Rapid Identification Kit for B.1.1.7 Lineage and B.1.351 Lineage of SARS-CoV-2 (Fluorescence RT-PCR)

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

Instructions for Use*

***Note: Instructions for use are being provided to assist laboratory researchers conducting research related to B1.1.7 lineage and B.1.351 lineage of SARS-CoV-2. This kit is for research use only, and these instructions should not be used to obtain clinical diagnostic results.**

【Product name】

Rapid Identification Kit for B.1.1.7 Lineage and B.1.351 Lineage of SARS-CoV-2 (Fluorescence RT-PCR)

【Package size】 50 tests/kit

【Intended use】

The kit is a qualitative in vitro nucleic acid amplification assay to identify B.1.1.7 lineage and B.1.351 lineage of SARS-CoV-2 from throat swab specimen or sputum confirmed positive SARS-CoV-2 by RT-PCR for research purposes.

Multiple SARS-CoV-2 variants are circulating globally. Several new variants emerged in the late 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 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 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 P681H and HV69-70del of B.1.1.7 lineage, both probably associated with increased transmissibility (i.e., more efficient and rapid transmission), and K417N of B.1.351 lineage, also a RBD mutation, which also increased the affinity of virus with human receptor.

This kit is intended for research use only and these instructions for use should not be used to obtain clinical diagnostic results.

【Principles of the procedures 】

The mutation detection is based on fluorescent RT-PCR (qPCR) method. For N501Y and P681H of B.1.1.7 lineage and K417N of B.1.351 lineage, sequence-specific primers and fluorescent probes were designed tailored for wild strain and variant, respectively. ARMS primer can specifically identify and differentiate wild strain from mutation using different fluorescent bands, enabling to differentiate wild strains (501N, 681P and 417K) from mutant strains (501Y, 681H and 417N) through 2 tests for each specimen in a single run. For HV69-70del of B.1.1.7 lineage, primers and probes are designed for mutant strain by using MGB probe.

In addition, internal reference is included in the kit to monitor the whole reactions, to avoid false negative detection results.

【Key contents】

Contents (50 tests/kit)	Specification	Quantity	Description
Reaction Mix for wild strain	1 mL /vial	1 vial	Reagent with primers and probe for amplification of wild strain and internal reference
Reaction Mix for mutant strain	1 mL /vial	1 vial	Reagent with primers and probe for amplification of mutant strain
Enzyme Mix	160 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 wild strain, mutant strain 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.

Table 1 Materials required but not provided.

Item	Validated products
Reagent	QIAamp Viral RNA Mini Kit (Cat. No. 52904 for 50 preps, 52906 for 250 preps) by QIAGEN
	TIANamp Virus RNA extraction Kit (Cat. No. YDP315-R) by TIANGEN
	MGIEasy Nucleic Acid Extraction Kit (Cat. No.1000020471 for 96 preps, 10000020261 for 1728 preps) by Wuhan MGI Tech Co., Ltd
	Solution for sputum decontamination
Extraction equipment *(Optional)*	DNA Sequencing Library Preparation System (MGISP-100RS) by Wuhan MGI Tech Co., Ltd
	High-throughput Automated Sample Preparation System (MGISP-960RS) 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 claimed storage condition. Unpacked kit should avoid repeated thaw-freeze (within 6 times). The kit can be transported at temperatures lower than -15 °C in dark conditions and is stable for 7 days.

The manufacture date and expiration date are provided on the product labels.

【Applicable instruments】

SLAN-96P PCR system, Applied Biosystems™ QuantStudio®5 Real-Time PCR Systems; Applied Biosystems™ ABI7500Fast PCR system; Roche 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. The operation of specimen collection 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 washing 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 not longer than 1 week and at temperature lower -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 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 assessed using the recommended RNA extraction kits by TIANGEN (DP315-R), QIAGEN(50 Preps: 52904, 250 Preps: 52906) and MGIEasy Nucleic Acid Ex-traction Kit (96 Preps: 1000020471, 1728 Preps: 1000020261) by Wuhan MGI Tech Co., Ltd. 140μL specimen is used by extraction kits from TIANGEN and QIAGEN. 200 μL specimen is needed for kit from MGI to extract nucleic acid manually and more than 160 μL specimen is needed for kit from MGI to extract nucleic acid automatically using High-throughput Automated Sample Preparation System(MGISP-960RS，Cat. No. 900-000154-00) or DNA Sequencing Li-brary Preparation System (MGISP-100RS, Cat. No. 900-000206-00).
- Sputum should be mixed with equivalent volume of solution for decontamination and shaken for 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.

