Instructions For Use
SARS-CoV-2 Real-time RT-PCR Kit

CE
IVD

[Product Name]
SARS-CoV-2 Real-time RT-PCR Kit
[Package Specification]
24 tests/kit
[Intended Use]

The SARS-CoV-2 Real-time RT-PCR Kit is a real-time reverse transcription polymerase chain reaction (rRT-PCR) test for the qualitative detection of ORF1ab/N gene from SARS-CoV-2 in upper and lower respiratory specimens (such as nasopharyngeal or oropharyngeal swabs, sputum) collected from individuals suspected of SARS-CoV-2 by their healthcare provider.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in respiratory specimens 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 do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease.

Negative results do not preclude SARS-CoV-2 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 to make decision.

This kit is intended for use by trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures. This kit is for in vitro diagnostic use only.

[Test Principles]

Steps	Number of cycles	Temperature (°C)	Time (min: sec)	Fluorescence measured
1	1	50	5:00	No
2	1	95	00:30	No
3	45	95	00:03	No
		60	00:20	Yes

This kit is a real-time reverse transcription polymerase chain reaction (rRT-PCR) test specially targeting at ORF1ab gene, N gene of SARS-CoV-2 The kit targets endogenous internal control (β- actin gene) to monitor the quality of the specimen collection and extraction process. The kit includes positive and negative control to monitor the quality of the detection process. The primer and probe sets are designed to detect RNA from SARS-CoV-2 in human nasopharyngeal or oropharyngeal swabs, sputum.

[Main Components]

Component Name	Main Component	Specification		
		3*8 Tests/Kit	2*12 Tests/Kit	1*24 Tests/Kit
Reaction Mix	Primer probe, amplification reagent dNTPs, etc. Lyophilized powder	3 tubes	2 tubes	1 tube
Positive Control	Lyophilized powder containing target genes (ORF1ab gene, N gene)	1 tube	1 tube	1 tube
Negative Control	RNase free water	1.5mL/tube*2	1.5mL/tube*2	1.5mL/tube*2

Note: The components from different batches of the kit cannot be used interchangeably.

[Storage Conditions and Expiry Date]

Unopened kit : Store between -25℃to 8℃ away from light,valid for 12months .

After tearing off the sealed pouch ,the lyophilized reagents in the tubes should be used immediately.

The Reaction mix after reconstitution with 120μL(8 Tests/tube)/180 μL(12 Tests/tube)/360 μL(24 Tests/tube) negative control (RNase free water): Store between -25°C to -15℃ away from light, valid for 3 months; repeated freeze-thaw no more than 3 times.

The Positive control after reconstitution with negative control (RNase free water): Store between -25°C to -15℃, valid for 3 months; repeated freeze-thaw no more than 3 times.

Transportation conditions : ≤37℃, stable for 2 months.

[Applicable Instruments]

This kit is applicable to be used with real-time PCR instruments such as ABI 7500, ABI Step-one Plus, Biorad CFX-96, Ultrafast QPCR Device FQ-8A.

[Sample Requirements]

1. Sample Collection

Freshly collected oropharyngeal swabs, nasopharyngeal swabs, sputum.

Sample collection of oropharyngeal swabs: Use sampling swab head to wipe the postpharyngeal flap and bilateral tonsils at the back of uvula (uvula palatina or lingula) with moderate force for many times without touching the tongue. Take it out and quickly dip the swab head into the sampling tube, break the swab handle at the point, discard the tail, and tighten the tube cover.

Sample collection of nasopharyngeal swabs: With the sampling swab, insert the swab head gently into the nasal canal at the nasopalatine, stay for a while, then slowly turn to exit; Quickly dip the swab head into the sampling solution, break the swab handle at the point, discard the tail, and tighten the tube cover.

Sputum collection: Ask the patient to rinse his/her mouth, take deep breaths several times and then cough up fresh sputum from his/her lungs. Collect it in a clean container, seal it and submit it for detection as soon as possible.

2. Precautions for Sample Collection

Contamination shall be avoided when the samples are collected, stored and transported. All samples shall be regarded as infectious when collected, and bio-safety containment shall be made during operation.

