

Quantiplus® Multiplex COVID-19 Fast PCR Kit

(Real Time Qualitative PCR Kit)


QL-MCF-100 : 100 Tests
IVD
PI/QLMCF-02

Intended Use

Quantiplus® Multiplex COVID-19 Fast PCR Kit is a Real-Time PCR based in vitro diagnostic assay for detection of COVID-19 in human nasopharyngeal swab extracts, deep cough sputum, etc. and lower respiratory tract specimens (alveoli irrigation fluid, etc.). The kit contains ready to use target specific Primer Probe Mix and Amplification Mix for cDNA synthesis and PCR amplification. The kit simultaneously detects two different genes of SARS-CoV-2, ORF1ab (FAM) and N gene (HEX/VIC) along with an endogenous B2M/ Beta-2 micro globulin (Texas Red).

Background Information

Coronaviruses are a group of viruses that cause diseases in mammals, including humans, and birds. In humans, the virus causes respiratory infections which are typically mild but, in rare cases, can be lethal. Coronaviruses are viruses in the subfamily Orthocoronavirinae in the family Coronaviridae, in the order Nidovirales. Coronaviruses are enveloped viruses with a positive-sense single-stranded RNA genome and with a nucleocapsid of helical symmetry.

Kit components

Color Coding (Caps)	Contents	Description	100 tests (QL-MCF-100)
Green	R Fast Core qPCR Mix (2X)	cDNA and PCR Amplification Mix	1 x 1.3 mL
Amber	Huwel Fast CON PPM	Target Specific Primer Probe Mix	1 x 200 µL
Red	Huwel Fast CON PC	DNA Positive Control	1 x 100 µL
White	MBG PW	Purified Water	1 x 500 µL

Note: 1. Please pay attention to the cap color coding and the tube contents.

2. Huwel Fast CON PC will serve as a common PC for both ORF1ab gene and N gene targets

MBGPW Molecular Biology Grade Purified Water

Storage and Transportation Conditions

The kit should be transported at temperature below $-20\text{ }^{\circ}\text{C}$. The kit is stable until the expiry date printed on the package, if the storage temperature is within $-20 \pm 5\text{ }^{\circ}\text{C}$. More than 4X freezing and thawing cycles reduces the assay sensitivity. For intermittent usage the reagents should be frozen in aliquots.

Technical Specification

Target Sequence	Conserved region <i>ORF1ab</i> and <i>N</i> gene
Specificity	100%
Limit of Detection	25 copies/reaction
External Quality Assessment	QCMD EQA Panels
Validated Specimen	Nasopharyngeal or oropharyngeal swabs

