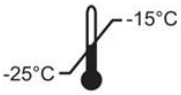
Genmark Sağlık Ürünleri
İTH. İHR SAN. TİC. LTD.

Instructions For Use

CE IVD *For In Vitro Diagnostic Use*



Store at -25ºC to -15ºC

REF nCoVM1-RT100

geneMAP™ 2019-nCoV N501Y & 69/70 Del

Detection Kit

For Real-Time PCR

(FAM, VIC, ROX & CY5 QuadruPlex)

Multiplex Real-time PCR System for detection of N501Y and 69/70 Del mutations in 2019 Novel Corona Virus (2019-nCoV).

Validated on:

* Biorad® CFX96,CFX384 Real-time PCR System (Bio-Rad)
* Life Technologies ABI Prism®- 7500
* Qiagen, RotorGene Q5/6,
* Bio Molecular Systems, MIC qPCR

Contents

1. Intended Use

The geneMAP™ 2019-nCoV N501Y & 69/70 Del Detection Kit is qualitative in vitro assay (Multiplex qRT-PCR) for the detection of mutation N501Y and 69/70Del of SARS-CoV-2 from Nasopharyngeal/Nasal aspirate, Nasopharyngeal swab, Oropharyngeal swab, Bronchoalveolar lavage, lower respiratory tract aspirates for people with or without clinical symptom associated with COVID-19.

The geneMAP™ 2019-nCoV N501Y & 69/70 Del Detection Kit usage specifically for trained scientists and lab techinicans in Healtcare and Medical Laboratories.

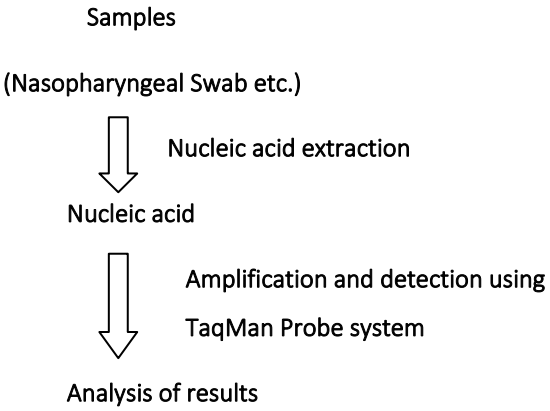
2. Principles and Procedure Overview

2.1 Principles

The polymerase chain reaction (PCR) is sensitive and specific TaqMan Probe technology with the use of DNA amplification technique, primer design and PCR optimization. The kit is, based on two main processes: nucleic acid extraction and PCR amplification of nucleic acid in the primer and probe mechanism of PCR machines by Real-time PCR. The kit is a real-time PCR test where Novel Coronavirus and Internal Control (IC) target is a multiplex realization that allows amplification of nucleic acids.

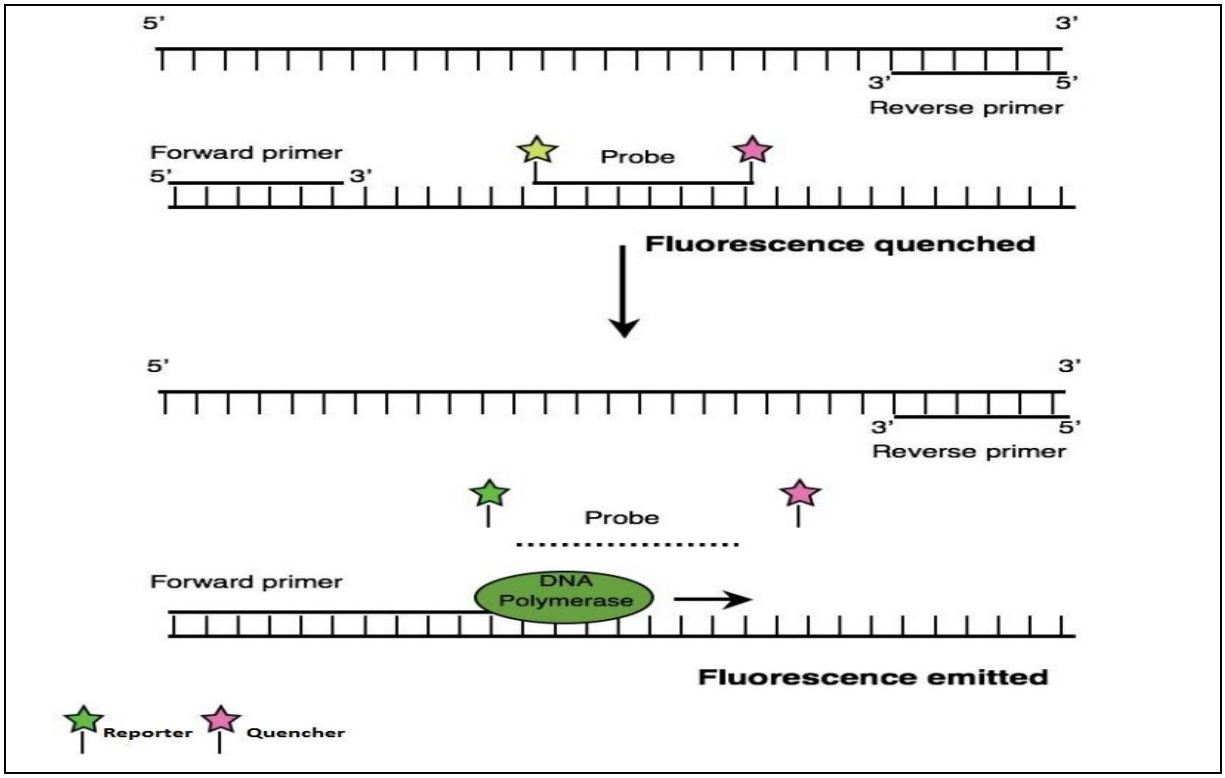
Targeted regions are RNA-dependent RNA polymerase (RdRp), Nucleocapside (N) ,Spike Protein (S) genes and plasmid as internal Control.

