GENERATION Biotech

SARS-CoV-2 Variant Detection Kit - "N501Y"

- For positive detection of wildtype and N501Y S gene mutation -

Multiplexed qRT-PCR Kit - For Research Use Only (RUO)

Instructions for Use

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1. Application

The SARS-CoV-2 Variant Detection Kit is a reagent system, based on quantitative reverse transcriptase (qRT) PCR technology, for the detection of RNA specific to emerging viral variants of concern (Severe Acute Respiratory Syndrome Coronavirus 2, SARS-CoV-2) that cause the Coronavirus Disease 2019 (COVID-19).

The "N501Y" multiplex assay of this kit specifically detects the viral mutation N501Y in a separate channel as a positive signal rather than as a negative drop-out, thereby increasing sensitivity and accuracy.

Quantitative polymerase chain reaction (qPCR) technology utilizes an enzyme (reverse-transcriptase, RT) to convert RNA into complementary DNA (cDNA), from which specific target sequences are then amplified and targeted with specific probes for the detection of their copy number (concentration) in the initial specimen. The detection probes are labelled with differently colored fluorescent reporter dyes, which enable the direct comparison of several genetic loci (M, N, S and RP) in a single assay.

For research use only (RUO). Not for use in diagnostic procedures.

2. Equipment and Consumables

Customer supplied equipment
Quantitative PCR Cycler / Digital PCR system
Calibrated single-channel pipettes
Vortex mixer
Benchtop microcentrifuge for 1.5 ml tubes

Customer supplied consumables	
Pipette tips (with aerosol barriers, nuclease-free)	General laboratory supplier
Quantitative PCR Plates / dPCR consumables	General laboratory supplier
1.5 ml tubes (low DNA binding, nuclease-free)	General laboratory supplier

3. Kit Components

Component	Tube Label
Buffer Mix	**BM**
Enzyme Mix	**EM**
Primer / Probe Mix	**PPM**
Positive Control	**CTR**
Nuclease-free water	**Water**

4. Storage

SARS-CoV-2 Variant Detection Kits are shipped on dry ice or with -80°C ice packs. The components of the kit should arrive frozen. If one or more components are not frozen upon receipt, or if tubes have been compromised during shipment, contact Generation Biotech for assistance.

All components should be stored between -25°C and -15°C upon arrival.

Repeated thawing and freezing of reagents should be avoided, as this might affect the performance of the assay. The reagents should be subdivided and frozen in aliquots if they are to be used intermittently.

Storage between +2°C and +8°C should not exceed a period of two hours.

Protect Primer/Probe Mixes from light.

5. Product Description

The **SARS-CoV-2 "N501Y"** multiplex assay detects **three SARS-CoV-2 genes (M, N and S)** with improved accuracy when compared to prior SARS-CoV-2 'wildtype' assays that detect only the M and N genes.

The additional S gene assay enables the specific detection of strains carrying the **N501Y** mutation that has been associated with an increased viral infectivity. This mutation is present in the variants B.1.1.7 ("UK-Variant"), B.1.351 ("South-African Variant") and B.1.1.28 ("Brazilian Variant"), amongst others.

The kit further includes a primer/probe set that detects the **human RNase P gene** as an internal control. This assay detects whether the patient specimen collection was properly carried out, thereby reducing false negative tests.

The SARS-CoV-2 "N501Y" Variant Detection Kit consists of:

- Buffer Mix
- Enzyme Mix (reverse transcriptase and heat-stable polymerase)
- Primer / Probe Mix (for SARS-CoV-2 genes M, N & S and for human RNase P gene RP)
- Positive Control (SARS-CoV-2 genes M, N & S, including N501Y, and RP gene)
- Water (nuclease-free)

The test consists of three processes in a single tube / single reaction assay:

- Reverse transcription of target RNA (from SARS-CoV-2) into cDNA
- PCR amplification of target cDNA (M, N & S genes) as well as of human control DNA (RP gene)
- Quantitative detection of PCR amplification products by fluorescent dye labelled probes

6. Procedure

6.1 Sample Preparation

RNA extracted from the specimen is the starting material for the SARS-CoV-2 Variant Detection Kit.

The quality of the extracted RNA has a profound impact on the performance of the entire test system. The time point(s) of sampling is important for the detection of SARS-CoV-2 RNA: the best time for an accurate identification of positive swab samples via qRT-PCR testing is 4 – 12 days after infection.

It is recommended to ensure that the system used for nucleic acid (RNA/DNA) extraction is compatible with the desired type of quantitative PCR technology and instrumentation.

If using a spin column-based sample preparation procedure that includes washing buffers containing ethanol, it is highly recommended to perform an additional centrifugation step for 10 min. at maximum speed, using a new collection tube and prior to the elution of the nucleic acid, to obtain reliable results.

For additional information and technical support regarding pre-treatment and sample preparation please contact our Technical Support (see Technical Assistance).

6.2 Reaction Setup

All reagents and samples should be thawed completely, mixed (by pipetting or gentle vortexing) and centrifuged briefly before use.

Reagents	Volume per Reaction
Buffer Mix (BM)	10 µl
Enzyme Mix (EM)	0.2 µl
Primer / Probe Mix (PPM)	2 µl
Sample or Positive Control (CTR)	5 µl
Nuclease-free Water	2.8 µl
Total Reaction Volume	**20 µl**

7. Programming the quantitative PCR Instrument

For basic information regarding the setup and programming of different quantitative PCR instruments, please refer to the user manual of the respective instrument.

