Kit Components

Item No.	Components	Quantity
1	Master Mix	1000μL×1
2	RT Mix	100μL×1
3	Primer and Probe Mix	300 μL×1
4	Positive Control (B117 + Wild)	100μL x1
5	Negative Control	100μL×1

[Storage Condition]
Recommend -70°C for long term storage. It is stable for 12 months stored at -20°C.

[Product Name] B117 RT-PCR
[Package Specifications] 100/500/1000 Tests

Intended Usage

Coronaviruses are a class of RNA viruses with viral cystic membranes and a positive linear single-stranded genome, about 80-120 nm in diameter. Currently, they only infect human, mouse, pig, cat, dog, and avian vertebrates. The new coronavirus (SARS-CoV-2) has been confirmed as a new variant that can cause viral pneumonia, fever and dry cough in mild cases, and breathing difficulties and even shock in severe cases.[1,2]

At present scenario, India is currently experiencing a massive COVID-19 surge. The daily case count has crossed the 100,000-mark, and the country has already reported the highest number of daily new cases in the world for the last two days – and all this despite limited testing. The reported positive rate is around 8% for the whole country and nearly 30% in Maharashtra. The growth in the number of new daily cases has been exponential and there is no doubt that the second wave is going to be more severe than it was last year, at least if the steep rise is any indication. In fact, since February 15, the number of cases has increased to more than 10-fold.

India's health ministry recently released data on genome-sequencing of 10,787 samples from 18 states, which showed 771 cases of known variants; 736 of B.1.1.7 (UK); 34 of B.1.351 (South Africa) and one of P.1 (Brazil). There were 336 samples from Punjab that tested positive for the B.1.1.7 variant. Officials also reported this strain in samples from Telangana (87), Delhi (65) and Andhra Pradesh.[3]

This kit is used for RNA detection of SARS-CoV-2 wild type and to differentiate it from UK variant (B.1.1.7), and the results can be used for auxiliary diagnosis of patients with new coronavirus infection or patients suspected of new coronavirus infection, providing molecular diagnosis for infected patients.

Principle

State-wise sequencing data revealing various established variants

State	Total UK strain (B.1.1.7)	South Africa Variant (B.1.351)	Brazil Variant (P1)	Total
ANDHRA PRADESH	17	3		20
DELHI	65	4		69
GOA	5			5
GUJARAT	39			39
HARYANA	6			6
KARNATAKA	30	3		33
KERALA	16			16
LADAKH	1			1
MADHYA PRADESH	11			11
MAHARASHTRA	56	5	1	62
ODISHA	3			3
PUNJAB	336			336
RAJASTHAN	22			22
TAMIL NADU	14	1		15
TELANGANA	87	17	0	104
UTTAR PRADESH	17			17
UTTARAKHAND	1			1
WEST BENGAL	10	1		11
Total	736	34	1	771

The SARS-CoV-2 detection kit (RT-PCR) is a real time reverse transcription followed by polymerase chain reaction (rRT-PCR) test. The SARS-CoV-2 primer and probe sets are designed to detect RNA from SARS-CoV-2 in respiratory specimens from patients who are suspected of SARS-CoV-2 infection by their healthcare provider. This kit is used for qualitative detection of the RdRp gene of SARS-CoV-2 RNA, and D3L mutation on the N gene of COVID-19 variants including UK B.1.1.7. The 3rd fluorescence probe, which is internal control, detects RNaseP for human sample.

After PCR reaction, fluorescent signal from target is acquired and further analyzed by a real time PCR instrument. As a result, genes specific to novel coronavirus SARS-CoV-2 can be detected with high precision.

In particular, SARS-CoV-2 RdRp probe contains HEX labeled dye, D3L (N) gene probe contains FAM and internal reference gene (RNaseP) probe contains Cy5 label.

The kits can provide information of N gene of SARS-CoV-2 UK (B.1.17) variants. The results indicate the presence or absence of SARS-CoV-2 UK variants.

Storage Conditions and Expiration Date

1. The reagents should be sealed from light and the reagent components should be stored below -20°C. The kit is valid for 12 months. Please see the outer box for manufacture date and expiration date.

2. The reagents can be stored stably within a valid period indicated on package. The repeated freeze-thaw cycles should not be more than 5. And unpacked kits should avoid repeated freeze-thaw cycles.

Instrument Compatibility

B117 RT-PCR is compatible with real time PCR instruments with FAM, HEX, RED/Cy-5 channels.

