SARS-CoV-2 Test Kit (Real-time PCR)

CE

This package insert must be read carefully prior to use and should be carefully followed. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

REF **801301**

48 Tests per Kit
***In Vitro* Diagnostic Medical Device**

For Professional Use Only

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1. INTENDED USE

This kit is used for in vitro qualitative detection of novel coronavirus (SARS-CoV-2) ORF1ab and N gene in samples, including nasopharyngeal swab, oropharyngeal swab, sputum, bronchoalveolar lavage fluid and stool, for diagnosis of the COVID-19 infection, the suspected cluster cases and of the patients who need differential diagnosis of COVID-19 infection.

2. PRINCIPLE OF THE PROCEDURES

This kit used a multiplex Taqman probe-based one-step reverse transcription polymerase chain reaction (RT-PCR), which enables simultaneous qualitative detection of ORF1ab and N gene of SARS-CoV-2 as well as a non-human internal control (Armored RNA for *SUC2*) in one reaction. For ease of storage and transportation, the amplification reagent is designed as a pre-distributed dry reagent .

3. REAGENTS AND MATERIALS SUPPLIED

The SARS-CoV-2 Test Kit contains reagents for 48 tests, components are tabulated as below.

COMPONENTS		MAIN INGREDIENT	QUANTITY
Amplification Reagents	SARS-CoV-2 RT-PCR tube	Primers, probes, dNTPs, MMLV Reverse Transcriptase, Taq polymerase	8 tests / strip (6 strips)
Control Reagents	SARS-CoV-2 internal control	Armored RNA for *SUC2*	51 tests / tube (1 tube)
	SARS-CoV-2 positive control	Armored RNA for ORF1ab and N	10 tests / tube (1 tube)
	SARS-CoV-2 negative control	No template control（Tris buffer）	10 tests / tube (1 tube)
Transparent Replacement tube cap		/	6 ranks

Note: Do not interchange reagents from one kit lot to another.

4. INSTRUMENT

The kit can be operated by the real-time PCR thermal cycler with FAM, HEX and ROX detection channels (e.g., Zeesan SLAN-96S).

5. MATERIALS REQUIRED BUT NOT PROVIDED

- Disposable powder-free gloves
- Zeesan Nucleic Acid Extraction System
- Adjustable pipettes and sterile filtered pipette tips, 1.5 mL and 2.0 mL microcentrifuge tubes
- Vortex mixer
- Hand-held centrifuge for 8-tube Strips or 96 plates as well as 1.5 mL microcentrifuge tubes

6. STORAGE AND EXPIRATION DATE

The kit should be stored at 2~8℃ away from light, temporarily valid for 12 months.
It is recommended to transport between -18℃ to 37℃. Production date and expiration date are shown on the package label.

7. SAMPLE REQUIREMENTS

Sample types: Upper respiratory tract sample includes nasopharyngeal swab and oropharyngeal swab, lower respiratory tract sample includes bronchoalveolar lavage fluid and sputum, and stool sample can be anal swab.

Storage conditions of samples: Samples collected in common saline buffer without disinfection should be submitted timely for testing, and those tested within 24 hours should be stored at 4℃ or below; For samples stored longer than 24 hours, preferably stored at -70℃ or below. Samples collected in special buffer with disinfection/stabilization of virus RNA, please refer to the instruction manual.

For extraction method of different sample types, please refer to "Specimen extraction and loading" in the test procedure.

8. TEST PROCEDURE

8.1 Reagent Preparation (Solution Preparation Area)

a. Take out the aluminum foil bag from the kit, tear the seal, open the self-sealing strip.

b. Open the blister packaging of the SARS-CoV-2 RT-PCR tube, take out n PCR tubes (n is determined according to the needs of the current experiment), the remaining reagents should be put back into the aluminum foil self-sealing bag and sealed tightly, and stored at 2~8℃ away from light.

8.2 Specimen Extraction and Loading (Extraction Area)

a. It is recommended that using the nucleic acid extraction kit of Xiamen Zeesan Biotech Co., Ltd. (Cat. No.606104) for extraction.

Add 20 μL of internal control and less than 2 mL of liquid sample to the extraction loading well, and carry out extraction according to the procedures in the instruction manual of the extraction kit. For quality control, 20 μL of SARS-CoV-2 positive control and 20 μL SARS-CoV-2 negative control are added into different extraction loading wells respectively with 20 μL of SARS-CoV-2 internal control, and start extraction in parallel with the samples to be tested.

NOTE: When start using SARS-CoV-2 positive control, SARS-CoV-2 negative control, and SARS-CoV-2 internal control, 200 μL, 200 μL and 1020 μL of sterilized purified water should be added respectively for dissolution. This is done by vortex for 20 seconds followed by a brief spin. They should be stored below -18℃, repeated thawing and freezing for not more than 5 times.

b. Open the new RT-PCR tubes and discard the tube lids, add 25 μL of sample extracts or SARS-CoV-2 positive / negative control extracts to each PCR tube containing lyophilized reagents, then immediately cover the tubes with the attached caps.

NOTE: This kit adopts positive control and negative control for quality control, which better to be used for each batch of testing.

c. The reaction tubes are subject to vortex for 20 s followed by a brief spin to remove any air bubbles.

d. Transfer the reaction tubes to the PCR amplification area.

8.3 PCR Amplification (Amplification Area)

The amplification program below is based on an automatic medical PCR analysis system (SLAN-96S).

a. The reaction program. (The reaction system of the kit is set as 25 μL)

Stage		Condition	Cycle number
PCR Reaction Program	RT reaction	50 ℃ 15 min	1
	Predegeneration	95 ℃ 2.5 min	1
	PCR cycle	94 ℃ 15 sec 60 ℃ 30 sec (FAM, HEX and ROX channels)	45

b. After the program is completed, remove the PCR tubes (closed) out and put them into a self-sealing (zip) bag and seal tightly and treat them as pollutant source.

