CE IVD

Detection Kit for 6 mutations in S gene of SARS-CoV-2(ARMS-PCR)

Instruction for Use

【Product name】

Detection Kit for 6 mutations in S gene of SARS-CoV-2(ARMS-PCR)

【Product specifications 】

50 tests/kit

【Catalogue number】

PGI030019

【Intended use】

The kit is a qualitative *in vitro* nucleic acid amplification assay to detection the mutations of N501Y, A570D, HV69-70del, K417N, K417T, and E484K in S gene of SARS-CoV-2 in B.1.1.7 lineage, B.1.1.28 lineage and B.1.351 lineage of SARS-CoV-2 in throat swab or sputum specimen confirmed as SARS-CoV-2 positive by RT-PCR.

Multiple SARS-CoV-2 variants are circulating globally. Several new variants emerged around the end of 2020, most notably, 20B/501Y.V1 or B.1.1.7 lineage in the United Kingdom (UK), and 20C/501Y.V2 or B.1.351 lineage in South Africa. B.1.1.7 lineage, B.1.1.28 lineage and B.1.351 lineage variants have been detected in numerous countries around the world. They both have mutation in the receptor binding domain (RBD) of the spike protein at position 501, where the amino acid asparagine (N) has been replaced with tyrosine (Y), N501Y, leading to a tight interaction of RBD with human receptor ACE2. Other mutations include A570D and HV69-70del, both probably associated with increased transmissibility (i.e., more efficient and rapid transmission), and K417N, K417T, E484K, also a RBD mutation, which also increased the affinity of virus with human receptor.

【Principles of the procedures 】

The kit is based on ARMS-PCR method. For N501Y, A570D, HV69-70del, K417N, K417T, and E484K of SARS-CoV-2, sequence-specific primers and fluorescence probes were designed tailored for variant strain, respectively. ARMS primers can specifically identify variant strain using different fluorescent bands, enabling to differentiate mutant strains (N501Y, A570D, HV69-70del, K417N, K417T, and E484K) from wild strain through 2 tests for each specimen in a single run. In addition, internal reference was developed in the kit to monitor the whole procedures including reagents, RNA extraction, and operation, to avoid false negative test results.

【Key contents】

Contents (50 tests/kit)	Specification	Quantity	Description
Reaction Mix A	1 mL/vial	1 vial	Reagent with primers and probe for amplification of ORF1ab , internal reference ,N501Y and K417N
Reaction Mix B	1 mL/vial	1 vial	Reagent with primers and probe for amplification of A570D, HV69-70del , K417T, and E484K
Enzyme Mix	240 μL/vial	1 vial	Taq DNA Polymerase, reverse transcriptase, UDG
Positive Control	750 μL/vial	1 vial	Mix solution of recombinant pseudo-viruses with target genes of mutant strain, ORF1ab and internal reference
Blank Control	750 μL/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
Reagent	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
	Solution for sputum decontamination
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
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 -15 °C in dark. It is stable with shelf-life for 9 months from date of production in the claimed storage condition. Unpacked kit should avoid repeated thaw-freeze cycle (within 6 times). The kit can be transported at temperature lower than -15 °C in dark stable for 7 days.

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

【Applicable instruments】

SLAN-96P PCR system; Applied Biosystems™ QuantStudio®5 Real-Time PCR Systems; Light Cycler® 480 Real time PCR System; Fluorescent Quantitative PCR Detection system FQD-96A; Real-Time Quantitative Thermal Cycler MA-6000.

【Specimen】

Sample collection

- Collect fresh specimen of throat swabs and sputum 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.
- **Sputum:** Collect sputum in the early morning after wash mouth. Take a deep breath. Hold the air for a few seconds. Breathe out slowly. Take another deep breath. Cough hard until sputum comes up in mouth. Spit the sputum into the sample bottle. Do this until there is enough sputum to cover the bottom of the bottle. Gas aspiration method can be used to collect sputum for those without sputum. Screw the tube cap tightly to ensure no leakage and seal the tube with film. The sputum should be delivered for testing immediately.

Storage

- The specimen should be kept in proper condition, at temperature lower than -15 ℃ for no longer than 1 week and at temperature lower -70 ℃ for no 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 bags.