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 Blank Control (1 tube), Positive Control (1 tube), and specimens prepared, respectively, for wild and mutant strains.
- Prepare 8-tube strips for PCR based on the estimated N of reactions for wild and mutant strains, respectively. PCR-Mix1 for testing wild strain should be prepared as ingredients in table 2 below. Table 3 below was ingredients for preparing PCR- Mix2 for testing mutant strain. Pipette 20 μL PCR-Mix1 per tube into the 8-tube strips prepared for testing wild strain and 20 μL PCR-Mix2 per tube prepared for testing mutant strain, respectively. Cap them tightly and transfer them to 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 wild strain

	Reaction Mix for wild strain (μL)	Enzyme Mix (μL)
PCR-Mix1	18.5×N	1.5×N

Table 3 PCR mix preparation for mutant strain

	Reaction Mix for mutant strain (μL)	Enzyme Mix (μL)
PCR-Mix2	18.5×N	1.5×N

Add sample

- Add 10 μL extracted RNA of specimens, Blank Control, and Positive Control respectively into the 8-tube strips prefilled with PCR-Mix1. Cap them tightly and centrifuge them at 2000 rpm for 10 seconds.
- Add 10 μL extracted RNA of specimens, Blank Control, and Positive Control respectively into the 8-tube strips prefilled with PCR-Mix2. Cap them tightly and centrifuge them at 2000 rpm for 10 seconds.
- Please refer to table 4 below for an example of PCR tube layout in PCR plate.

Table 4 Example of PCR tube in 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.

  FAM channel (Reporter: FAM, Quencher: None) for 23063A (501N) in wild strain and 23063T (501Y) in mutant strain of SARS-CoV-2 S-gene.
  CY5 channel (Reporter: CY5, Quencher: None) for 23604C (681P) in wild strain and 23604A (681H) in mutant strain of SARS-CoV-2 S-gene.
  VIC/HEX channel (Reporter: VIC/HEX, Quencher: None) for 22813G (417K) in wild strain and 22813T (417N) in mutant strain of SARS-CoV-2 S-gene.
  ROX channel (Reporter: ROX, Quencher: None) for internal reference in PCR-Mix1 and HV69-70del (21767-21772del6) in mutant strain of SARS-CoV-2 S-gene in PCR-Mix2.
  Reference Dye: None.
  Sample Volume: 30 μL.

- Configure PCR protocol

Step	Cycles	Temperature	Duration	Fluorescent signal collection
1	1 cycle	50 ℃	5 min	No
2	1 cycle	95 ℃	1 min	No
3	45 cycles	95 ℃	5 sec	No
		55 ℃	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. 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 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. In 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].

- Applied Biosystems™ ABI7500Fast 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 all channels.

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

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

- Positive Control:

  Standard curves at channel FAM, CY5, VIC/HEX and ROX channels are all in S-shape with Ct values not higher than 35 in both PCR-Mix1 and PCR-Mix2.

- Above requirements should be met in a single test. Otherwise, the test is invalid and may be retested in line with the package insert.

<table>
<tr><th colspan="2">Quality control metrics</th><th>FAM (Observation)</th><th>CY5 (Observation)</th><th>VIC/HEX (Observation)</th><th>ROX (Observation)</th><th>Interpretation</th></tr>
<tr><td rowspan="2">Blank Control</td><td>PCR-Mix1</td><td rowspan="2">No amplification</td><td rowspan="2">No amplification</td><td rowspan="2">No amplification</td><td>No amplification or Ct value is >38</td><td rowspan="3">Pass. Proceed to sample analysis</td></tr>
<tr><td>PCR-Mix2</td><td>No amplification</td></tr>
<tr><td rowspan="2">Positive Control</td><td>PCR-Mix1</td><td rowspan="2">Sigmoidal amplification curve and Ct value is ⩽35.</td><td rowspan="2">Sigmoidal amplification curve and Ct value is ⩽ 35.</td><td rowspan="2">Sigmoidal amplification curve and Ct value is ⩽35.</td><td rowspan="2">Sigmoidal amplification curve and Ct value is ⩽35.</td></tr>
<tr><td>PCR-Mix2</td></tr>
<tr><td rowspan="2">Blank Control</td><td>PCR Mix1</td><td rowspan="2">Sigmoidal amplification curve and Ct value is ⩽41.</td><td rowspan="2">Sigmoidal amplification curve and Ct value is ⩽ 41.</td><td rowspan="2">Sigmoidal amplification curve and Ct value is ⩽41.</td><td>Sigmoidal amplification curve and Ct value is ⩽38.</td><td rowspan="3">Failed. Repeat the Run</td></tr>
<tr><td>PCR Mix2</td><td>Sigmoidal amplification curve and Ct value is ⩽41.</td></tr>
<tr><td rowspan="2">Positive Control</td><td>PCR Mix1</td><td rowspan="2">No amplification or Ct value is >35.</td><td rowspan="2">No amplification or Ct value is >35.</td><td rowspan="2">No amplification or Ct value is >35.</td><td rowspan="2">No Amplification or Ct value is >35.</td></tr>
<tr><td>PCR Mix2</td></tr>
</table>

【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 501, 681 and 417 are Ct values lower than 41 in both wild and mutant strain. The identification of wild or mutant strain of 501, 681 and 417 should be determined by Ct

values in combination with ΔCt. Cut-off value for positive HV69-70del mutant is Ct values lower than 41.