Procedure Overview;

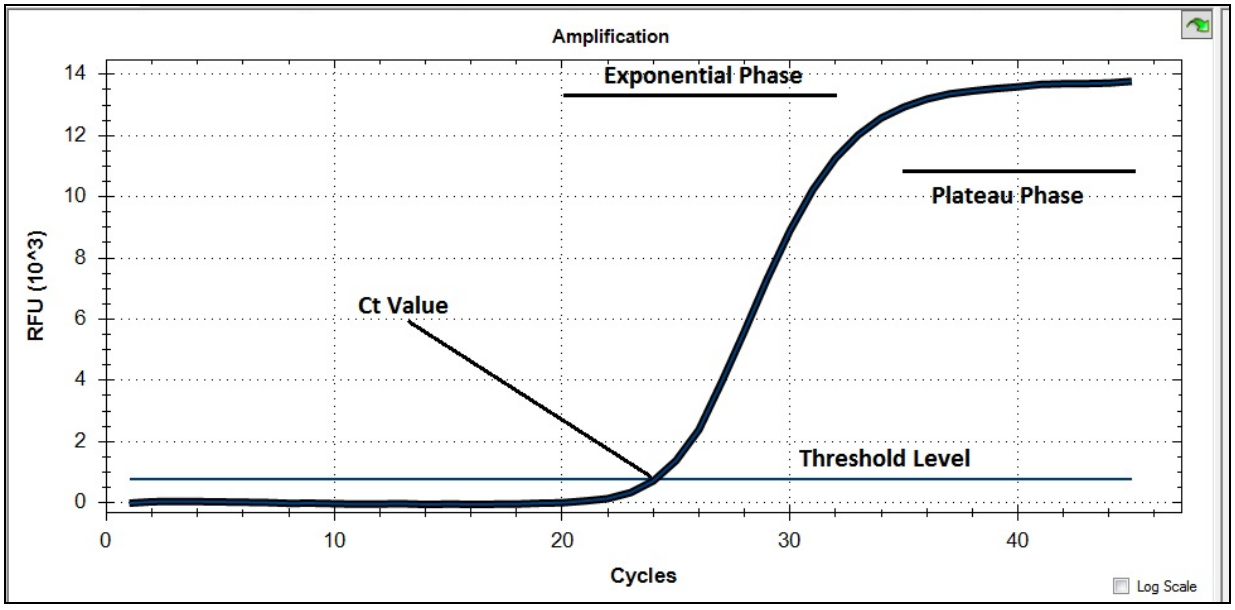


2.2 Technology

Hydrolysis (TaqMan) probes are the most common form of qPCR probes and are widely used in human, veterinary, and environmental diagnostics. These probes utilize a fluorescent dye at one end of the DNA oligonucleotide and a quencher at the other. During PCR, the probe specifically anneals to the target DNA sequence (from sample), which is flanked by the two primers. As DNA polymerase extends the new DNA strand, the probe is degraded by the 5' to 3' exonuclease activity of the polymerase, resulting in the fluorophore being separated from the quencher and emitting fluorescence. The more DNA present in the reaction, the earlier the fluorescence reaches a detectable level resulting in earlier Ct values.



