COVID-19 Real-Time PCR Kit

Ref: HBRT-COVID-19

Instruction Protocol

For Professional Use Only

Content

Application

The COVID-19 Real-time PCR Kit (HBRT-COVID-19) is designed for in vitro detection of any suspected infection by novel coronavirus that may induce severe coronavirus pneumonia (CNP) by using the targeting genes of ORFlab and N targeting genes, with B2M gene as internal control. Nasopharyngeal swab, Sputum and Alveolar lavage fluid samples from suspected patient could be used for testing.

Specification:

24 Tests/Kit

Principle of the test:

With use of multiplex real-time PCR technology, this kit can detect the presence or absence of RNA of COVID-19 (ORF1ab) and N region. Signals can be detected from the fluorescent reporter dye once it is separated from the quencher dye during the amplification process.

Kit Contents:

This kit is available for 24 tests.

Table 1: Composition of the kit

Kit contents	**Specification(tube)**	**Key Contents**
COVID-19 RT-PCR Mix	564µl	Probes, dNTP, $MgSO_4$
COVID-19 Enzyme Mix	36µl	Hot Start DNA Polymerase, Reverse transcriptase
Positive Control	50µl	COVID-19, B2M
Negative Control	50µl	Distilled water without RNA enzyme

Storage and Period of Validity:

Storage and Transportation:

The kit should be stored below -15℃. Repeated freeze/thaw more than 5 times should be avoided. Transporting the kit with both dry ice and frozen bags are suggested.

Period of Validity:

9 months period from the manufacturing date stated on the box

Compatible Instrument:

Real-Time PCR with FAM, HEX/JOE two detection channels. (e.g.: ABI 7500)

Sample Requirement:

Sample Collection

(1) Nasopharyngeal swab: Use a sterile swab to wipe the nasal and pharyngeal secretions and place them in a sterile test tube with 400-500 μL physiological saline or PBS, the test tube is plugged tightly with a sterile cotton ball for sample testing.

(2) Sputum: Rinse the mouth with clean water 3 times, and cough the sputum and collect with an aseptic Sputum collector. The sputum volume cannot be less than 1mL. Before extraction, liquefaction the sputum by adding equal volume of acetylcysteine (10g / L) to the sample, mixing at room temperature for 30 minutes, and then perform extraction.

(3) Alveolar lavage fluid: The bronchoalveolar lavage fluid was collected and sent for sample testing.

Sample storage

The samples can be stored for 24 hours at 2 ° C ~ 8 ° C, and can be stored for 3 months at -70 ° C.

Sample transportation

All samples must be transported with ice cool / ice-gel box and securely sealed and handled. Handling procedure should comply with the state's regulations for type 2 pathogens on biosafety regulations.

2. Sample Preservation and Delivery

If the specimen is not processed immediately, stored at 2℃~8℃, and perform tests within 24 hours. Storing at below -70℃, and perform tests within 8 months will be another option. Frequent thawing and freezing should be avoided.

Test Procedures:

The whole test is divided into 6 major parts as follow:

1. RNA Extraction
2. PCR Amplification
3. RT-PCR
4. Baseline and threshold value setting

1. RNA Extraction

Automatic nucleic acid extraction system from Qiagen, Thermofisher, Zinexts are compatible with our real-time PCR test kit. For other extraction system or manual extraction method, please consult with the technical support of Hybribio before using the test kit.

2. PCR Amplification

2.1 Take out COVID-19 RT-PCR Mix and COVID-19 Enzyme Mix from -15℃. Store at 4℃ after thawing. Mix contents well before use. Centrifuge at 8000r.p.m for 10 seconds.

2.2 COVID-19 Reaction MIX calculation and aliquot according to Table 2

No. of tests	RT-PCR Mix	Enzyme Mix
1 test	23.5μl	1.5μl
10 tests	235μl	15μl

2.3 Mix well PCR Reaction Mix and centrifuge the tubes at 8000r.p.m for 10sec. Aliquot 25ul PCR reaction solution into PCR tubes one by one. Adding 5ul extracted RNA sample into PCR reaction solution, and spin down.