3. Sample Preservation

Samples used for virus isolation and nucleic acid detection should be detected immediately. If samples cannot be detected immediately, they should be stored below -70℃. Samples should avoid repeated freeze-thaw cycles.

4. Sample Transportation

The short-term transportation of samples shall be protected and sealed in ice pot and foam box with ice or dry ice, and the transportation of samples shall comply with the relevant national biosafety regulations on Class II pathogens.

[Test Procedure]

1. Sample Processing

1.1 Nucleic acid extraction and purification

When extracting freshly collected oropharyngeal swabs, nasopharyngeal swabs and sputum, the quality of RNA and the quantity of RNA meeting the experimental needs should be ensured, and the extracted RNA samples should be immediately detected. Sputum samples need to be liquefied before RNA extraction. It is recommended to use the virus RNA extraction kit produced by Baicare (Tianjin) Biotechnology Co., Ltd. and finish the extraction strictly according to the instructions.

1.2 Positive control

Totally 200μL of negative control was added to the positive control tube for re-dissolution, and totally 200μL was extracted with the above extraction kit.

2. RT-PCR Protocol

2.1 According to the experimental requirements, prepare the corresponding PCR reaction tube (n= the number of samples to be tested+ one positive control + one negative control).

2.2 Take out the lyophilized reaction mix, centrifuge it for 15s to the bottom of the tube to avoid the add lyophilized reaction mix flying out when the cap is opened. According to different specifications, add the corresponding negative control to reconstitute the reaction mix (add 120μL negative control to 8 tests/tube, add 180μL negative control to 12 tests/tube, add 360μL negative control to 24 tests/tube). After fully dissolved, divide the reconstituted reaction mix solution into 8-tubes PCR strip at 15 μL /tube. then add 5 μL of the RNA sample /extracted positive control/ negative control into each tube and mix well. After all tubes are mixed well, centrifuge briefly or flick the liquid to the bottom of the tube (if use Ultrafast QPCR Device for the detection, please add one drop paraffin oil into each tube cover the reaction mix, and do not mix paraffin oil with reaction mix), then cover the tube with lip tightly.

Note: If many impurities in the specimen inhibit the PCR reaction, it is recommended to reduce the loading volume of the RNA sample and add water to make up reduced volume.

2.3 Place the tubes in the regular real-time PCR instrument. Program and run the instrument using the following thermal cycling parameters.

Note: If use ABI 7500 Real-time PCR system, be sure to select "none" in both the passive reference and quencher. Save the file and run the program.

2.4 Place the tubes in the Ultrafast QPCR device. Program and run the instrument using the following thermal cycling parameters.

Steps	Number of cycles	Temperature (°C)	Time (sec)	Fluorescence measured
1	1	50	300s	No
2	1	92	30s	No
3	40	92	3s	No
		55	20s	Yes

Note: Please add one drop (15μL -25μL) paraffin oil into each tube cover the reaction mix, and do not mix paraffin oil with reaction mix, then cover the tube with lip tightly.

2.5 Selection of fluorescence channels: set up to measure four-color fluorescence like FAM, VIC/HEX and ROX in each well.

Fluorescence channel	Target gene
FAM	N Gene
VIC/HEX	ORF1ab
ROX	IC

3. Analysis of Results

Baseline and Threshold Setting: Baseline is usually based on the baseline automatically set by the instrument. Principle for baseline adjustment: Select the area where the fluorescence signal is relatively stable before exponential amplification, the start avoids the signal fluctuation in the initial stage of fluorescence collection, and the End reduces the Ct/Cq value of the sample with the earliest exponential amplification by 1-2 cycles. The principle for threshold setting is that the threshold line just above the highest point of the detection fluorescence curve of negative control.

After setting the baseline and threshold, click Analyze, and the sample CT value will be automatically analyzed and calculated by the recording apparatus.

4. Quality Control

4.1 Negative control: All four channels have no Ct value or the Ct value is undet.

4.2 Positive control: ROX channel detection has no Ct value or the Ct value is undet, and the other three channels show typical S-shaped curves with Ct value ≤ 32.

The above requirements need to be met at the same time in the same experiment, otherwise, the experiment shall be regarded as invalid and needs to be carried out again.

