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

For Emergency Use Only

Instructions for Use

Catalog：

50 reactions

For *In-vitro* Diagnostic (IVD) Use

Rx Only

Shanghai BioGerm Medical Technology Co.,LTD
Address: Room 1302-1307,1309,Building 3,No.1588,Huhang Road, Fengxian District,Shanghai,China.

1 Intended Use

SARS-CoV-2 Nucleic Acid Detection Kit contains the assays and controls for a real-time reverse transcription polymerase chain reaction (RT-PCR) test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in nasopharyngeal swab, oropharyngeal swab, sputum specimens from individuals suspected of COVID-19. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high complexity tests.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in nasopharyngeal swab, oropharyngeal swab, sputum during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results are indicative of active infection. Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.

Negative results do not preclude 2019-nCoV infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

The SARS-CoV-2 Nucleic Acid Detection Kit is intended for use by qualified and trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures. SARS-CoV-2 Nucleic Acid Detection Kit is only for use under the Food and Drug Administration's Emergency Use Authorization.

2 Product descriptions

The oligonucleotide primers and probes for detection of SARS-CoV-2 were selected from regions of the virus ORF1ab gene and nucleocapsid (N) gene. The panel is designed for specific detection of the SARS-CoV-2 (two primer/probe sets). An additional primer/probe set to detect the human RNase P gene (RNP) in control samples and clinical specimens is also included in the panel.

The SARS-CoV-2 Nucleic Acid Detection Kit is a molecular in vitro diagnostic test that aids in the detection and diagnosis of 2019-nCoV and is based on widely used nucleic acid amplification technology. The product contains oligonucleotide primers and dual-labeled hydrolysis probes and control material used in RT-PCR for the in vitro qualitative detection of SARS-CoV-2 RNA in nasopharyngeal swab, oropharyngeal swab and sputum specimens.

3 Contents and storage

Table 1 SARS-CoV-2 Nucleic Acid Detection Kit

Kit components	Quantity per kit	Reagent ingredients
RT-PCR Reaction Mix	600 μL	Tris-HCl, KCl, $MgCl_2$, dNTPs
RT-PCR Enzyme Mix	200 μL	DNA polymerase, Reverse transcriptase, RNase inhibitor
SARS-CoV-2 reaction mix	200 μL	ORF1ab, N gene and RNase P specific Primers and probes

Positive Control	500 μL	ORF1ab specific fragment pseudovirus particle, N gene specific fragment pseudovirus particle, RNase P virus-like particles
Negative Control	500 μL	0.9% saline solution

- Store all the kit components at -15~-25°C.
- Always check the expiration date prior to use. Do not use expired reagents.
- Protect SARS-CoV-2 reaction mix from light.
- RT-PCR Reaction Mix, RT-PCR Enzyme Mix, and SARS-CoV-2 reaction mix must be thawed and kept on cold block at all times during preparation and use.

4 Required materials not supplied

- Biological safety cabinet
- Refrigerated microcentrifuge
- Cold blocks
- Vortex mixer
- 1.5 ml screw-capped microcentrifuge tubes
- Tube racks
- Dedicated adjustable pipettes and aerosol barrier tips
- Disposable Plasticware: The use of sterile, disposable polypropylene tubes is recommended throughout the procedure. These tubes are generally RNase-free and do not require pretreatment to inactivate RNases
- Dedicated laboratory coat for each area
- Disposable booties
- Biohazard bag for tip and tube disposal
- Powder-free latex, vinyl or nitrile gloves
- 20% (v/v) bleach solution (2.0% w/v sodium hypochlorite in water)
- 70% ethanol
- 96-well PCR plates
- Optical caps
- Applied Biosystems 7500 Fast DX Real-Time PCR Instrument
- Qiagen extractor EZ1 Advanced XL and EZ1 DSP Virus kit (62724)
- Nuclease-free water

5 Procedures

5.1 Sample Preparation

The quality of the RNA from the sample extraction is essential for the performance of SARS-CoV-2 Nucleic Acid Detection Kit. The extraction protocol should be performed following manufacturer's instructions or an internally validated protocol. The suitability of the nucleic acid extraction procedure for use with SARS-CoV-2 Nucleic Acid Detection Kit must be validated by the user.

Select appropriate nucleic acid extraction kit to extract nucleic acid from specimens, positive control and negative control. Operate according to the corresponding kit instructions.

5.2 **Prepare the Reaction Mix**

When preparing Reaction Mix, clean all working surfaces with a fresh 10% bleach solution

followed by 70% ethanol, or another equivalent method of cleaning that disinfects and degrades nucleic acids.

