Introduction

Corona virus belongs to the family of Coronaviridae, in the order of Nidovirales. Novel Corona virus (SARS-CoV-2) is a single stranded positive RNA (+ssRNA) virus that translates four structural proteins (S, M, E and N) which are essential for virion assembly and infection to the host.1 Spikes on surface (S-protein) are responsible for host Angiotensin Converting Enzyme 2 (ACE2) receptor attachment and serine protease TMPRSS2 priming. Viral pathogenesis is carried out by E-protein. Viral replication is enhanced by N- protein with the help of nsps (non-structural proteins).2 Analyses of viral specific genes by reverse transcription polymerase chain reaction (RT-PCR) are suitable diagnostic modality for qualitative analysis with high sensitivity and specificity.

Intended use

COVID-19 RT-PCR DIRECT is a real-time RT-PCR test intended for the qualitative detection of SARS-CoV-2 in nasopharyngeal or oropharyngeal swabs in Preservative media, Normal saline or Viral Transport media (VTM) without the extraction of RNA and provide molecular diagnostic basis for infected patients. This test is also for the qualitative detection of RNA extracted from the SARS-CoV-2 of upper respiratory swab specimens that were collected using individual vials containing transport/preservative media from individuals suspected of COVID-19 by their healthcare provider. The assay is intended for use by qualified and trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR.

The test results of this kit are for clinical reference only and should not be used as the only standard for clinical diagnosis. It is recommended to conduct a comprehensive analysis by combining the test results with patients' symptoms and other laboratory tests.

Principle

COVID-19 RT-PCR DIRECT test includes primer and probe mix that adopts the dual target gene design, which detects the specific conserved sequence encoding the nucleocapsid, N1 and N2 gene. The viral clinical sample is heated to expose the RNA genome and with the PCR master mix & RT mix, the amplification of the template can be monitored by the increasing fluorescence signal detected by real time PCR instrument. The primer-probe set specific to detect RNase P (RP), is included to serve as an endogenous internal reference. The result of internal control provides the accuracy of sampling and RNA exposure, in order to avoid false negative results.

Components of the kit

COVID-19 RT-PCR DIRECT kit includes following components

Components	Volume (100 Tests)	Volume (500 Tests)	Volume (1000 Tests)
RT Mix	150 µl	750 µl	1.5 mL
Master Mix	1.0 mL	5.0 mL	10.0 mL
Primers & probes set	300 µl	1.5 mL	3.0 mL
Positive control	100 µl	200 µl	300 µl
Nuclease Free Water	200 µl	500 µl	1.0 mL

Controls to be used & provided with the COVID-19 RT-PCR DIRECT

1. A negative control (Nuclease free water) is needed to verify the possibility of sample contamination on the assay run and is used on every assay run.

2. A positive control (COVID-19_P & RNase P) is needed to verify that the assay run is performing as intended and is used on every assay run at a concentration.

3. An Internal control (RNase P) targeting human RNase P gene is needed to verify that nucleic acid is present in every sample and is used for every sample processed to ensure that samples resulting as negative contain nucleic acid for testing.

Equipment and Consumables Required (But Not Provided)

Biosafety cabinet, Presrvative media, normal saline or VTM tube & Swabs, RT-PCR instrument, Water bath, Vortex mixer, Micro centrifuge and micro centrifuge tubes, Micropipettes and aerosol barrier pipette tips, Gloves & Personal Protective Equipment (PPE)

Storage and Shelf life

All components of COVID-19 RT-PCR DIRECT diagnostic kit must be stored at -20°C with protection from light. Reagents are stable for 12 months when stored at the recommended conditions. Repeated freezing and thawing may lead to inaccurate results. Do not use the kit after expiry date or if the pack is damaged.

Instrument Compatibility

COVID-19 RT-PCR DIRECT kit is compatible with real time PCR instruments with FAM, HEX, RED/Cy-5 channels.

Sample Collection and Storage

Sample Type: Nasopharyngeal, Oropharyngeal or Nasal swab specimen in virus preservative buffer/normal saline/ VTM.

Sample Collection: Collect the sample in accordance with conventional sample collection method. Specimen collection should avoid possible contamination according to regulatory guidelines. Refer to Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Patients Under Investigation (PUIs) for 2019 Novel Coronavirus (2019-nCoV).3

Sample Storage & Transportation: Sample to be tested can be processed tested immediately after collection or it may be stored at 2-8°C for up to 24 hours before testing or at -20°C for up to 72 hours. The specimen should be shipped in low temperature conditions using refrigerant packs or dry ice.