[Cut-off Value or Reference Interval]

The cut-off value detected by the kit is analyzed by ROC curve analysis method.

(1) With regular PCR systems testing, it is confirmed that the cut-off values of ORF1ab, N genes of SARS-CoV-2 detected by this kit are Ct value ≤ 37 .

(2) With Ultrafast QPCR Device testing, it is confirmed that the cut-off values of ORF1ab, N genes of SARS-CoV-2 detected by this kit are Ct value ≤ 36.

[Interpretation of Test Results]

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Test with regular PCR systems: under the condition of normal quality control, the gene detection results are determined as follows:

1. If the two channels amplification curves of FAM, VIC/HEX of the sample to be tested show S-shaped curves with the Ct values are ≤ 37, the sample is detected to be positive.

2. If the amplification curves of any one channel of FAM, VIC/HEX of the sample to be tested show S-shaped curves with the Ct values are ≤ 37, and the other channel shows no amplification curve, the sample is detected to be positive.

3. If the two-channel amplification curves of FAM, VIC/HEX of the sample to be tested do not show S-shaped curves, and the Ct value of ROX channel detected is ≤ 37, and the sample is detected to be negative.

4. If the amplification curves of FAM and VIC/HEX channels of the samples to be tested are not S-shaped curves, the sample Ct value is >37 or undet and the internal control (ROX) is detected Ct value >37 or undet, then there is a problem in the quality of the samples, or there is a problem in the operation, and the samples have to be re-sampled for sample extraction and detection.

According to the testing results of the above genes, the results are analyzed as follows:

Situation	Ct value			Interpretation
	ORF 1ab (VIC/HEX)	N gene (FAM)	IC (ROX)	
1	⩽37	⩽37	No request	Positive for SARS-CoV-2
2	⩽37	＞37 or undet	No request	Positive for SARS-CoV-2
3	＞37 or undet	⩽37	No request	Positive for SARS-CoV-2
4	＞37 or undet	＞37 or undet	⩽37	Negative for SARS-CoV-2
5	＞37 or undet	＞37 or undet	＞37 or undet	Invalid test, it is recommended to retest or re-sample before retest.

Test with Ultrafast QPCR Device: under the condition of normal quality control, the gene detection results are determined as follows:

1. If the two channels amplification curves of FAM, VIC/HEX of the sample to be tested show S-shaped curves with the Ct values are ≤ 36, the sample is detected to be positive.

2. If the amplification curves of any one channel of FAM, VIC/HEX of the sample to be tested show S-shaped curves with the Ct values are ≤ 36, and the other channel shows no amplification curve, the sample is detected to be positive.

3. If the two-channel amplification curves of FAM, VIC/HEX of the sample to be tested do not show S-shaped curves, and the Ct value of ROX channel detected is ≤ 36, and the sample is detected to be negative.

4. If the amplification curves of FAM and VIC/HEX channels of the samples to be tested are not S-shaped curves, the sample Ct value is >36 or undet and the internal control (ROX) is detected Ct value >36 or undet, then there is a problem in the quality of the samples, or there is a problem in the operation, and the samples have to be re-sampled for sample extraction and detection.

According to the testing results of the above genes, the results are analyzed as follows:

Situation	Ct value			Interpretation
	ORF 1ab (VIC/HEX)	N gene (FAM)	IC (ROX)	
1	⩽36	⩽36	No request	Positive for SARS-CoV-2
2	⩽36	＞36 or undet	No request	Positive for SARS-CoV-2
3	＞36 or undet	⩽36	No request	Positive for SARS-CoV-2
4	＞36 or undet	＞36 or undet	⩽36	Negative for SARS-CoV-2
5	＞36 or undet	＞36 or undet	＞36 or undet	Invalid test, it is recommended to retest or re-sample before retest.

[Limitations of the Test Method]

1. The test results of this kit are for clinical reference only. The clinical diagnosis and treatment of patients should be comprehensively considered in combination with their symptoms/signs, medical history, other laboratory tests and therapeutic reactions.

2. Unreasonable sample collection, transportation and processing, improper experimental operation and experimental environment may lead to results of false negative or false positive.

