COVID-19 One-Step RT-PCR Kit

100 Tests Kit

(This kit is for in vitro diagnostic (IVD), for professional use only.)

Catalogue No.: PT.COVID.100

PISHTAZ TEB DIAGNOSTICS

Introduction

This kit is used for nucleic acid detection of novel coronavirus (SARS-CoV-2), the results can be used for auxiliary diagnosis of patients with new coronavirus infection or patients suspected of new coronavirus infection. The test results of this kit are suitable for clinical reference and should not be used as the sole criterion for clinical diagnosis. It is recommended to conduct a comprehensive analysis combining with clinical manifestations of the patient and other laboratory tests.

Test Principle

The primer-probe of this kit adopts the dual-target gene design, which targets the specific conserved sequence encoding the RdRp (RNA-dependent RNA polymerase) region and the nucleocapsid protein N region. With the provided reaction buffer, the amplification of template can be qualitatively monitored by the increasing fluorescence signal detected by a real time PCR instrument.

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

Packaging Specifications: 100 tests/kits

Materials Provided with The Kit

	Component	Amount
1	COVID-19 Enzyme Mix (Lyophilized)	100 tests/vial
2	COVID-19 Primer-Probe Mix	100 µL/vial
3	RT-PCR Buffer (5×)	400 µL/vial
4	COVID-19 PCR Positive Control	90 µL/vial
5	COVID-19 PCR Negative Control	90 µL/vial

Storage Conditions

1. Shelf-life of reagent kit is 12 months. Manufacture date is indicated on the box.

2. Reagents should be stored in the dark at -20 ± 5°C.

3. Repeated thawing and freezing should be no more than 10 times.

4. The reconstituted liquid reagent should be used up at once. Leftover reagents should be stored at 4°C for no longer than 2 week.

Instrument Compatibility

This kit is compatible with real time PCR instruments with FAM, HEX/VIC, RED/ROX channels.

Sample Requirement

1. **Sample Type:** Bronchoalveolar lavage fluid, sputum, throat & nasal swabs, virus preservation buffer and others

2. **Sample Collection:** Collect in accordance with conventional sample collection methods

3. **Sample Storage & Transportation:** Sample to be tested can be processed immediately, or stored at -20 ± 5°C for 3 months, -70°C for long term. Avoid repeated thawing and freezing. Sample should be transported with refrigerant packs in sealed Styrofoam box or ice chest.

Preparation before Testing

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, and is compatible with DSP Viral RNA Kit (Qiagen), High Pure Viral Nucleic Acid kit (Roche Life Science) and other commercial Viral extraction kits.

Detection Method

1. Reagent Preparation
(Perform in Reagent Processing Area)

1.1. **Master Mix Preparation:**

Take out the components from the box and let it thaw at room temperature until equilibrated. 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. 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 2 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 × volume required		
	For 5 µL Sample	**For 10 µL Sample**
Resuspended master mix	9 µL	9 µL
RdRp/N/ICON Primer & probe (FAM/HEX/ROX)	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. Aliquot 15 µL (or 10 µL, depending on sample volume) of the above reaction mix into the PCR plate of the chosen PCR platform. Aliquot 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:

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:

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 (RdRp gene) and HEX (N gene) channels to detect SARS-CoV-2 RNA.

b) Select ROX (RNase P) channel to detect internal control.

3.3. Enter the amplification program. Recommended as below:

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

※ Save the file after settings and run the reaction.

Note: Please set the fluorescence internal control of the instrument to "None". For example, for ABI series instruments, set "Passive Reference" to "None".

4. Result Interpretation

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

4.1. After the reaction is completed, the results are automatically saved and the amplification curves of the detected target RNA and the internal control are analyzed separately.

4.2. 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 3~15, 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.

5. Quality Control

5.1. COVID-19 PCR Negative Control:

None of the FAM, HEX & Internal Control (ROX) channels have a Ct value or Ct > 40.

5.2. COVID-19 PCR Positive Control:

FAM, HEX & Internal Control (ROX) channels Ct ≤ 35

5.3. 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:

a) If a typical S-type amplification curve is detected by the FAM and/or HEX channel, with Ct ≤ 40, it indicates that SARS-CoV-2 virus is positive.

b) If FAM and HEX channels do not detect a typical S-type amplification curve (No Ct) or Ct > 40, it indicates that SARS-CoV-2 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 (SARS-CoV-2) 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. Operators should strictly follow the manufacturer's instructions in performing the test.

Product Performance

1. Specificity

The primers and probes provided in this kit are designed based on the conserved sequence of the novel coronavirus (SARS-CoV-2), 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: 200 copies / mL

Warnings and Precautions

1. This product is only used for in vitro detection. Please read this manual carefully before use.

2. 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.

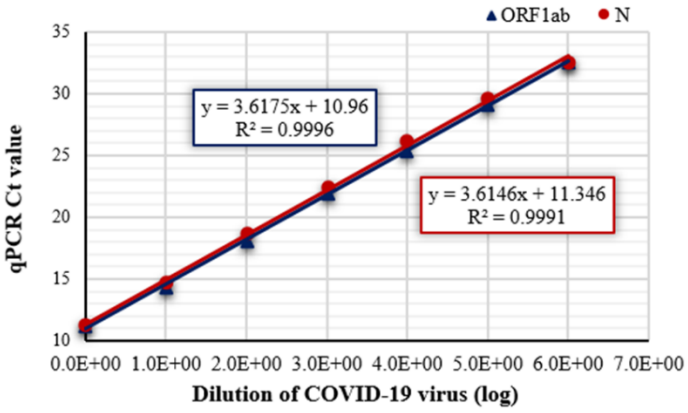
3. All samples should be regarded as potentially infectious materials. Laboratory workers should wear appropriate personal protective equipment (PPE) which includes disposable gloves, laboratory coat or grown. Gloves should be changed regularly to avoid cross-contamination between samples.

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.

Evaluation Report

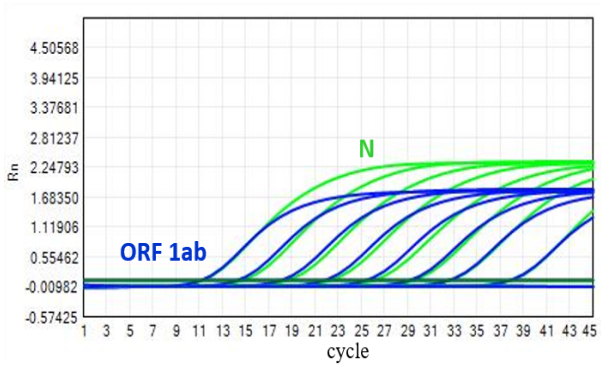
1. Qualitative test consistency

1.1. Linearity



The figure demonstrates the linearity of Ct values of Pishtaz Teb COVID-19 RT-PCR Kit for detecting different dilutions of SARS-CoV-2 virus (10-fold dilution, initial factor ~ 106 dilution). RdRp gene is shown in blue, whereas N gene is shown in red. Pishtaz Teb COVID-19 RT-PCR Kit showed a linear correlation of R2 > 0.9990 with the virus diluents.

1.2. qPCR Amplification Plot



The figure above demonstrate amplification plots and of Pishtaz Teb COVID-19 RT-PCR Kit for detecting different dilutions of SARS-CoV-2 virus (initial factor ~ 107 dilution). RdRp gene is shown in blue, whereas N gene is shown in green. The corresponding Ct values are within 11 to 40, baselines are flat and linear, and typical S-type amplification curves are observed.

CE IVD

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