

IHU Méditerranée Infection, Marseille, France

<https://www.mediterranee-infection.com/>

In-house one-step real-time reverse transcription-PCR (qPCR) assay that specifically detects SARS-CoV-2 N501Y variants.

This qPCR system targets nucleotide position 23,063 [in reference to SARS-CoV-2 genome GenBank Accession no. NC_045512.2 (Wuhan-Hu-1 isolate)] within S gene where A>U leads to N501Y.

Sequences of primers and probe

Name	Sequence (5'-3')	Positions *
<i>Primers:</i>		
Pri_IHU_N501Y_F1	ATCAGGCCCGGTAGCACAC	22,980-22,997
Pri_IHU_N501Y_R1	AAACAGTTGCTGGTGCATGT	23,116-23,135
<i>Probe (6FAM-labelled):</i>		
Pro_IHU_C_GB_1_MBP	<u>CCACTTATGGTGGTGGTTACCAA</u>	23,058-23,080

* in reference to SARS-CoV-2 genome GenBank Accession no. NC_045512.2 (Wuhan-Hu-1 isolate).

The nucleotide carrying the mutation is covered by the probe and underlined.

qPCR conditions

The qPCR can be performed by adding 5 µL of extracted viral RNA to 15 µL of reaction mixture containing 5 µL of 4X TaqMan Fast Virus 1-Step Master Mix (Thermo Fisher Scientific, Grand Island, NY, USA), 0.5 µL of forward primer (10 pmol/µL), 0.5 µL of reverse primer (10 pmol/µL), 0.4 µL of probe (10 pmol/µL), and 8.6 µL of water. PCR conditions are as follows: reverse transcription at 50°C for 10 min, then a hold at 95°C for 20 sec followed by 40 cycles comprising a step at 95°C for 15 sec and a step at 60°C for 60 sec. This qPCR was run on a LC480 thermocycler (Roche Diagnostics, Mannheim, Germany).