Dynamiker Biotechnology (Tianjin) Co., Ltd.

Novel Coronavirus (2019-nCoV) RT-PCR Kit

Catalogue No.: DNK-1418-1

User Manual / 50 tests

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1. INTENDED USE

Novel Coronavirus (2019-nCoV) RT-PCR Kit is a polymerase chain reaction IVD test to detect RNA of 2019-nCoV in samples like upper respiratory tract samples (throat swabs, nasal swabs, etc), lower respiratory tract samples (respiratory tract extracts, bronchial lavage fluid, alveolar lavage fluid, deep sputum, etc), conjunctival swabs, stool specimens, anticoagulant and serum. These samples were taken from human patients which were considered at risk for the new coronavirus pneumonia. This assay has high sensitivity and specificity, and is of great significance for the early diagnosis and treatment of new coronavirus infections.

2. PRODUCT INTRODUCTION

2.1 Novel Coronavirus-2019

Coronaviruses are systematically classified as Coronaviridae (Coronaviridae). Coronaviruses are single-stranded positive-stranded RNA viruses with an envelope, and are a large class of viruses widely found in nature.

On January 12, 2020, the World Health Organization (WHO) has named the new coronavirus that caused the pneumonia epidemic in Wuhan as 2019 New Coronavirus (2019-nCoV), and its full name is 2019 Novel Coronavirus, hereinafter referred to as new coronavirus. The new coronavirus (2019-nCoV) is transmitted through respiratory droplets and can also be transmitted through contact. The population is generally susceptible and the virus may be mutated. The current basic infection number R0 is about 2.2 (90% high-density interval 1.4-3.8), indicating that there is a strong infectious capacity between people. The most common symptoms were fever, cough, myalgia or fatigue, all patients had pneumonia, and a chest CT scan revealed abnormalities.

New methods for diagnosing coronavirus infections include imaging, real-time PCR and gene sequencing. The use of PCR technology to diagnose new coronavirus pneumonia is due to its advantages in speed, sensitivity, and specificity.

The Novel Coronavirus (2019-nCoV) RT-PCR Kit is a multiple RT-PCR method for detecting novel coronavirus RNA extracted from clinical samples like upper respiratory tract samples (throat swabs, nasal swabs, etc), lower respiratory tract samples (respiratory tract extracts, bronchial lavage fluid, alveolar lavage fluid, deep sputum, etc), conjunctival swabs, stool

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specimens, anticoagulant and serum. The test provides fast and reliable detection to assist early diagnosis, and quality control ensures that users can have full confidence in the quality and reproducibility of the test.

This product uses a new type of coronavirus RNA as a template to reverse transcribe into cDNA using reverse transcriptase, and then uses the synthetic cDNA as a template to continue to amplify with gene-specific primers and detect with fluorescent probes. The entire process from reverse transcription to qPCR can be completed in the same reaction system. During the amplification process, the amount of fluorescence is directly proportional to the amount of amplification product, so the amount of sample nucleic acid can be determined by the amount of fluorescence.

2.2 Kit Components

The number of reagents can be used for 50 tests.

Reagent	Volume (μL)	Cap color	Cap number
PCR Master Mix	1050	Orange	1
PCR PP	350	Brown	2
PCR Water	800	White	3
Positive Control	30	Red	4
Negative Control	5	Blue	5

3. STORAGE AND EXPIRATION DATE

3.1 Inspection on Arrival

Check the Novel Coronavirus (2019-nCoV) RT-PCR Kit on arrival. If the packaging is damaged the kit must not be used. In addition, the reagents should be transported at -25°C-15 °C or lower temperature.

3.2 Storage Space and Temperature

The kit should be stored in an amplicon free laboratory. Positive Control (PC) and Negative Control (NC) in the kit should be stored in the area where the Nucleic acid template/sample template is stored. Should be kept in-25 °C to -15 °C, valid for 6 months.