Specimen Requirements

1. Applicable specimen types: upper respiratory tract specimens (throat swabs or nasal swabs). Nasopharyngeal, Oropharyngeal or Nasal swab specimen collected in virus preservative buffer, Normal saline, VTM or MTM can be used for the assay.

2. Specimen storage and transportation: Specimens is recommended to immediately process. Specimens should be tested within 96 hours if stored at 2-8°C. Specimens that cannot be tested within 96 hours should be stored at - 70°C or below (in the absence of -70°C storage conditions, specimens can be stored below -20°C). Multiple freeze-thaw cycles should be avoided. Specimens should be transported in a sealed frozen pitcher with ice or in a sealed foam box with ice.

Assay Method

1. RNA Extraction
Please follow manufacturer's instruction to extract virus RNA from clinical sample using RNA extraction kit. Extracted RNA can be used directly for PCR detection. Otherwise, keep RNA sample at -70°C if not in use. Avoid repeated thawing and freezing. This product does not contain an RNA extraction kit, and is compatible with Qiagen/Thermo Viral RNA Kit and any other commercial kits. The RNA is subjected to use as template for the RT-PCR reaction.[4,5]

Or Sample processing

The VTM/Saline tube containing specimen is directly heated at 70°C in water bath for 10 minutes. The specimen is then subjected to centrifuge the for 5-10 seconds and used as template for the RT-PCR reaction.

2. Reagent Preparation
Aliquot (n+1) x 10µl of the B117 RT-PCR Master mix , (n+1) x 3 µl of Primer & probe mix and (n+1) x 1 µl RT Mix (n is the number of reaction tubes, including specimens, negative control and positive control) into a centrifuge tube, shake and mix thoroughly, and centrifuge at 3000 rpm for 1 minute.

1 x Volume Required	
Component	**Volume (For 5.5 µl sample)**
Master Mix	10.0 µL
RT Mix	1.0 µL
Primers & Probe set	3.0 µL
Total Volume	14 µL

3. Selection of Fluorescence channel

Gene	Dye	Color
B117 N (D3L) Gene	FAM	Green
RdRp gene	HEX	Yellow
RNase P	Cy5	Red

4. Specimen/template addition
Aliquot 14 µl of the above mixed solutions into each PCR tube, and then add 6 µl each of the specimens in extracted RNA or processed specimen to each PCR tube, 6 µl positive control, or negative control to one PCR tube, mix and spin. Immediately perform the PCR amplification reaction.

5. PCR Amplification
The PCR reaction tube is placed in a Real Time PCR instrument. The recommended thermal cycling protocol is set as follows:

RT-PCR procedure				
Steps		Temperature	Time	Cycles
1	Reverse transcript	50°C	10 min	1
2	Initial Denaturation	95°C	1 min	1
3	PCR cycling	95°C	15 s	45
		60°C	30 s	

6. Set baseline and threshold
Take fluorescence signals from 3-20 cycles for baseline adjustment. The threshold setting principle is based on the threshold line just exceeding the highest point of the DEPC-H2O fluorescence detection curve.

7. Quality Control

the following criteria. If negative control shows result other than described in the table below, it indicates contamination of reagents or specimens. All specimen results need to be invalidated and results must not be reported. It is recommended to decontaminate the PCR lab and use a new box of un-opened reagents before repeating specimen testing. If positive control shows result other than described in the table below, it indicates the failure of RT-PCR reaction. All specimen results need to be invalidated and results must not be reported. The specimens are required to be re-tested.

Control	FAM Ct (D3L N)	HEX Ct (RdRp gene)	Cy5 Ct (Internal Reference)
Negative control	UNDET or >36	UNDET or >36	UNDET or >36
Positive control	≤36	≤36	≤36

8. Examination and Interpretation of Patient Specimen Results

Assessment of clinical specimen test results should be performed after the positive and negative controls have been examined and determined to be valid and acceptable. If the controls are not valid, the patient results cannot be interpreted. Table above describes the results interpretation of specimens concerning the use of the controls provided with the test. The Ct cutoff value of this kit is set as 36 for D3L (N) B.1.17 variant gene (FAM), RdRp gene (HEX) and IC (Cy5). And the end user is required to review fluorescent curves before final interpretation. All positive curves should be typical S-shape amplification curves.

Interpretation of Specimen Results

D3L (N) gene B117 variant (FAM)	RdRp (HEX)	IC (Cy5)	Results
+	+	+	SARS-CoV-2 positive (UK B.1.1.7)
+	-	+	SARS-CoV-2 positive (UK B.1.1.7)
-	+	+	SARS-CoV-2 Positive (Wild type)
-	-	+	SARS-CoV-2 Negative
-	-	-	Invalid

Result of (-): Ct value >36 or Undetermined for D3L (N) gene B117 variant (FAM), RdRp (HEX) and IC (Cy5), and Ct value >36.