Sample processing before testing
50 µl of the specimen collected in virus preservative buffer/ VTM/Saline is incubated at 70°C for 10 minutes and is used directly as template in the PCR reaction.4

Assay Setup

1. In the reagent set-up room, clean the biosafety cabinet, place BGX RT mix, Master Mix and Primer/Probes on ice or cold-block.

2. Determine the number of reactions (N) to set up per assay. It is necessary to make excess reaction mix for the negative & positive controls and specimens for pipetting error.

3. Prepare the reaction mix by combining the BGX Master Mix, BGX RT Mix and BGX Primers & Probe set as described below:

1 x Volume Required	
Component	**Volume (For 5.5 µl sample)**
Master Mix	10.0 µL
RT Mix	1.5 µL
Primers & Probe set	3.0 µL
Total Volume	14.5 µL

4. Add 5.5 µL of the processed specimen and controls to the appropriate wells/tubes.

5. Centrifuge for 5-10 seconds and set up reaction tubes or plates in a cooler rack. The samples are now ready for thermal cycling.

Selection of Fluorescence channel

Gene	Dye	Color
N Gene	FAM	Green
N2 gene	HEX	Yellow
RNase P	Cy5	Red

Thermal Cycling
Enter the amplification program. Recommended as below:

Step	Cycles	Temperature	Time
cDNA Synthesis	1	50 ^{0}C	10 min
Initial Denaturation	1	95 ^{0}C	2 min
PCR cycling	45	95 ^{0}C	5 sec
		60 ^{0}C	30 sec

6. Result Interpretation: After the reaction is completed, the results are automatically saved and the amplification curves of the detected target DNA and the internal control are analyzed separately.

7. According to the analysis, the amplification plot will adjust the Start value, End value and Threshold value of the Baseline (Users can adjust the values according to the actual situation. Start value can be set within 3~15, End value can be set within 5~20; Users can adjust the amplification curve of negative control to make it linear or below the threshold line). Click "Analyze" to perform the analysis and the parameters should meet the following requirements mentioned in "Quality Control". Lastly, record the qualitative results in the Plate window.

Quality Control

All test controls should be examined prior to interpretation of patient results. If the controls are not valid, the patient results cannot be interpreted.

COVID-19 RT-PCR DIRECT – Positive, Negative and Internal Controls

The expected results generated from each control and acceptance criteria are as follows:

- Covid-19 PCR Negative Control – No amplification should be observed in FAM, HEX & Cy5 channels before Ct value 36. If a false positive amplification is observed with any channel in the no template control (NTC) reactions, sample contamination may have occurred and repeat testing is recommended.

- Covid-19 PCR Positive Control – FAM & HEX channels, Ct ≤ 36 and Cy-5 channel, Ct ≤ 36.

- Internal Control (RNase P) - Internal Control (Cy-5) channels, Ct ≤ 36.

If controls do not perform as described above, the run is considered invalid and the specimen must be reanalyzed, and the test should be repeated from the sample processing step.

Quality Control			
Control	N gene (FAM)	N2 gene (HEX)	Internal control-RNase P (Cy-5)
Negative Control	No Ct value or Ct > 36	No Ct value or Ct > 36	No Ct value or Ct > 36
Positive Control	Ct ≤ 36	Ct ≤ 36	Ct ≤ 36
Internal Control (RNase P)	-	-	Ct ≤ 36

Examination and Analysis of Results

a) If a typical S-type (sigmoidal) amplification curve is detected by the FAM and HEX channel, with Ct ≤ 36, it indicates that COVID-19 virus is positive.

b) If FAM and HEX channels do not detect a typical S type (sigmoidal) amplification curve (No Ct) or Ct > 36, it indicates that COVID-19 virus is negative.

c) If both FAM and HEX channels detect a typical S-type (sigmoidal) amplification curve with Ct ≥ 36 and ≤40, sample shall be considered as suspected. User should repeat the experiment. If upon repetition, Ct value appears in the same Ct range, the sample shall be considered presumptive positive.

Cut-off for all targets

Target	Ct value	Result
N gene (FAM)	≤ 36	SARS-CoV-2 N1 positive
N2 gene (HEX)	≤ 36	SARS-CoV-2 N2 positive
Internal Control – RNase P (Cy-5)	≤ 36	Internal Control

Assessment of clinical specimen test results should be performed after the positive, negative (no template) and internal controls have been examined and determined to be valid and acceptable. If the RNase P assay does not produce a positive result for human clinical specimens, interpret as follows:

(i) If N1 and N2 are positive even in the absence of a positive RNase P, the result should be considered valid. It is possible that some samples may fail to exhibit RNase P growth curves due to low cell numbers in the original clinical sample. A negative RNase P signal does not preclude the presence of SARS CoV-2 virus RNA in a clinical specimen.

