Atlas Medical

COVID-19 PCR kit

IVD *For In-Vitro diagnostic and professional use only*

Store at -20 °C

Σ **96 Tests**

INTRODUCTION

Coronaviruses are a group of related viruses that cause diseases in mammals and birds. In humans, coronaviruses cause respiratory tract infections that can range from mild to lethal. They are enveloped viruses with single-stranded RNA genome and a nucleocapsid of helical symmetry. Coronaviruses are zoonotic, meaning they are transmitted between animals and people. Human coronaviruses were first discovered in the late 1960s. Other members of this family have since been identified, including SARS-CoV in 2003, HCoV NL63 in 2004, HKU1 in 2005, MERS-CoV in 2012, and COVID-19 in 2019.

The COVID-19 virus spreads primarily through droplets of saliva or discharge from the nose when an infected person coughs or sneezes. Most people infected with the COVID-19 virus will experience mild to moderate respiratory illness and recover without requiring special treatment. Older people and those with underlying medical problems like cardiovascular disease, diabetes, chronic respiratory disease, and cancer are more likely to develop serious illness. Common signs of infection include respiratory symptoms, fever, cough, shortness of breath and breathing difficulties. In more severe cases, infection can cause pneumonia, severe acute respiratory-syndrome, kidney failure and even death.

TEST PRINCIPLE

The primer and probe mix for this kit adopts the dual-target gene design, which targets the specific conserved sequence encoding the ORF 1ab gene and the nucleoprotein N gene. With the PCR reaction mix provided, the amplification of template can be quantitatively monitored by the increasing fluorescence signal detected by a real time PCR instrument.

The PCR detection system includes an endogenous internal control primer and probe mix. The result of internal control provides the accuracy of sampling and extraction process, in order to avoid false negative results.

MATERIALS

1. COVID-19 Enzyme Mix (Lyophilized) :
   One bottle, 96 tests.
2. COVID-19 Primer-Probe Mix
   One vial, 100 µL.
3. Enzyme Mix Buffer (5X)
   1 vial, 400 µL.
4. COVID-19 PCRPositive Control:
   1 vial, 90 µL.
5. COVID-19 Negative Control (DEPC-treated H_2O)
   1 vial, 90 µL.
6. Package insert.

WARNINGS AND PRECAUTIONS

1. *For in vitro diagnostic and professional use only*.
2. This package insert must be read completely before performing the test.
3. Follow Good Laboratory Practices, wear protective clothing, use disposal gloves, do not eat, drink or smoke in the area. Gloves should be changed regularly to avoid cross-contamination between samples.
4. Clinical laboratories involving manipulation of potentially infected specimens should be performed in a certified Class II Biological Safety Cabinet (BSC) in a BSL-2 facility. Diagnostic tests should follow standard laboratory practices, including Standard Precautions, when handling potential patient specimens. For laboratory waste, follow standard procedures associated with other respiratory pathogens.
5. The reagent kit is transported for 7 days in sealed foam box with refrigerant packs, and the temperature is not higher than 20°C, which will not affect the shelf life of the product.
6. Laboratory personnel should be trained and familiar with the operation procedures and precautions of the instrument before the experiment. Quality control should be performed for each experiment.
7. Laboratory management should be strictly in accordance with the regulations of PCR gene amplification laboratories. Laboratory personnel must be professionally trained and the experimental process should be strictly divided into sections. All consumables should be used only once after sterilization. Instruments and equipment should be assigned to each stage of the experiment and cannot be used alternatively.

STORAGE

1. Store all components at -20 °C ±5°C in the dark area. Do not freeze.
2. The components of the kit will remain stable through the expiration date indicated on the label and package.
3. The reconstituted liquid reagent should be used Up at once. Leftover reagents should be stored at 4 °C for no longer than 1week.

SPECIMEN COLLECTION AND PREPARATION

- Serum, throat swab, virus preservation buffer and other can be used with this assay.
- Blood should be drawn using standard venipuncture techniques and serum separated from the blood cells as soon as possible. Care should be taken to ensure that the serum specimens are clear and not contaminated by microorganisms.
- Testing should be performed immediately after the specimens have been collected. Do not leave the specimens at room temperature for prolonged periods. Specimens may be stored at -20°C for up to 3 months. For long-term storage, specimens should be kept below -70°C.

