

## SARS Coronavirus – 2 Real Time RT-PCR

### Manufacturer – BGI

### Real time Fluorescent RT-PCR kit for detecting 2019-nCoV

As per kit protocol

Target gene – ORF1ab for SARS CoV-2

Duplex assay (ORF1ab and Internal Control)

### Master-mix preparation:

All reagents provided in the kit. Master-mix prepared in a single tube.

30 µl reaction volume

	Component	Volume for 1 reaction
1	2019-nCoV Reaction Mix	18.5 µl
2	2019-nCoV Enzyme Mix	1.5 µl
	Template RNA	10 µl
	Total	<b>30 µl</b>

Kit content- Blank control and Positive control (pooled PC for 2019-nCoV ORF1ab and IC)

### Target dyes:

	Target gene	Dyes used
Duplex assay	ORF1ab	FAM
	Internal Control	VIC/ HEX

Passive reference - None

### Cycling condition:

Reverse Transcription	50°C for 20 min
Initial denaturation	95°C for 10 min
PCR amplification (40 cycles)	95°C for 15 sec 60°C for 30 sec *(data collection)

### Analysis & Interpretation:

Positive Control - Ct value ≤ 32 and sigmoid curve

Internal Control - Ct value ≤ 32 and sigmoid curve

Test specimen – Positive - Ct value < 38 and sigmoid curve