3.3 Stability

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The Novel Coronavirus (2019-nCoV) RT-PCR Kit showed that under the condition of more than 10 freeze-thaw cycles, its performance is not affected. All reagents contained within the kit have been marked on the kit label and must be used within this effective period.

4. MATERIALS REQUIRED BUT NOT PROVIDED

4.1 General Equipment

Real-time quantitative PCR instrument (over four channels)

Vortex mixer

Mini centrifuge

Microplate processing centrifuge

Refrigerator (2-8 °C) and freezer (-25 °C to -15 °C)

Micropipettes for volumes of 1 to 1000 µL

4.2 Reagents

Recommended nucleic acid extraction reagent

4.3 Consumables

DNase/RNase free plastics were used in the preparation of PCR

DNase/RNase free 1 to 1000 µL pipette tips

Disposable gloves, powderless

Disposable face mask

5. SAMPLE COLLECTION AND DNA EXTRACTION

The quality of extracted nucleic acid is essentially related to the sensitivity of real-time PCR detection, so samples must be collected and stored according to the following guidelines.

According to the recommendations for sample collection of the novel Coronavirus-infected pneumonia prevention and control plan (Six edition), extract the nucleic acid from samples like upper respiratory tract samples (throat swabs, nasal swabs, etc), lower respiratory tract

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samples (respiratory tract extracts, bronchial lavage fluid, alveolar lavage fluid, deep sputum, etc), conjunctival swabs, stool specimens, anticoagulant and serum.

Precise experimental practice recommends including at least one positive and negative control extraction in each analysis.

Extracted samples should be stored at -80°C for long-term storage, in -25 °C to -15 °C for temporary storage.

Recommended RNA extraction kit:

- QIAamp® Viral RNA Mini Kit
- QIAamp® MinElute Virus Spin Kit
- RNasy®Mini Kit (QIAGEN)
- EZ1 DSP Virus Kit (QIAGEN)
- Roche MagNA Pure Compact RNA Isolation Kit
- Roche MagNA Pure Compact Nucleic Acid Isolation Kit
- Roche MagNA Pure96 DNA and Viral DNA Small Volume Kit
- Invitrogen ChargeSwitch®Total RNA Cell Kit

6. TEST PROCEDURE

6.1 PCR Instrument Setting

It is recommended that a clean laboratory to set up all PCR reactions. To avoid any risk of contamination, users should always follow standard laboratory practices and isolate operational processes properly.

Before starting the PCR setting, the user should prepare the required consumables such as 96-well plates, the sample will be precisely assigned to the appropriate wells in advance. The information should be recorded at any time.

6.1.1 PCR Reaction Procedure 1

Remove the Novel Coronavirus (2019-nCoV) RT-PCR Kit from the refrigerator and allow the reagents to thaw.

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Prepare PCR reaction Mix by referring to the reagents and volumes shown in table 1. The volume of each reaction well should be multiplied by the number of reaction wells. At the same time taking into account the number of samples in the analysis. The prepared PCR reaction Mix should be fully mixed and centrifuged for 10 seconds.

Table 1 Preparation of PCR reaction volume without adding clinical sample

Reagent component	PCR addition volume (μL)
PCR Master Mix	15
PCR PP	5
Total volume（per well）	20

For clinical samples, in each reaction well add 20 μL PCR reaction Mix firstly (see table 1) and in each reaction well add 5 μL extracted Sample (see table 2).

Table 2 Final PCR reaction volume after adding clinical samples

Reagent component	PCR addition volume (μL)
Total volume（per well）	20
Sample	5
Final volume（per well）	25

For the Positive Control and Negative Control, prepare PCR reaction Mix according to the reagent and volume shown in table 3 and the prepared PCR reaction Mix should be fully mixed and centrifuged for 10 seconds. In each reaction wells add 20 μL PCR reaction Mix respectively. (see table 3).