Prepare sufficient quantity of the following reagent mix for the number of samples and controls being tested. All volumes include 10% overage for pipette error.

Reagent	Volume/Sample (μL)	Volume for N Sample plus 2 controls (μL)
RT-PCR Reaction Mix	12	12×（N+2）μL
RT-PCR Enzyme Mix	4	4×（N+2）μL
SARS-CoV-2 reaction mix	4	4×（N+2）μL
Total volume	20	-

5.3 Set up the reaction plate

Add 20 μL of the Reaction Mix prepared in step 5.2 into each well of a Plate or tubes then combine with the Sample or the Control according to the following table.

Coponent	Volume/reaction (μL)		
	Sample reaction	Purified Positive Control	Purified Negative Control
Reaction Mix	20	20	20
Purified Sample nucleic acid	5	-	-
Purified Positive Control	-	5	-
Purified Negative Control	-	-	5
Total volum	25	25	25

5.4 Set up and run the reaction

See the Applied Biosystems™7500 Fast Dx Real-Time PCR Instrument Reference Guide (Pub. No. 4406991) for detailed instructions. The instrument must be calibrated for FAM, HEX/VIC, ROX. And confirm the run settings: 1) Assay: Standard Curve (Absolute Quantitation); 2) Run mode: Standard 7500; 3) Passive reference: None; 4) Sample volume: 25 μL.

Using the Detector Manager in the tools menu create the following detectors with the quencher set as none. The detector name must be an exact match with the names shown in the table below.

Reporter dye	detector
FAM	ORF1ab
VIC/HEX	N gene
ROX	RNase P

Program with the cycling conditions below:

step	Temperature (℃)	Time	Cyclers
Reverse transcription	50	10 minutes	1
Activation	95	5 minutes	1
Denaturation	95	10 seconds	45
Anneal / extension	55	40 seconds	

6 Data analysis

6.1 Quality Control

Positive control: the amplification curve was S shaped, and Ct value≤30.
Negative control: Ct value > 38 or not detected.
Negative Control and Positive Control must be performed correctly; otherwise the sample results are invalid.

6.2 Data Analysis and Interpretation

Detection Channel(Target Gene)			Results Analysis
FAM(ORF1ab)	VIC/HEX(N)	ROX (RNase P)	
Ct≤38	Ct≤38	Ct≤38 or undetermined	SARS-CoV-2 positive
Ct>38 or Undetermined	Ct>38 or Undetermined	Ct≤38	SARS-CoV-2 negative
Ct>38 or Undetermined	Ct≤38	Ct≤38	Re-extract and repeat RT-PCR*
Ct≤38	Ct>38 or Undetermined	Ct≤38	
Ct>38 or Undetermined	Ct>38 or Undetermined	Ct>38 or Undetermined	Invalid

*Strongly recommend to collect samples from lower respiratory tract such as sputum specimens. If the repeat result remains inconclusive, additional confirmation testing should be conducted if clinically indicated.

7 Assay limitations

• The use of this assay as an In vitro diagnostic under the FDA Emergency Use Authorization (EUA) is limited to laboratories that are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. § 263a, to perform high complexity tests.

• The SARS-CoV-2 Nucleic Acid Detection Kit performance was established using nasopharyngeal swab, oropharyngeal swab, sputum specimens only. Other specimen types have not been evaluated and should not be tested with this assay.

• Samples must be collected, transported, and stored using appropriate procedures and conditions. Improper collection, transport, or storage of specimens may hinder the ability of the assay to detect the target sequences.

• Extraction and amplification of nucleic acid from clinical samples must be performed according the specified methods listed in this procedure. Other extraction approaches and processing systems have not been evaluated.

• False-negative results may arise from:
– Improper sample collection
– Degradation of the viral RNA during shipping/storage
– Specimen collection after nucleic acid can no longer be found in the specimen matrix
– Using unauthorized extraction or assay reagents
– The presence of RT-PCR inhibitors
– Mutation in the SARS-CoV-2 virus
– Failure to follow instructions for use

• False-positive results may arise from:
– Cross contamination during specimen handling or preparation
– Cross contamination between patient samples
– Specimen mix-up
– RNA contamination during product handling

• The impacts of vaccines, antiviral therapeutics, antibiotics, chemotherapeutic or immunosuppressant drugs have not been evaluated. The SARS-CoV-2 Nucleic Acid Detection Kit

cannot rule out diseases caused by other bacterial or viral pathogens.

- Negative results do not preclude infection with SARS-CoV-2 virus, and should not be the sole basis of a patient management decision.
- Laboratories are required to report all positive results to the appropriate public health authorities.