华大基因
BGI

CE IVD **Real-time fluorescent RT-PCR kit for detecting 2019-nCoV**

【Generic product name】

Real-time fluorescent RT-PCR kit for detecting 2019-nCoV

【Package size】

50 tests/kit

【Catalogue Number】

MFG030010

【Intended use】

The kit is a qualitative in vitro nucleic acid amplification assay to detect the new coronavirus identified in China in 2019 using Reverse transcription PCR in specimen of throat swab and Bronchoalveolar Lavage Fluid (BALF)from suspects.

In end of 2019, some pneumonia cases were reported in Wuhan, China and the pathogen was confirmed as a new strain. World Health organization has named the newly identified coronavirus as 2019-nCoV. Although more intensive researches must be conducted later to well understand the virus, in response to the emergency in disease control, simple and rapid kit is necessary to identify the virus timely and implement efficient interventions to contain the spread. The kit will qualitatively detect the nucleic acid of 2019-nCoV in specimen from suspects enabling to assess the infection situation of 2019-nCoV in suspects in clinical and public health practice.

【Principle of the procedures】

The kit is based on in vitro RT-PCR combining fluorescent probing. Primers and a sequence-specific fluorescence probes were designed tailored to high conservative region in 2019-nCoV genome. The probes are oligonucleotide attached fluorophores at the 5' end with FAM as reporter and 3' end with quencher. In a meantime, specific primers and probes were developed as internal reference with fluorophores VIC/HEX attached at 5' end as reporter. 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. Monitoring the fluorescence intensities during Real Time allows the qualitative detection of 2019-nCoV in specimens.

【Key contents】

Item（50 tests/kit）	Specification	Quantity	Description
2019-nCoV Reaction Mix	1mL /vial	1 vial	Composed of reagent for amplification and probes and primers of target gene and internal reference
2019-nCoV Enzyme Mix	80μL /vial	1 vial	Taq polymerase, Reverse transcriptase and UDG
2019-nCoV Positive control	750μL/vial	1 vial	Mix solution of pseudo-virus with target virus genes and internal reference
2019-nCoV Blank control	750μL/vial	1 vial	DNase/RNase free water

Materials required but not provided

- Reagents：TIANamp Virus RNA extraction Kit (DP315-R) manufactured by TIANGEN, or QIAamp Viral RNA

Mini Kit (52904) by QIAGEN.

- 1.5 mL RNase/DNase-free microcentrifuge tube, RNase/DNase-free tips for pipettes, 0.2mL 8-tube strips for real-time PCR, Bench centrifuge, Vortex mixer.

- Notes：Components contained within a kit are intended to be used together. Do not mix components from different kit lots.

【Storage and shelf-life】

- The RT-PCR Kit should be stored at temperature lower than -18°C in dark. It is stable with shelf-life at 2-8 °C for 5 days and at -18°C for 6 months（tentative）. Unpacked kit should avoid repeated thaw-freeze cycle (within 4 times)

- The PCR Kit can be transported at -18°C in dark stable for 5 days. The manufacture date and shelf life would be 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 and BALF 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.

- **Throat swabs**: Carefully take out the swab from package and quickly rotate it around two sides of fauces, throat and tonsil a few times applying 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.

- **BALF**: Collect 3ml of unprocessed BALF in sterile, dry and clean DNase/RNase free Cryotubes. Screw the tube cap tightly to ensure no leakage and seal the tube with film.

Storage

- The specimen should be kept in proper condition, at -18℃ for not longer than 1 weeks and at -70℃ for not longer than 6 months.

- Frozen specimen should be thawed thoroughly while avoiding repeated thaw-freeze cycle.

Transportation

- The specimen should be shipped in low temperature condition using dry ice or ice bag.

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

Sample processing

- The fresh specimen should be collected to ensure the qualified RNA in terms of quality and quantity for the

assay. RNA should be extracted using Nucleic Acid extracting Kit in line with the manufacturer's instruction. Equivalent volume of positive control and blank control should be processed simultaneously. The assay was validated by the recommended RNA extraction kits by TIANGEN （DP315-R）and QIAGEN（52904）.