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

Sample processing

- The fresh swab 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 instructions. Equivalent volumes of positive control and blank control should be processed simultaneously. The assay was validated by the recommended RNA extraction kits by TIANGEN (YDP315-R), QIAGEN (52904) and Nucleic Acid Extraction Kit (96 Preps: 1000021042, 1728 Preps: 1000021043) by Wuhan MGI

Tech Co., Ltd. 140 μL specimen is used by extraction kits from TIANGEN and QIAGEN. 160 μL specimen is needed by kit from MGI to extract nucleic acid manually or automatically using High-throughput Automated Sample Preparation System (MGISP-960, Cat. No. 900-000165-00) or DNA Sequencing Library Preparation System (MGISP-100, Cat. No. 900-000207-00).

- Sputum should be added equivalent volume of solution for decontamination and vibrated about 30 minutes at ambient temperature followed by nucleic acid extraction.
- The extracted RNA should be tested immediately or stored at temperature lower than -70 °C for test later, and the storage time does not exceed 7 days

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 on ice continuously.
- Estimate the number of reactions (N) in the test, which includes the Blank Control (1 tube), Positive Control (1 tube), and specimens prepared, respectively.
- Prepare 8-tube strips for PCR based on the estimated N of reactions for ORF1ab and mutant strains, respectively. PCR-Mix1 for testing ORF1ab, internal reference , N501Y and K417N. should be prepared as shown in Table 2 below. Table 3 below was the ingredients for preparing PCR-Mix2 for testing E484K, A570D, K417T and HV69-70del. Pipette 20 μL of PCR-Mix1 per tube into the 8-tube strips prepared for testing ORF1ab, internal reference, N501Y and K417N and 20 μL of PCR-Mix2 per tube prepared for testing E484K,A570D ,K417T and HV69-70del , respectively. Cap them tightly and transfer them to the sample processing area. The remaining nucleic acid reaction mix and Enzyme Mix should be stored at temperature lower than -15 °C immediately.

Table 2 PCR mix preparation for ORF1ab, internal reference , N501Y and K417N.

	Reaction Mix A (μL)	Enzyme Mix (μL)
PCR-Mix1	18×N	2×N

Table 3 PCR mix preparation for E484K, A570D, K417T and HV69-70del.

	Reaction Mix B (μL)	Enzyme Mix (μL)
PCR-Mix2	18×N	2×N

Add sample

- Add 10 μL of the extracted RNA of specimens, Blank Control, and Positive Control respectively into the 8-tube strips prefilled with PCR-Mix1and PCR-Mix2. Cap them tightly and centrifuge them at 2000 rpm for 10 seconds.
- Please refer to Table 4 below for the example of PCR tube layout on a PCR plate.

Table 4 Example of PCR tube on a PCR plate

	1	2	3	4	5	6	7	8	9	10	11	12
	PCR-Mix1	PCR-Mix2	PCR-Mix1	PCR-Mix2	PCR-Mix1	PCR-Mix2	PCR-Mix1	PCR-Mix2	PCR-Mix1	PCR-Mix2	PCR-Mix1	PCR-Mix2
A	BC	BC	Sample7	Sample7								
B	PC	PC	Sample8	Sample8								
C	Sample1	Sample1										
D	Sample2	Sample2										
E	Sample3	Sample3										
F	Sample4	Sample4										
G	Sample5	Sample5										
H	Sample6	Sample6										

Note: BC-Blank Control; PC-Positive Control

Real-time PCR

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

  PCR-Mix1：

  1) FAM channel（Reporter： FAM， Quencher： None）for ORF 1ab；
  2) VIC/HEX channel（Reporter： VIC/HEX， Quencher： None）for N501Y mutant strain；
  3) ROX channel（Reporter： ROX， Quencher： None）for K417N mutant strain ；
  4) CY5 channel（Reporter： CY5， Quencher： None）for internal reference；

  PCR-Mix2：

  1) FAM channel（Reporter： FAM， Quencher： None）for E484K mutant strain；
  2) VIC/HEX channel（Reporter： VIC/HEX， Quencher： None）for HV69-70del mutant strain；
  3) ROX channel（Reporter： ROX， Quencher： None）for K417T mutant strain；
  4) CY5 channel（Reporter： CY5， Quencher： None）for A570D mutant strain ；

  Reference Dye: None.
  Sample Volume: 30 μL.

- Configure PCR protocol

  Table 5 Configure PCR protocol

Step	Cycles	Temperature	Duration	Fluorescent signal collection
1	1 cycle	50 ℃	10 min	No
2	1 cycle	95 ℃	1 min	No
3	45 cycles	95 ℃	5 sec	No
		58 ℃	15 sec	Yes

Data analysis

- 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. When 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 all channels.

