Real-time Fluorescent RT-PCR Kit for Detecting SARS-CoV-2, Influenza A Virus and Influenza B Virus

For Research Use Only. Not for use in diagnostic procedures.

Instructions For Use*

*Note: Instructions for use are being provided to help laboratory researchers validate BGI Patho-Genesis's test and provide feedback to BGI Patho-Genesis regarding the test's performance. This kit is for research use only, and these instructions should not be used to obtain clinical diagnostic results.

Test kits are not for commercial use or distribution, and are intended only for laboratory confirmation of test validity.

【Product Name】

Real-time Fluorescent RT-PCR Kit for Detecting SARS-CoV-2, Influenza A Virus and Influenza B Virus

【Packing Specifications】 50 tests/kit

【Catalogue Number】

PGI030004

【Intended Use】

The kit is a qualitative in vitro nucleic acid amplification assay to detect and differentiate SARS-CoV-2, influenza A virus (IFV A), and influenza B virus (IFV B) in throat swab specimen from individuals suspected of respiratory viral infection consistent with COVID-19, suspicious clustering cases and those under investigation.

Corona Virus Disease 2019 (COVID-19) is caused by a novel coronavirus (SARS-CoV-2). The SARS-CoV-2 belongs to the coronavirus of genus β. COVID-19 is an acute respiratory infectious disease, and the population is generally susceptible. The known infection sources are COVID-19 patients and asymptomatic carrier of SARS-CoV-2. Based on the current epidemiological data, the incubation period of COVID-19 is estimated to be between 1 and 14 days, mostly 3-7 days. Typical clinical symptoms of COVID-19 include fever, dry cough and fatigue. Symptoms such as nasal congestion, runny nose, sore throat, myalgia and diarrhea were also reported in a few patients.

Symptoms of respiratory viral infection due to SARS-CoV-2 and influenza can be similar. Influenza (flu) is an acute contagious respiratory illness caused by influenza viruses in winter and spring. Flu viruses spread mainly by droplets made when people with flu cough, sneeze or talk. There are three types of influenza (flu) virus, Types A, B and C. All influenza viruses are the family of orthomyxovirus consisting of single-stranded, segment RNA. Influenza A viruses are the only influenza viruses known to cause flu pandemics, current subtypes of influenza A viruses that routinely circulate in people include A(H1N1) and A(H3N2). Influenza B viruses are classified into two lineages B/Yamagata and B/Victoria. Influenza type B viruses change only by the more gradual process of antigenic drift. Influenza B viruses generally change more slowly in terms of their genetic and antigenic properties than influenza A virus. Influenza can also spread in human causing illness and epidemics.

【Detection Principle】

The kit is based on in vitro RT-PCR combining fluorescent probing. Primers and sequence-specific fluorescence probes were designed tailored to high conservative and specific region in SARS-CoV-2, IFV A and IFV B genome. The probes were attached by fluorophores at the 5' end as reporter with FAM for ORF1ab, with ROX for IFV B, and with CY5 for IFV A, and at 3' end with quencher, respectively. In a meantime, specific primers and probes were developed using human housekeeping gene as internal reference with fluorophores VIC/HEX attached at 5' end as reporter. RNA is reverse transcribed to cDNA. During the PCR procedures, the DNA polymerase cleaves the probe at the 5' end and separates the reporter dye from the quencher dye when the probes hybridize to the target DNA. This cleavage results in the fluorescent signal generated by the cleaved reporter dye, which is monitored real-time by the PCR detection system. Measuring the fluorescence intensities during Real Time PCR allows the qualitative detection of SARS-CoV-2, IFV A and IFV B in specimens.

The internal reference in the kit is used to monitor the whole procedures including reagents, RNA extraction and operation, to avoid false negative test results.

【Key contents】

Component (50 tests/kit)	Specification	Quantity	Description
SARS-CoV-2/IFV A/IFV B Reaction Buffer	800μL /vial	1 vial	Buffer for amplification reaction and dNTP
SARS-CoV-2/IFV A/IFV B Primers and Probes Mix	195μL /vial	1 vial	Probes and primers of SARS-CoV-2, IFV A, IFV B and internal reference
SARS-CoV-2/IFV A/IFV B Enzyme Mix	80μL /vial	1 vial	Taq polymerase, Reverse transcriptase and UDG
SARS-CoV-2/IFV A/IFV B Positive Control	750μL /vial	1 vial	Mix solution of recombinant pseudo-viruses with target genes of viruses and internal reference
SARS-CoV-2/IFV A/IFV B Blank Control	750μL /vial	1 vial	DNase/RNase free H_2O

Notes: Components contained within a kit are intended to be used together. The reagents with different lot numbers cannot be mixed.