Hydrolysis (TaqMan) Probe Technology.



Typical Amplification Plot of Real-time PCR in Linear Scale Graphic.

3. Background Information

Coronaviruses are a large family of viruses found in both animals and humans. Some infect people and are known to cause illness ranging from the common cold to more severe diseases such as Middle East Respiratory Syndrome (MERS) and Severe Acute Respiratory Syndrome (SARS). A novel coronavirus (nCoV) is a new strain of coronavirus that has not been previously identified in humans. The new, or "novel" coronavirus, now called SARS-CoV-2, had not previously detected before the outbreak was reported in Wuhan, China in December 2019.

Variant of Concern 202012/01, abbreviated VOC-202012/01 (also known as 20I/501Y.V1, lineage B.1.1.7, or commonly as the UK variant or British variant; see § Names), is a variant of SARS-CoV-2, the virus that causes COVID-19. One of several variants believed to be of particular importance, it is estimated to be 30%–80%more transmissible than wild-type SARS-CoV-2 and was detected in November 2020 from a sample taken in September, during the COVID-19 pandemic in the United Kingdom; it began to spread quickly by mid-December, and is correlated with a significant increase in SARS-CoV-2 infections in the country. This increase is thought to be at least partly because of one or more mutations in the virus's spike protein. The variant is also notable for having more mutations than normally seen.

The key mutations of this variant are N501Y and 69/70del,

As of January 2021, more than half of all genomic sequencing of SARS-CoV-2 was carried out in the UK. This has given rise to questions as to the variant's origins, and how many other important variants may be circulating around the world.

4. Reagents

Reagents contained in a kit are sufficient for 100 reactions.

Contents	Volume	Description
B.1.1.7 (UK) Reaction Mix	1500 µL	Primer Probe Mix (PPM), Buffer containing dNTPs), ddH2O
OneStep Enzyme Mix	165 µL	Taq Polymerase, Reverse Transcriptase and Uracil-DNA Glycosylase (UDG)
B.1.1.7 (UK) PC	100 µL	Positive Control (PC): Recombinant Viral RNA
Negative Control	100 µL	ddH2O

5. Storage and Handling

All components of the kit must be stored at between -15°C /-25°C. All components are stable under the recommended storage conditions until the expiration date indicated on the label on the box. The performance of the kit components are not affected until 5 freeze and thaw. If reagents are to be used only intermittently, they should be stored in aliquots.

This product is shipped on frozen blue ice packs (+4 °C) and may thawed upon arrival.

Expiry date of the kit is one year from manufacter date.

6. Materials Required But Not Provided

- Disposable powder-free gloves (latex ornitrile)
- Pipettes (adjustable) and sterile pipette tips
- 1.5 mL microcentrifuge tubes
- Desktop centrifuge
- Vortex mixer
- Clean bench
- For Biorad CFX Instruments;
    - 96-Well Skirted PCR Plate, White well (Cat. No. HSP-9655, Biorad)
    - Permanent Clear Heat Seal (Cat. No. 1814035, Biorad) or Microseal 'B' Film (Cat. No:MSB1001)
    - Low-Profile PCR Tubes 8-tube strip, white
    - Optical Flat 8-Cap Strips for 0,2ml tube strips/plates TCS0803

For the other instruments please use 96 well plates and tubes recommended by device manufacturers.

7. Protocol

7.1 Specimen Collection, Storage, and Transport

All samples should be considered as potentially infectious material. Only sample materials collected, stored and transported in accordance with the following rules and instructions are permitted.

To ensure a high sample quality, samples should be transported as quickly as possible. The samples should be transported at the specified temperatures.

7.1.1 Specimen Collection

Nasopharyngeal swab and Oropharyngeal swab samples are examined for routine detection of common respiratory pathogens. The samples can be collected with flocked nylon swabs such as COPAN, Italy or Puritan (U.S).

Kit is validated on following mediums;

-Virus Transport Medium (VTM),
-Universal Transport Medium (UTM),
-Phosphate Buffer Saline (PBS),
-Saline Solution
-Steril Distilled Water samples.

7.1.2 Specimen Storage and Transport

Specimen	Storage*		Transport**	Note
	Temp.	Duration	Temp.	
Nasopharyngeal aspirate	2-8°C	3 days	2-8°C	Store any leftover specimens at ≤-20°C
Nasopharyngeal swab				
Oropharyngeal swab				
Bronchoalveolar lavage				

*: Performance may be affected by routine freezing or prolonged storage of specimens.

