SARS-CoV-2 RT-qPCR Detection Kit (Dual Gene OFT1a/N)

LS-K1073-25, -50 (25, 50 Tests) • **See Storage Conditions Below**
(Szybio Life Origin Biotech Device Number 20100488)

LSBio
LifeSpan BioSciences, Inc.

For research use only. Intended for use by laboratory professionals.

For use with ABI 7500, Stratagene Mx3000P/Mx3005P, and Roche LightCycler® 480 Real-Time Fluorescence Quantitative PCR Instrument.

Introduction

A novel pneumonia epidemic broke out in Wuhan, China, at the end of 2019. On February 11, 2020, the World Health Organization (WHO) named the causative virus SARS-CoV-2 (2019 Novel Coronavirus). This assay kit contains pairs of specific primers and TaqMan probes for the conserved regions of the SARS-CoV-2 N gene and ORF1ab gene, which were recently posted on the Global Initiative on Sharing All Influenza Data (GISAID) webpage (www.gisaid.org). In this assay, the SARS-CoV-2 RNA in specimens is analyzed qualitatively using one-step Real-Time PCR detection technology. Detection of two target genes reduces the potential for false positive and false negative results. The kit also amplifies the human-derived ribonuclease P (RNP) gene as an internal control to monitor the sample collection and extraction processes in order to avoid false negatives.

Sample Types

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

Components

Component	K1073-25	K1073-50
	25 Tests	50 Tests
N/ORF1ab RT-PCR Mix	425 µl	850 µl
N/ORF1ab Primer and Probe Mix	50 µl	100 µl
N/ORF1ab Enzyme Mix	25 µl	50 µl
Negative Control	30 µl	30 µl
N/ORF1ab Positive Control	30 µl	30 µl
Instructions	1 copy	1 copy

Note: The components of different kit lots cannot be used interchangeably.

Storage Conditions

1. Kits are shipped on blue ice, and the temperature should not exceed 8°C. Upon arrival, all components should be stored at -20°C and protected from light. The reagents are valid for 12 months. The expiration date is printed on the outer packaging.
2. Repeated free-thaw cycles should be avoided (no more than 5 times).
3. The kit should be used before the expiration date, which is printed on the outer packaging.

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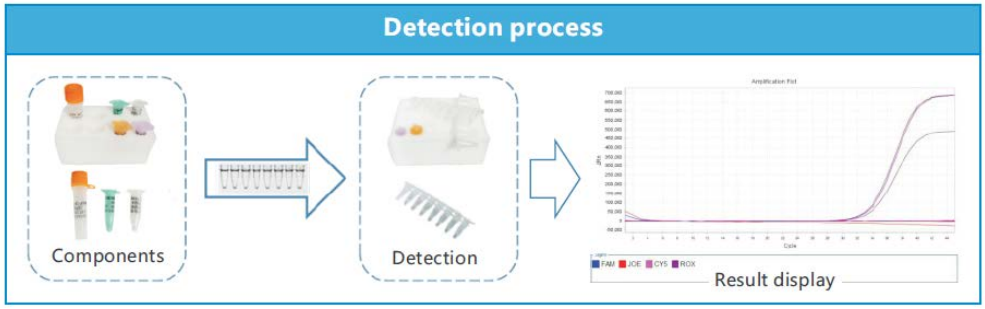
Applicable Instruments

1. Suitable for ABI 7500, Stratagene Mx3000P/Mx3005P, and Roche LightCycler® 480 Real-Time Fluorescence Quantitative PCR Instrument.
2. Instrument models not listed above may be applicable, but compatibility has not been tested. Please contact LSBio Technical Support (technicalSupport@LSBio.com) if you have questions.