Materials required but not provided

The assay was validated by the recommended materials as table 1 below.

Table 1 Materials required but not provided

Item	Validated products
RNA extraction kit	QIAamp Viral RNA Mini Kit (Cat. No. 52904) by QIAGEN
	TIANamp Virus RNA extraction Kit (Cat. No. YDP315-R) by TIANGEN
	Nucleic Acid Extraction Kit (Regulatory No. 20200167) by Wuhan MGI Tech Co., Ltd
Extraction equipment *(Optional)*	DNA Sequencing Library Preparation System (MGISP-100) by Wuhan MGI Tech Co., Ltd
	High-throughput Automated Sample Preparation System (MGISP-960, MGISP-960B) by Wuhan MGI Tech Co., Ltd
Sample collection	Virus sampling tube (HBPT8661) from Qingdao Haibo Biotechnology Co., Ltd.,
	Virus sampling tube (MT0301-3) from Youkang Hengye Biotechnology (Beijing) Co., Ltd.,

	Sample collection tube (Cat. No. 163441) of Jiangsu Kangjian Medical Supplies Co.,Ltd
	Consumables recommended by WHO
Consumables	RNase/DNase-free tips for pipettes
	disposable gloves
	RNase/DNase-free microcentrifuge tube, 8-tube strips for real-time PCR

【Storage and shelf-life】

The kit should be stored at temperature lower than -18°C in dark. It is stable with shelf-life for 9 months from date of production in claimed storage condition. Unpacked kit should avoid repeated thaw-freeze (within 4 times). The kit can be transported at temperature lower than -18°C in dark stable for 7 days.

The manufacture date and expire date are provided in the labelling.

【Applicable instruments】

Applied Biosystems™ Real time PCR system 7500; SLAN-96P PCR system

【Specimen】

Sample collection

- Collect fresh specimen of throat swabs from suspects. The operation of specimen should avoid possible contamination in collection, storage and transportation. The specimen should be presumed contagious and be operated according to related regulations of biosafety.
- It is recommended to use the virus sampling tube in Table 1 or suggested by WHO or other authority.
- **Throat swabs**: Carefully take out the swab from package and quickly rotate the head around two sides of fauces, throat and tonsil a few times with pressure to collect as much secretions as possible. Avoid touching tongue. Break the swab stick and put the head into sampling solution in specimen tubes. Screw the tube cap tightly to ensure no leakage.

Specimen Storage

- The specimen should be kept at temperature lower than -18°C for not longer than one week or at lower than -70°C for not longer than 6 months.
- Frozen specimen should be thoroughly thawed before operation while avoiding repeated thaw-freeze cycle more than 4 times

Specimen Transportation

- The specimen should be shipped in low temperature condition (lower than -18°C) using dry ice or ice bag for less than 7 days.

【Laboratory procedures】(Please read the procedures carefully before your operation)

Reagent preparation

- Take out all the reaction Mix and thaw them thoroughly at ambient temperature. Vortex and centrifuge briefly. The enzyme mix should be kept on ice after thawed and vortexed.
- Estimate the number of reactions (N) in the test, which includes Samples (n tubes), Blank control (1 tube), Positive control (1 tube). Prepare 8-tube strips for PCR based on the estimated N of reactions and develop the PCR mix as ingredients in following table. Pipette 20μL PCR Mix per tube into the 8-tube strips. Cap them tightly and transfer them to sample processing area. The

remaining Reaction Buffer, Primers and Probes Mix, and Enzyme Mix should be stored at -18°C immediately.

	SARS-CoV-2/IFV A/IFV B Reaction Buffer (μL)	SARS-CoV-2/IFV A/IFV B Primers and Probes Mix (μL)	SARS-CoV-2/IFV A/IFV B Enzyme Mix (μL)
PCR-Mix (μL)	15×N	3.5×N	1.5×N

Sample processing

- The specimen should be inactivated in a water bath at 56℃ for 30minutes after arrived at the laboratory.

- The collected throat swab specimen was operated to extract RNA in line with the manufacturer's instructions. The extracted RNA should be tested immediately or stored at temperature lower than -70°C for test later (less than 7 days). In parallel, equivalent volume of Positive Control and Blank Control should be also processed in nucleic acid extraction.

- The assay was validated by the recommended RNA extraction kits in Table 1 above mentioned. The sample extraction volume should be in accordance with manufacturer's instructions. 140μL sample is used by extraction kits from TIANGEN and QIAGEN. 200μL sample is needed by MGI's extraction kit to extract nucleic acid manually, 160μL sample is needed for automatically extraction using High-throughput Automated Sample Preparation System or DNA Sequencing Library Preparation System.