**: Specimens should also adhere to local and national instructions for transport of pathogenic material.

7.2 Nucleic Acid Extraction

Various manufacturers offer nucleic acid extraction kits. Use the correct protocol according to the manufacturers' protocol. The following extraction kits have been validated for use with this kit.

7.2.1 geneMAP™ Viral RNA Extraction Buffer (Optional)

1- Transfer 50 µL Viral RNA Extraction Solution Buffer into a new PCR tube (1,5 mL).
2- Add 50 µL patient sample (Nasopharyngeal swab, oropharyngeal swab medium or aspirate.) into the tube contains RNA Extraction buffer.
3- Pipette the mixture at least 5 times (up and down) and incubate for 5 minutes at room temperature.
4- 100uL mixture is ready to use in qPCR

Note: Short term storage at +4°C, long term storage at -20°C /-70°C)

7.2.2 Manual Nucleic Acid Extraction Kits

* Please use the recommended volumes of specimen and elution as indicated below. For all others, refer to the manufacturer's protocol.

Extraction Kit	Manufacturer	Cat. No	Recommended Vol.
QIAamp® MinElute® Virus Spin Kit*	QIAGEN	57704	Specimen:190µL Elution:40 µL
Ribo_spinvRD* (Viral RNA/DNA Extraction Kit)	GeneAll	302-150 SG1701	Specimen:290 µL Elution:40 µL

7.3 Preparation for Real-time PCR

* The correct tubes and caps must be used (see MATERIALS REQUIRED BUT NOT PROVIDED).
* Aerosol resistant filter tips and tight gloves must be used when preparing one-step RT-PCR reactions. Use extreme care to ensure no cross-contamination.
* Completely thaw all reagents in room temperature
* Briefly centrifuge the reagent tubes to remove drops from the inside of the cap.

B.1.1.7 (UK) Reaction Mix	13,5 µl
Enzyme Mix	1,5 µl
Total volume of PCR Master Mix	15µl

Note: PCR Master Mix is stable at 2-8 C for 24 hours. Master mix should never be frozen.

* Calculate the necessary amount of each reagent needs based on the number of reactions (samples + controls).

1. Mix by inverting the tube 5 times or quick vortex, and centrifuge briefly.
2. Aliquot 15 µL of the one-step RT-PCR Master Mix into PCR tubes.
3. Add 5 µL of each sample's nucleic acids into the tube containing the one-step RT-PCR Master Mix.

PCR Master Mix	15 µl
Sample's nucleic acid	5 µl
Total volume of reaction	20 µl

* Use a new sterile pipette tip for each sample.
* For Negative Control (NC), use 5 µL of RNase-free Water instead of sample's nucleic acid.
* For Positive Control (PC), use 5 µL of PC.
* Please be careful not to cross-contaminate the one-step RT-PCR Master Mix and samples with the Positive Control.
* The PCR tubes must be mixed by pipetting and centrifuged before running PCR reaction. It needs to be checked that liquid containing all PCR components is at the bottom of each PCR tube.

8. Real-time PCR Instrument Setup and Results Analysis

8.1 Real-time PCR System

8.1.1 Pre-settings for Data Analysis

A. Pre-settings for Fluorophore Selection and Thermal Cycling Conditions

Target	Reporter Fluorophore
RdRp & N Gene	ROX (Orange)
S Gene 69-70 Del	CY5 (Red)
S Gene N501Y Mutation	FAM (Green)
Internal Control	VIC/HEX (Yellow)

For Biorad CFX96 & CFX384 (Duration 44 min. & 58 min)

Temperature	Time	Cycles	Data Collection
50˚C	10 min	1	
95˚C	2 min	1	
95˚C	1 sec	40	
60˚C	1 sec		ROX, CY5, FAM and VIC/HEX

For ABI7500 (Standard) (Duration 58 min.)

Temperature	Time	Cycles	Data Collection
50˚C	10 min	1	
95˚C	2 min	1	
95˚C	1 sec	40	
60˚C	30 sec		ROX, CY5, FAM and VIC/HEX

For Rotorgene Q5/Q6 (Duration 58 min.)

