SARS-CoV-2 RT-qPCR Fluorescence Detection Kit

(ORF1ab & N Genes)

CE

Instruction for Use

IVD

For Medical Professional Use Only!

Please read the instruction for use carefully before performing the test or use after consulting your doctor.

INTENDED USE

The novel coronaviruses belong to the β genus. COVID-19 is an acute respiratory infectious disease. People are generally susceptible. Currently, the patients infected by novel coronavirus are the main source of infection: asymptomatic infected people can alse be an infectious sourse. Based on the current epidemiological investigation, the incubation period is 1 to 14 days, mostly 3 to 7 days. The main manifestations include fever, fatigue and dry cough. Nasal congestion,runny nose,sore throat, myalgia and diarrhea are found in a few cases.

This kit is suitable for qualitative detection of SARS-CoV-2 nucleic acid. The test results are for clinical reference only and cannot be used as the basis of diagnosis and treatment alone.

MODEL

50 tests/kit.

COMPOSITION

Main materials： PCR Buffer, dNTPS, Primers and Taqman Probes for SARS-CoV-2, RNase inhibitor, UDG, Reverse Transcriptase, Taq DNA polymerase, RNA pseudovirus containing target gene, DEPC-Treated Water

PRINCIPLE

The dual-target gene design is based on the target regions of SARS-CoV-2 ORF1ab and the specific conserved sequence encoding of nucleocapsid protein N genes. Detect SARS-CoV-2 RNA virus by real-time PCR system with PCR mix.

PRECAUTIONS

1) For in vitro diagnostic use only.
2) The test results should not be used as a sole basis for diagnosis or exclusion of COVID-19.
3) Please read the instruction inserts carefully before performing the test.
4) All samples and wastes shall be treated as infectious materials.
   The kit should be stored at -25±5℃.

MATERIAL

Material provided:

Components	Amount	Ingredient
PCR Mix	Freeze-dried powder (add 670 μL ddH2O before use) ×1 tube	RNase inhibitor, UDG, Reverse Transcriptase, Taq DNA polymerase, Buffer, dNTPs, Primers,Probes
Positive Control	200 μL×1 tube	RNA pseudovirus containing target gene
Negative Control	500 μL×1 tube	DEPC-Treated Water

APPLICABLE EQUIPMENT

ABI 7500 Real-Time PCR , SLAN Real-Time PCR.

STORAGE AND STABILITY

1) The test kit should be stored at -25℃±5℃.
2) The shelf-life of the kit is 12 months.

SAMPLE REQUIREMENTS

The applicable sample used for kit: upper respiratory tract samples (including throat swabs, nasal swabs, nasopharynx extracts, expectoration fluid); lower respiratory tract samples (including respiratory tract extracts, bronchoalveolar lavage fluid, alveolar lavage fluid, lung tissue biopsy samples); tissue culture samples, etc.

TEST PROCEDURE

1) Reagents Preparation

   Prepare PCR mix in Reagent Preparation Area according to the following table. It is recommended to prepare 110% of the calculated amount of PCR mix to account for pipetting carryovers.

Component	Volume/test	Volume for n samples and 2 controls	110% of volume
PCR mix	12 μL	12 ×(n+2) μL	13.2×(n+2) μL

   Completely vortex the prepared PCR mix, aliquot 12 μL into each PCR tube or each well of a 96-well PCR plate.

2) Sample Preparation

   The extracted nucleic acid were added to the reaction pores, each with 8 μL. Controls: 8 μL; The total volume per reaction is 20 μL. Close lids for the PCR tubes or seal PCR plates with an appropriate film, vortex the tubes and briefly centrifuge them to get rid of bubbles.

3) PCR Amplification(PCR Area)

   The reaction tubes or PCR plates were placed in the qPCR apparatus in order, and the reaction was performed according to the following conditions. FAM : ORF1ab gene; ROX: N gene, VIC/HEX: IC.(For ABI system, please set the “Quencher Dye” and “Passive Reference” to "None")

Program	Step	Temperature	Constant time	Number of cycles
1	Reverse transcription	55℃	15 mins	1
2	Pre degeneration	95℃	30 sec	1
3	degeneration	95℃	10 sec	45
	Annealing, elongation and fluorescence detection	60℃(Read)	30 sec	

INTERPRETATION OF RESULTS

Baseline Threshold adjustment

Adjust the Baseline and Threshold according to the image after analysis.

