SARS-CoV-2 Nucleic Acid Detection Kit (PCR-Fluorescent Probe Method)

[Product Name]

SARS-CoV-2 Nucleic Acid Detection Kit (Polymerase Chain Reaction - Fluorescent Probe Method)

[Packing Specification]

Catalog No.	Package Size
CoV2-32	32 T/kit

[Intended Use]

SARS-CoV-2 is a member of the coronavirus β genus. It has envelope and round or oval particles in diameter of 60 ~ 140 nm. Its genetic characteristics are significantly different from SARS-CoV and MERS-CoV. Current researches show that it has more than 85% homology with Bat SARS-like Coronavirus (Bat-SL-CoVZC45). When cultured in vitro, SARS-CoV-2 can be found in human respiratory epithelial cells in about 96 hours. It can cause symptoms such as viral pneumonia and dyspnea.

This kit is used in qualitative detection of SARS-CoV-2 RNA in nasal /throat swab, alveolar lavage fluids specimens from suspected CoVID-19 cases, suspected clustered cases, other cases requiring SARS-CoV-2 diagnosis or differential diagnosis. It can specifically detect the target genes (ORF 1ab and N genes) of SARS-CoV-2 and is applied in clinical diagnosis of SARS-CoV-2 infection.

The test results are only for clinical reference, rather than the only standard for clinical diagnosis and treatment. It is recommended to conduct comprehensive analysis of the patients' conditions in combination with their clinical manifestations and other laboratory diagnostic results.

[Test Principle]

This product qualitatively detects SARS-CoV-2 RNA in the specimen through One Step Real-Time RT-PCR method. The specimen detection process mainly consists of two steps:

① **Isolation and Preparation**

Isolation and preparation of SARS-CoV-2 RNA can be conducted manually or in semi-automatic or in full-automatic instruments. (See the nucleic acid extraction kit instructions for details).

② **Reverse Transcription and PCR Amplification**

Applicable instruments (see [Applicable Instruments] for detailed models) is used for detection. The SARS-CoV-2 RNA prepared in step ① is reverse-transcribed to generate complementary DNA (cDNA) in RT-PCR reaction system. And it combines with specific primers and probes (primers and probes are designed according to the conserved sequence of ORF 1ab and N gene) for PCR amplification. Qualitative detection of SARS-CoV-2 is realized by monitoring the change of fluorescence signal intensity during RT-PCR amplification.

UNG-dUTP is set to minimize the possibility of contamination cause by PCR amplification products during detection. In the meantime, with the combination of exogenous positive control, it can avoid false negative result in PCR amplification.

[Main Components]

No.	Components	Main Constituents	Volume
1	SARS-CoV-2 PCR Reaction Solution	Tris-HCl Buffer, dNTPs, Mg^{2+}, primers and probes	256 μL
2	SARS-CoV-2 Enzyme Solution	Reverse Transcriptase, Taq DNA polymerase, Uracil N-glycosylase(UNG)	64 μL
3	SARS-CoV-2 Negative Control	0.9%(w/v) NaCl	800 μL
4	SARS-CoV-2 Positive Control	Armored virus contained SARS-CoV-2 target fragment sequence	800 μL
5	SARS-CoV-2 Internal Control	Armored virus contained internal control fragment sequence	32 μL

1. Note: Do not use the reagents in different lots within one experiment.
2. Consumables required but not provided: nucleic acid extraction and purification reagent and matched equipment; Centrifuge tubes, 0.2 mL PCR tubes (or PCR 8-strip tubes, 96-well plates), filter tip (10 μL、200 μL、1000 μL) and other consumables.

[Storage and Validity]

1. Stored at -25～-15℃ for 12 months, protecting from direct sun light.
2. Stored at 2～8℃ for 10 days once opened. Do not refreeze more than 5 times (≤ 5 times).
3. Transported in sealed cooler box or styrofoam box with dry ice for 7 days.

[Applicable Instruments]

ABI 7500 Real-Time PCR System, Bio-Rad CFX96, Real-Time and other PCR systems embedded with FAM, VIC/HEX and ROX channels. Instruments except for ABI 7500 Real-Time PCR System, Bio-Rad CFX96 Real-Time PCR System, need to be applicably adjusted before use.

[Sample Requirements]

1. Acceptable Specimens: Nasopharyngeal / oropharyngeal swabs.
2. Specimen Sampling: Technicians engage in collecting specimen of SARS-CoV-2 should receive biosafety training (qualified by training) and are entitled with corresponding experimental skills.

Personal protective equipment (PPE) requirements: N95 masks, goggles, protective gowns, nitrile / latex gloves, waterproof boots.

3. Sample Handling

1) Oropharyngeal Swab: Take 2 plastic-rod swabs with polypropylene fiber tip to simultaneously swab the bilateral pharyngeal tonsils and posterior pharyngeal wall. Dip the swab tips into a tube containing 3 mL specimen solution (or 0.9% NaCl), discard the tails and tighten the tube cap.
2) Nasopharyngeal Swab: Gently insert a plastic-rod swab with polypropylene fiber tip into the nasopalatine in the nasal canal, hold for a moment, then slowly rotate it and take it out. Take a plastic-rod swab with polypropylene fiber tip and collect specimen from another nostril in the same way. Immerse two swabs in the same tube containing 3 mL specimen solution (or 0.9% NaCl), discard the tails and tighten the tube cap.