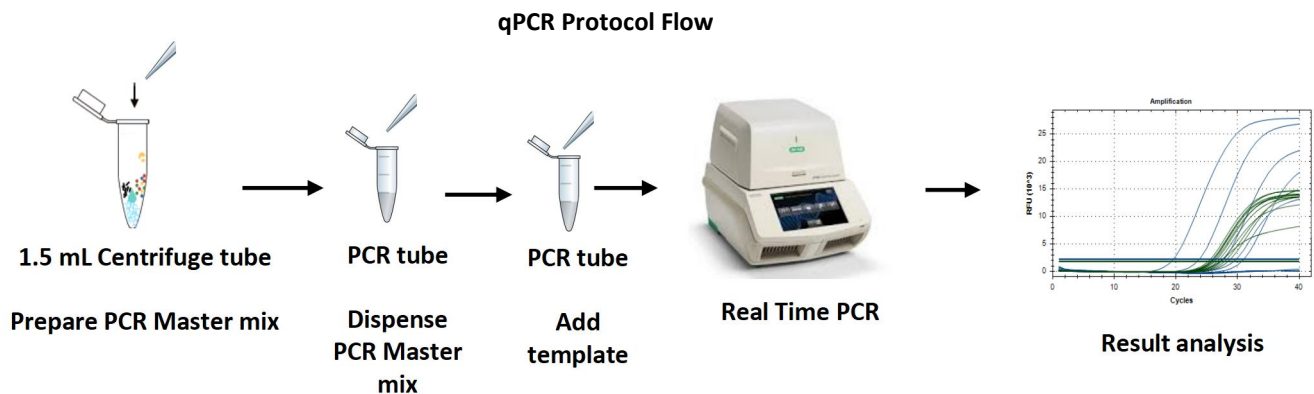
Assay Procedure

RNA Extraction

Quantiplus® Multiplex COVID-19 Fast PCR Kit has been validated using the following Viral RNA extraction kits:
 Recommended sample volume for extraction and elution are as follows:

S. No.	Name of the Extraction Kit	Recommended Sample volume for Extraction	Recommended Final Elution volume
1.	Huwel Nucleic Acid Extraction Kit - Version 2.0 (Cat. No. HL-NAX-100)	200 µL	100 µL
2.	Roche High Pure Viral Nucleic Acid kit (Cat. No. 11858874001)	200 µL	100 µL

Note: Customer can also validate their own extraction process using other Viral RNA extraction Kits.



Preparation of Reaction Master mix

Components	Volume per reaction (25 µL)
R Fast Core qPCR Mix (2X)	13.0
Huwel Fast CON PPM	2.0
Extracted RNA/ Huwel Fast CON PC/ MBG PW	10.0

It is necessary to keep all components at +2°C to +8 °C during the PCR preparation. Close the tubes and centrifuge briefly before proceeding to thermal cycler.

Cycling Conditions

Steps	No. of cycles	Temperature (°C)	Time
1 (cDNA Synthesis)	1	53	5 min.
2 (Initial denaturation)	1	95	1 min.
3 (PCR cycling)	5	95	05 sec.
		60	10 sec.
4 (PCR cycling)	35	95	01 sec.
		60*	01 sec.

*Plate Read/Data Acquisition in **FAM, YAKIMA YELLOW/ HEX/ VIC** and **Texas Red/ROX** Channel

Note: Consider the first 5 cycles also in the Ct determination. Total number of PCR cycles in the program = 5+35=40.

Sample analysis and Interpretation

Interpret the values for unknown samples based on the observations as described in the following table. There should be no amplification signal in negative control. IC should show signal in all the samples to identify possible PCR Inhibitions. ≤ 38 Ct of unknown samples should be considered for result interpretation.

(Note: Cut off Ct used for result interpretation includes the 5 cycles in the 1st PCR cycling step. Total number of PCR cycles in the program = 5+35=40).

ORF1ab gene (FAM)	N gene (YAKIMA YELLOW/ HEX/VIC)	B2M (Texas Red)	Interpretation
Positive	Positive	Positive	Covid -19 positive
Positive	Negative	Positive	Inconclusive (Repeat the assay. If the result is still inconclusive recommend collection of new specimens from the patient)
Negative	Positive	Positive	
Negative	Negative	Positive	Covid -19 negative
Negative	Negative	Negative	In Valid Result Repeat from Extraction. If the repeated results remain same, recommend collection of new specimen from patient

Note: All the Target channels (FAM, HEX/VIC and Texas Red/ROX) to be analyzed individually.

Target	Ct Value	Interpretation
ORF1ab gene (FAM)	Ct \leq 38	ORF1ab gene- positive
N gene (HEX/VIC)	Ct \leq 38	N gene - positive
B2M IC (Texas Red/ROX)	Ct \leq 38	Internal control – positive

Validated Instruments

- Thermo QS5 Real-Time PCR System
- Bio-Rad™ CFX 96



HLSS Manufacturing Pvt Ltd
 Plot No's M14, M15, M16, TSIC Medical device park
 Sultanpur villiage, Ameenpur Mandal,
 Sangareddy Dist, TS-502319