Sample addition

- Add 10μL the extracted RNA of specimens, Blank Control and Positive Control respectively into the 8-tube strips prefilled with PCR Mix. Cap them tightly and centrifuge briefly.

Real time PCR

- Set the fluorescent channels and reaction volume: please refer to the manufacturer's instructions of thermocycler for detailed information on channel setting.

  1) FAM channel (Reporter: FAM, Quencher: None) for SARS-CoV-2;
  2) ROX channel (Reporter: ROX, Quencher: None) for IFV B;
  3) CY5 channel (Reporter: CY5, Quencher: None) for IFV A;
  4) VIC/HEX channel (Reporter: VIC/HEX, Quencher: None) for internal reference;
  5) Reference Dye: None (only for ABI PCR system);
  6) Reaction Volume: 30μL;

- Configure PCR protocol

Step	Cycle	Temperature	Time	Fluorescence measured(Y/N)
1	1 cycle	50℃	20 min	N
2	1 cycle	95℃	5 min	N
3	45 cycles	95℃	15 sec	N
		55℃	30 sec	Y

Data analysis

- Applied Biosystems™ ABI7500 PCR system

Baseline starting point at 3 and ending at 15.

The threshold of each fluorescent channel should be set separately. In setting the threshold for a channel, the blank control should be selected firstly and click off the Automatic standard curve by changing the option from "☑Auto" to "□Auto". Set the threshold manually just above the maximum level of blank control curve (random noise curve) at FAM, ROX, CY5 and VIC/HEX channels.

- SLAN-96P real time PCR system

The starting and ending points of baseline should be set as 6 and 12 respectively.

The threshold of each fluorescent channel should be set separately. In setting the threshold for a channel, change the configuration of baseline optimization in basic parameter from automatic to manual. Then, manually set the threshold just above the maximum level of blank control curve (random noise curve) at FAM, ROX, CY5 and VIC/HEX channels.

Quality control

- Blank control: Ct values at FAM, CY5 and ROX channels are no data available, Ct values at VIC/HEX channel are higher than 35 or no data available.
- Positive control: Standard curves at FAM, ROX, CY5 and VIC/HEX channels are in S-shape with Ct values not higher than 35.
- Above requirements should be met in a single test. Otherwise, the test is invalid. Please operate the retest strictly according to the instructions to users.

Quality control metrics	**VIC (Internal reference)**	**FAM (ORF1ab)**	**ROX (IFV B)**	**CY5 (IFV A)**	**Interpretation**
Blank control	No amplification Ct value is >35 or no data.	No data	No data	No data	Pass; proceed to sample analysis
Positive control	Sigmoidal amplification curve and Ct value is ≤35.	Sigmoidal amplification curve and Ct value is ≤35.	Sigmoidal amplification curve and Ct value is ≤35.	Sigmoidal amplification curve and Ct value is ≤35.	
Blank control	Sigmoidal amplification curve and Ct value is ≤35.	Sigmoidal amplification curve and Ct value is ≤35.	Sigmoidal amplification curve and Ct value is ≤35.	Sigmoidal amplification curve and Ct value is ≤35.	Failed; repeat of Run
Positive control	No amplification or Ct value is >35.	No amplification or Ct value is >35.	No amplification or Ct value is >35.	No amplification or Ct value is >35.	

【Positive threshold and reference range】

- The cut-off value of the kit was determined through ROC curve analysis on basis of clinical specimens. The positive threshold of Ct values was not higher than 40 for SARS-CoV-2, IFV A and IFV B and the positive threshold of Ct values was not higher than 35 for internal reference.

【Testing result interpretation】

- The specimen is positive SARS-CoV-2, IFV B or IFV A, if standard curves at FAM, ROX or CY5 channel are in S-shape with Ct values not higher than 40, while standard curve at VIC/HEX channel is in S-shape with Ct value not higher than 35.
- The specimen is negative SARS-CoV-2, IFV B or IFV A, if standard curves at FAM, ROX or CY5 channels are not in S-shape with Ct at FAM, ROX or CY5 no value or higher than 40, while standard curve at VIC/HEX channel are in S-shape with Ct values not higher than 35.
- Specimen test was invalid and should be tested if Ct value of internal reference is higher than 35. If the Ct value of internal reference in the retesting specimen is higher than 35 again, specimen should be recollected and retested.

