

Instruction of SARS-CoV-2 Nucleic Acid Dual-Detection Kit(Real-Time PCR Method)

【Product Name】

Common name: SARS-CoV-2 Nucleic Acid Dual-Detection Kit(Real-Time PCR Method)

【Packing Specification】 25 tests/pack、50 tests/pack

【Intended Use】

An unexplained pneumonia epidemic broke out in Wuhan at the end of 2019. On February 11, 2020, the World Health Organization (WHO) has named the virus as SARS-CoV-2 (2019 Novel Coronavirus).

The product is used for clinical qualitative detection of SARS-CoV-2 nucleic acid in specimens such as throat swabs, nasal swabs, the extract of nasopharyngeal, sputum, the extract of respiratory tract, bronchial perfusate, alveolar lavage fluid, lung tissue, stool, urine, blood or serum.

【Test Principle】

The kit designs pairs of specific primers and Taqman probes for the conserved regions of the SARS-CoV-2 (2019 Novel Coronavirus) N gene and ORF1ab gene sequence which recently announced on the GISAID. The SARS-CoV-2 nucleic acid in the specimens is qualitatively analyzed by using one-step Real-time PCR detection technology after RNA extraction. The kit uses human-derived ribonuclease P (RNP) as an Internal control gene, which can monitor the sample collection and extraction process to avoid false negative generation to the greatest extent.

【Main Components】

Component	25T	50T
1. N/orf1ab RT-PCR Mix	1vial (425μL)	1vial (850μL)
2. N/orf1ab primer and probe Mix	1vial (50μL)	1vial (100μL)
3. N/orf1ab Enzyme Mix	1vial (25μL)	1vial (50μL)
4. Negative control	1vial (30μL)	1vial (30μL)
5. N/orf1ab positive control	1vial (30μL)	1vial (30μL)
6. Instruction	1 copy	1 copy

Note: The components of different batches of kits can not be used interchangeably.

【Storage and Validity】

1. All the reagents should be stored below -15℃ in dark. The term of validity is 12 months. The foam box which contain biological ice bag is sealed for transportation, and the temperature should not exceed 8℃; the production date and expiring date are printed on the packing box.

2. Repeated free-thaw should be avoided (<5 times)

3. After unsealing, it should be stored below -15℃ in dark, and used before the expiring date.

【Applicable Instruments】

1. Suitable for ABI7500、Stratagene 3000P/3005P、Roche lightcycler480 Real-time fluorescence quantitative PCR Instrument

2. No listed other models have not conducted or completed relevant experiments with the kit. If the users need to use this kind of instruments to carry out detection with this kit, please contact the technical support department of our company for relevant supports.

【Sample Requirement】

1. Nasal swab and Throat swab. The concrete operating procedure:

1) Nasal swab: Insert the wet sterile swab parallel to the upper jaw to one side nostril into the inner nasal palate of the nasal canal with gently rotating the swab. Generally, when there is resistance to the swab insertion, stay the swab for 2~3s and then slowly rotate to exit.

2) Throat swab: Hold a tongue spatula against the posterior root of tongue, the other hand hold the root of the wet sterile swab, and then quickly and

vigorously scrape the from both sides of the tonsil and the posterior wall of the pharynx with both sides and the head of the swab

3) Place the nasal swab and the Throat swab together in a centrifuge tube containing 1.0ml of normal saline.

2. Blood or serum sample: Draw the sample with sterile injection and place in a centrifuge tube for testing.

3. Avoid cross contamination between samples.

4. Samples should be tested in time, or stored at -20 ± 5℃ waiting for Detection. Long-time storage should be placed below -70℃.

【Detection Procedures】

1. Reagent preparation: (Reagent preparation area)

1) Remove the kit from the refrigerator below -15℃ to balance at room temperature (20~25℃), shake and centrifuge at low speed for 10s after complete dissolution.

2) Check the reaction number (n), and calculate the test dose according to the reaction system preparation method from Table 1.

$$N = \text{Negative control (1T)} + \text{Positive control (1T)} + \text{Error reserve} + \text{Samples}$$

Table 1: Reaction system preparation method

Single reaction system formulation	N/orf1ab RT-PCR Mix	17μL
	N/orf1ab primer and probe Mix	2μL
	N/orf1ab Enzyme Mix	1μL

3) Add the reagents into a sterile centrifuge tube with appropriate volume, centrifuge at a low speed of 2000rpm for 10s after fully mixing. And divide into eight-link PCR reaction tubes at 20 μL/tube.

