

Covid-19 Real Time PCR Kit v3

Cat. No: 114R-10-03



INTRODUCTION

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)** and **Nucleocapsid protein gene (N)** regions of SARS-CoV-2 with high sensitivity and specificity.

In the autumn of 2020, several mutations were detected in Sars-Cov-2, different variants called the UK variant (B.1.1.7), the South Africa variant (B.1.351) and the Brazilian (B.1.1.28) variant occurred. All three variants contain the **N501Y** mutation in the spike protein gene. The kit also detects the N501Y mutation and determines whether the positive sample is a variant or not.

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**” format for one step RT-PCR mastermix which has been specifically adapted for cDNA and 5' nuclease PCR. The test system is designed for use with sequence specific primers and probes. The fluorescence of RdRp Gene is FAM dye, Nucleocapsid Gene is Texas RED and N501Y mutation is HEX dye. Also mastermix contains an internal control labelled with CY5 dye. Regions 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 : Regions and Related Dyes.

Tube	Regions	Dyes
Covid-19 Master Mix	RdRp Gene	FAM
	Nucleocapsid Gene	TEXAS RED
	N501Y	HEX
	Internal Control	Cy5

KIT CONTENTS

Reagents	100 Rxn
Covid-19 Master Mix	1000 µL
SARS-CoV-2 Positive Control	60 µL
Variant SARS-CoV-2 Positive Control	60 µL
User Manual	1

STORAGE

- All reagents should be stored at – 20 °C and dark.
- All reagents can be used until the expired date mentioned on the box label.
- Repeated thawing and freezing (≥ 5X) should be avoided, as this may reduce the sensitivity of the assay.

RNA EXTRACTION

- Human nasopharyngeal, oropharyngeal, anterior nasal and mid-turbinate nasal swab as well as nasopharyngeal wash/aspirate or nasal aspirate specimens and sputum samples should be collected with appropriate sterile swab into viral transport fluid. Inactivation & Transport Fluid Kit (Cat# 23S-04) for safe transport of specimens is a recommended product.
- The system is optimized for any RNA Isolation System such as Spin Column RNA Extraction, Automated RNA Extraction, salt extraction and phenol/chloroform RNA Extraction. We advise the SNP Viral Extraction Kit (Cat# 21S-04) especially developed for the SNP Covid-19 Real Time PCR Kit.

PROCEDURE

- Before starting work, mix the mastermixes gently by pipetting.
- For each sample, pipet **10 µl mastermix** with micropipets of sterile filter tips to each optical well PCR tubes. For reliable amplification results, we recommend the use of white Real-Time PCR tubes/strips/plates (for all 96/48 well equipments).
- Add **10 µl RNA** into each tube.
- Mix gently by pipetting
- Run with the programme shown below.

42 °C	10 Min.	cDNA Syntesis
96 °C	25 Sec.	Holding
96 °C	2 Sec.	40 Cycles
60 °C	35 Sec.	

Fluorescent dyes are FAM, Texas Red, CY5 and HEX.

Real Time PCR time is 63 minutes for the Biorad CFX96. This time may differ slightly depending on the device.

The following settings are valid for the Biorad CFX96 device. It may require different settings on different real time devices. For detailed information, please contact us; info@snp.com.tr

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 use with;

Bio-Rad CFX96
ABI 7500/7500 Fast
Miq qPCR Cycler
LongGene Q

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DATA ANALYSIS

After the run is completed, data are analysed using the software with all dyes. The below results were studied with Bio-Rad CFX96. If white tubes are used, the threshold values should be 500 for all dyes and 50 if a clear tube is used. The CT value of internal controls should be $x \leq 35$ (Figure: 1). Amplification plots in FAM and/or Texas RED dyes should be accepted as "Positive" for COVID-19 (Figure 2). Amplification plot in HEX dye should be accepted N501Y variant of SARS-CoV-2 (Figure3). You can give "Negative" results to samples that are no amplification plots in FAM, TEXAS RED and HEX dyes for COVID-19 (Figure 4).

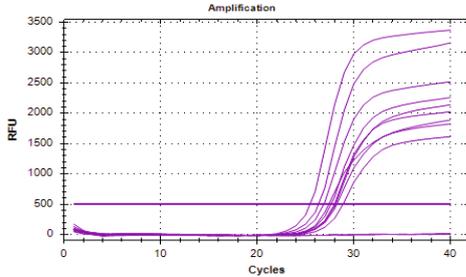


Figure 1: Internal Control Plots (CY5 dye)

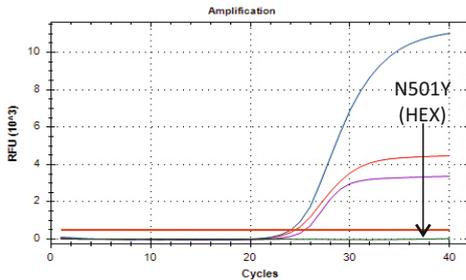


Figure 2: SARS-CoV-2 Positive Sample (FAM/TEXAS RED/HEX/CY5 dyes)

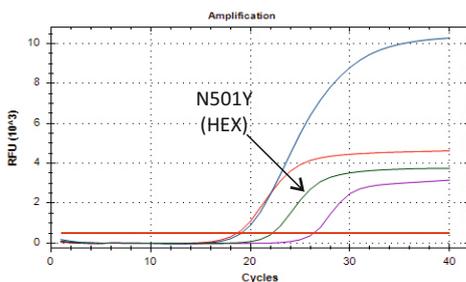


Figure 3: Variant SARS-CoV-2 Positive Sample (FAM/TEXAS RED/HEX/CY5 dyes)

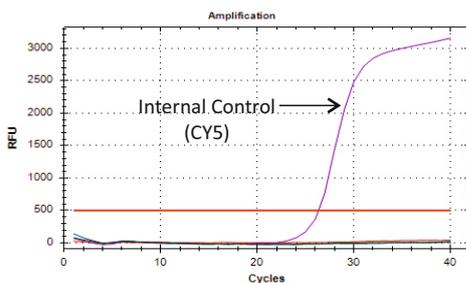


Figure 4: Covid-19 Negative Sample (FAM/TEXAS RED/HEX/CY5 dyes)

Table 2: Evaluation of Results

Sample Type	Internal Control (CY5)	RdRp Gene (FAM)	N Gene (Texas Red)	N501Y Variant (HEX)	Results	Interpretation	
Case 1	+/-	+	+	+	Variant SARS-CoV-2 Positive	All results are valid SARS-CoV-2 RNA is detected	
Case 2	+/-	-	+	+			
Case 3	+/-	+	-	+			
Case 4	+/-	+	+	-	SARS-CoV-2 Positive		
Case 5	+/-	-	+	-			
Case 6	+/-	+	-	-			
Case 7	+	-	-	-	SARS-CoV-2 Negative		All results are valid SARS-CoV-2 RNA is not detected
Case 8	-	-	-	-	Invalid		Dilute to extracted RNA or re-extract clinic sample
Case 9	-	-	-	-			

TROUBLE SHOOTING

If internal control doesn't work

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

If target 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. Please dilute RNA by adding 1 to 1 PCR grade water.

Please contact us for your questions. tech@snp.com.tr

CAUTIONS

- In case of contact, it may irritate skin.
- Do not use without gloves.
- In case of contact, immediately wash skin with copious amounts of water.
- 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 pipetting before use.
- Shelf-life of PCR mastermix is 12 months. Please check the manufacturing date before use.
- Only use in vitro diagnostics.

Date of issue : 19/03/2021