Interpret the testing results of SARS-CoV-2

	VIC/HEX (Internal Reference)	FAM (orf1ab)	Interpretation
Sample 1	Sigmoidal amplification curve and Ct value is ≤35.	Sigmoidal amplification curve and Ct value is ≤40	Positive for SARS-COV-2 RNA; amplification detected in both channels and Ct is below threshold.
Sample 2	No Ct or Ct>35	Any	Repeat extraction and RT-PCR operation.
Sample 3	Sigmoidal amplification curve and Ct value is ≤35.	Ct>40 or No Ct data	SARS-CoV-2 negative

Interpret the testing results of IFV A and IFV B

	VIC/HEX (Internal reference)	ROX (IFV B)	CY5 (IFV A)	Interpretation
Sample 1	Sigmoidal amplification curve and Ct value is ≤35.	Sigmoidal amplification curve and Ct value is ≤40	Sigmoidal amplification curve and Ct value is ≤40.	Positive for IFV B and IFV A. Amplification detected in both channels and Ct is below threshold.
Sample 2	Sigmoidal amplification curve and Ct value is ≤35.	No Ct data or Ct value >40.	Sigmoidal amplification curve and Ct value is ≤40.	Positive for IFV A. Amplification detected in CY5 channels and Ct is below threshold.
Sample 3	Sigmoidal amplification curve and Ct value is ≤35.	Sigmoidal amplification curve and Ct value is ≤40.	No Ct data or Ct value >40.	Positive for IFV B. Amplification detected in ROX channels and Ct is below threshold.
Sample 4	Sigmoidal amplification curve and Ct value is ≤35.	Ct>40 or No Ct data	Ct>40 or No Ct data	Negative for IFV A and IFV B
Sample 5	No Ct or Ct>35	Any	Any	Repeat extraction and RT-PCR operation.

【Limitation of the assay】

- This kit is for research use only, and these instructions should not be used to obtain clinical diagnostic results. Test kits are not for commercial use or distribution, and are intended only for laboratory confirmation of test validity.

- Incorrect results can be caused by improper operations in sample collection, transportation or processing, very low concentration of target virus in the specimens, mutations within the viral genome covered by the kit's primers and/or probe, and unproved external interference factors, such as PCR inhibitor.

【Performance characteristics】

- The kit package is intact and liquid contents are clear, transparent and no sediments. All contents are in correct quantity as the package insert listed.

- The kit was validated by manufacturer's positive references with Coincidence rates 100%.

- Positive control is positive with standard curves at FAM, ROX, CY5 and VIC/HEX channel in S-shape and Ct values not higher than 35. Blank control is negative without Ct values at FAM, ROX, CY5 and VIC/HEX while Ct values at VIC/HEX channels is higher than 35 or no data.

【Warning and precautions】

- For Research Use Only. Not for use in diagnostic procedures.
- The relevant laboratory management standards shall be strictly implemented in accordance with the regulations on the management of gene amplification testing laboratories promulgated by the administrative department.
- Specimens are potentially infectious, and the appropriate operations of specimen, including collection, storage and transportation, and laboratory test, should be strictly manipulated in line with relevant regulations of biosafety and molecular laboratory management.
- The false positive or negative testing result can be led by poor quality of specimen, incorrect operations in sample collection, transportation or laboratory processing, or the quality of extracted RNA, or limitation of the technology. Operator should understand well the principles of the laboratory procedures and its limitation in performance in advance and avoid any potential mistakes intentionally.
- The kit should be stored and transported in claimed conditions. Thaw all kit components thoroughly and centrifuge them briefly before starting an assay. Avoid repeated thaw-freeze cycle.
- All contents in a kit are prepared dedicatedly and validated for the intended testing purpose. Replacing any of them will affect the testing performance of the kit. Components contained within a kit are intended to be used together. The reagents with different lot numbers cannot be mixed.
- Separate laboratory areas are recommended to performing predefined procedures of the assay.
  a) 1st Area: Preparation Area—Prepare testing reagent;
  b) 2nd Area: Sample processing—Process specimens and controls;
  c) 3rd Area: Amplification Area—PCR conducted

  All materials used in one area should always be remained in the area and should not be moved or used in other areas. After the assay procedures, the workbench and lab supplies should be

cleaned and disinfected timely.