(ii) If N1, N2 and RNase P are negative for the specimen, the result should be considered invalid for the specimen. If residual specimen is available, repeat the extraction procedure and repeat the test. If all markers remain negative after retest, report the results as invalid and a new specimen should be collected if possible. If the controls are not valid, patient results cannot be interpreted.

N Gene (FAM)	N2 gene (HEX)	RNase P (Cy5)	Result	Report	Actions (Specimen from clinical sites)
+	+	±	SARS-CoV-2	SARS-CoV-2 Positive	Report results to sender and appropriate public health authorities
-	-	+	SARS-CoV-2 not detected	SARS-CoV-2 Negative	Report results to sender
If any one of the two targets is positive		+	SARS-CoV-2 detected	SARS-CoV-2 Positive	Report results to sender and appropriate public health authorities
If any one of the two targets is positive		-	Invalid	Invalid	Sample is repeated, if second failure occurs, report to sender as invalid; recommend recollection of sample.
-	-	-	Invalid	Invalid	Sample is repeated, if second failure occurs, report to sender as invalid; recommend recollection of sample.

If there is no typical S-shape amplification curve or Ct > 36 or No Ct detected for N1, N2 or Ct > 36 for RNase P, it indicates that the specimen concentration is too low, 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.

Limitations of Detection Methods:

- The test results of this kit are for clinical reference only. The clinical diagnosis and treatment of patients should be considered in combination with their symptoms, medical history, other laboratory tests and treatment response.
- Improper sample collection, processing & transportation may cause false negative results.
- Improper reagent storage and cross contamination during sample processing may cause false positive results.
- This assay should be performed according to good laboratory practice (GLP) regulation. Operators should strictly follow the manufacturer's instructions in performing the test.

Warnings and Precautions

This product is only used for in vitro detection. Please read this manual carefully before use.

Laboratory personnel should be trained and familiar with the operation procedures and precautions of the instrument before the experiment. Quality control should be performed for each experiment.

Laboratory management should be strictly in accordance with the regulations of PCR gene amplification laboratories. Laboratory personnel must be professionally trained and the experimental process should be strictly divided into sections. All consumables should be used only once after sterilization. Instruments and equipment should be assigned to each stage of the experiment and cannot be used alternatively. All samples should be regarded as potentially infectious materials. Laboratory workers should wear appropriate personal protective equipment (PPE) which includes disposable gloves, laboratory coat or grown. Gloves should be changed regularly to avoid cross-contamination between samples.

Clinical laboratories involving manipulation of potentially infected specimens should be performed in a certified Class II Biological Safety Cabinet (BSC) in a BSL-2 facility. Diagnostic tests should follow standard laboratory practices, including Standard Precautions, when handling potential patient specimens. For laboratory waste, follow standard procedures associated with other respiratory pathogens.

References

1. Satarker, S., & Nampoothiri, M. (2020). Structural Proteins in Severe Acute Respiratory Syndrome Coronavirus-2. Archives of medical research, 51(6), 482–491.

2. Cong, Y., Ulasli, M., Schepers, H., Mauthe, M., V'kovski, P., Kriegenburg, F., Thiel, V., de Haan, C., & Reggiori, F. (2020). Nucleocapsid Protein Recruitment to Replication- Transcription Complexes Plays a Crucial Role in Coronaviral Life Cycle. Journal of virology, 94(4), e01925-19.

3. https://www.fda.gov/media/135659/download.

4. Smyrlaki, I., Ekman, M., Lentini, A. et al. Massive and rapid COVID-19 testing is feasible by extraction-free SARS-CoV-2 RT-PCR. Nat Commun 11, 4812 (2020).

Catalog No.	Description
CRTD009-1	100 Test COVID-19 RT-PCR DIRECT
CRTD-009-5	500 Test COVID-19 RT-PCR DIRECT
CRTD-009-10	1000 Test COVID-19 RT-PCR DIRECT

Index of Symbols

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

CE-IVD

Manufactured by:
BioGenex Lifesciences Pvt. Ltd.
Plot No.4, survey #656/A,
TSIIC SEZ for Aerospace & Precision Engineering,
Adibatla, R. R. District, Telangana, India-501510.

For technical information or questions,
E-mail: customerservice@biogenex.com,
internationalcs@biogenex.com,
T: +91-9100032862

InGenuity Diagnostics Inc.
48810, Kato Rd, Suite 100 E,
Fremont, CA 94538, United States

For technical information or questions
customerservice@ingenuityd.com
internationalcs@ingenuityd.com
Tel: +1 510-824-1417