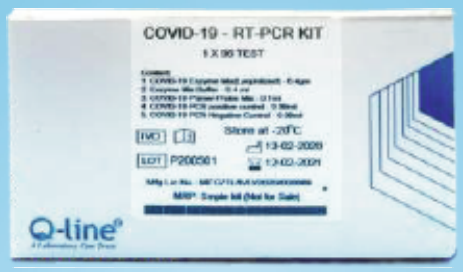
Q-LINE COVID-19 RT-qPCR

Detection Kit

Q-line® Molecular



SARS-COV-2 Detection

- Specific detection of SARS-Cov-2 ORF-1ab gene and nucleoprotein N gene with extremely high sensitivity and specificity using Multiplex PCR-fluorescent probe technology combined with fast one-step RT-PCR technology.
- One tube reaction for identification and detection of 2019-CoV (ORF1ab and N genes).
- One-step Multiplex Real-Time RT-PCR.
- Reliable endogeneous Internal control, UNG enzyme and dUTP wee used to reduce risk of contamination and false negative results.
- Limit of detection (LOD) : 500 copies/ml.
- Repeatability and reproducible results.
- No cross-reactivity with other respiratory viruses.
- Short detection time : 65 Mins assay.
- Specificity & Sensitivity : 100%

Specimen

- Nasopharyngeal (NP) swab
- Oropharyngeal (OP) swab
- Serum & others

Compatible with most RT-PCR instruments

Thermocycler	Manufacture
ABI 7500/7500 Fast	Thermo Fisher
Roche Light Cycler 480	Roche
Qiagen Rotor Q5	Qiagen
CFX96	Bio-Rad
Other System	Any with FAM,HEX/VIC,RED/ROX Channels

Material provided

Kit Contents	Cap Color	Volume (96 tests)
COVID-19 Master Enzyme mix	White	One Vial (Lyophilised)
COVID-19 Primer-Probe mixture	Green	100 µL
Enzyme Mix Buffer (5x)	Pink	400 µL
COVID-19 PCR Positive Control	Yellow	90 µL
COVID-19 PCR Negative Control	White	90 µL

Ordering Information

Product Code	Product description	Pack Size
COVIDM96PS	Corona Virus (COVID-19) RT-PCR Kit	1 x 96 Test

Marketed By

SMS
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Manufactured By

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Complete Medical Solution

Q-line® *Molecular*

Coronavirus(COVID-19) RT-PCR Kit

Reference / Pack Sizes
COVIDM32PS 1 x 32 Test
COVIDM96PS 1 x 96 Test

INTENDED USE

Q-Line® *Molecular* Novel Coronavirus (COVID-19) RT-PCR Kit is designed to detect COVID-19 using real time PCR. The results can be used to assist diagnosis of patients with COVID-19 infection, and provide molecular diagnostic basis for infected patients. The test results of this kit are for clinical reference only and should not be used as the only standard for clinical diagnosis. It is recommended to conduct a comprehensive analysis by combining the test results with patients' symptoms and other laboratory tests.

SUMMARY

Coronavirus belongs to the family of Coronaviridae, in the order of Nidovirales. It is formed by a positive-sense single-stranded RNA, usually appears spherical with a size of 80-120nm and with crown-like spikes on the surface. This large family of virus is commonly circulating among vertebrates, such as camels, cats and bats. Novel coronavirus (COVID-19) has been identified as a new strain of coronavirus. It can cause viral pneumonia and dyspnea in humans.

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.

KIT CONTENTS

This kit contains real time PCR amplification reagents, composed of the following:

	Component	Amount	
1	COVID-19 Enzyme Mix (Lyophilized)	96 tests/Kit	32 tests/Kit
2	COVID-19 Primer-Probe Mix	100 µL/vial	32 µL /Vial
3	Enzyme Mix Buffer (5X)	400 µL/vial	128 µL /Vial
4	COVID-19 PCR Positive Control	90 µL/vial	30 µL /Vial
5	COVID-19 PCR Negative Control (DEPC-treated H2O)	90 µL/vial	30 µL /Vial

MATERIALS REQUIRED BUT NOT PROVIDED

- RNA extraction kit
- RT-PCR System FAM, HEX/VIC, RED/ROX channels

WARNINGS AND PRECAUTIONS

1. This product is only used for in vitro detection. Please read this manual carefully before use.
2. 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.
3. 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.
4. 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.
5. 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.
6. 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.

STORAGE & STABILITY

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 1 week.

INSTRUMENT COMPATIBILITY

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

SAMPLE REQUIREMENT

1. Sample Type: Serum, throat 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.
4. Sample transportation : Sample should be transported with refrigerant packs in sealed Styrofoam box or ice pack.

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 Qiagen DSP Viral RNA Kit and other commercial kits.

DETECTION METHOD (TEST PROCEDURE)

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 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 × volume required		
	For 5 µL Sample	**For 10 µL Sample**
Resuspended master mix	9 µL	9 µL
ORF1ab/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:
Dilute positive control with 5 µL DEPC-treated water to total volume of 10 µL. 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:

SN	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. 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 DNA 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

Internal Control	ORF 1ab gene	N gene	Conclusion	Remark
Ct<40	Has amplification curve; Ct<40	Has amplification curve; Ct<40	Positive	
Ct<40	No amplification curve	No amplification curve	Negative	If again getting one gene positive result, then need collecting the sample again
Ct<40	Has amplification curve ; Ct<40	No amplification curve	Suspected; need retesting	If again getting one gene positive result, then need collecting the sample again
Ct<40	No amplification curve	Has amplification curve; Ct<40	Suspected; need retesting	If again getting the same result, then the result is positive
Ct<40	Has amplification curve ; Ct>40	Has amplification curve; Ct>40	Suspected; need retesting	
Ct>40			Invalid; Need collecting sample again	

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 or HEX channel, with Ct ≤ 40, it indicates that COVID-19 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 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.

Product Performance

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 copies / mL.

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

SYMBOLS:-

IVD	In vitro diagnostic medical device use		Single Use
	Manufacturer	Σ	Number of tests in the pack
	Date of Manufacturing		Do not use if pouch or kit damaged
	Expiry Date		This side Up
LOT	Lot Number		Read package insert before use
	Store at - 20 ± 5°C		

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