Detection Kit for Coronavirus Disease 2019 (COVID-19)

(PCR Fluorescence Probe) Instruction

【Product Name】

Detection Kit for Coronavirus Disease 2019 (COVID-19) (PCR Fluorescence Probe)

【Packing Specification】

48 tests/ kit

【Intended use】

This kit is used to further determine the specific infection strain among people who have been diagnosed with COVID-19, the test results are for clinical reference and cannot be used alone as a basis for confirming or excluding cases.

This kit uses real-time PCR fluorescence technology to detect the COVID-19 nucleic acid of patients with convection like symptoms, patients with severe pneumonia and patients with asymptomatic infection.

The operators shall have professional training of gene amplification or molecular biological method detection, have the relevant experimental operation qualification, and the laboratory shall have reasonable biological safety prevention facilities and protection procedures.

【Test Principle】

This kit based on real-time PCR fluorescence technology, and the specific primers and corresponding fluorescent probes target to the conserved region of COVID-19genome was designed for highly specific detection. By detecting the fluorescent signals changes, the COVID-19 nucleic acid from a throat swab or nasopharyngeal secretion specimen can be qualitatively detected.

【Main Components】

Items	Name	Spec.	Constituents
PCR amplification kit	Reactive lyophilized powder	6 ×8 strip-tubes	Primers, Probes, dNTPs, PCR tubes, caps, inner control
	Resolution buffer	1200μL×1 Tube	PCR enhancer
	PC	100μL×1 Tube	target gene fragment
	NC	100μL×1Tube	target gene fragment

Notes:

The components from different batches should not be mixed.

The PCR tube with good thermal conductivity can achieve better experimental results, and even reduce the reaction time to less than 30 minutes.

【Storage conditions and the period of Validity】

The kits should be stored at 2-8℃with a validity period of 24months. In 15-30℃ the kit only can store with a validity period of 60 days.

【Applicable instruments】

Four channel instruments such as Singuway 9600 and BioRad cfx96 can be used in this experiment; But it takes nearly an hour. The rapid fluorescent PCR instrument, such as Singuway 9600PRO, is recommended and can complete the experiment in 30 minutes.

A real-time PCR instrument containing at least four colors fluorescence channels. Because of different PCR instruments are suitable for different PCR 8-strip tubes, We provide two types of PCR lyophilization tube:

PCR001FDY: Use 0.2ml 8-strip tubes which can suit for Thermofisher ABI7500/ Quant Studio3/ Quant Studio 5 Real Time PCR System, Slan 96P, Singu9600 PRO

PCR001FDYL: Use 0.1ml 8-strip tubes which can suit for Singuway Singu9600, Bio rad CFX96, Roche LightCycle 480.

【Sample requirement】

1. Applicable sample type: Throat swab (Oropharyngeal swab/Nasal swab),
2. Sample collection

2.1 Sample time: The reagent is not affected by the specific clinical symptoms of patients, and can be sampled at each stage of the course of the disease.

2.2 The test performance of the reagent is not affected when using the drugs described in the analysis performance evaluation, and the sample can be taken normally. It is better to take samples before using other unverified drugs

2.3 Throat swab requirement: It's better to use long handle swab with polypropylene fiber head or wood handle swab, please select the preservation solution suitable for cells and viruses. It is better to directly select the commercial throat swab sampling tube.

3. Sampling method:

3.1 Oropharyngeal swab: Wipe both tonsils and the posterior pharyngeal wall with a swab with moderate force to avoid touching the tongue.

3.2 Nasal swab: Use the swab to penetrate the nasal cavity for 2-3cm, and rotate it for 2-3times.

【Preservation and delivery of samples】

The samples for detection should be stored at under the following conditions: less than 24hours at 2-8℃, less than 1 month at -20℃, or a longer time at -70℃. And repeated freezing and thawing shall be avoided (freezing and thawing shall not exceed 3 times).

An ice transfer box was used for specimen transportation.

【Test method】

1. Nucleic acid extraction (In the sample processing area of the lab)

1.1 It is recommended to use the virus nucleic acid extraction kit, centrifugal column or magnetic bead method by Singuway Company. Nucleic acid was extracted from about 200μLsamplerefer to the instructions of the nucleic acid extraction kit.

1.2 Centrifugation column method, magnetic bead method and common methods of nucleic acid purification can be used in this experiment, while direct lysis method and other methods that cannot purify nucleic acid are also suitable for this experiment

2. PCR reagent preparation (In the reagent preparation area)

2.1 Prepare PCR reaction solution according to the following composition 20μL Resolution buffer in Reactive lyophilized powder

3. Add samples (In the sample processing area)

5μL of the treated samples (positive control and negative control direct add 5μL), added to the PCR reaction tube containing the PCR reaction solution. Cover the cap, mixed well and performed short spin to allow the reaction

solution to sink to the bottom of the tube.