Result of (+): Ct value ≤ 36 for D3L (N) gene B117 variant (FAM), RdRp (HEX) and IC (Cy5), and Ct value≤ 36.

Invalid Result: There is no typical S-shape amplification curve or Ct value >36 or Undetermined for D3L (N) gene UK variant (FAM), RdRp (HEX) and IC (Cy5), and Ct value >36 of positive control, indicating that the specimen concentration is below detection limit, or there are interfering substances that inhibit the reaction. If upon retest, the result is invalid again, another fresh sample should be collected and tested.

Interpretation of test results

Laboratory environment pollution, reagent contamination, and specimen cross-contamination will cause false positive results; improper reagent transportation, storage, or inaccurate reagent preparation may result in a decline in the reagent detection efficiency, false negatives or inaccurate quantitative detection. There is no typical S-shape amplification curve or Ct value >36 or Undetermined for D3L (N) gene UK variant (FAM), RdRp (HEX) and IC (Cy5), and Ct value >36 of positive control, indicating that the specimen concentration is below detection limit, or there are interfering substances that inhibit the reaction. If upon retest, the result is invalid again, another fresh sample should be collected and tested. If Ct value ≤36 for D3L (N) gene UK variant (FAM), RdRp (HEX) and IC (Cy5), Ct value ≤36 of negative control, indicating the reagent or the testing environment is contaminated. Decontamination is required before running new tests.

Limitation of the test method

1. Test results are affected by how the specimen is collected, processed, transported and stored. Loss of control in any of the steps will lead to incorrect results.

2. Improper specimen collection, transport and processing may lead to false negative results; mutation of target sequence may lead to false positive or false negative results.

This kit is applicable to specified specimen types and detection system, including Real Time PCR instruments, nucleic acid extraction reagent, detection method and etc. Validation is required before applying any new specimen types or detection system.

Product performance specification

Detection limit: 50 copies/uL

Precautions

Please read the entire manual carefully before starting test.

1. The entire testing process is suggested to be performed in three separated areas:

a)Reaction system preparation and reagent preparation area;
b)Specimen processing and specimen adding area;
c)PCR amplification, fluorescence detection and result analysis area.

Reagent preparation and specimen processing should use ultra-clean workbenches (negative pressure) or anti-pollution covers to prevent environmental pollution; Instruments, equipment, consumables and work clothes used in each area shall be used independently;

Clean the workbench immediately after the experiment. Pipettes, centrifuges, PCR amplifiers and other instruments should be disinfected with 10% hypochlorous acid or 75% alcohol, UV lamps or ozone.

2. Operators should be professionally trained and have corresponding operating skills, certain experimental experience, and good safety precautions.

3. Non-fluorescence contaminated disposable gloves, disposable centrifuge tubes, and disposable pipettes tips with filters should be used throughout experiments.

4. To ensure the success and accuracy of experiment:

5. Each experiment should include negative and positive controls. Reagents should be equilibrated to room temperature before use, and fully melted and mixed.

References

1. Rambaut A., et al. Preliminary genomic characterisation of an emergent SARS-CoV-2 lineage in the UK defined by a novel set of spike mutations. https://www.cogconsortium.uk.

2. Brookman S., et al. Effect of the new SARS-CoV-2 variant B.1.1.7 on children and young people. doi.org/10.1016/S2352-4642(21)00030-4.

3. Coronavirus Variants in India: 771 Cases of COVID-19 Variants Detected Across 18 States in the Country, Check State-Wise Details (yahoo.com)

4. Bruce, A.E., et al. RT-qPCR detection of Sars-Cov-2 RNA from patient nasopharyngeal swab using Qiagen RNeasy kits or directly via omission of an RNA extraction step. BioRxiv, doi.org/10.1101/2020.03.20.001006.

5. Ambrosi, C., et al. SARS-CoV-2: Comparative analysis of different RNA extraction methods. Journal of Virological Methods 287 (2021) 114008.

Index of Symbols

IVD In Vitro Diagnostics only	Consult Instructions for use
Don't use if package is damaged	Store at -20°C
REF Catalogue Number	LOT Batch Number
Expiry Date	Manufacturer

CE-IVD

Manufactured by:
BioGenex Lifesciences Pvt. Ltd.
Plot No.4, survey #656/A,
TSIIC SEZ for Aerospace & Precision Engineering,
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