



Ultra-rapid Real-time RT-PCR Assay for COVID-19

Instruction Manual

(For research use only)

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[Product Name]

Ultra-rapid Real-time RT-PCR Assay for COVID-19

[Packaging Specifications]

48 test / box

[Intended Usage]

This kit is used to detect the viral pneumonia outbreak and related pathogen – the Novel Corona virus COVID-19, through in vitro qualitative nucleic acid test (NAT) on a qPCR device based on fluorescence detection. Through the RT-qPCR detection of COVID-19, this qualitative nucleic acid test, can assist the diagnostics of COVID-19 and epidemiology surveillance of the Novel Coronavirus COVID-19.

The Novel Coronavirus COVID-19 can cause acute infectious pneumonia. When a person is infected with coronavirus, the typical signs of infection are respiratory symptoms, fever, cough, shortness of breath and dyspnea. In more severe cases, the infection can lead to pneumonia, severe acute respiratory syndrome, kidney failure, and even death.

Currently, there is no clinically approved treatment for patients infected by the Novel Coronavirus.

Laboratory operators should have received professional training in gene amplification or biophysical methods and have the relevant qualifications for laboratory operations. The laboratory should have reasonable facilities and protective procedures for personnel safety. With this kit, negative results cannot exclude the Novel Coronavirus infections, and should not be used as the sole source for diagnosis, treatment, or other management decisions. A positive result does not exclude bacterial or other viral infections.

[Principle of the Test]

This kit integrates the nucleic acid extraction and the real time PCR process into a single-step process. This kit does not need for separate extraction and purification steps of nucleic acid. Patient's sample can be mixed directly with the real time PCR reagents and tested on the qPCR devices.

This kit can also distinguish the differences and similarities of ORF1ab gene and N-gene among various strains of coronavirus.

Specific primer probes are designed for this single-step RT-qPCR kit. A primer probe using human β -Globin gene sequence as a template is used for internal control. This kit uses four fluorescence dyes, ORF1ab novel coronavirus gene and S (Spike) gene use are labeled with FAM, Coronavirus N gene is labeled with VIC, internal control is labeled with Cy5.



Main Components

Kit components are listed in the table below.

Item #	Label	Ingredients	Quantity
1	PCR mix	primer probe, DNA polymerase, reverse transcriptase, nucleoside triphosphate, magnesium ion, purified water	2-tubes (950µL / tube)
2	Positive Control	Target gene TE solution	1 tube (200µL / tube)
3	Negative Control	internal control TE solution	1 tube (200µL / tube)

[Storage conditions and expiration date]

The packaged kit should be kept in a tightly closed container at -20 ° C, protected from light. The validity period is tentatively set at 6 months. Use foam boxes with dry ice or ice packs for transportation. It should not be placed at a high temperature (for example, at 37 ° C) for more than 48hr. Repeated freezing and thawing up to 7 times will not affect the detection result of the kit, and the effect of more than 7 times has not been tested.

After the bottle is opened, it can be stored at 4 °C for no more than three (3) days. (More than 3 days has not been tested). It is recommended to be one-time use. After the packaging is opened, it needs to be stored at -20°C

The production date and expiration date can be found on the product label.

[Applicable Instrument]

This kit is designed for portable 4-plex real-time quantitative PCR device such as Maverick qPCR by Anitoa Systems.

[Sample requirements]

1. The sample is collected from patients with unknown cause of viral pneumonia.
2. Sample Type: Nasal swab and throat swab, plasma, or serum



3. Storage conditions: samples should be collected and tested immediately. If not tested immediately, it should be tested within 24 hr. provided the sample is preserved at 4°C or preserved at -70°C if test will be done longer than 24hr. Avoid repeated freeze-thaw cycles.

[Test Method]

1 Reagent preparation

Divided PCR reaction solution into 38 µL per PCR tube.

2 Add the sample

Immerse the pharyngeal swab or nasal swab containing the patient's specimen in 200-300 µL salinity solution and press the swab tip 7-10 times. Pipette 12 µL of swab and wash solution and add to the PCR reaction tube (including positive / negative quality control) containing the PCR reaction solution.

3 Testing in qPCR device

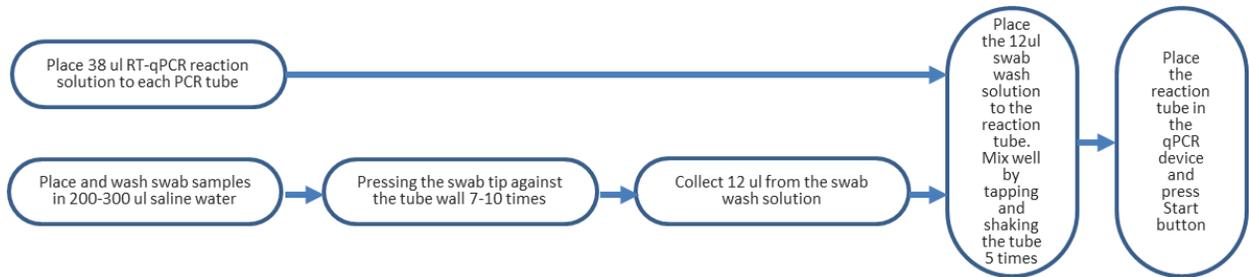
Place the reaction tube into the fluorescence PCR device. The thermal cycling parameters are set as follows:

number of steps	cycle	temperature (°C)	reaction time (min: sec)
1	1	50	15:00
2	1	96	01:30
3	45	96	00:02
		58	00:10

The collection of fluorescence signals is set to FAM and VIC / HEX, ROX and Cy5, and the data collection is set to 58 ° C. Under normal circumstances, there is no need to set up or change the pre-determined protocol in the qPCR device since Maverick qPCR is pre-configured with the protocol for the test kit. Press the start button on the Maverick qPCR to begin the test.