7.1 Settings

Define the following settings as described below.

Settings	
Reaction Volume	20 µl
Ramp Rate	Default
Passive Reference	(None)

7.2 Fluorescence Detector

Define the fluorescence detectors as described below.

Target	Reporter Dye	Quencher Dye
SARS-CoV-2 gene N1	FAM	(none)
SARS-CoV-2 gene M	JOE	(none)
Human RNase P gene	ROX	(none)
SARS-CoV-2 gene S N501Y	Cy5	(none)

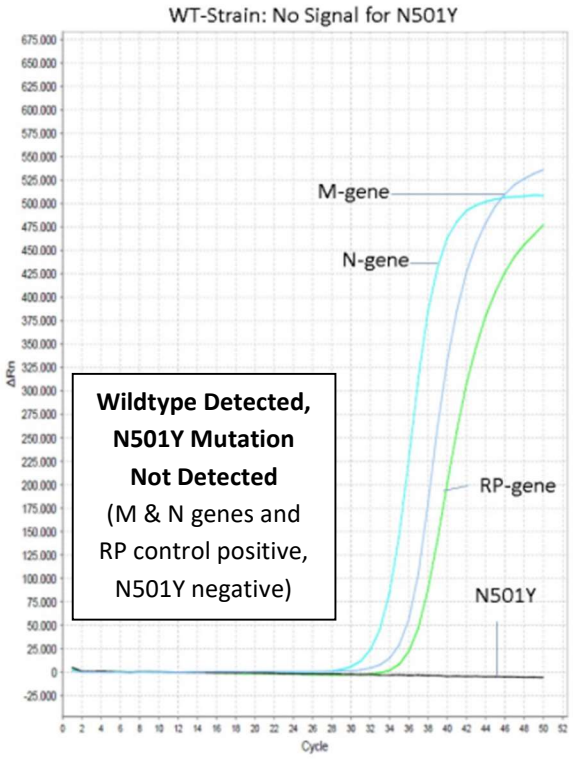
7.3 Temperature Profile

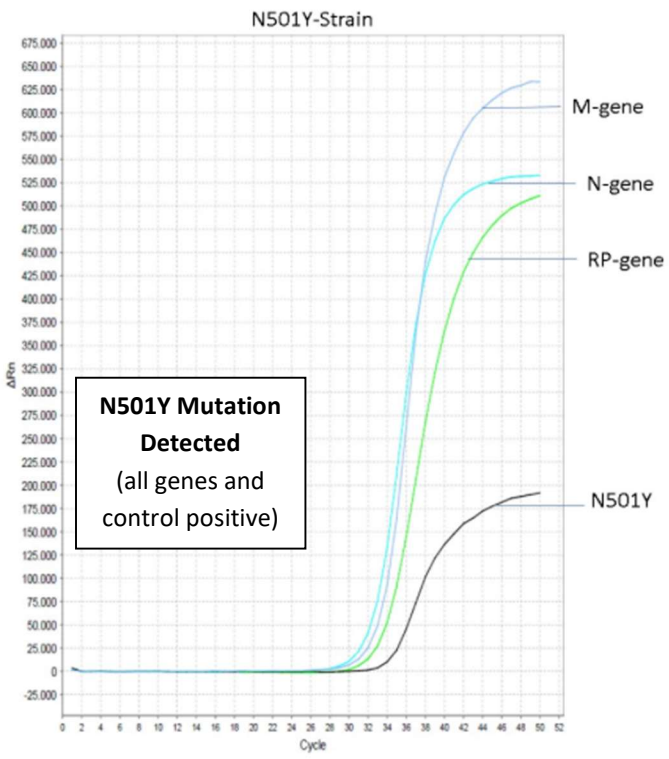
Incubate the reaction as described below.

Step	Stage	Cycles	Acquisition	Temp	Time
RT	Hold	1	-	50 °C	10 min
Denaturation	Hold	1	-	95 °C	2 min
Denaturation	Cycling	45	-	95 °C	5 sec
Amplification			yes	60 °C	30 sec

8. Data Analysis / Interpretation of Results

SARS-CoV-2 genes M/N	Human RNase P	SARS-CoV-2 gene S N501Y	Status	Result	Action
NEG	NEG	NEG	Invalid	NA	Repeat Test
NEG	POS	NEG	Valid	SARS-CoV-2 Not Detected	Report Results
POS	POS or NEG	POS or NEG	Valid	SARS-CoV-2 Detected	Report Results
POS	POS or NEG	POS	Valid	N501Y Mutation Detected	Report Results
POS	POS or NEG	NEG	Valid	N501Y Mutation **NOT** Detected	Report Results





For detailed instructions regarding the analysis of the data generated with the SARS-CoV-2 Variant Detection Kit on different quantitative PCR instruments please contact our Technical Support.

9. Technical Assistance

For customer support, please contact our Technical Support:

e-mail: info@generationbiotech.com

phone: 609-637-0878

10. Disclaimer

For Research Use Only. Not for use in diagnostic procedures. Generation Biotech does not assume any responsibility for your use of these materials, the accuracy of any documentation or the safety of user protocols. Use standard laboratory safety precautions for infectious specimens. Use at your own risk.

Take appropriate precautions to avoid false positive results. Routine use of nucleic acid high amplification technologies such as PCR carries a high risk of contamination. Perform nucleic acid extraction and reaction setup in an environment free from the presence of SARS-CoV-2 genes M, N & S and RNase P gene RP.

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