

SARS-CoV-2 Multiplex RT-qPCR Protocol



#COVID-19 M/Plex COVID-19 (SARS-CoV-2) Multiplex RT-qPCR Kit 500 tests

Introduction

At the end of December 2019, the WHO was informed of a new virus causing pneumonia in Wuhan, China. A novel coronavirus was isolated that was closely related to the SARS coronavirus first identified in 2003. This new virus was termed SARS-CoV-2 and causes an affliction known as COVID-19. Multiple tests have been developed but are mostly in singleplex formulation. Multiplex assays (multiple targets in the one reaction) greatly increase throughput.

Internal controls are usually mandated for molecular diagnostic assays and ideally should provide sample adequacy information by detecting a constitutive target present in the intended sample (e.g. human DNA in the case of swabs and sputum)

Kit Contents	Volumes	Storage
20X primer & probe mix	0.52 mL	4°C for up to 6 months / -20°C
PCR Grade Water	2.5 mL	room temperature
2X Blue Mastermix	1.74 mL (3 tubes)	-20°C (not affected by freeze/thaw)

Targets

FAM	E gene (detects SARS-CoV-2, 2003 SARS-CoV and some bat coronaviruses)
HEX	RdRP (newly-designed primers, and probe - specific to SARS-CoV-2)
ROX	Internal Control, modified RNaseP (CDC recommended)

Required Items

- Extraction reagents
- qPCR instrument capable of resolving three channels (FAM, VIC/HEX and ROX/Red610)
- qPCR plates/tubes as required by instrument, along with sealing foils or caps
- 1.5ml microcentrifuge tubes, vortex mixer, mini centrifuge and pipettes/filter tips

Extraction

Viral RNA may be extracted by a number of methods, including rapid lysis methods due to the increased tolerance of clinical inhibitors by the reagents in this test. Rapid lysis, column-based, 96 well plate and magnetic particle options are available from dnature.

Reaction Mix

Prior to starting, warm reagents to room temperature and briefly **vortex** (3 seconds) / **pulse centrifuge** both the 20X oligo mix tube and the 2X mastermix tube.

- Make up a reaction cocktail as follows (adjust volumes for larger reaction sizes if desired)
- Per 20µl reaction (allow extra for pipetting error – additional volume is supplied in the kit):

PCR-Grade Water	4 µl
20X COVID Oligo Mix	1 µl
2X Blue Mastermix	10 µl
- Briefly **vortex** (3 seconds) and **pulse centrifuge** working mastermix
- Dispense 15 µl working mastermix per reaction well and 5µl extracted RNA (or positive control and water for 'no template control').
- Seal tubes/plates, centrifuge (if required) and run on qPCR instrument

qPCR Protocol

BMS Mic

Reverse Transcription	50 °C	10 minutes	
Denaturation	95 °C	1 minute	
Cycling	95 °C	5 seconds	} 45 cycles
	60 °C	10 seconds	

Data (FAM, HEX and ROX) should be acquired at the end of the 60°C step

For plate-based instruments (e.g. 7500, QuantStudio, CFX, LightCycler 480)

Reverse Transcription	50 °C	10 minutes	
Denaturation	95 °C	1 minute	
Cycling	95 °C	10 seconds	} 45 cycles
	60 °C	30 seconds	

Analysis

Automated analysis may be performed on some instruments, to account for internal control performance (e.g. 'Identifier' on the BMS Mic).

Refer to your instrument manual to analyse data for the FAM channel (E gene), HEX channel (RdRp) and ROX channel (internal control, human nucleic acid).

Cq limits of detection should be established in the user's laboratory

Interpretation

E gene result	RdRp result	Internal Control	Result
Not detected	Not detected	Not detected	Invalid: sample inhibited. Repeat extraction and RT-qPCR
Not detected	Not detected	Detected	COVID-19 not detected
Detected	Detected	Detected or not	Sample positive for COVID-19
Only one marker detected		Detected or not	Inconclusive – repeat test (see notes)

Notes

- Ensure ROX normalisation is turned off/NONE (if ROX is an option on your instrument)
- E-gene marker detects the original SARS, SARS-CoV-2 (COVID-19) and some other Sarbeco coronaviruses i.e. it is not specific to the COVID-19 virus. Therefore it is possible in a rare instance, that an E gene positive sample does not confirm with the RdRp marker.

Disclaimer

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