- ΔCt calculated using formula: Ct value in reaction of wild strain- Ct value in reaction of mutant strain.

ΔCt	Fluorescent signal	Allele	ΔCt value
ΔCt1	FAM	501	Ct_{wild}- Ct_{mutant}
ΔCt2	CY5	681	Ct_{Wild}- Ct_{mutant}
ΔCt3	VIC/HEX	417	Ct_{Wild}- Ct_{mutant}

- Reference range for different alleles

Allele	Δ Ct value	Result
501	Δ Ct<-4	501N（wild strain）
	Δ Ct>4	501Y（mutant strain）
681	Δ Ct<-4	681P（wild strain）
	Δ Ct>4	681H（mutant strain）
417	Δ Ct<-4	417K（wild strain）
	Δ Ct>4	417N（mutant strain）

- Cut-off value for internal reference was determined as 38, not higher than 38 as positive.

【Testing result interpretation】

- Testing results should be interpreted as below (45 should be used as Ct value for the channel of either wild strain reaction or mutant strain reaction without amplification curve in calculating ΔCt). Result of HV69-70del is judged directly without calculating ΔCt.

- In case that amplification curve present in only one allele of either wild strain or mutant strain with Ct value not lower than 41, the specimen was in low concentration of virus if Ct value of internal control at ROX not higher than 38. The sample with Ct value of internal reference higher than 38 at ROX should be re-extracted and retested.

- No amplification curve for detecting allele 501 at FAM or 681 at CY5 or 417 at VIC/HEX in both mutant and wild strain was present, the test was valid if Ct value for internal reference at ROX is not higher than 38. Otherwise, the specimen should be re-tested.

Example for Result interpretation

Sample	Target (FAM/CY5/VIC) and ROX(HV69-70)				Internal Reference (ROX)	Interpretation
	Channel	Wild strain	Mutant strain	ΔCt		
Sample 1	FAM/CY5/VIC	Sigmoidal amplification curve and Ct value is <41.	Any	<-4	Any	Wild strain
		Any	Sigmoidal amplification curve and Ct value is <41.	>4		Mutant strain
	ROX (HV69-70del)	/	Sigmoidal amplification curve and Ct value is <41.	/		HV69-70del mutant strain
Sample 2	FAM/CY5/VIC	Sigmoidal amplification curve and Ct value is <41.	Sigmoidal amplification curve and Ct value is <41.	-4<=Δ Ct<=4	Any	Reextracted and retest. If the retest results are still in this range, other methods are recommended for

						further identification.
Sample 3	FAM/CY5/VIC	No Ct or Ct value is >=41.	No Ct or Ct value is >=41.	/	Sigmoidal amplification curve and Ct value is <=38.	Low concentration of virus RNA.
	ROX(HV69-70del)	/	No Ct or Ct value is >=41.	/		HV69-70del wild strain; or low concentration of HV69-70del mutant strain
Sample 4	FAM/CY5/VIC	No Ct or Ct value is >=41.	No Ct or Ct value is >=41.	/	0, no Ct or Ct value is >38.	Invalid test. Reextracted and retest.
	ROX(HV69-70del)	/	No Ct or Ct value is >=41.	/		

【Limitation of the assay】

- This kit is intended for research use only for research involving the development of B.1.1.7 lineage and B.1.351 lineage of SARS-CoV-2 from positive SARS-CoV-2 throat swab specimen or sputum. These instructions should not be used to obtain clinical diagnostic results.
- 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 virus genome not covered by the kit's primers and/or probes, and uncontrolled external interference factors, such as PCR inhibitor. If necessary, other methods are recommended for further identification.

【Performance characteristics】

- The package is intact and liquid contents are clear, transparent and no sediments. All contents are in correct quantity as listed in the package insert.
- Positive Control is positive at FAM, CY5, VIC/HEX and ROX channel in testing while Blank Control is negative at all channels with Ct of internal reference at ROX higher than 38 or no value.

【Warning and precautions】

- For Research Use Only. Not for use in diagnostic procedures. Please contact BGI Sales in the event of damage to the protective packaging.
- 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 and validated for the intended research purpose. Replacing any of them will affect the 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 performing 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—Conduct PCR.

- 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 capped fasten 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. Mineral oil should be added immediately, and 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.
- 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.

【Disclaimer】

- These kits are distributed for research use only, not for clinical diagnostic purposes.

【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] Starr, Tyler N., Allison J. Greaney, Sarah K. Hilton, Daniel Ellis, Katharine H. D. Crawford, Adam S. Dingens, Mary Jane Navarro, et al. 2020. Deep Mutational Scanning of SARS-CoV-2 Receptor Binding Domain Reveals Constraints on Folding and ACE2 Binding. Cell 182 (5): 1295–1310.e20.
[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.

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