- To prevent the contamination from exogenous RNA, blank control should be added firstly in sample addition followed by specimen RNA and positive control in order. RNase/DNase-free filtered tips should be used separately in preparing reagent and RNA template addition.
- Reaction tubes for real time PCR should be capped tightly and transferred to specimen processing area immediately after addition of Nucleic Acid reaction Mix. Ensure to pipette the samples exactly into the reaction mix in PCR tubes and avoid sticking the samples to the inside tube wall. The tubes should be capped tightly immediately after the addition.
- After the protocol of amplification is done, remove PCR tubes from the thermal cycler and discard them in a sealable plastic bag for autoclave and decontamination.
- All pipette tips and centrifuge tubes in the assay should be DNase/RNase-free. The workbench and lab supplies should be cleaned and disinfected regularly using 1% Sodium hypochlorite, 75% ethanol or UV light.
- Operator should receive professional training for Real-time PCR before operating.
- Please contact BGI sales for the most up-to-date information in the event of damage to the protective packaging.
- The damaged kit should not be used for laboratory testing of clinical specimen and should be discarded after autoclaved.

【Disclaimer】

- The kits are sent for validation, not for commercial use or distribution, and are intended only for laboratory confirmation of test validity.

【References】

[1] LU Rou-jian, ZHANG Ling-lin, TAN Wen-jie, ZHOU Wei-min, WANG Zhong, PENG Kun, RUAN Li. Development and Comparison of Real-Time and Conventional RT-PCR Assay for Detection of Human Coronavirus NL63 and HKU1[J]. CHINESE JOURNAL OF VIROLOGY, 2008(4).

[2] NIU Pei-hua, LU Rou-jian, LAN Jia-ming, LIU Gao-shan, WANG Wen-lin, TAN Wen-jie. Development of Novel Multiplex Real-time RT-PCR Assays for Detection of MERS-CoV Infection[J]. CHINESE JOURNAL OF VIROLOGY, 2016(3).

[3] CHEN Yu-jing. Development of two-panel reactions of real-time PCR for detection of 18 types/subtypes of respiratory viruses[D]. 2015

[4] CHEN Ji-ming, GUO Yuan-ji, WU Kun-yu, GUO Jun-feng. The Origin and Evolution of Two Genetic Lineages of Influenza B Virus. Chinese Journal of Virology 2001(4).

[5] XU Cui-ling, XIANG Ni-juan, ZHANG Ye, WEN Le-ying, ZHAO Xiang, LI Zi, GUO Jun-feng, WANG Min, YU Hong-jie, YANG Wei-zhong, GUO Yuan-ji, SHU Yue-long. Antigenic and genetic study of influenza virus (H1N1) circulated in China in 2004-2005. Chinese J Exp Clin Virol, 2006.

[6] Wang Min, Guo Yuan-jie. Hemagglutinin protein antigenicity and gene characteristics of influenza B virus. Chinese Journal of Experimental and Clinical Virology.1995(3).

[7] Novel Swine-Origin Influenza A (H1N1) Virus Investigation Team. Emergence of a Novel Swine-Origin Influenza A (H1N1) Virus in Humans. N Engl J Med, 2009.

[8]M W Shaw, R A Lamb, and P W Choppin. Proc Natl Acad Sci U S A. 1982; 79(22).

[9] Yamashita M, Krystal M, Fitch WM, Palese P. Influenza B virus evolution: co-circulating lineages and comparison of evolutionary pattern with those of influenza A and C viruses. Virology. 1988;163(1).

【Release date of the user manual】

This manual was released on 2020-07-06

【Language edition】

For the requirements of Instruction for Use in other languages, please contact BGI PathoGenesis Pharmaceutical Technology Co.,Ltd.

【Key to symbols used】

	**MANUFACTURER**
	**USE BY DATE**
	**BATCH CODE**
	**DATE OF MANUFACTURE**
	**CATALOGUE NUMBER**
	**CAUTION**
	**UPPER LIMIT OF TEMPERATURE**
	**CONSULT INSTRUCTIONS FOR USE**
	**KEEP AWAY FROM SUNLIGHT**
	**KEEP DRY**
	**DO NOT RE-USE**
	**POSITIVE CONTROL**
	**CONTAINS SUFFICIENT FOR N TESTS**

【Contact details】

BGI PathoGenesis Pharmaceutical Technology Co.,Ltd.
Building No.7, BGI Park, No.21 Hongan 3rd Street, Yantian District, Shenzhen 518083, China

Manufacturing Site: Jiangsu Macro & Micro-Test Med-Tech Co., Ltd.

Site Address: No.888, Zhujiang Road, Juegang Sub-district, Rudong County, Jiangsu Province

Please Contact: BGI PathoGenesis Pharmaceutical Technology Co., Ltd.

Service Hotline:

Shenzhen, China: (+86) 400-706-6615

Email: P_pathoservice@genomics.cn

Website：www.bgi.com

【Revision history】

Version	Chapter	Revision contents	Revision	Date
V1.0	All	First release		