[Test Procedure]

Below is the flow diagram for test procedure



[Interpretation of the test results]

After the reaction is over, the instrument automatically saves the results, reads the Ct displayed by the software, and performs the following analysis:

In the same test, the following conditions need to be met at the same time, otherwise the PCR reaction was considered ineffective and needed to be retested. The details are as follows:

1. The negative control target genes should be no Ct (negative);
2. The positive control target gen should show a typical amplification curve, and the $Ct \leq 32.0$;
3. The internal control of all test wells should show a typical amplification curve, and the $Ct \leq 34.0$;

When the above conditions are satisfied at the same time, the following judgments are made:

Select each fluorescent channel and read the Ct.

When the FAM channel (ORF1ab/S) displays a typical amplification curve and $Ct \leq 38.5$, it should be judged as positive COVID-19. When it shows a typical amplification curves and $38.5 < Ct \leq 42$, it should be judged as suspected positive COVID-19.

When the VIC channel (N gene) shows a typical amplification curves with $Ct \leq 38.5$, it should be judged as COVID-19 positive. When it shows a typical expansion with an amplification curve and $38.5 < Ct \leq 42$, it should be judged as suspected positive COVID-19;

For suspected positive samples, it should be tested again. If the retest result is positive or suspected to be positive, it should be judged as positive for infection, otherwise it is judged as negative.

Refer to the following table to determine test results and recommended actions:



Ct Value	Fluorescence Channel	Ct ≤ 38.5	38.5 < Ct ≤ 42	Ct > 42 or no typical amplification curve
Results	FAM	Positive (+)	Suspected positive (+/-)	Negative (-)
	Hex/VIC	Positive (+)	Suspected positive (+/-)	Negative (-)

ORF1ab gene (FAM)	S gene (FAM)	N gene (Hex/VIC)	Internal control (CY5)	Result Interpretation	Recommended actions
+		+	+/-	COVID-19 Positive	Report results to sender and appropriate public health authorities.
+		+/-	+/-	COVID-19 Positive	Report results to sender and appropriate public health authorities.
+/-		+	+/-	Suspected COVID-19 Positive	For suspected positive samples, it should be tested again. If the retest result is positive or suspected to be positive, it should be judged as positive for infection, otherwise it is judged as negative.
+/- (or at least one target gene shows suspected positive)		+/- (or at least one target gene shows suspected positive)	+/-	Suspected COVID-19 Positive	For suspected positive samples, it should be tested again. If the retest result is positive or suspected to be positive, it should be judged as positive for infection, otherwise it is judged as negative.
-		-	+	COVID-19 Negative	Report negative results to sender.



-	-	-	Invalid result	Sample is repeated once. If a second failure occurs, it is reported to sender as invalid and recommend recollection if patient is still clinically indicated.
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[Limitation of the test method]

The test results of this kit are for clinical reference only. The clinical diagnosis and treatment of patients should be based on the combination of patient's symptoms/signs, medical history, other laboratory tests, and existing medical treatment. Sample test results are related to the quality of sample collection, processing, shipping, and storage; any errors in each step will lead to false negative results. If cross-contamination is not controlled during sample processing, false positive results may occur. For kits containing internal control, the internal control amplification will fail when the prepared sample concentration is too high.

[Product performance index]

Minimum detection limit: Not less than 500 copies / mL.

Notes:

1. Throughout the testing process of the COVID-19 pathogen should be kept the test apparatus, equipment and work clothing isolated. The biological protection of the operators should follow P3 level to protect operators from infection. This also prevents contamination of the specimen.
2. During the operation, the operator should always pay attention to avoid RNase and DNase contamination. The operator should use disposable gloves that do not contain fluorescent substances, disposable thin walled 200 µL PCR tubes, and pipette head (with filter tip). Do not touch the reaction tube directly by hand.
3. A biological safety cabinet should be used when processing specimens to ensure the safety of operators and prevent environmental pollution. Harmful and toxic specimens and reagents in the experiment should be properly placed and kept by special persons; bio-waste should be properly disposed in special containers. Instruments and equipment such as operating platforms, pipettes, centrifuges, and amplification instruments should be with 1.0% sodium hypochlorite and/or 70% wipe and disinfect ethanol. The lab room and ultra-clean workbench should be treated with UV light regularly and after each test.



4. Before placed into the centrifuge, the reagents should be fully thawed in the tube, then mixed and centrifuged for a few seconds to concentrate the liquid at the bottom of the centrifuge tube. When preparing the reaction system, it should be noted that mixing of all liquids should be performed on a vortex mixer as much as possible, without pipetting to avoid air bubbles. The reaction system is prepared by centrifugation at low speed for several seconds. Use the kit within the warranty period. Do not mix reagents of different batches.

[Reference]

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