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4. Specimen Preparation and Storage:

1) Specimen should be collected in biosafety cabinet of BSL-2 laboratory after sampling.
2) All specimens should be stored in freezing-resistant specimen collection tube of appropriate size with gasket at screw caps. Tighten lids of tube and mark number, type, name and collecting date on.
3) Cover sample tubes with medical cling film. One film individually for one sample tube.

5. Specimens should be detected ASAP, or stored at 2-8℃ for no more than 24 h; -20±5℃ for no more than 3 months. If a delay in extraction is expected, specimens should be stored at -70℃ or lower, avoid refreezing.

6. Specimen Transporting:

1) Specimen should be sent to the laboratory as soon as possible after collection. If long-distance transportation is expected, it requests full sealed pack with ice bag or dry ice refrigeration to remain temperature.
2) Packaging requirements: The transport package of SARS-CoV-2 strain or other potentially infectious biological materials is categorized as category A and its corresponding UN number is UN2814. The packaging should conform to PI602 classification and packaging requirements in Technical Instructions for the Safe Transport of Dangerous Goods by Air (Doc 9284) formulated by International Civil Aviation Organization. The environmental sample is categorized as category B and its corresponding UN number is UN3373. The packaging should conform to PI650 classification and packaging requirements in Technical Instructions for the Safe Transport of Dangerous Goods by Air (Doc 9284) formulated by International Civil Aviation Organization. Other means of transportation can follow standard packaging showed above.
3) Transportation requirements: Should be subject to national, federal, state, and local transportation regulations for pathogens.

[Application Procedures]

1. Nucleic Acid Extraction (Conducted in sample processing zone)

Nucleic Acid Extraction Kit（*Magnetic Bead Method*）(YXB20180096) manufactured by Zybio Inc is highly recommended to make extraction and preparation of SARS-CoV-2 RNA, manual, semi-automatic and automatic processes are available for preparation of a sample volume of 200 μL.

SARS-CoV-2 Internal Control is used in nucleic acid extraction, it is required be added to **[protease K]** in nucleic acid extraction kit in proportion with 1μL/test, then mix thoroughly before extraction. For detailed extraction process, please follow insert.

Nucleic acid extraction is simultaneously conducted in SARS-CoV-2 Negative Control and SARS-CoV-2 Positive Control.

2. Detection Capacity

Nucleic Acid Extraction Kit (*Magnetic Bead Method*) (YXB20180096) manufactured by Zybio Inc. is used to make extraction and preparation of SARS-CoV-2 RNA manually or in semi-automatic or full-automatic instruments. Different processing methods and timing are shown as below:

Processing Method	Processing Time (96 tests)	PCR Amplification Time (96 tests)	Tests/12 h
Manual	180 min	70 min	300
Semi-automatic instrument	60 min	70 min	>800
Full-automatic instrument	60 min	70 min	>800

Note: Detection time varies slightly among different instruments.

3. PCR Reagent Preparation (conducted in reagent preparation zone)

Take out SARS-CoV-2 PCR Reaction Buffer from the kit and melt it at room temperature, then mix and centrifuge it for several seconds. Calculate the number N of reagents required (N= Number of samples + Number of Negative Control + Number of Positive Control). Prepare the reaction solution according to the table below:

Constituents	**Volume（μL）/ Test**
SARS-CoV-2 PCR Reaction Solution	8
SARS-CoV-2 Enzyme Solution	2
Total	10

Mix the prepared reaction solution and centrifuge it for several seconds. Transfer 10 μL of solution into

each PCR reaction tube with filter tips, transfer them to sample processing zone.

4. Sample preparing (conducted in sample processing zone)

Add respectively 10 μL of nucleic acid sample, SARS-CoV-2 Negative Control and SARS-CoV-2 Positive Control into each PCR reaction tube with filter tips, and cover the caps, then transfer them to the amplification detection zone after transient centrifugation to avoid producing bubbles in tubes.

5. PCR Amplification Detection (conducted in amplification detection zone)

Put PCR reaction tubes into fluorescent PCR instrument and record the number and sequence of the sample, and set PCR amplification parameters up as shown in the table below (take ABI 7500 for example):

Steps		Temperature	Time	Cycle
1	UNG reaction	37℃	1 min	1
2	Reverse transcription	50℃	5 min	1
3	Initial denaturation	95℃	2 min	1
4	Denaturation	95℃	5 sec	45
5	Amplification and fluorescence detection	60℃	30 sec	
Fluorescence Detection: Step 5 Report Fluorescence Setting: FAM, ROX, VIC; Quenching Fluorescence Setting: None; Passive Reference Setting: None.				