2.4 The total volume of a reaction system in a PCR tube is 30ul, and set up a blank control and a positive control in a plate.

2.5 The total volume of a reaction system in a PCR tube is 25ul, and set up a blank control and a positive control in a plate.

Fluorescence detecting channel

Detector Name	Target	Reporter Dye	Quencher
FAM	COVID-19 ORF1ab	FAM	none
HEX	COVID-19 N region	HEX/JOE	none
Cy5	B2M	Cy5	none

Programs setting on Real-Time PCR

Program	Number of cycles	Temperature	Constant time	Sampling mode
1	1	55℃	15min	none
2	1	95℃	30sec	none
3	45	95℃	10 sec	none
		60℃	35 sec	Signal Taken
4	1	38℃	30 sec	none

3. Baseline and threshold value setting

Please consult instructions of companies for detail setting procedure.

For threshold selection: the threshold should be adjusted above the amplification line of negative control

Result Interpretation

1. Quality Control

1.1 The Ct value in any fluorescent detection channel of blank control should be ＞40 or Undet.

1.2 The Ct value in any fluorescent detection channel of positive control should be ≤34.

If above two situations achieved, this test is deemed to be valid.

2. Sample Result

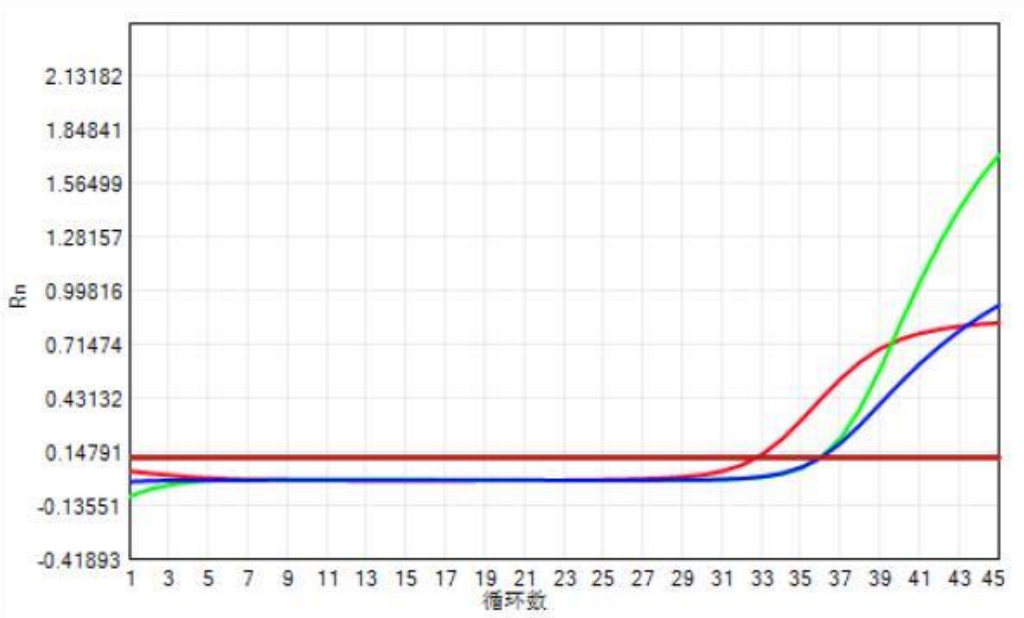
2.1 If the Ct value of a sample in FAM and HEX/JOE detection channel displayed as Undet, and the Ct value of internal control B2M in the fluorescent Cy5 channel is ≤40, considered the result to be negative.

2.2 If the Ct value of a sample in FAM and HEX/JOE detection channel is ≤40, and amplification curve presents, considered the result to be positive.