Temperature	Time	Cycles	Data Collection
50˚C	10 min	1	
95˚C	2 min	1	
95˚C	1 sec	40	
60˚C	1 sec		Orange, Red, Green, Yellow

Note1*: Please use only 72-well carousel, 36-well carousel does not recommended*

Note2: *Please perform Auto-Gain optimisation befor first acquisition. (Auto-Gain optimisation tube should be PC)*

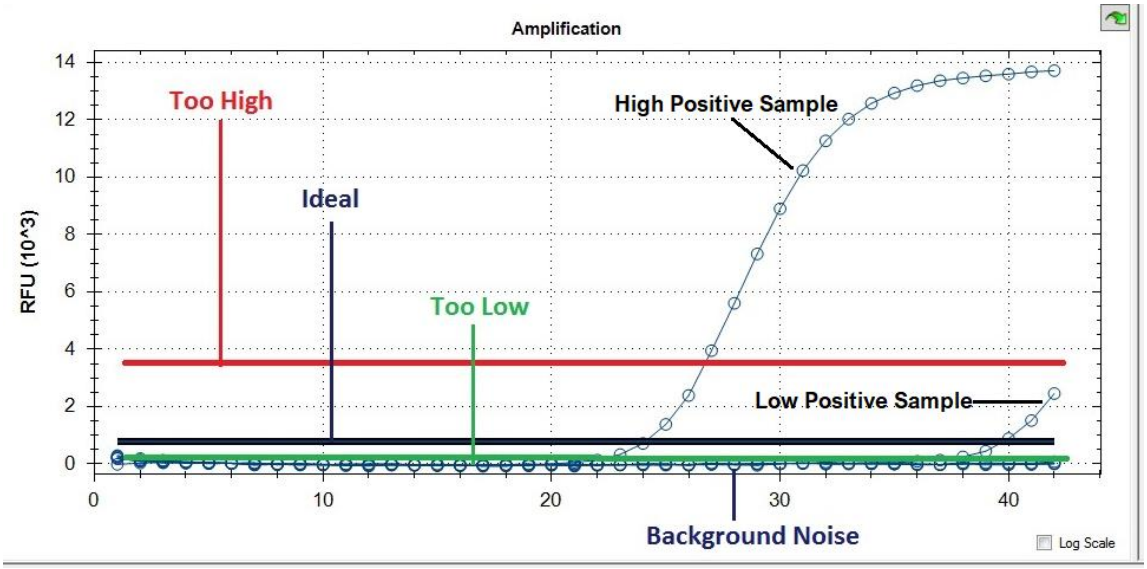
9. Results

9.1 General Rules of the Threshold Settings Manually

Normally the software-based methods will select a proper threshold, but in cases where the curves do not conform to the assumptions made by the algorithm, an incorrect threshold may be calculated but manually adjusting the threshold is highly recommended.

Ideally, the threshold should be set in the region where the just above the background noise. The threshold should not be so high that it crosses any of the plots where they are starting to plateau and are no longer linear.

To adjust the threshold for each dye collected must be set separately.



Example of ideal threshold level.

If the threshold is too high, it gives false negative.

If the threshold is too low, it gives false positive.