Sample Collection

1. Procedure for nasal swabs and throat swabs:
   a) Nasal swab: Insert wet, sterile swab parallel to the upper jaw into one nostril against the inner nasal palate of the nasal canal while gently rotating the swab. Generally, if there is resistance to the swab insertion, leave the swab in place for 2 to 3 seconds and then slowly rotate to exit.
   b) Throat swab: Hold a tongue depressor against the root of the tongue, hold a wet sterile swab with the other hand, and then quickly and vigorously scrape both sides of the tonsils and the posterior wall of the pharynx with both sides of the swab.
   c) Place the nasal swab and the throat swab together in a centrifuge tube containing 1.0 ml of normal saline.
2. Blood or serum samples: Draw the sample with a sterile syringe and place it in a centrifuge tube for testing.
3. Avoid cross contamination between samples.
4. Samples should be tested at time of collection or stored at -20°C ± 5°C. Store at -70°C for long-term storage.

Assay Procedure



1. Reagent preparation:
   a) Remove the kit from the freezer and thaw at room temperature (20-25°C). After components have thawed, mix each tube by shaking or flicking and then centrifuge each tube at 2000 rpm for 10 seconds.

b) Determine the number of reactions required for the assay (N) and calculate the test doses according to the reaction Master Mix preparation instructions in Table 1.

N = Negative Control (1T) + Positive Control (1T) + Error Reserve + Number of Samples

Table 1: Reaction Master Mix preparation.

Amount of reagent needed for each PCR tube	N/ORF1ab RT-PCR Mix	17 µl
	N/ORF1ab Primer and Probe Mix	2 µl
	N/ORF1ab Enzyme Mix	1 µl

c) To make the Master Mix, add the appropriate volume of each reagent to a sterile centrifuge tube, mix well by pipetting, and centrifuge at 2,000 rpm for 10 seconds. Add 20 µl of Master Mix to each PCR tube.

d) Cap the 8-Strip PCR tubes tightly and pay attention to the identification. Mark the protruding sites at both ends of the 8-Strip PCR tube cap. Do not mark the middle of the 8-Strip PCR tube cap to avoid affecting signal collection. Place the remaining reagents into the freezer (-20°C).

2. Sample preparation:

a) RNA Extraction: Extract RNA of the test samples according to the instructions for the extraction kit (kit not provided)

b) Addition of sample:

i. Centrifuge the previously prepared reagents at 2000 rpm for 10 seconds.

ii. Open the cap of the PCR tube and add 5 µl of sample to each experimental tube.

iii. Add 5 µl of N/ORF1ab Positive Control to the positive control tube and 5 µl of Negative Control to the negative control tube. The total for each tube should be 25 µl.

iv. Cap the PCR tubes, record the order of the added samples, and then centrifuge at 2000 rpm for 10 seconds.

Note: Contamination should be avoided during the extraction of sample RNA and sampling. If the extracted RNA template cannot be tested immediately, it should be stored at -70°C.

3. PCR:

a) Turn on the machine and check the performance of the instrument.

b) Place the PCR tubes into the PCR machine and record the order of placement. Ensure all reaction tubes are capped tightly before loading to prevent aerosols from contaminating the instrument and work environment.

c) Carry out the PCR amplification and set the instrument according to the parameters outlined in Table 2.

Table 2: Nucleic acid amplification parameters

Volume	Reaction volume is 25 µl		
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 for 30 min	1
	Pre-denaturation	95°C for 3 min	1
	PCR	95°C for 5 sec	45
		55°C for 30 sec (Collect the fluorescence signal at the end of this phase)	

Note: For ABI fluorescence quantitative PCR instruments, do not select ROX correction. For quenching group, select "None".

Analysis of Results

Results should be saved automatically after the amplification is completed, including the analyzing curve regulating the Start Value, End Value, and Threshold Value of baseline. Review your data. On the basis of run results, the Start Value can be in the range of 3 - 15, and 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 the Report interface.