4) Cover the eight-link PCR tubes tightly and pay attention to the identification (Please mark the protruding sites at both ends of eight-link PCR tube cap. Do not mark the middle of eight-link PCR tube cap to avoid affecting signal collection.). Place the PCR tube to the sample preparation area. Put the remaining reagents back to the refrigerator below -15℃.

2. Sample preparation: (Sample preparation area)

1) RNA extraction:

Take 100μL sample on the basis of the procedure in the extraction kit instructions.

2) Sampling:

A. Remove the prepared reagents from the reagent preparation area, centrifuge at low speed for 10s.

B. Open the cap of PCR tube and add 5μL of corresponding sample template to each tube. The same 5μL would be added for Positive and negative control

C. Cover the PCR tube, record the template adding order, then centrifuge at low speed for 10s.

D. Place the PCR tube to the nucleic acid amplification region for loading.

Notes: Contamination should be avoided during the extraction of sample RNA and sampling. If the extracted RNA template can not be test immediately, it is recommended to store below -70℃.

3. PCR: (Nucleic acid amplification region)

1) Warm up the machine and check the performance of the instrument.

2) Take place the PCR tube from sample preparation area to the sample tank (Ensure all reaction tubes are covered tightly before loading to avoid aerosol made by the leak of PCR products contaminate instrument and environment). And record the order of placement.

3) Carry on the PCR amplification and set the instrument according to parameters of relevant nucleic acid amplification in Table 2.

Table 2: Nucleic acid amplification parameters

System	Reaction system is 25μL
Signal Collection	SARS-CoV-2 (N gene) — FAM channel collects the fluorescence signal;

	SARS-CoV-2 (ORF1ab gene) — HEX/VIC/JOE channel collects the fluorescence signal; RNP—ROX channel collects the fluorescence signal.		
PCR Reaction Conditions	Phase	Condition	Cycle number
	Reverse transcription	50°C: 30min	1
	Predegeneration	95°C: 3min	1
	PCR	95°C: 5s 55°C: 30s (Collect the fluorescence signal at the end of this phase)	45

Notes: ABI fluorescence quantitative PCR instrument do not select ROX correction, quenching group select None.

【Results Analysis】

The result would be automatically saved after reaction, according to the analyzing curve regulating the Start Value, End Value and Threshold Value of baseline (Adjust by physical truth, the Start Value can be in the range of 3~15, the End Value can be in the range of 5~20, adjust the curve of the negative control to be straight or below the threshold). Click Analysis to automatically obtain the results, and check them in Report interface.

【Reference Range】

1. Quality Control:

- 1) Positive control: N gene and ORF1ab gene, Ct value ≤ 38 , with significant exponential growth.
- 2) Negative control: N gene and ORF1ab gene, Ct value > 41 or no Ct value; amplification result of Internal control gene, Ct value ≤ 40 .
- 3) The above requirements must be in the same experiment, otherwise the test is invalid.

2. Determination:

- 1) FAM channel: The Ct value ≤ 38 of the sample, with an obvious exponential growth period, which was judged to be positive. The Ct value in the range of 38-41 of the sample detection results, the sample should be tested repeatedly. If the Ct value ≤ 41 of the repeated test results and there is an obvious exponential growth, it is judged as positive, otherwise it is negative. The Ct value > 41 or no Ct value of the sample, and the Ct value ≤ 40 of the amplification result of the Internal control gene, which was judged to be negative.
- 2) HEX/VIC/JOE channel: The Ct value ≤ 38 of the sample, with an obvious exponential growth period, which was judged to be positive. The Ct value in the range of 38-41 of the sample detection results, the sample should be tested repeatedly. If the Ct value ≤ 41 of the repeated test results and there is an obvious exponential growth, it is judged as positive, otherwise it is negative. The Ct value > 41 or no Ct value of the sample, and the Ct value ≤ 40 of the amplification result of the Internal control gene, which was judged to be negative.
- 3) If the test results of the two channels of the sample are negative, and the Ct value > 40 of the amplification result of the Internal control gene, then re-sampling, extraction and detection to be required.

【Interpretation of Result】

	Channels and result			Result and explanation
	FAM	HEX/VIC/JOE	ROX	
1	+	-	±	N gene of SARS-CoV-2 in sample
2	-	+	±	ORF1ab gene of SARS-CoV-2 in sample
3	+	+	±	N gene and ORF1ab gene of SARS-CoV-2 in sample
4	-	-	+	No SARS-CoV-2 in sample
5	-	-	-	Re-sampling, extraction and detection

Notes: 1. The conclusion of sample testing should be determined according to *The Technical Guidelines for Laboratory Testing of Pneumonia Caused by SARS-CoV-2 Infection*.