PREPARATION OF REAGENT

Please follow manufacturer's instruction to extract virus RNA from clinical sample using RNA extraction kit. Extracted RNA can be used directly for PCR detection. Otherwise, keep RNA sample at -70°C if not in use. Avoid repeated thawing and freezing.

Note: This product does not contain an RNA extraction kit.

PROCEDURE

1. Reagent Preparation (Perform in Reagent Processing Area)

1.1. **Master Mix Preparation:**

1.1.1. Bring all reagents and specimens to room temperature before beginning the assay.

1.1.2. Resuspend the Lyophilized Enzyme Mix in 400 µL Enzyme Mix Buffer. Add 500 µL RNase-free water and gently pipette up and down. Avoid generating air bubbles.

1.1.3. Wash the wall of tube by pipetting to prevent lyophilized powder from remaining. Place the tube aside for 30 min.

Note: The reconstituted liquid reagent should be used up at once. Leftover reagents should be stored at 4°C for no longer than 1 week.

1.2. **Reaction Mix Preparation:**

The recommended sample volume used in the reaction is 5 µL or 10 µL. Refer to one of the columns below to prepare the reaction mix:

1 x volume required		
	For 5 µL sample	For 10 µL sample
Resuspended master mix	9 µL	9 µL
Pimer & Probe	1 µL	1 µL
RNase-free water	5 µL	---
Total volume	15 µL	10 µL

*Multiply the numbers according to the number of tests.

1.3. Transfer 15 µL (or 10 µL, depending on sample volume) of the above reaction mix into the PCR plate of the chosen PCR platform. Transfer into wells according to the number of samples to be tested, include one well for the positive control and one well for the negative control. Transfer the reaction mix to Sample Processing Area.

2. **Sample Adding (Perform in Sample Processing Area)**

2.1. For 5 µL sample:

2.1.1. Add 5 µL of the following into the appropriate wells according to plate setup: Sample(s), Positive Control, Negative Control

2.2. For 10 µL sample:

2.2.1. Dilute positive control with 5 µL DEPC-treated water to total volume of 10 µL.

2.2.2. Add 10 µL of the following into the appropriate wells according to plate setup: Sample(s), Diluted Positive Control, Negative Control

2.3. After adding the samples, cover the lid immediately. Spin down briefly using a centrifuge to remove air bubbles. Transfer the mixture to amplification area.

3. PCR Amplification (Perform in Amplification and Analysis Area)

3.1. Place the tubes on the sample holder in the instrument. Set up the test panel according to the positions of positive control, negative control and RNA samples.

3.2. Select the detection channels as following:
a) Select FAM (ORF-1ab gene) and HEX (N gene) channels to detect COVID-19 RNA.
b) Select ROX channel to detect internal control.

3.3. Enter the amplification program. Recommended as below:

Step	Temp.	Time	Cycle
Reverse Transcription	50°C	15 min	1
cDNA Initial Denaturation	95°C	3 min	1
Denaturation	95°C	15 sec	45-50
Annealing, Extension and Fluorescence measurement	55°C	40 sec	45-50
Cooling	25°C	10 sec	1

Save the file after settings and run the reaction. Please set the fluorescence internal control of the instrument to "None". For example, for ABI series instruments, set "Passive Reference" to "None".

INTERPRETATIONS OF THE RESULTS

(Please refer to the user manual of instrument for setting, the following analysis uses ABI series instruments as an example)

1. After the reaction is completed, the results are automatically saved and the amplification curves of the detected target DNA and the internal control are analyzed separately.
2. According to the analysis, the amplification plot will adjust the Start value, End value and Threshold value of the Baseline (Users can adjust the values according to the actual situation. Start value can be set within 315, End value can be set within 5~20; Users can adjust the amplification curve of negative control to make it linear or below the threshold line). Click "Analyze" to perform the analysis and the parameters should meet the following requirements mentioned in "Section 5. Quality Control". Lastly, record the qualitative results in the Plate window.