Table 3 Final PCR reaction volume after adding Positive Control/Negative Control

Reagent component	PCR addition volume (μL)
Total volume（per well）	20
Positive/ Negative Control	5
Final volume（per well）	25

For each Positive Control, add 5 μL Positive Control to the PCR reaction Mix. Similarly, for each Negative Control, add 5 μL Negative Control to the PCR reaction Mix. Include at least one

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Positive Control and one Negative Control.

Then the entire 96-well plate is placed in the centrifuge to ensure that the sample is centrifuged rapidly. The 96-well plate is placed into a PCR instrument to initiate the PCR process.

7. PROCEDURE SETTING

7.1 LightCycler 480 II PCR protocol

Please refer to the instruction on how to operate LightCycler 480 II Real-Time PCR instrument and data analysis.

A detailed list about different detection channels corresponding to the detection targets on Table 7. Set these dyes to report dyes.

Table7 Detector channels used to detect the corresponding of the 2019-nCoV targets using the LightCycler 480 II

Dye Channel	FAM	HEX/VIC	Cy5
Targets	ORF1ab	N gene	Actin

Set the detection format and use LightCycler 480 II to set the following settings:
Reaction volume: 25 μL. The test procedure is shown in table 8. Data of 45 cycles should be collected.

Table 8 PCR parameters for the Novel Coronavirus (2019-nCoV) RT-PCR Kit

Step	Temperature (℃)	Data collection	Time	Number of cycles	Analysis Mode
Reverse transcription	50	None	15min	1	None
Pre-denaturation	95	None	3 min	1	None
Extension	95	None	15 sec	45	Quantification
	60	Single	45 sec		
Cooling	37	None	10 sec	1	None

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7.2 ABI7500 PCR protocol.

Please refer to the instruction on how to operate ABI 7500 Real-Time PCR instrument and data analysis.

A detailed list about different detection channels corresponding to the detection targets on Table 6. Set these dyes to report dyes. ORF1ab gene and N gene from 2019-nCoV, Actin beta gene from human as Internal Control.

Table 6 Detector channels used to detect the corresponding of the 2019-nCoV targets using the ABI 7500

Dye Channel	FAM	HEX/VIC	Cy5
Targets	ORF1ab	N gene	Actin

Set the detection format and use ABI 7500 to set the following Settings:

Reaction volume: 25 μL. The test procedure is shown in table 10. Data of 45 cycles should be collected.

Table 10 PCR parameters for the Novel Coronavirus (2019-nCoV) RT-PCR Kit

Step	Temperature (℃)	Data collection	Time	Number of cycles	Analysis Mode
Reverse transcription	50	None	15min	1	None
Pre-denaturation	95	None	3 min	1	None
Extension	95	None	15 sec	45	Quantification
	60	Single	45 sec		

8. INTERPRETATION OF RESULTS

8.1 Positive and Negative Controls

At least one positive and negative control provided in the kit should be included in each analysis. Each negative or positive control should be prepared and tested in the same way as the patient samples.

Negative control that produce positive test result indicating samples contamination problem. A new "negative control material" should be repeated to ensure proper decontamination of the operating area and equipment.

Positive control that produce negative result indicating problems with the reagent or a wrong sample addition. Ensure that all reagents are stored correctly and tested within their expiry dates.

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Detection of two targets, ORF1ab gene and N gene, respectively. Only when the Ct value of both targets is <37, the test result is positive. Single Ct value<37 cannot be interpreted as positive, repeated test is recommended. Table 6 summarizes the results possible with the Novel Coronavirus (2019-nCoV) RT-PCR Kit.

Table 12 Analysis of detection results for the Novel Coronavirus (2019-nCoV) RT-PCR Kit

ORF1ab gene C_t Value	N gene C_t Value	Actin C_t Value	Result interpretation
< 37	< 37	>10 and <35	Positive
N/A or > 37	N/A or > 37		Negative
< 37	N/A or > 37		Retest
N/A or > 37	< 37		Retest
		<10 or >35	Retest

9. TROUBLESHOOTING

1. The Positive Control is not within the normal range

Reasons：The kit did not store in storage section of this Instruction for Use (IFU), or the kit has expired. One of the components was not added or incorrect PCR profile/programming.