- The extracted RNA should be tested immediately or stored at -70°C for test later.

Reagent preparation

- Take out all the kit contents and thaw them thoroughly at ambient temperature. Vortex and centrifuge briefly. The Enzyme Mix should be kept in ice continuously.

- Estimate the number of reactions (N) in the test, which includes the number of Blank control (1 tube), Positive control (1 tube), and specimens prepared. Prepare 8-tube strips for PCR based on the estimated N of reaction and develop the PCR mix as ingredients in following table. Pipette 20μL PCR Mix per tube into the 8-tube strips. Capped them fastened and transfer them to sample processing Area. The remaining Nucleic acid reaction Mix and Enzyme Mix should be stored at -18°C immediately.

	2019-nCoV Reaction Mix(μL)	2019-nCoV Enzyme Mix(μL)
PCR-Mix (μL)	18.5×N	1.5×N

Add sample

- Add 10μL the extracted RNA of specimens, Blank control and Positive controls respectively into the 8-tube strips prefilled with PCR Mix. Capped them fastened and centrifuge them at 2000rpm for 10 seconds. Place the tubes into thermal cycler and record the exact location of controls and every specimen.

Real time PCR

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

FAM channel (Reporter: FAM, Quencher: None) for RNA of 2019-nCoV;

VIC/HEX channel（Reporter: VIC/HEX, Quencher: None） for internal reference;

Reference Dye：None（only for ABI PCR system）;

Sample Volume: 30.

- Configure PCR protocol

Step	Cycle	Temperature	Duration	Fluorescence measured(Y/N?)
1	1 cycle	50℃	20minutes	N
2	1 cycle	95℃	10minutes	N
3	40cycles	95℃	15 seconds	N
		60℃	30 seconds	Y

Data analysis

- Baseline and threshold for 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 channel.

- Data from SLAN-96P 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/ VIC(HEX).

Quality control

- Blank control: Ct values at FAM and VIC/HEX channels are 0 or no data available.
- Positive control：Standard curves at channel FAM and VIC/HEX channels are in S-shape with Ct values not higher than 32.
- Testing specimen: Standard curves at VIC/HEX channel is in S-shape with Ct not higher than 32.
- Above requirements should be met in a single test. Otherwise, the test is invalid. Please operate the retest strictly in line with the package insert.

【Threshold and reference range】

- Cut-off value of the kit was determined based on the Receiver Operator characteristic curve from testing clinical samples. Ct value for 2019-nCoV positive by the kit is not high than 38.

【Testing result interpretation】

- The specimen is positive of 2019-nCoV if standard curve at FAM channel is in S-shape with Ct value not higher than 38.
- The specimen is negative of 2019-nCoV　if standard curve at FAM channel is not in S-shape with Ct at FAM as 0 or no data available while Ct at VIC/HEX not higher than 32.
- The specimen should be retested if standard curve at FAM is in S-shape with Ct higher than 38. The specimen can be reported on basis of retesting results as positive of 2019-nCoV for Ct higher than 38 and as negative of 2019-nCoV for standard curve not in S-shape and Ct of internal reference not higher than 32 at VIC/HEX.
- In case that standard curve at FAM is not in S-shape with Ct value as 0 or no data available, the specimen should be retested if Ct at VIC is higher than 32 or no data available.

【Limitation of the assay】

- The Results of the test is just for information in clinical practices to assess infection condition of patients combining

with clinical presentations and other laboratory markers.