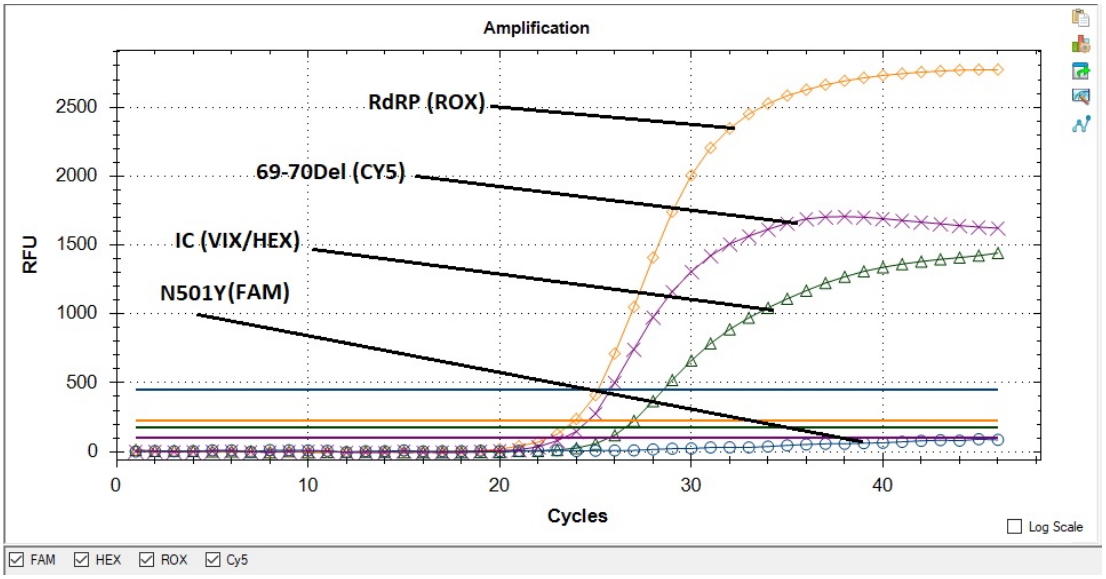
9.2 Interpretation of Results

Target	Reporter Fluorophore	Sample	
		Ct Value	Result
RdRp & N Gene	Orange	<36	Positive (+)
		≥36 or NA	Negative (-)
*S Gene 69/70 Del	CY5	<36	Negative (-)
		NA	Positive (+)
S Gene N501Y Mutation	FAM	<35	Positive (+)
		≥35 or NA	Negative (-)
Internal Control	VIC/HEX	<35	Positive (+)
		≥35 or NA	Negative (-)

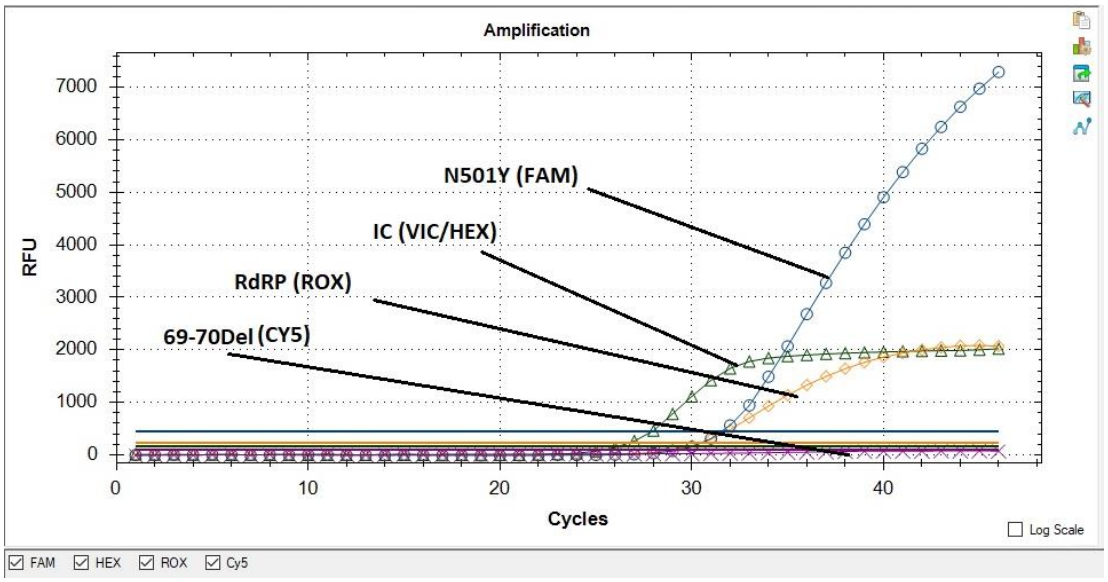
*If there is S gene 69/70 Del mutation Cy5 amplification does not generate positive amplification curve.

Interpretation	RdRp & N Gene	S gene 69/70 Del	S gene N501Y	Internal Control	Reporting
Case 1	+	+	-	+/-	2019-nCoV Positive
Case 2	-	-	-	+	2019-nCoV Negative
Case 3	+	+	+	+/-	B1.1.7 (UK) Positive
*Case 4	+	-	+	+	2019-nCoV & N501Y positive
Case 5	-	-	-	-	Invalid

*Go for further mutation analysis for Brazil and South Africa strains.



2019-nCoV Wildtype: There are only ROX, HEX (Internal Control) and CY5 amplification curves.



2019-nCoV B.1.1.7 Strain: There are FAM, HEX and ROX amplification curves. There is no amplification curve for CY5 (69-70 Del) due to the deletion.