For ABI system, the Baseline can be set to 3-15 by default (the Baseline can vary within a certain range according to the actual situation, the Start value can be 3-15, and the End value can be 5-20).

For SLAN system, the default setting value of the instrument (starting cycle number 6, ending cycle number 12, threshold line 0.12) can be adopted, or adjusted according to the result.

Quality Control

Negative control : no typical s-type amplification curve, Ct value of FAM/ROX＞40 or no Ct value.

Positive control : typical s-type amplification curve, Ct value of FAM/VIC/ROX channels≤33.

If the above two conditions are satisfied at the same time, the result is credible.

Interpretation of Results

If the criteria of QUALITY CONTROL is met, analysis the data of sample as follows

Ct		Result interpretation
IC(VIC/HEX)	ORF1ab(FAM),N(ROX)	
≤35	Both targets Undet or Ct＞40	SARS-CoV-2 not detected
/	Both targets ≤38	SARS-CoV-2 detected
/	One of the targets ≤38	SARS-CoV-2 detected
＞35 or Undet*	Both targets Undet or Ct＞38	Invalid result, specimen needs to be re-tested from re-extraction or re collected from patient for test

/: No requirements on the Ct value. Undet: Undetermined

*If the Ct value of HEX/VIC (Internal Control) channel is > 35 or Undetermined , it may indicate that the detected specimen contains lower concentration of cells, extracted nucleic acid was degraded or certain inhibitors were present in the reaction. It is recommended to re-collect specimen or change the collection location, then test the specimen again.

No requirement for HEX/VIC channel test results, if the sample is extracted from virus culture.

INVALID

If the criteria of QUALITY CONTROL is no met,review the procedure and repeat the test .

LIMITATION OF THE PROCEDURE

1. The detection result of this product is only for clinical reference, and it should not be used as the only evidence for clinical diagnosis and treatment. The clinical management of patients should be considered in combination with their symptoms/signs, history, other laboratory tests and treatment responses. The detection results should not be directly used as the evidence for clinical diagnosis, and are only for the reference of clinicians.
2. Don`t define all negative results as no COVID-19 infection, because whichever of elements of lower concentration of the content than the detection limit, undue sample collecting and transferring and processing, improper operating process and experimental environment could lead to false positive or false negative results.
3. The assay targets specific genomic regions of SARS-CoV-2: ORF1ab and N genes.However, target sequence variations may lead to false negative re sult.

PERFORMANCE

（1） Check the positive and negative standard products of the enterprise, and the compliance rate is 100%.

（2） Lowest detection limit: 10 copies/reactions.

（3） Cross Reactivity: No cross reactivity were observed with following microorganism positive sample: HCoV-OC43, HCoV-NL63, HCoV-229E, HCoV-HKU1,MERS,SARS, H1N1,H3N2,H5N1,H7N9,Influenza B,RSV, Parainfluenza, Adeno virus.Mycoplama Pneumonia, Chlymedia Pneumonia,Rhinovirus, EBV,CMV,HIV,HBV,HCV.

（4） Interference: No interference for following drugs were observed: Beclomethasone, dexamethasone, flunisolide, triamcinolone acetonide, budesonide, mometasone, fluticasone, Alpha-interferon, zanamivir, ribavirin, oseltamivir, paramivir, lopinavir, ritonavir, abidor,Levofloxacin, azithromycin, ceftriaxone, meropenem, Tobramycin**.**

（5） Precision: For low-concentration positive quality control products, 10 consecutive tests were repeated, and the CV of Ct value ≤ 5%.

Index of CE Symbols

IVD	For in vitro diagnostic use only		Do not reuse
	Expiry date		See instruction for use
	Warning, please refer to the instructions in the annex		Manufacturer
	Manufacture Date	LOT	Batch number
	Biological risks		Tests per kit
REF	Catalog #		Keep away from sunlight
	Don't use the product when the package is damaged		
CE	The product meets the basic requirements of European in vitro diagnostic medical devices directive 98/79/EC		

Manufacturer:

Zhengzhou Fortune Bioscience Co.,Ltd.

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