2. N/ORF1ab double target are positive, can be judged as the SARS-CoV-2 positive; If the single target is positive, it is recommended to re-sample for testing. If the retest result is still positive for the same target, it can be judged as positive for the SARS-CoV-2.

【Performance Index】

1. Minimum detection limit: The detection limit of this kit for testing treated sample is 1.0×10^3 copies/ml.
2. Cross reaction: The specificity test showed that this reagent had no cross reaction with chosen specific references, for instance, influenza A virus H1N1, seasonal influenza A (H3N2) virus, influenza B virus /Yamagata, influenza B virus /Victoria, respiratory adenovirus type 3, human coronavirus OC43. The result of SARS-CoV-2 diagnosis would not be significantly affected in blood (5%), snot (5%), saliva (25%), mucin (0.35 mg/ml), leukocyte (5.0×10^5 unit/ml), cephalosporin (1 mg/ml), budesonide (1.28mg/ml), Jinying (0.1 g/ml), hydroxymethazoline (0.5 mg/ml), beclomethasone (1.54 mg/ml).
3. Precision: the coefficient of variation (CV%) is less than 5%.

【Limitation】

1. The test results of this kit are for clinical reference only. The clinical diagnosis and treatment of the patient should be considered in combination with their symptoms/signs, medical history, other laboratory tests and treatment response.
2. The improper operation of the tested samples in the process of collection, transportation, storage and nucleic acid extraction can easily lead to RNA degradation and false negative results.
3. False negative results may occur when the concentration of nucleic acid detected in the sample is less than the minimum detection limit.
4. If cross contamination occurs during sample collection and preparation, false positive results are easily obtained.
5. A large number of dead viruses appear in the samples of some infected patients due to the antiviral drug administration. At this time, the test results of this kit are strong positive and the culture method are negative. Then, the patient should be inquired about the recent drug administration.
6. Mutations or other reasons caused of sequence changes may lead to false negative results.
7. For emergent novel viruses, the optimal sample type and the optimal sampling time after infection may not have been confirmed. Therefore, the possibility of false negative results will be reduced if samples are collected in the same patient at different times and multiple sites.

【Precautions】

1. Laboratory management shall be carried out in strict accordance with the "laboratory management measures for clinical gene amplification test in medical institutions" issued by the general office of the ministry of health.
2. The experimenter must have professional training and experience.
3. The experimental process should be carried out in different areas (reagent preparation area, sample processing area, nucleic acid amplification area). Special instruments and equipment should be used at each stage of the experimental operation. There should be strict requirements on the flow of people and air in each section to minimize cross-contamination.
4. There should be reasonable cleaning and quality control procedures for consumables used in experiments (such as centrifugal tube and tips) to avoid false positive results caused by contamination or false negative results caused by amplification reaction inhibitors.
5. Before use, the instrument and the supporting power supply system should be preliminarily checked to ensure the normal operation of the instrument after the reagent is put on the machine.
6. The tips used in the experiment, please directly discard into the waste tank containing 10% sodium hypochlorite with other waste items.
7. Tables and various laboratory items should be often disinfected with 10% sodium hypochlorite, 75% alcohol and ultraviolet lamps.
8. The fluorescent PCR instrument should be used to be corrected, and clean the plate holes frequently.
9. To prevent fluorescence interference, avoid contact with the eight-link PCR tube and tube cap with hand directly.

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10.The positive control in this kit is not infectious and will not harm the human body. However, it is recommended to treat it as a potentially infectious substance.

11.The samples should be deemed to be infectious substances, and should be handled and disposed in accordance with the *general guidelines for biosafety in microbiology and biomedical laboratories* and *the regulations on medical waste management* of the ministry of health.

【Basic Information】

Registrant /Manufacturer: Wuhan Life Origin Biotech Joint Stock Co., Ltd.

Address: Wuhan Hi-tech Medical Devices Park, Building B11, #818 Gaoxin Road, Donghu Hi-Tech Development Area, Wuhan, Hubei Province 430206, P.R. China

Contact : 027-87196282

Aftersales Service: Wuhan Life Origin Biotech Joint Stock Co., Ltd.

Contact: 027-87926888

Manufacturing Site: Wuhan Hi-tech Medical Devices Park, Building B11, #818 Gaoxin Road, Donghu Hi-Tech Development Area, Wuhan, Hubei Province 430206, P.R. China

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【Modification Date】

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