GENE BHARAT

CORONA VIRUS (COVID-19)

Real Time PCR Detection Kit

KIT NAME	KIT SIZE	CAT. NO
Gene Bharat –Corona Virus (Covid-19) RT-PCR Kit	100T	PCRCOV1100T

GENUINE BIOSYSTEM

Introduction

COVID-19, the new coronavirus, is on the verge of spreading across the world. Large clusters of cases are emerging outside China and new cases are springing out daily in our country. Coronaviruses (CoVID-19) are a large family of viruses that cause illness ranging from the common cold to more severe diseases such as Middle East Respiratory Syndrome (MERS-CoV) and Severe Acute Respiratory Syndrome (SARS-CoV). Coronavirus disease (COVID-19) is a new strain that was discovered in2019 and has not been previously identified in humans.

Unboxing of Kit

Once the kit reaches users facility, carefully open the box. Each kit contains the following components mentioned in the below table which illustrates the volume/quantity of component and storage conditions.

***Kindly report back immediately if there are any issues in packing or if any component of kit is missing after the kit is unpacked

Kit Contents:

S. No	Component	Volume	Number of Vials
1	2x Master mix	1.25 ml	2
2	E-Gene Probe Mix	0.25 ml	1
3	RdRp Probe mix	0.25 ml	1
4	Enzyme Mix	0.4 ml	1
5	Positive Control	0.7 ml	1
6	Water (PCR Grade)	1.0 ml	1

Storage and Stability

- The GENE BHARAT'S Corona Virus (COVID-19) RT-PCR Detection Kit is shipped on dry ice. The components of the kit should arrive frozen. If one or more components are not frozen upon receipt, or if tubes have been compromised during shipment, contact GENE BHARAT Biotechnologies for assistance.
- GENE BHARAT's Coronavirus (COVID-19) Real-Time PCR kit should be stored in the original packaging and is stable for up to 1 year if stored at -20°C, but should not be used past the "use by" date as indicated on the pack label and individual tube labels.
- All components should be stored between -25°C and -15°C upon arrival.
- Repeated thawing and freezing of Master Mix reagents (more than twice) should be avoided, as this might affect the performance of the assay. The reagents should be frozen in aliquots, if they are to be used intermittently.
- Protect Primer probe from light. Components may be aliquoted into smaller volumes after resuspension, if necessary.

Product Description

GENE BHARAT's Corona virus (COVID-19) Coronavirus Real-time RT-PCR (RT-qPCR) Detection Kit (CVPD) is designed to detect the presence of SARS-CoV-2 Coronavirus in respiratory specimens and serum samples following CDC guidelines. Please read carefully and follow these guidelines for biosafety precautions when dealing with possible infectious samples and for obtaining quality data. Included in the kit are Two primer/probe that target the conservative regions of coronavirus Envelop (E) gene and RdRp gene and the Human RPP gene primer/probe set that targets exon 1 of human RPP gene and serves as a control to assess specimen quality. For assessing reverse transcription.

➢ Positive Control

The Positive Control Template (PCT) contain standardized concentrations of E-gene and RdRp gene specific sequence in concentration of **1.0 x 10^5** copies per 7 µl. To ensure PCR run validity, the PCT should produce C_T value ≤27 in the FAM channel.

➢ Internal Control

Human ribonuclease P (RPP) gene is used as internal control. RPP being an endogenous gene control in Real-time PCR assays it would exhibit a good quantity of RPP RNA to ensure the assay performance.

➢ Real-Time PCR Instruments Compatibility

The GENE BHARAT Corona Virus (COVID-19) RT-PCR Detection Kit can be used with the following real time PCR instruments:

- Mx 3005P™ QPCR System (Stratagene)
- ABI Prism® 7500 SDS (Applied Biosystems)
- Rotor-Gene® 6000 (Corbett Research)
- Rotor-Gene® Q5/6 plex Platform (QIAGEN)
- CFX96™ Real-Time PCR Detection System (Bio-Rad)
- CFX96™ Dx System (Bio-Rad)
- LightCycler® 480 Instrument II (Roche)

➢ RNA Extraction Kits

Extracted RNA is the starting material for the GENE BHARAT's Corona Virus (COVID-19) RT-PCR Detection Kit. The quality of the extracted RNA has a profound impact on the performance of the entire test system. It is recommended to ensure that the system used for nucleic acid extraction is compatible with real-time PCR technology.

If using a spin column-based sample preparation procedure including washing buffers containing ethanol, it is highly recommended to perform an additional centrifugation step for 10 min at approximately 17000 x g (~ 13000 rpm), using a new collection tube, prior to the elution of the nucleic acid.

CAUTION *If your sample preparation system is using washing buffers containing ethanol, make sure to eliminate any traces of ethanol prior to elution of the nucleic acid. Ethanol is a strong inhibitor of real-time PCR.*

Warning and Precautions

Handling and Procedural Requirements

- Always wear disposable gloves when handling kit components.
- Use separated working areas for specimen preparation, reaction set up and amplification.
- When mixing reagents by pipetting up and down this should be done with a volume roughly equal to 50% of the total component volume.

