

COVID-19
REAL TIME PCR KIT



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Coronaviruses are enveloped non-segmented positive-sense RNA viruses belonging to the family Coronaviridae and the order Nidovirales and broadly distributed in humans and other mammals. In December, 2019, a series of pneumonia cases of unknown cause emerged in Wuhan, Hubei, China, with clinical presentations greatly resembling viral pneumonia. Deep sequencing analysis from lower respiratory tract samples indicated a novel coronavirus, which was named 2019 novel coronavirus (SARS-CoV-2). The disease caused by this virus is called COVID-19.

The System can detect RNA-dependent RNA polymerase gene (RdRP), Envelope protein gene (E) and Nucleocapsid protein gene (NA and NC) regions of SARS-CoV-2 with % 100 sensitivity and % 100 specificity.



PRINCIPLE OF THE SYSTEM

Reverse Transcriptase component (M-MLV) of the kit is active at 45 or 50 °C, a half life of 230 minutes, and the activity of RNase H was reduced. HotStart Taq DNA Polymerase enzyme is a mixture of enzyme and Anti-Taq monoclonal antibodies to ensure specificity and thermostability. Buffer contains 0.2 mM of each dNTP and 3 mM MgCl₂. System can be used directly both with DNA and RNA samples.

During the PCR reaction, the DNA polymerase cleaves the probe at the 5' end and separates the reporter dye from the quencher dye only when the probe hybridizes perfectly to the target DNA. This cleavage results in the fluorescent signal which is monitored by Real-Time PCR detection system. An increase in the fluorescent signal (CT) is proportional to the amount of the specific PCR product.

PRODUCT SPECIFICATION

The kit provides reagents in a "ready-to-use" mastermix format which has been specifically adapted for 5' nuclease PCR. The test system is designed for use with sequence specific primers and probe. The fluorescence of COVID-19 analysis is FAM. Also each mastermix contains an internal control labelled with HEX dye. Diseases and related dyes can be seen in Table 1.

The limit of detection (LOD) in Covid-19 Real Time PCR Kit was determined between 1-10 Copies/Rxn.

Table 1 : Tubes- Viruses/Diseases- dyes.

Tube	Viruses	Dye
Mix	Internal Control	HEX
	COVID-19	FAM

SYSTEM CONTENTS

Reagents	100 rxns
• COVID-19 Master Mix	1500 µl
• Positive Control	50 µl
• Negative Control	50 µl

STORAGE

- All reagents should be stored at – 20 °C and dark.
- All reagents can be used until the expiration date on the box label.
- Repeated thawing and freezing (>4X) should be avoided, as this may reduce the sensitivity of the assay.

RNA EXTRACTION

Nasopharyngeal samples should be collected in appropriate sterile swab and can be stored at +4°C up to one week. For more than one week specimen should be stored at -20°C. The system is optimized for any RNA Isolation System.

PROCEDURE

- Before starting work, mix the mastermixes gently by pipetting.
- For each sample, pipet 15 µl mastermix with micropipets of sterile filter tips to each optical white strips or tubes.
- Add 10 µl (~1-100 ng) RNA into each tube.
- Mix gently by pipetting
- Run with the programme shown below.

PCR PROGRAMME

45 °C	30 Min.	cDNA Synthesis
95 °C	3 Min.	Holding
95 °C	15 Sec.	45 Cycles
60 °C	1 Min.	

Fluorescent dyes are FAM and HEX.

If you use;

- ABI Prism® system, please choose "none" as passive reference and quencher.
- Mic qPCR Cycler, please adjust gain settings, "Green Auto Gain" to 20 and "Yellow Auto Gain" to 10.

This system can be used with;

Bio-Rad CFX96
 ABI Prism ® 7500/7500 Fast
 Roche LightCycler® 480 System
 Rotor Gene Q
 Mic qPCR Cycler



DATA ANALYSIS

After the run is completed data are analysed using the software with both dyes. The below results were studied with Bio-Rad CFX96.

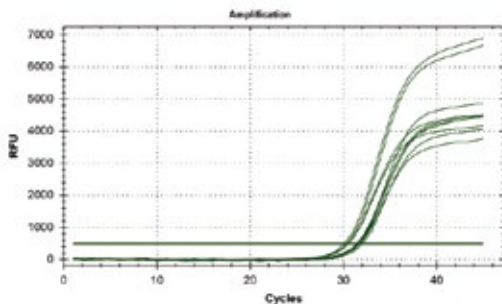


Figure 1: Internal Control Plots (HEX Dye)

Internal control amplification plots must be seen in all wells except NTC and has been labelled with HEX dye. The CT value of internal controls should be $25 \leq X \leq 35$ (Figure 1)

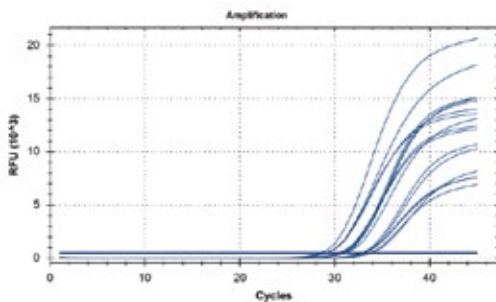


Figure 2 : COVID-19 – Positive samples (FAM Dye)

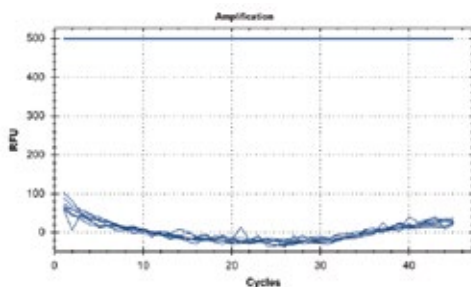


Figure 3 : COVID-19 Negative samples (FAM Dye)

TROUBLE SHOOTING

If internal control doesn't work properly,

- Unloaded well
- Sample is containing RNA inhibitor(s)

If plots start late,

- Compare positive control and sample. If there is no problem in positive control,
- The amount of target RNA may be low
- Target RNA quality is not good.
- "Elution Buffer", used in obtaining target RNA, contains more than 5 mM Tris-HCl.

Please contact us for your questions.

CAUTIONS

- All reagents should be stored at suitable conditions.
- Do not use the PCR mastermixes forgotten at room temperature.
- Thaw PCR mastermix at room temperature and slowly mix by inverting before use.
- Shelf-life of PCR mastermix is 12 months.
- Please check the manufacturing data before use. Only use in vitro diagnostics.



TESSCORN SEASIA PTE. LIMITED

596b Ang Mo Kio Street 52
#17-327, Singapore (562596)

enquiries@tesscorn-seasia.com

TESSCORN USA LLC.

3895 Lawson Dr.,
Troy, MI 48084

Cell: +1 734 658 0450
raghu@tesscorn-usa.com

TESSCORN SYSTEMS INDIA PVT LTD

1285, 5th Main, 17th Cross
HSR Sector 7, Bangalore 560068, India

Cell: +91 8861751155, +91 9900240316
info@tesscorn.com