- The incorrect result can be caused by incorrect 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 package is intact and liquid contents are clear, transparent and no sediments. All contents are in correct quantity as the package insert listed.
- Positive control is positive at both FAM and VIC/HEX channel in testing while blank control is negative at both channels.
- Limitation of Detection (LOD) of the kit is 100 copies/mL for detecting 2019-nCoV.
- The kit was validated by national positive and negative standards.
- A potential cross-reactivity of the RT-PCR Kit was tested and none of the tested pathogens and human gene have been reactive. The tested pathogens include 54 pathogens, such as human coronavirus includes OC43,229E, HKU1 and NL63(HCoV-OC43, HCoV-229E, HCoV-HKU1, HCoV-NL63) and other pathogens.
- The reproducibility of the assay was validated by manufacturer's precision standards (CV1 and CV 2), LOD standard and negative standard. All samples were tested repeatedly for 20 times, respectively. Coefficient of variance (CV) for Ct values were analyzed to evaluate the variability of inter- and intra-batches, within day and day-to-day operation. The CVs are all less than 5% respective (n=20).
- The repeatability of assay was validated by manufacturer's repeatability standards, LOD standard and negative standard repeatedly for 20 times. Coefficient of variance (CV) for Ct values were analyzed to evaluate the inter-batch variability. They are all less than 5%.
- Interference trial shows that performance of the kit is stable with endogenous and exogenous interfering substances such as some anti-microorganism drugs, nasal sprays and nasal drops in specimen. Specimen with elevated level of mucoprotein at a concentration of 60 mg / mL and other substances do not influence the kit performance at virus concentration higher than Limit of Detection.

【Warning and precautions】

- FOR IN VITRO TEST ONLY. Please read the package insert carefully before your operation. The appropriate operations from specimen collection, storage and transportation, and laboratory test should be strictly manipulated in line with relevant regulations of biosafety and molecular laboratory management. Please contact BGI sales for the most up-to-date information in the event of damage to the protective packaging
- 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 limitation of the technology. Operator should understand well the principles of the procedures and its limitation in performance in advance and avoid any potential mistakes intentionally.
- 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 the specimen and controls；

c) 3rd：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.
- All contents in the package are prepared dedicatedly for the intended testing purpose and validated. Replacing any of them will affect the testing performance of the kit. Components contained within a kit are intended to be used together. Do not mix components from different kit lots.
- Thaw all kit components thoroughly and centrifuge them briefly before starting an assay. Avoid repeated thaw-freeze cycle.
- 8-tube strips for real time PCR capped fasten and transferred to specimen processing area immediately after addition of Nucleic Acid reaction Mix.
- To prevent the contamination from exogenous RNA, sample addition should follow the sequence of negative control, specimen RNA and positive control. Filtered tips should be prepared and used separately in preparing reagent and sample addition.
- 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 fasten 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.
- Ensure no foam or bubbles present in the tubes when aliquoting nucleic acid Mix. All PCR tubes capped fasten before loading them into the thermal cycler to avoid any possible leakage and contamination.
- The workbench and lab supplies should be cleaned and disinfected regularly using 75% ethanol or UV light.
- All pipette tips and centrifuge tubes in the assay should be DNase/RNase-free. The used centrifuge tubes and pipette tips should be discarded in waste bin with Clorox (84) disinfectant and disposed with other laboratory wastes after decontamination.
- Operator should receive professional training before operating.

【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 P, LU R, LAN J, LIU G, WANG W, TAN W. 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

【Contact details】

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【Language edition】

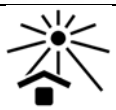
For the requirements of Instruction for Use in other languages, please contact BGI Europe A/S.

【Release date of the user manual】

This manual was released on 2020-02-26

【Key to symbols used】

	**IN VITRO DIAGNOSTIC MEDICAL DEVICE**
	**MANUFACTURER**
	**USE BY DATE**
	**BATCH CODE**
	**DATE OF MANUFACTURE**
	**CATALOGUE NUMBER**

	**CAUTION**
	**UPPER LIMIT OF TEMPERATURE**
	**CE MARK**
	**CONSULT INSTRUCTIONS FOR USE**
	**KEEP AWAY FROM SUNLIGHT**
	**KEEP DRY**
	**DO NOT RE-USE**
	**POSITIVE CONTROL**
	**CONTAINS SUFFICIENT FOR N TESTS**