Preventing Template Contamination

- Positive control template is provided in a vial contains a high copy number of templates. It should be opened and processed away from test samples and kit components to avoid cross-contamination.
- After each run has been set up and performed, clean work surfaces and equipment with a DNA/RNA remover.
- Handle post-amplification plates with care to ensure that the seal is not broken. For further instruction for disposal.

Prevention of Nucleases Contamination

- Use DNase/RNase free disposable plasticware and pipettes reserved for DNA/RNA work to prevent cross-contamination with DNases/RNases from shared equipment.
- Use DNase/RNase free filter tips throughout procedure to prevent aerosol and liquid.

Procedure

RNA Extraction Procedure

- Please use the proper RNA extraction kit/ system as per the product guideline. The quality of RNA is important for the PCR sensitivity.
- It is recommended to ensure that the system used for nucleic acid extraction is compatible with real-time PCR technology.

The following kits and systems are suitable for nucleic acid extraction:

- GB's PURE SPIN RNA Isolation Kit
- GB's AURA PURE MAGNETIC RNA Isolation Kit
- QIAamp® Viral RNA Mini Kit (QIAGEN)

Master Mix Setup

All reagents and samples should be thawed completely, mixed (by pipetting or gentle vortexing) and centrifuged briefly before use.

- With test samples, two control samples should be run concurrently, the non-infectious DNA control (Negative control) and No Template Control (NTC).
- Two separate reaction to be set up for each sample to be analyzed each reaction with E gene, RdRp gene detected using FAM Channel.
- It is also recommended to use E-gene assay as screening assay and RdRp gene assay as confirmatory test.
- Human RPP (ribonuclease P) gene used as Internal control (IC) of the assay and probe (HEX) and primers added along with target gene thus we should detect the IC using HEX Channel.

For the two tube Real-time PCR, the following components to beadded into single reaction tube:

E-gene master Mix

Name	One Reaction	10 Reactions
2X Mater mix	12.5 µl	**125 µl**
E-Gene Probe Mix	2.5 µl	**25 µl**
Enzyme Mix	2.0 µl	**20 µl**
Nuclease free water	1.0 µl	**10 µl**
Total Volume	**18.0 µl**	**180 µl**

RdRp-gene Master Mix

Name	One Reaction	10 Reactions
2X Mater mix	12.5 µl	**125 µl**
RdRp Probe Mix	2.5 µl	**25 µl**
Enzyme Mix	2.0 µl	**20 µl**
Nuclease free water	1.0 µl	**10 µl**
Total Volume	**18.0 µl**	**180 µl**

Reaction setup

- Pipette 18 µl of the prepared Master Mix into each required well of an appropriate optical 96-well reaction plate or an appropriate optical reaction tube.
- Add 7 µl of the sample (eluate from the nucleic acid extraction) or 7 µl of the controls (Positive Control).

Reaction Setup	
Mater mix	18.0 µl
Sample or Control	7.0 µl
Total Reaction volume	**25.0 µl**

- Make sure that each Positive Control and Negative Control is used per run. Thoroughly mix the samples and controls with the Master Mix by pipetting up anddown.

Temperature Profile and Dye Acquisition

- Define the temperature profile and dye acquisition in the instrument settings panel as per following profile:

Storage		Cycle Repeats	Acquisition	Temperature (°C)	Time (min: sec)
Reverse Transcription	Hold	1	-	44	20:00
Denaturation	Hold	1	-	95	3:00
Amplification	Cycling	40	- yes	95 60	00:10 00:45

Fluorescence Channel Detectors (Dyes) for E gene Tubes

Target	Detector Name	Reporter
Corona virus (COVID-19)	**Target E-gene**	**FAM**
Internal Control	**Target RPP**	**HEX**

Fluorescence Channel Detectors (Dyes) for RdRp gene Tubes

Target	Detector Name	Reporter
Corona virus (COVID-19)	**Target RdRp gene**	**FAM**
Internal Control	**Target RPP**	**HEX**

Data Analysis

For basic information regarding data analysis on specific real-time PCR instruments, please refer to the user manual of the respective instrument. Before interpreting sample results, it is necessary to verify the success of the run. If the following criteria are not satisfied, then testing needs to be repeated:

- NTC is free from amplification
- PCT produces a C_T of ≤27

For detailed instructions regarding the analysis of the data generated with the GENE BHARAT'S Corona Virus (COVID-19) RT-PCR Detection Kit on different real-time PCR instruments please contact our Technical Support.

Interpretation of Results

Table: 4 CV kit target sample test results interpretation when control results are as expected.

E Gene FAM	RdRp Gene FAM	IC HEX	Results Interpretation
+	+	±	Corona virus detected
If only E gene or RdRp Gene is positive		±	Inconclusive result
-	-	+	Corona virus not detected
-	-	-	Invalid result

Detection of the Internal Control in the HEX detection channel is not required for positive results either in the FAM detection channel. If both FAM channels shows positive then it is considered as positive sample. Sometime a high corona virus (target E gene) and (target RdRp gene) RNAload in the sample can lead to reduced or absent Internal Control signals.

- **Please manually inspect amplification curves for all samples assigned a C_T value to verify the positive amplification**

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