9. REFERENCE RANGE

The controls in the kit must meet the following requirements, otherwise the experiment will be considered invalid. The ranges of Ct values in each test channel (FAM, HEX, ROX) are as follows：

Control	Test Channel		
	FAM	HEX	ROX
SARS-CoV-2 positive control	≤ 32	≤ 32	≤ 27
SARS-CoV-2 negative control	No Ct or ≥ 40	≤ 32	No Ct or ≥ 40

10. EXPLANATION OF RESULT

Ct values of each channel are used to determine whether there are corresponding targets to be tested.

Target Gene	Test Channel	Critical Ct value	Results
ORF1ab	FAM	≤ 37	+
		> 37 or No Ct	-
N	ROX	≤ 35	+
		> 35 or No Ct	-
SUC2	HEX	≤ 34	+
		> 34 or No Ct	-

According to the test results of the above three channels, there may be several conclusions for each sample:

Test Results			Conclusions
ORF1ab	**N**	***SUC2***	
+	+	+	SARS-CoV-2 positive
+	+	-	SARS-CoV-2 positive
+	-	+	Suspected SARS-CoV-2 positive; It is suggested to retest or adopt other methods for verification.
+	-	-	The sample may be suppressed and should be resampled and retested.
-	+	+	Suspected SARS-CoV-2 positive; It is suggested to retest or adopt other methods for verification.
-	+	-	The sample may be suppressed and should be resampled and retested.
-	-	+	SARS-CoV-2 negative
-	-	-	The sample may be suppressed and should be resampled and retested.

Note：“+” refers to positive；“-” refers to negative.

11. LIMITATION OF THE METHOD

1) PCR product contamination might occur in the laboratory, reagent preparation and cross-contamination of samples and will produce false positive results. The component of the test kit may decline due to improper transportation, storage or inaccurate preparation and will produce false negative results.
2) Low viral load and excessive degradation in the samples may cause negative results. Thus a negative result cannot completely exclude the existence of SARS-CoV-2 in the sample.

12. PERFORMANCE CHARACTERISTICS

1) Repeatability: the precision references were tested, the results were all SARS-CoV-2 positive, and the CV of Ct values is no higher than 5%.
2) Specificity: a) Cross-reaction: human genomic DNA and a total of 29 other pathogens (*Streptococcus pneumoniae, Haemophilus influenzae, Klebsiella pneumoniae, Cryptococcus, Citrobacter, Serratia marcescens, Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, Acinetobacter baumannii, Candida albicans, Streptococcus pyogenes, Streptococcus salivarius, Streptococcus oralis,* Influenza A virus H1N1 - 2009, Influenza A virus H3N2, Influenza B Virus Yamagata, Influenza B Virus Victoria, Respiratory syncytial A virus, Respiratory syncytial B virus, Coronavirus OC43, Coronavirus NL63, Coronavirus 229E, Coronavirus HKU1, Parainfluenza virus (type I), SARS, MERS and adenovirus) were tested, and there were no cross-reaction. b) Interference test: the common interfering substances in the samples, such as Oxymetazoline, Dexamethasone, Zanamivir, Mupirocin, etc, have no effect on the test results.
3) The limit of detection for the kit is: 200 copies/mL.
4) A small clinical study enrolled 280 patients including 240 common cold and 40 COVID-19 infection was carried out between February 6 to 20, 2020 in three hospitals of Xiamen, China. The kit correctly identified all of the 40

COVID-19 infection confirmed patients, reaching a clinical sensitivity of 100%. None of the 240 non-COVID-19 patients who were confirmed to be common cold was positive, showing a clinical specificity of 100%.

13. WARNING AND PRECAUTIONS

1) This kit is only used for in vitro diagnosis. Operators must be trained and have certain experience. Please read the instructions carefully before using the kit.
2) Please conduct the test operation in strict accordance with the management standards of gene amplification test laboratory: for example, the PCR test shall be strictly divided into different sections; There should be special gloves and pipettes in each district, and they should not be cross-used to avoid contamination; Operators should follow the principle of one-direction from zone one to zone two, and each working area is relatively isolated; The work table and related items for PCR test should be sterilized and disinfected regularly with 1% sodium hypochlorite, 75% alcohol, or 1 mol/L hydrochloric acid (for used pipette tips) or ultraviolet lamp.
3) Consumable items for test operation shall be used in one time and treated aseptically before use.
4) The controls in the kit should be fully thawed before use, well mixed and briefly centrifuged. Bubbles should be avoided. The PCR reaction tube should be centrifuged instantaneously after adding the template. Avoid shaking the PCR reaction tube before starting the machine and in order to avoid contaminations, pay careful attention to the capped tubes and make sure each tube is sealed tightly.
5) The PCR reaction mixture should be kept away from light; negative control and positive control should be run for each test.
6) Do not mix reagents of different batches. Please use the kit within the validity period.
7) After the reaction, remove the PCR tubes (closed) and put them into a self-sealing (zip) bag and seal tightly, treat as pollutant source.

14. BIBLIOGRAPHY

1. Zhu N, Zhang D, et al. A novel coronavirus from patients with pneumonia in China, 2019 [J]. N. Engl. J. Med, 2020.
2. Huang C, Wang Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. [J]. Lancet, 2020.

Key to symbols used in the package:

Catalogue Number

In Vitro Diagnostic Medical Device

Manufacturer

CE Mark

Store at 2°C ~8°C

Expiration Date

Do Not Reuse

CAUTION

Consult Instructions for Use

Contains Sufficient for 48 tests

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