- Applied Biosystems™ QuantStudio®5 Real time PCR system

Baseline is set as default.
Threshold: In most cases, the auto threshold line function yields satisfying results. In some cases, it can be adjusted manually. When setting threshold, click [Show Plot Setting], select the target gene to view and the "Show: Threshold" as ☑. Adjust the threshold through dragging it by mouse or inputting values directly, then, click [Analyze].

- Light Cycler® 480 Real time PCR system

Baseline is set as default.

Threshold: In most cases, the auto threshold line function yields satisfying results. In some cases, it can be adjusted through slightly improving the standard curve error value by manually moving the threshold line up or down, fitting the line to the exponential portion of the amplification curve, higher than while horizontally paralleling the amplification curve of Blank control. Click [Analysis] to get results and [Report] to present them.

- Fluorescent Quantitative PCR Detection system FQD-96A

Baseline is set as default.
Threshold: In most cases, the auto threshold line function yields satisfying results. In some cases, it can be adjusted manually. When setting threshold, click [Analysis settings], select the target gene to view and the "Automatic threshold" as □. Adjust the threshold by inputting values directly, then, click [Save and analyze].

- Real-Time Quantitative Thermal Cycler MA-6000

Baseline is set as default.
Threshold: In most cases, the auto threshold line function yields satisfying results. In some cases, it can be adjusted manually. When setting threshold, click[Analysis], select the target gene to view and the "Automatic threshold" as □. Adjust the threshold by inputting values directly, then, hit the Enter key on the keyboard.

【Quality control】

- Blank Control:

  PCR-Mix1: Ct values at FAM, VIC/HEX and ROX channels are 0 or no data available. Ct value at CY5 channel is 0, no data available or higher than 38.

  PCR-Mix2: Ct values at FAM, CY5, ROX and VIC/HEX channels are 0 or no data available.

- Positive Control:

  PCR-Mix 1&2: Ct values at FAM, CY5, ROX and VIC/HEX channels are all in S-shape with Ct values no higher than 35 in both PCR-Mix1and Mix2.

- 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】

- Reference range of the kit was determined based on the Receiver Operating Characteristic curve and percentile method. Cut-off values for positive N501Y, A570D, HV69-70del, K417N, K417T, and E484K are Ct values no higher than 41. The identification of mutant strain of N501Y, HV69-70del, E484K, A570D, K417N and K417T should be determined by Ct values in combination with Δ Ct.
- Δ Ct calculated using formula: Ct value of mutant strain- Ct value of ORF1ab.

Table 6 Δ Ct calculation method

Δ Ct	Allele	Fluorescent signal	Δ Ct value
/	ORF1ab.	FAM(PCR-MIX1)	/
ΔCt1	N501Y	VIC(PCR-MIX1)	Ct_{N501Y} - Ct_{ORF1ab}
ΔCt2	K417N	ROX(PCR-MIX1)	Ct_{K417N} - Ct_{ORF1ab}
ΔCt3	E484K	FAM (PCR-MIX2)	Ct_{E484K} - Ct_{ORF1ab}
ΔCt4	HV69-70del	VIC/HEX(PCR-MIX2)	$Ct_{HV69-70del}$ - Ct_{ORF1ab}
ΔCt5	K417T	ROX(PCR-MIX2)	Ct_{K417T} - Ct_{ORF1ab}
ΔCt6	A570D	CY5(PCR-MIX2)	Ct_{A570D} - Ct_{ORF1ab}

Table 7 Reference range for different mutation .

Allele	Δ Ct value	Result
N501Y	Δ Ct1≤6	N501Y（mutant strain）
	Δ Ct1>6	Non-N501Y mutant
K417N	Δ Ct2≤6	K417N（mutant strain）
	Δ Ct2>6	Non-K417N mutant

E484K	Δ Ct3≤6	E484K（mutant strain）
	Δ Ct3>6	Non-E484K mutant
HV69-70del	Δ Ct4≤6	HV69-70del（mutant strain）
	Δ C4t>6	Non- HV69-70del mutant
K417T	Δ Ct5≤6	K417T（mutant strain）
	Δ Ct5>6	Non-K417T mutant
A570D	Δ Ct6≤6	A570D（mutant strain）
	Δ Ct6>6	Non-A570D mutant

- Cut-off value for internal reference was determined as 38, no higher than 38 as positive.