Advices：Please check correct kit storage conditions has been followed. Check the expiry date of the kit box. Ensure that all components have been added and check your work steps procedure and check calibration of real-time PCR machine.

2. The Negative Control has generated a positive signal in one or more channels.

Reasons：Contamination occurred during the adding process.

Advices：Taking extra care when adding the templates, in particular the Positive Control. Make sure that the work area and instruments are properly decontaminated before and after use.

3. Very weak fluorescence signals

Reasons：Incorrect instrument settings. Incorrect real-time PCR mix

Advices：Check channel settings. Check if the PCR mixtures are prepared according to the protocol. Check expiry date and storage conditions.

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10. PERFORMANCE CHARACTERISTICS

The low limit detection of this product is 2×10^2copies/mL. The detection range was 2×10^2copies/mL~2×10^8 copies/mL.

The coefficient of variation (CV) values of intra batch error of this kit are less than 5%.

11. RISK AND SAFETY INFORMATION

The kit contains no harmful substances. The composition of all reagents in the kit pose no specific risk to the user or his property. Other chemicals and materials may be required for the procedures used in this instruction. Read carefully any warnings, instructions or material safety data sheets provided by the supplier and comply with general safety regulations when handling chemicals, biological hazards or other materials.

11.1 General Precautions

1. The product is ONLY for IVD use.

2. Do not use the kit if the label seal is broken upon receipt of the product. Before beginning the test, the packaging and sealing of the product as well as its shelf life must be checked. The product cannot be used after the specified period of validity.

3. Reagents from different test tubes or kits should not be mixed at will even if they come from the same batch. Also do not replace reagents from different manufacturers.

4. Long-term storage of low-concentration DNA is unstable, so the storage time of samples should be shortened as much as possible.

5. The samples tested by the kit are whole blood, plasma and serum.

6. The testing procedure should be carried out in accordance with the requirements of this instruction.

7. Ensure all required consumables are DNase/RNase free.

11.2 Biological risks

The Novel Coronavirus (2019-nCoV) RT-PCR Kit involves potentially dangerous and spreadable biological materials. Personnel using this kit must read and follow all

necessary health and safety precautions.

Technicians engaged in the collection of novel coronavirus detection specimens should be trained in biosafety and possess the appropriate experimental skills, and adopt third-level biosafety-level personal protection. It is very important to wear appropriate personal protective equipment during the operation; medical protective masks or N95 masks, double-layer latex gloves, face shields, goggles, protective clothing for work clothes, double-layer medical protective caps, and hand hygiene should be used. If necessary, double-layer masks (external medical protective masks, inner N95).

12. DISPOSAL

Proper handling this production will help to conserve natural resources. Be sure to recycle it in a way that prevents potential negative impacts on the environment and human health.

General disposal

Please dispose of unused reagents, wastes and transport materials in accordance with national and local regulations.

Packaging disposal

Please dispose of all packaging according to local recycling regulations.

13. MANUFACTURER

Company Name: Dynamiker Biotechnology (Tianjin) Co., Ltd.

Registration Address: No. 101-2, 14th Building, Ecological Science Park No. 2018 Zhongtian Revenue, Eco-City TEDA, Tianjin 300467, P. R. China

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Company Name: Wellkang Ltd.

Address: Suite B, 29 Harley Street, LONDON W1G 9QR, England, United Kingdom

[SYMBOLS USED]

The following symbols may appear on the label of the Novel Coronavirus (2019-nCoV) RT-PCR Kit or in the instructions for use.

Symbol	Description
	Use By
	Batch Code
	Manufacturer
	Keep Away from Sunlight
	Temperature Limitation
	In Vitro Diagnostic Medical Device
	Product reference number
	Authorized Representative in the European Community
	CE Mark

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