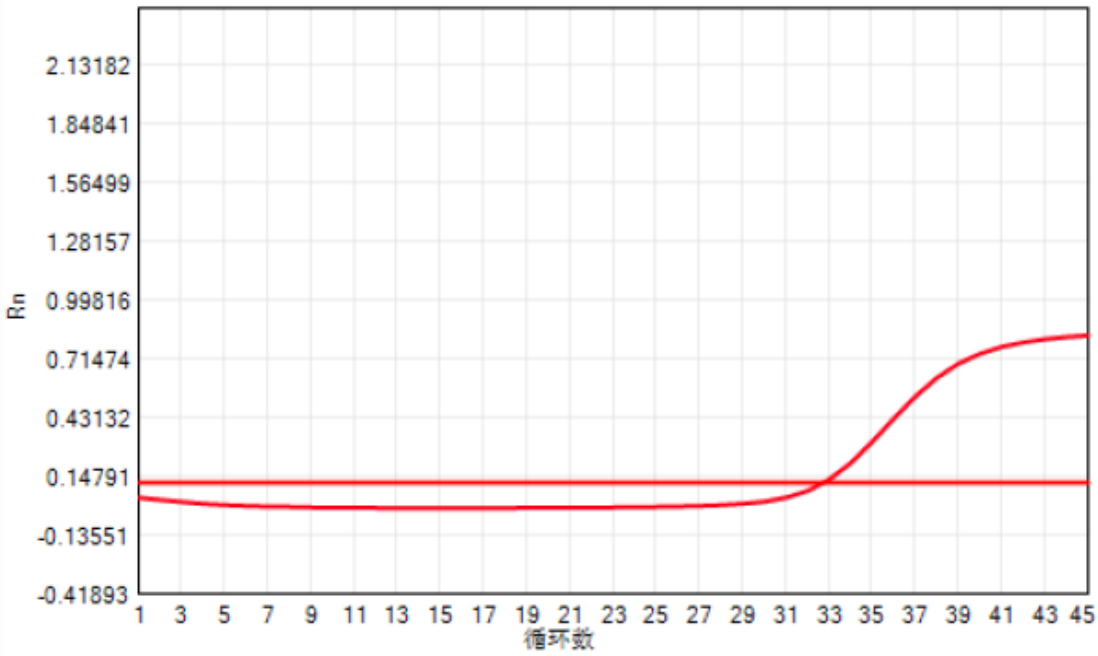
2.3 If amplification curve presents in either FAM or HEX/JOE detection channel, and the Ct value of a sample is ≤40, suggest repeating the test. If the Ct value is ≤40 and amplification curve presents then considered the result to be positive, otherwise it is negative.

Below are some examples of results:

Positive Sample



Negative Sample



Limitations of detection method

1. The kit is only used in detecting COVID-19(ORF1ab) and N region. It cannot be used in testing SARS
2. Don`t define all negative results as no COVID-19 infection, because whichever of elements of lower concentration of the content than the detection limit, undue sample collecting and transferring and processing, improper operating process and experimental environment could lead to false positive or false negative results.
3. The detection results and report should be considered as a part of strategy of patient management and other relative clinical examination.

Analytical Performance Characteristics

Lowest detection limit: 1×10^3copies/ml

Precise between two batch: CV% of CT value ≤ 5.0%

Precise within a batch: CV% of CT value ≤ 5.0%

When testing with other virus: (Including: influenza A virus, influenza B virus, respiratory syncytial virus, adenopathy Virus, parainfluenza virus, Middle East Respiratory Syndrome Coronavirus (MERSr CoV), human crown HCoV 229E, human coronavirus HCoV HKU1, human coronavirus HCoV NL63, Human coronavirus HCoV OC43, EB virus, human cytomegalovirus, etc.), there is no cross-reactivity.

Precautions:

1. All PCR preparation MUST be performed in PCR workstation ONLY.

2. NEVER bring extracted RNA samples or amplified PCR products into PCR workstation.

3. Spin all reagents down to the bottom of the tube WITHOUT any bubbles.

4. All PCR reagents must be stored in a pre-PCR area at -20℃ immediately when not in use.

5. Thawing the RNA Taq polymerase is not necessary and return it back to the freezer promptly after use.

6. Repeating freeze/thaw cycle of reagents should be avoided.

7. To prevent contamination of RNA amplification, DO NOT touch the mouth of the PCR reaction tube.

8. Clean all working bench before and after the test with 70% alcohol and 10% sodium hypochlorite (bleach).

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