10. Performance Characteristics

10.1 Assay Specificity

10.1.1 *In Silico* Studies

In silico studies are summarized below;

No	Organism	In silico Analysis for % Identity target: RdRp gene	In In silico Analysis for % Identity target: N gene
1	Human respiratory syncytial virus	No alignment found	No alignment found
2	Influenza A virus	No alignment found	No alignment found
3	Influenza B virus	No alignment found	No alignment found
4	Human coronavirus HKU1	No alignment found	No alignment found
5	Human adenovirus (e.g C1 Ad.71)	No alignment found	No alignment found
6	Human rhinovirus	No alignment found	No alignment found
7	Human metapneumovirus	No alignment found	No alignment found
8	Human parainfluenza virus1,2,3 &4	No alignment found	No alignment found
9	Enterovirus (e.g. EV68)	No alignment found	No alignment found
10	SARS-CoV-1	No alignment found	No alignment found
11	Middle East Respiratory Syndrome (MERS)	No alignment found	No alignment found
12	Epstein Barr Virus (EBV)	No alignment found	No alignment found
13	Human bocavirus	No alignment found	No alignment found

14	Streptococcus (Taxid: 1301)	No alignment found	No alignment found
15	Streptococcus pyogenes	No alignment found	No alignment found
16	*Mycobacteria* (Taxid:85007)	No alignment found	No alignment found
17	*Mycoplasma* (Taxid:2085)	No alignment found	No alignment found
18	*Legionella* (Taxid: 445)	No alignment found	No alignment found
19	*Bordetella pertussis*	No alignment found	No alignment found
20	*Pneumocystis jirovecii (PJP)*	No alignment found	No alignment found

10.1.2 Clinical Studies

Cross-reactivity of geneMAP™- 2019-nCoV N501Y & 69/70 Del Detection Kit was tested using 21 viruses and bacteria as indicated below.

No	Organism	Source	Results
1	Respiratory syncytial virus A	TURKEY ISOLATE	Not Detected
2	Respiratory syncytial virus B	TURKEY ISOLATE	Not detected
3	Influenza A virus (H3N2)	TURKEY ISOLATE	Not detected
4	Influenza A virus (H1N1)	TURKEY ISOLATE	Not detected
5	Influenza B virus	TURKEY ISOLATE	Not detected
6	Human coronavirus NL63	TURKEY ISOLATE	Not detected
7	Human coronavirus OC43	TURKEY ISOLATE	Not detected
8	Human coronavirus 229E	TURKEY ISOLATE	Not detected
9	Human coronavirus HKU1	TURKEY ISOLATE	Not detected
10	Adenovirus	TURKEY ISOLATE	Not detected
11	Human rhinoviruses	TURKEY ISOLATE	Not detected
12	Human metapneumovirus	TURKEY ISOLATE	Not detected
13	Parainfluenza 1	TURKEY ISOLATE	Not detected
14	Parainfluenza 2	TURKEY ISOLATE	Not detected
15	Parainfluenza 3	TURKEY ISOLATE	Not detected
16	Mycoplasma pneumoniae	TURKEY ISOLATE	Not detected
17	Human bocavirus	TURKEY ISOLATE	Not detected
18	Streptococcus pneumoniae	TURKEY ISOLATE	Not detected
19	Mycoplasma pneumoniae	TURKEY ISOLATE	Not detected
20	Haemophilus influenzae	TURKEY ISOLATE	Not detected
21	Chlamydophila pneumoniae	TURKEY ISOLATE	Not detected

10.2 Assay Sensitivity and Intra-Assay Reproducibility

geneMAP™ 2019-nCoV N501Y & 69/70 Del Detection Kit assay sensitivity is 500 copies/mL for 2019-nCoV but the viral load must be above than 1000 copies/ml for reliable mutation detection. (ROX Ct<33)

10.3 Clinical Evaluation

The clinical performance of the geneMAP™ 2019-nCoV N501Y & 69/70 Del Detection Kit was established in one site clinical evaluation. Fresh clinical Nasopharyhngeal Swab (NPS) and Orapharyngeal Swab (OPS) specimens were tested with geneMAP™ 2019-nCoV N501Y & 69/70 Del Detection Kit and Sanger Sequencing Method has chosen as comparator. Results are summarized below.

Test Name		Sanger Sequencing		Total
		Positive	Negative	
geneMAP™ 2019-nCoV N501Y & 69/70 Del Detection Kit	Positive	24	0	24
	Negative	0	162	162
Total		24	162	186

Positive Agreement Rate: 24/24x100%= 100%
Negative Agreement Rate: 162/162x100%= 100%

10.4 Reactivity/Inclusivity

An in silico inclusivity analysis of the geneMAP™ 's primers and probes was performed. All primer sets designed for detection of the RdRP and N gene were tested against the complete available SARS-CoV-2 genome sequence. The analysis demonstrated that the regions recognized by the designed primers and probes have 100% homology with all available SARS-CoV-2 sequences from the National Center for Biotechnology Information (NCBI).

Database	Identity		
	RdRP Gene Primers/Probe %	N Gene Primers/Probe	S gene Primer/Probe
NCBI	50/50 (100%)	50/50 (100%)	50/50 (100%)

11. Limitations

Mutation in the target sequence of SARS-CoV-2 or change in the sequence due to Virus Evolution can lead to false negative results.