Reference Range

1. Quality Control
   a) Positive control: N gene and ORF1ab gene, Ct value ≤ 38 with significant exponential growth
   b) Negative control: N gene and ORF1ab gene, Ct value > 41 or no Ct value; amplification result of internal control gene, Ct value ≤ 40
   c) The above requirements must be met in the same experiment, otherwise the test is invalid
2. Determination
   a) FAM channel: A Ct value ≤ 38 for the sample with an obvious exponential growth period is considered a positive result. If the Ct value is in the range of 38-41 of the sample detection results, the analysis should be repeated. If the Ct value for the repeat test is ≤ 41 **and** there is an obvious exponential growth, it is considered positive. If there is no obvious exponential growth, then it is considered negative. If the Ct value is > 41 or no Ct value of the sample and the Ct value ≤ 40 of the amplification result of the internal control gene, it is considered to be negative.
   b) HEX/VIC/JOE channel: The Ct value ≤ 38 of the sample, with an obvious exponential growth period is a positive result. If the Ct value is in the range of 38-41 of the sample detection results, repeat the testing of the sample. If the Ct value ≤ 41 of the repeated test results and there is an obvious exponential growth, it is considered as

positive, otherwise it is negative. If the Ct value > 41 or if there is no Ct value for the sample **and** the Ct value ≤ 40 for the internal control gene, the result is considered to be negative.

c) If the test results for the two channels for the sample are negative **and** the Ct value > 40 for the internal control gene, then resampling, extraction, and detection are required.

Interpretation of Results

	Channels and Results			Results 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	-	-	-	Resampling, extraction, and detection

Notes:

If both the N and ORF1ab genes are positive, the sample can be scored as SARS-CoV-2 positive. If a single target is positive, we recommend retesting the sample. If the retest result is positive for the same target, it can be scored as positive for 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 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%), nasal secretions (5%), saliva (25%), mucin (0.35 mg/ml), leukocyte ($5.0x10^5$ unit/ml), cephalosporin (1 mg/ml), budesonide (1.28 mg/ml), Jinying (0.1 g/ml), Oxymetazoline hydrochloride (0.5 mg/ml), Beclomethasone (1.54 mg/ml).
3. Precision: The coefficient of variation (CV%) is less than 5%.

Limitation

1. This test is for **RESEARCH USE ONLY** and not for diagnostic/clinical use.
2. Improper handling of samples during 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 present in the sample is below the minimum detection limit.
4. If cross-contamination occurs during sample collection and preparation, false-positive results may occur.
5. A large number of dead viruses appear in the samples of some infected patients due to antiviral drug administration. This test would yield a strong positive result, while culture results would be negative. If this is the case, the patient should be asked about recent drug administration.

6. Mutations or damage leading to 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. The possibility of false-negative results is reduced if samples are collected in the same patient at different times and from multiple sites.

Precautions

1. We recommend that the experimental process be carried out in different areas (reagent preparation area, sample processing area, nucleic acid amplification area) to reduce the likelihood of contamination. Special (designated) instruments and equipment should be used at each stage of the process. There should be strict requirements on the flow of people and air in each section to minimize cross contamination.
2. 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.
3. Before use, the instrument and the supporting power supply system should be preliminarily checked to ensure the normal operation of the instrument.
4. The pipette tips used in the experiment should be discarded into a waste container containing 10% sodium hypochlorite (bleach).
5. Tables and various laboratory items should be disinfected regularly with 10% sodium hypochlorite (bleach), 75% alcohol, or ultraviolet irradiation.
6. The fluorescent PCR instrument should be calibrated regularly and the plate holes cleaned frequently.
7. To prevent fluorescence interference, avoid direct contact of ungloved hands with the 8-Strip PCR tube and tube cap.
8. The positive control in this kit is not infectious and will not harm the human body. However, we recommend that it be treated as a potentially infectious substance.
9. Samples should be considered potentially infectious substances and should be handled and disposed of in accordance with OSHA, CDC, and local guidelines.

Manufacture

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

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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

Medical Device Manufacturer License Number: Hubei SFDA Medical Device Number 20100488.

LifeSpan BioScience, Inc. is an authorized distributor of this product.

Version: V.06.04.2020

These kits are sold for research purposes only, and have not been tested or approved for use in human diagnostics.

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