

COVID-19 REAL TIME PCR KIT

Cat # 114R-10-01

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.

PRINCIPLE OF THE SYSTEM

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.

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- Virus - Dyes.

Tube	Virus	Dye
Mix	Internal Control	HEX
	COVID-19 (RDRP and N genes)	FAM

SYSTEM CONTENTS

Reagents **100 rxns**

- COVID-19 Master Mix 1000 µl
- Positive Control 60 µl
- Negative Control 60 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

- 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-04-01) for safe transport of specimens is a recommended product.
- Sample can be stored at +4°C up to one week in viral transport fluid.
- Sample can be transported at RT.
- For more than one week sample should be stored at -20°C.
- 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 white strips or tubes.
- Add **10 µl RNA** into each tube.
- Mix gently by pipetting
- Run with the programme shown below.

PCR PROGRAMME

42 °C	8 Min.	cDNA Syntesis
96 °C	1 Sec.	40 Cycles
60 °C	25 Sec.	

Fluorescent dyes are FAM and HEX.

- Real Time PCR time is **55 minutes for Bio-Rad CFX96**. This time may differ slightly depending on the device.

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 Prism® 7500/7500 Fast
- Rotor Gene Q
- Roche Lightcycler 480
- 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.

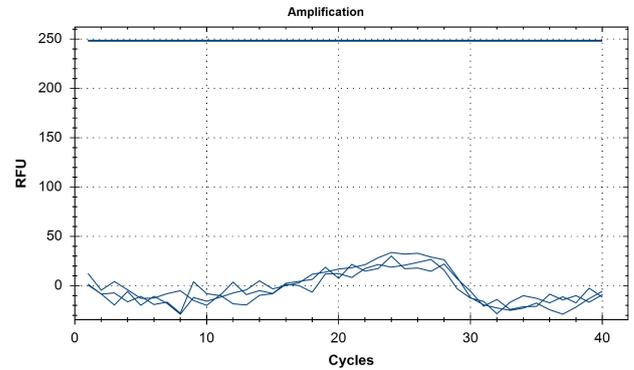


Figure 3 : COVID-19 Negative samples (FAM Dye)

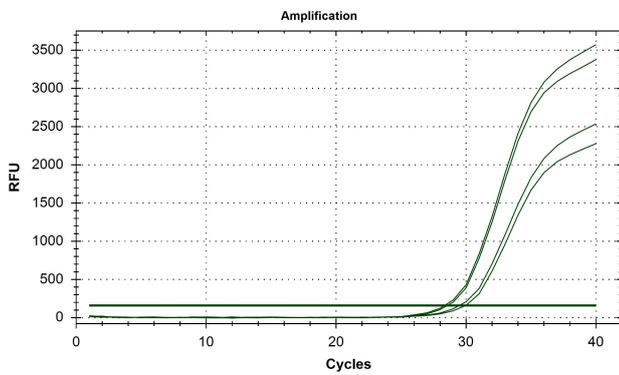


Figure 1: Internal Control Plots (HEX Dye)

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 $X \leq 36$ (Figure 1).

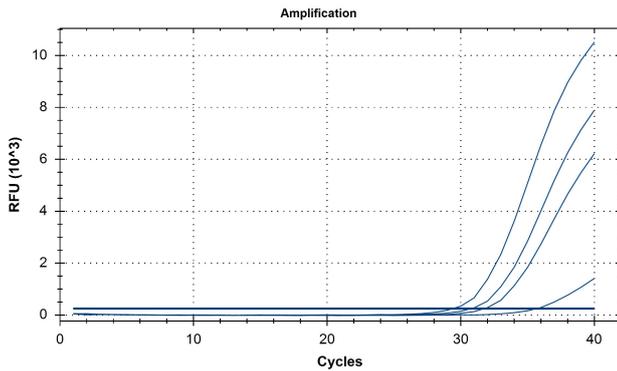


Figure 2 : COVID-19 – Positive samples (FAM Dye)

TROUBLE SHOOTING

If internal control doesn't work 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.

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.