3. There is a risk of false negativity in case that the detected virus has variations in nucleic acid sequence.

[Performance Indicators of the Product]

1. Accuracy: The results of enterprise's reference products detected by this kit are correct.

2. Specificity: No cross-reaction with influenza A virus (H1N1), influenza B virus, respiratory syncytial virus, adenovirus (Types 1, 2, 3, 4, 5 and 7), parainfluenza virus (1-Type 3), mycoplasma pneumoniae, chlamydia pneumoniae, bacillus pertussis, streptococcus pneumoniae, rhinovirus (Type A), legionella pneumophila, Middle East Respiratory Syndrome Coronavirus (MERSr-CoV), human coronavirus HCoV-229E, human coronavirus HCoV-HKU1, human coronavirus HCoV-NL63, human coronavirus HCoV- OC43, influenza A virus (H3N2), influenza A virus (H5N1), influenza A virus (H7N9), human interstitial pneumovirus, EB virus, measles virus, human cytomegalovirus, rotavirus, norovirus, Staphylococcus aureus, cryptococcus neoformans, streptococcus pyogenes, acinetobacter baumannii, pneumocystis, klebsiella pneumoniae, streptococcus pneumoniae, haemophilus influenzae, Pseudomonas aeruginosa, klebsiella pneumoniae and streptococcus pyogenes.

3. Minimum LOD (limit of detection): 200-1000copies/mL

4. Precision: For simulated samples or nucleic acids with the concentration of 500,00 copies/mL and 5,000 copies/mL, the CV of Ct within and between batches is less than 5%.

5. Interference test: It is verified that the endogenous interfering substances that may exist in the sample like 30% blood, 10 mg/mL mucin, 1.5 mg/L of hydroxymetazoline, 6 mg/L of dexamethasone, 100 U/mL of interference, 8μg/mL of azithromycin, 20 μg/mL of cephalexin, etc, have no obvious interference to this kit.

[Attentions]

1. This kit is only used for in vitro diagnosis.
2. The whole detection process should be strictly divided into three areas: preparation area of PCR reaction system; sample processing and sample loading area; area for PCR amplification, fluorescence detection and result analysis. Instruments, equipment, supplies and work clothes used in each area shall be independent and exclusive. Please clean and disinfect the workbench immediately after the experiment.
3. Pay attention to safety protection during operation. Personnel contacting pathogens shall wear suitable protective clothing, disposable gloves and masks and work in specified experimental site. All items directly exposed to the pathogens shall be disposed or reused after strict disinfection.
4. Please strictly follow the relevant management standards on gene amplification and testing laboratory issued by the competent administrative department of the industry.
5. Operators must be trained and qualified for their jobs.
6. Do not use expired reagents.
7. Various factors during the storage, transportation and use of reagents may lead to performance changes, such as improper storage and transportation, irregular operation of sample collection, sample processing and detection processes, etc., so please strictly follow the instructions. Considering the characteristics of sample collection processes with swabs and that of the course of viral infection, false negative results are possibly caused by insufficient sample size and other reasons. Therefore, a comprehensive identification should be made in combination with other clinical diagnosis and treatment information, and another detection should be taken when necessary.
8. After the nucleic acid extraction of the sample, it is recommended to carry out the next step of the experiment immediately, otherwise please keep it at -70℃ and complete the detection within one week.
9. After the experiment, 50ppm- 2000ppm sodium hypochlorite or 75% alcohol and ultraviolet lamp shall be used to clean and disinfect the workbench and pipette.
10. The disposal of disinfected wastes in biosafety laboratories shall conform to relevant national, regional and local regulations. When designing procedures for the treatment, transportation and disposal of biohazardous wastes, you must refer to the latest version of relevant documents. All wastes (including contaminated padding, used cleaning heads, etc.), other treated substances and clothes to be washed shall be disinfected with high-pressure steam sterilizer, and treatable wastes shall be incinerated.

[Explanation of the Symbol]

CE	CE Mark	LOT	Batch code
	Consult instructions for use	IVD	In vitro diagnostic medical device
	Store between -25～8℃	REF	Catalogue number
	Manufacturer		Date of manufacture
EC REP	Authorized representative in the European Community		Use-by date
Σ	Contains sufficient for <n> tests		Biological risks
	Caution		Do not use if package is damaged

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EC REP

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