6. Result Analysis

Results are automatically saved after the reaction, while target curves and corresponding internal standard curves are analyzed separately. Start value, End value, and Threshold value of Baseline are adjusted according to analyzed image (e.g. Start value range 3 ~ 15, END value range 5 ~ 20) and make amplification curve of the negative control straight or below the threshold line. Click “Analyze” to obtain analysis results and make parameters meet the requirements in “7. Quality Control Procedure” below, then record the detection results in “Plate” window.

7. Quality Control Procedure

No	Controls	Control Standards
1	Positive Control	Target (FAM, ROX) and internal control (VIC): The amplification curves are both in typical S shape and Ct<40
2	Negative Control	Target (FAM, ROX): Ct =45 or not detected Internal control (VIC): Ct <40
All the above must be met in the test, otherwise the results are invalid.		

[Cut-off Value]

Result is clamed positive when the Ct of two targets (FAM, ROX) <40.

[Test Results Explanation]

Results	Criteria
Positive	The amplification curves of FAM and ROX fluorescence channels are typical S-shaped and Ct <40, suggesting SARS-CoV-2 is positive. FAM indicates N gene, ROX indicates ORF 1ab test result.
Negative	FAM and ROX fluorescence channels are not detected or Ct = 45, and VIC channel Ct <40, suggesting SARS-CoV-2 is negative.
Gray Zone	FAM or ROX fluorescence channel 40 ≤ Ct values <45, and VIC channel Ct values < 40, indicating that the result is in gray zone and need re-test. If the results are same and show typical S-shaped curves, it is judged as positive, otherwise it is negative.
Invalid	Ct values = 45 or no value in FAM and ROX fluorescence channels, and Ct values ≥ 40 or no value in VIC channel, indicating that the result is invalid, and re-test is needed.

[Limitations of Test Methods]

1. The test results of this kit are for clinical reference only. Clinical diagnosis and treatment should be referenced in combination with patient’s symptoms / signs, medical history, other laboratory tests, and therapeutic reactions.
2. Inappropriate sample collection, transfer and process, improper experimental operation, and experimental environment may cause false negative or false positive results.

3. Virus mutation may lead to false negative results.
4. Negative results only indicate that the sample is below the detection limit and infection is not completely excluded.

[Product Performance Indicator]

1. **Minimum detection limit**: 200 copies/mL. Dilute positive samples which are traceable to national reference material (NCRM,) (No.GBW (E) 091089) to the lowest detection limit concentration, and the positive detection rate ≥95% (n≥20).

2. **Precision:** Three samples with different concentration level (negative, critical positive and moderate positive samples) were tested 12 times within 4 days. Each sample was tested with 3 replicates, and the results all met: the test results of negative samples were all negative. The test results of critical positive samples were all positive. The coefficient of variation of Ct for the test results of moderate positive samples was less than 5%.

3. **Specificity:** Primer and probe of this product are designed based on conservative region of SARS-CoV-2 genome. It shall not have cross reaction with other respiratory coronaviruses (NL63, HKU1, 229E, OC43), SARS-CoV, MARS-CoV, Influenza A Virus, Influenza B Virus, Respiratory Syncytial Virus, Adenovirus, Parainfluenza Virus, *Klebsiella pneumoniae, Streptococcus pneumoniae, Haemophilus influenza, Pseudomonas aeruginosa, Legionella pneumoniae, Bordetella pertussis, Staphylococcus aureus, Mycoplasma pneumoniae and Chlamydia pneumoniae*.

[Warnings and Precautions]

1. Clinical laboratories should strictly follow the management regulations of molecular biology laboratories and clinical gene amplification laboratories in *Measures for the Management of Clinical Gene Amplification Laboratories in Medical Institutions*.
2. Operators should have knowledge of molecular biological detection or get training for PCR skills.
3. The positive control and internal control should be regarded as infectious substances. Processing and treatment must meet the requirements of relevant regulations.
4. After the experiment, all the used instruments and equipment must be treated with 10% hypochlorous acid or 70% ethanol and UV lamps. Discarded tips must be disposed in waste tanks containing 10% hypochlorous acid to prevent contamination.

[References]

1. *Guidelines on Laboratory Detection Techniques for SARS-CoV-2 (second edition)*. Bureau of Disease Control and Prevention in National Health Commission of The People's Republic of China, 2020.
2. *Etiology and Prevention of Acute Respiratory Virus Infection*. China Union Medical University Press. Hou Yunde, 2005.
3. *Real-Time Fluorescent PCR Technology (second edition)*. Science press, Li Jinming, 2016.
4. *Multiplex real-time PCR for detection of respiratory tract infections*. Journal of Clinical Virology, Brittain-Long R, Nord S, Olofsson S, et al. 2008, 41(1):53-56.

[Explanations on Symbols]

Symbol	Explanation	Symbol	Explanation	Symbol	Explanation
IVD	IN VITRO DIAGNOSTIC MEDICAL DEVICE		USE-BY DATE	CE	EUROPEAN CONFORMITY
LOT	BATCH CODE		TEMPERATURE LIMIT	EC REP	AUTHORIZED REPRESENTATIVE IN THE EUROPEAN COMMUNITY
	CONSULT INSTRUCTIONS FOR USE		MANUFACTURER	REF	CATALOGUE NUMBER

[Manufacturer Information]

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