4. PCR amplification (In the amplification test area)

4.1 Load the PCR reaction tubes into the real-time PCR instrument for amplification.

4.2 Amplification program setup:

ABI7500 ：

Step	Cycle	Temp. (℃)	Time	Collect fluorescence signal
1	1	50	5:00	No
2	1	95	0:30	No
3	40	95	0:02	No
		60	0:30	Yes

Roche 480，Q5，7500fast：

Step	Cycle	Temp. (℃)	Time	Collect fluorescence signal
1	1	50	5:00	No
2	1	95	0:30	No
3	40	94	0:01	No
		60	0:13	Yes

Instrument detection channel selection: FAM is ORF1a/b region amplification signal, HEX/VIC is E gene region amplification signal, ROX is N gene amplification signal, CY5 is Inner control amplification signal.

1. QC standard

Negative control: FAM/HEX/ROX results are negative

COVID-19 positive: FAM/HEX/ROX ： at least 2 of them are positive and 24＜ Ct＜30

The above items must be met at the same time in one experiment, otherwise, this experiment is invalid and the experiment should be repeated.

【Result judgement】

1. Negative result determination:
   If the FAM/VIC/ROX channel amplification curve has no significant logarithmic growth curve; at the same time, the CY5 channel has logarithmic growth curve, and the internal control result Ct value is less than 36, the result is negative. If there is no significant logarithmic growth curve in the CY5 channel, the samples should be rechecked.
2. Determination of positive results:
   If the FAM/VIC/ROX channel have logarithmic growth curve, and the Ct value is ≤38, the result determined that COVID-19 is positive.
3. Experimental gray area:
   If the FAM/VIC/ROX channel has only logarithmic growth curve, and 38<Ct value<45, the result is judged as located in the experimental gray area, and the sample should be rechecked. If there is obvious logarithmic growth curve in the retest result, the result can be judged positive; if there is no obvious logarithmic growth curve, the result is negative.

4.The “recheck” operations mentioned in steps 1 and 3are as follows:

It is recommended to concentrate the sample: take 1ml well-mixed sample; centrifuge12000rpmfor 5min at 4℃; carefully remove the supernatant and leave about 200μLbottom layer as the sample. Then perform the extraction and amplification same as the general sample.

Table 1. The judgement of different results.

Gene ORF（FAM）	Gene E（HEX/VIC）	Gene N（ROX）	Inner Control（Cy5）	Negative Control	Positive Control	Interpretation
+	+	+	+/-	-	+	SARS-COV-2 POSITIVE
-	-	-	+/-	-	+	SARS-COV-2 NEGATIVE
+	+	-	+/-	-	+	SARS-COV-2 POSITIVE
+	-	+	+/-	-	+	SARS-COV-2 POSITIVE
-	+	+	+/-	-	+	SARS-COV-2 POSITIVE
+	-	-	+/-	-	+	SARS-COV-2 POSITIVE
-	-	+	+/-	-	+	SARS-COV-2 POSITIVE
-	+	-	+/-	-	+	SARS-COV-2 Presumably POSITIVE
+	+	+	+	+	+	NO VALID
-	-	-	-	-	-	NO VALID

【Reference value (reference ranges)】

The threshold reference Ct value of this kit is equal to 38.

【Product performance index】

1. **Minimum detection limit (analysis sensitivity):**

The assay sensitivity of this reagent was 500copies/mL

2. **Enterprise reference test**

Ten factory positive reference sample and 10 negative reference samples were tested, and the coincidence rate was 100%.

One enterprise limited reference was performed for 20 tests, the result should be at least 17are positive.

One enterprise precision reference with 10^4 copies/mL pseudo virus was performed for 10 tests respectively, and the CV of Ct value was less than or equal to 5%.

3. **Analysis specificity**

3.1 Cross reaction:

Viruses below the following concentrations have no cross reaction with this kit.