QUALITY CONTROL

1. COVID-19 PCR Negative Control:None of the FAM, HEX & Internal Control (ROX) channels have a Ct value or Ct >40.
2. COVID-19 PCR Positive Control: FAM, HEX & Internal Control (ROX) channels Ct ≤35
3. The above requirements must be met at the same time in the same experiment. Otherwise, this experiment is invalid and needs to be repeated.

POSITIVE THRESHOLD

According to the study of the reference value, the Ct reference value for the target gene detected by this kit is 40, and the Ct reference value of internal control is 40.

RESULT ANALYSIS

1. First to analyze the amplification curve of internal control ROX channel. If Ct ≤ 40, it indicates that the detection is valid, and users can continue the subsequent analysis:

1.1. If a typical S-type amplification curve is detected by the FAM or HEX channel, with Ct ≤40, it indicates that COVID-19 virus is positive.

1.2. If FAM and HEX channels do not detect a typical S-type amplification curve (No Ct) or Ct > 40, it indicates that COVID-19 virus is negative.

2. If the internal control ROX channel failed to detect Ct or Ct > 40, it indicates that the concentration of the tested sample is too low or there is an inhibitory reaction from the interfering substance. Users have to repeat the experiment.
3. For positive samples and virus cultures, there is no requirement of the internal control results. For negative samples, the internal control should be positive. If the internal control is negative, the test result of the sample is invalid. The cause should be found and eliminated. Users should redo sampling and repeat the experiment. (If the retest result is still invalid, please contact the manufacturer.)
4. Determination of grey area results: If the fluorescence signal of a sample has a significant increase in the FAM and HEX channels, but the Ct value is greater than 40, the sample is in the grey area and needs to be re-examined. If the retest result is still in the grey area, it is judged as positive.

LIMITATIONS OF DETECTION METHODS

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, medical history, other laboratory tests and treatment response.
2. Analysis of possibility of false positive & negative results:

2.1. Improper sample collection, processing & transportation, and low sample concentration may cause false negative results.

2.2. Variations in the target sequence of the novel coronavirus (COVID-19) or sequence changes caused by other reasons may lead to false negative results.

2.3. Improper reagent storage can lead to false negative results.

2.4. Other unproven interferences or PCR inhibitors may cause false negative results.

2.5. Cross-contamination during sample processing may cause false positive results.

2.6. This assay should be performed according to Good Laboratory Practice (GLP) regulation.

2.7. Operators should strictly follow the manufacturer's instructions in performing the test.

PERFORMANCE CHARACTERISTICS

1. Specificity

The primer and probe provided in this kit is designed based on the conserved sequence of the novel coronavirus (COVID-19), and has a high detection rate of the target gene fragment. This kit has no cross-reactions among positive samples of Coronavirus (NL63, HKU1, 229E, OC43), Influenza A virus, Influenza B virus, Respiratory syncytial virus, Adenovirus, Parainfluenza virus, Klebsiella pneumoniae Streptococcus pneumoniae, Haemophilus influenza, Pseudomonas aeruginosa, Legionella pneumophila, Pertussis, Staphylococcus aureus, Mycoplasma pneumoniae, Chlamydia pneumoniae. The negative and positive rates of detecting commercial reference materials were 100%.

2. **Minimum detection limit:** 500 IU / mL

ATLAS Medical
Ludwig-Erhard Ring 3
15827 Blankenfelde-Mahlow
Germany
Tel: +49 - 33708 – 3550 30
Email: Info@atlas-medical.com

PPI2014A01
Rev A (30.03.2020)

REF	Catalogue Number		Temperature limit
IVD	In Vitro diagnostic medical device		Caution
Σ	Contains sufficient for <n> tests and Relative size		Consult instructions for use (IFU)
LOT	Batch code		Manufacturer
	Fragile, handle with care		Use-by date
	Manufacturer fax number		Do not use if package is damaged
	Manufacturer telephone number		Date of Manufacture
	Keep away from sunlight		Keep dry