplification curve, Ct value ≤32.

3. The above requirements shall be met at the same time in the same experiment, otherwise, the experiment is invalid and needs to be carried out again.

6. Interpretation of results

1. According to the below table, determine the detection results of the new coronavirus.

Test Possibility	Test results		Test results and interpretation
	N gene (FAM channel)	ORF1ab (VIC channel)	
a	+	-	Suspected positive, repeat test
b	-	+	Suspected positive, repeat test
c	+	+	Positive
d	-	-	Negative

2. Samples with a and b items that test positive for a single target need to be re-sampled. If the re-test result is positive for a single target or double targets, the sample test result is positive. If the retest result is negative, the sample test result is negative.

3. If the internal standard Cy5 of the negative sample has no amplification curve or Ct> 40, the test result of this sample is invalid. The cause should be found out and eliminated, and the test repeated for this sample.

[Positive Value Determination]

1. Positive result: The fluorescent quantitative curve has amplification curve, Ct value ≤40.

2. Negative result: The fluorescent quantitative curve has no amplification curve or Ct value> 40.

[Test Results Interpretation]

1. Negative and positive controls shall be tested in each test and the test result can be determined only when the controls meet the quality control requirements.

2. When FAM and VIC test channels are positive, Cy5 channel result may be negative.

3. When the internal standard result is negative, if the FAM and VIC test signals of the test sample are also negative, the test result of the sample is invalid, the cause should be found and eliminated and the test repeated for this sample.

[Product Performance Index]

1. **Specificity**: α-interferon, Zanamivir, Ribavirin, Oseltamivir, Paramivir, Lopinavir, Ritonavir (Litonavir) Abidor (Abidol), Levofloxacin, Azithromycin, Ceftriaxone , Meropenem, Tobramycin, Histamine hydrochloride, Phenylephrine (Benzolin), Oxymetazoline (Hydroxymezoline), Sodium chloride were selected to test their interference with this kit and the results did not reveal any interfere with this kit.

2. **Cross-reaction**: Other pathogens that are similar to the new coronavirus 2019 (COVID-19) species or cause similar symptoms (new H1N1 influenza virus (2009), seasonal H1N1 influenza virus), influenza A H3N2, H5N1, H7N9, influenza B Yamagata , Type B influenza Victoria, respiratory syncytial virus type A, respiratory syncytial virus type B, parainfluenza type I, parainfluenza type II, parainfluenza type III, Adenovirus 1, 2, 3, 4, 5, 7, 55 Types, Enteroviruses A, B, C, D, Epstein-Barr virus, Measles virus, Human cytomegalovirus, Rotavirus, Norovirus, Mumps virus, Varicella-zoster virus, Mycoplasma pneumoniae, Chlamydia pneumoniae, Legion Bacteria, Pertussis, Haemophilus influenzae, Staphylococcus aureus, Streptococcus pneumoniae, Streptococcus pyogenes, Klebsiella pneumoniae, Mycobacterium tuberculosis and human genomic DNA did not cross-react.

3. **Precision:** The coefficient of variation Intra/ Inter-batch, Intra/Inter-day among different operators shall not be higher than 5.0%.

4. **Coincidence rate of negative positive controls**: The coincidence rate of 7 positive controls and 10 negative controls is 10

5. **Limit of Detection**: The minimum detection limit of this kit is 500 copies/mL.

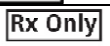
[Test Method Limitations]

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 lead to false negative results.
3. When the detected nucleic acid 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 their using antiviral drugs. At this time, there may be strong positive results detected by this kit and negative results detected by the culture method. In such case, 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 cause of sequence changes may lead to false negative results.
7. For a new type of 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 testing 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 operation, and supplies should not be used in each stage. There should be strict requirements to minimize cross-contamination.
4. 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 test shall be directly put into the waste tank containing 10% sodium hypochlorite and discarded together with other waste products.
7. The workbench and various testing 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.

[Symbols Legend]

Consult Instructions for Use	
Batch Number	
Manufacture Date	
Prescription Use Only	
Manufacturer	
In Vitro Diagnostic Device	
Expiration Date	
Caution, consult accompanying documents	
Keep Dry	
Protect from light	
Catalog Number	

[Technical Support]

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