Sample name	Concentration	Sample name	Concentration
Flu A（H1N1）	1.1×10^5TCID50/ml	A stream (H3N2)	2×10^6TCID50/ml
Flu B	3.2×10^8TCID50/ml	Respiratory Syncytial Virus Type B	8.9×10^8TCID50/ml
Adenovirus (Type 3)	3.2 x 10^5 TCID50/ml	Rhinovirus Type 1A	3×10^7TCID50/ml
Adenovirus (type 7)	1.1 x 10^6 TCID50/ml	Chlamydia pneumoniae	6×10^5CFU/ml
Human Coronavirus HCOV-OC43	3.7×10^7 TCID50/ml	Human Coronavirus HCOV-HKU1	3.8×10^5PFU/ml
Cytomegalovirus	1.9×10^6 PFU/ml	Mycoplasma pneumoniae	1.2×10^5CFU/ml
Enterovirus EV71	2.1×10^5 PFU/ml	Mycobacterium tuberculosis	1×10^8CFU/ml
Human parainfluenza virus (type 1)	1.7 x 10^7TCID50/ml	Legionella pneumophila	3.3×10^9CFU/ml

3.2 Interfering substances:

Endogenous interfering substances, such as blood and nasopharynx secretion, do not interfere with the detection of this kit. Zanamivir, epinephrine, budesonide, sulfur, Jinying, menthol, mupirocin and tobramycin, which are common therapeutic drugs, do not interfere with the test results of this kit.

3.3 Genotype detection capability

This kit have the ability to detect conventional strains and common mutants such as α(b.1.1.7), γ（P.1）, δ（b.1.617）

4. **CLINIC study**

		Normal RT-PCR		
		Positive	Negative	Total
Singuway	Positive	30	0	30
	Negative	0	220	220
	Total	30	220	250

【Notice】

1. This product is only used for in vitro diagnosis; please read this manual carefully before use.
2. In order to avoid any potential biological hazard in the sample, the test sample shall be regarded as infectious substance to avoid contact with skin and mucous membrane; it is recommended to operate the sample in the bio-safety cabinet which can prevent the outflow of air mist, and the sample operation and treatment shall meet the requirements of relevant laws and Regulations: General guidelines for bio-safety of microbiological biomedical laboratory and medical waste management issued by the Ministry of health Regulations.
3. The positive control substance of the kit is artificial synthetic nucleic acid which has no potential infectivity; the negative control substance of the kit is cell culture medium, which belongs to human source substance. Although it has passed the tests of HBs-Ag, HIV1/2-Ab, HCV-Ab and other items, until now, there is no test can ensure absolute safety, so the above components should be treated as infectious substance during operation.
4. The experimental operators shall have received the professional training of gene amplification or molecular biological method detection, and have the relevant experimental operation qualification. The laboratory shall be equipped with proper biosafety prevention facilities and protection procedures, and the laboratory management shall be carried out in strict accordance with the management specifications of relevant national molecular biology laboratories and clinical gene amplification laboratories. The experimental process shall be carried out in different areas such as reagent preparation area, sample preparation area, amplification and product analysis area. Special instruments and equipment shall be used in each stage of the experimental operation, and supplies in each stage of each area shall not be used interchangeably. Strict requirements shall be set for personnel flow and air flow in each section to avoid cross contamination to the greatest extent. Consumables, such as centrifugal pipe, suction head, etc. for the experiment shall be proper cleaning and quality control procedures to avoid RNase contamination or amplification of reactive inhibitors resulting in false negative results.
5. Each component of the kit should be thoroughly melted and shaken before use, but repeated freezing-thawing

should be avoided. The reagent in the centrifuge tube should be centrifuged for several seconds before use.

6. After the nucleic acid extraction of the sample is completed, it is recommended to conduct the next experiment immediately. Otherwise, please store the sample at -20℃ and complete the test within one week. The sample pyrolysis products stored at -20℃ should be thawed at room temperature before sample addition and used after a short time centrifugation.

7. When reactants are packaged separately, bubbles should be avoided as far as possible. Before loading, please pay attention to check whether the cover of each reaction tube is tight, so as to avoid leakage of fluorescent substances and contamination of the instrument.

8. After the experiment, 50ppm~2000ppm sodium hypochlorite or 75% alcohol and ultraviolet lamp are needed to treat the table and pipette.

9. According to the current experience of the epidemic, the virus may survive for a long time in the intestine. It is suggested to measure the anal swab at the same time.

10. Medical waste generated in the test should be treated in accordance with local regulations.

【label instruction】

Label	Instruction	Label	Instruction	Label	Instruction
IVD	IVD medical devices		Important warning information		Do not use if package is damaged
	Refer to instructions for use		Do not reuse		Stacking limit by number
	Potential biological risks associated with medical devices		Avoid humidity and keep dry		Storage and transportation temperature range
	Fragile items, handle with care		Manufacturer	EC REP	Authorized representative in the European Community
	Upward	CE	Meet EU directives 98/79 EEC	REF	Catalogue number

【Basic information】

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