【Testing result interpretation】

- When Ct value of mutation strain is not higher than 41, ΔCt should be calculated as Table 6 above (45 should be used in calculating ΔCt when Ct value of ORF1ab is 0 or no Ct). Testing results should be interpreted as Table 7 above .
- When Ct value of mutation strain is higher than 41 and Ct value of ORF1ab gene is not higher than 41, the specimen is not a mutant strain of target gene .
- When Ct value of the mutation strain and ORF1ab gene are both higher than 41, the specimen was in low concentration of SARS-CoV-2 if Ct value of internal reference at CY5 not higher than 38. The sample with Ct value of internal reference higher than 38 at CY5 should be re-extracted and retested.

Example for Result interpretation

Sample	Target (FAM/VIC/ROX/CY5(A570D))			Internal Reference (CY5)	Interpretation
	Mutant strain	ORF1ab	ΔCt		
Sample 1	Sigmoidal amplification curve and Ct value is ≤41	Any	≤6	Any	Mutant strain
Sample 2	Sigmoidal amplification curve and Ct value is ≤41	Any	>6	Any	Non mutant strain of target gene
Sample 3	0, no Ct or Ct value is>41	Sigmoidal amplification curve and Ct value is ≤41	/	Any	Non mutant strain of target gene
Sample 4	0, no Ct or Ct value is>41	0, no Ct or Ct value is>41	/	Sigmoidal amplification curve and Ct value is ≤38	Low concentration of virus RNA.
Sample 5	0, no Ct or Ct value is>41	0, no Ct or Ct value is>41	/	0, no Ct or Ct value is >38	Invalid test . Reextracted and retest.

【Limitation of the assay】

- This kit is for identifying N501Y, A570D, HV69-70del, K417N, K417T, and E484K Mutation detection of SARS-CoV-2 in samples confirmed positive SARS-CoV-2 by RT-PCR. The results should not be used to obtain clinical diagnostic results.
- 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 virus genome not

covered by the kit's primers and/or probes, and unproved external interference factors, such as PCR inhibitor.

【Performance characteristics】

- The package is intact and the liquid contents are clear, transparent and with no sediments in it. All contents are in correct quantity as the package insert listed.
- Positive Control is positive at FAM, CY5, ROX and VIC/HEX channel in testing while Blank Control is negative at all channels with Ct of internal reference at CY5 higher than 38 or no value.
- The kit was validated by manufacturer's positive references and negative references all positive references were positive, all negative references were negative.
- Limit of Detection (LOD) of the kit was 1000 copies/mL.

【Warning and precautions】

- For IN VITRO TEST ONLY . Please read the package insert carefully before 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.
- 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 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.
- Separate laboratory areas are recommended to perform predefined procedures of the assay.

  a) 1st Area: Preparation Area—Prepare testing reagent.

  b) 2nd Area: Sample processing Area—Process the specimen 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, 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.
- 8-tube strips for real time PCR should be capped tightly and transferred to specimen processing area immediately after addition of nucleic acid reaction mix. Pipette the samples exactly into the reaction mix in PCR tubes and avoid sticking the samples on the inside wall of the tube. Mineral oil should be added immediately, and the tubes should be capped tightly immediately after the addition.
- After the amplification is done, remove PCR tubes from the thermal cycler and discard them in a sealable plastic bag for autoclave and decontamination.
- 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】

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[5] Gu, Hongjing, Qi Chen, Guan Yang, Lei He, Hang Fan, Yong-Qiang Deng, Yanxiao Wang, et al. 2020. Adaptation of SARS-CoV-2 in BALB/c Mice for Testing Vaccine Efficacy. Science 369 (6511): 1603–7.

【Release date of the user manual】

This manual was released on 2021-3-1. Version 1.0.

【Language edition】

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

【Key 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**
	**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: BGI Biotechnology (Wuhan) Co.,Ltd.

Site Address: Building B2, Zone B/C/D, Wuhan National Bioindustry Base, NO.666 Gaoxin Avenue, East Lake High-tech Development Zone, Wuhan

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

Company： SUNGO Europe B.V.

Address：Olympisch Stadion 24, 1076DE Amsterdam, Netherlands

Contact Person： SUNGO Secretary

Tel /Fax： +31(0)2021 11106

【Revision history】

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V1.0	Released	2021-3-1