False positive and false negative results can be caused by poor sample quality, incorrect sample collection, incorrect handling, incorrect laboratory processing, or restriction of testing technology.

The kit performance has not been validated specimens from Urine, stool etc.

Interacting agents or PCR inhibitors can lead to false negative or Invalid results.

The viral load must be above than 1000 copies/ml for reliable mutation detection. (ROX Ct<33)

12. Interference Studies

Interference Studies study was performed to demonstrate that potentially interfering substances that may be found in the upper respiratory tract in symptomatic subjects (including over the counter medications) do not cross-react or interfere with geneMAP™ 2019-nCoV N501Y & 69/70 Del Detection Kit Each substance was tested in triplicate in the absence or presence of SARS-CoV-2 at 5 x LoD. The final concentration of the substances tested are documented in the Table below.

No	Interfering Substance	Source	Test Concentration	Interference (Yes/No)
1	Blood	Human	5%	No (negative 3/3, positive 3/3)
2	Mucin (bovine submaxillary gland, type I-S)	Sigma-Aldrich (Cat.No.M3895)	60 μg/ml	No (negative 3/3, positive 3/3)
3	Mupirocin (Antibiotic, nasal ointment)	Sigma-Aldrich (Cat.No.1448901)	6.5 mg/ml	No (negative 3/3, positive 3/3)
4	Oxymetazoline	Sigma-Aldrich (Cat.No.O2378)	15% (v/v)	No (negative 3/3, positive 3/3)
5	Tobramycin	Sigma-Aldrich (Cat.No.T4014)	5.0 μg/mL	No (negative 3/3, positive 3/3)
6	Zanamivir (Anti-viral drug-Relenza)	Sigma-Aldrich (Cat.No.SML0492)	3.5 mg/mL	No (negative 3/3, positive 3/3)
7	Oseltamivir (Anti-viral drug-Tamiflu)	Sigma-Aldrich (Cat.No.1479304)	25 mg/mL	No (negative 3/3, positive 3/3)

13. Revision History

14. Troubleshooting

geneMAP™ 2019-nCoV N501Y & 69/70 Del Detection Kit		
OBSERVATION	**POSSIBLE CAUSES**	**SOLUTION**
No signal in any flororphore	Fluorophores incompatible with protocol for data analysis	Select the correct fluorophores.
	Incorrect setting of real-time thermal cycler	Please check the thermal cycling conditions and repeat the test under the correct settings.
	Incorrect storage or past expiry date of the test kit	Please check the storage condition and the expiry date (refer to label) of the test kit and use a new kit if necessary.
	Presence of inhibitor	Repeat the test with the new extracted nucleic acid.
Amplification signals in Negative Control	Cross Contamination	Decontaminate all surfaces and instruments with sodium hypochlorite and ethanol. Only use filter tips and throughout the procedure and change tips between tubes. Repeat entire procedure from nucleic acid extraction with the new set of reagents.
No amplification signal in Positive Control	The fluorophores for data analysis does not comply with the protocol	Please select the correct fluorophores for data analysis.
	Incorrect setting of real-time thermal cycler	Please check the thermal cycling conditions and repeat the test under the correct settings.
	Incorrect PCR mixture	Confirm that all components are added to the PCR mixture. Sensitivity is compromised with pre-composed premix. All reagents must be homogenized and spun down before use.
	Did not add sample's nucleic acid	Please carefully repeat the test.
	Error in adding nucleic acid to corresponding PCR tubes	Check the sample numbers of tubes containing nucleic acid and make sure to add nucleic acid into the correct PCR tubes and carefully repeat the test if necessary.
	Incorrect storage or past expiry date of the test kit	Please check the storage condition (See page 6) and the expiry date (refer to label) of the test kit and use a new kit if necessary.
	Error in nucleic acid extraction	Please check the nucleic acid extraction procedure and re-extract the nucleic acid. If the original specimen is not available, a new specimen must be collected.

15. Symbols Used

The following symbols used on labels and packaging of this product conform to the harmonized standard EN980.

Catalog Number

Lot/Batch Number


Expiration Date

Storage Conditions


Manufactured by

Intended Use

16. Contact Information

Genmark Sağlık Ürünleri

İthalat İhracat ve Ticaret Limited Şirketi

Kaptanpaşa Mah. Darülaceze Cad. No:45 Famas İş Merkezi A Blok Kat:2 Daire:6 34384 Okmeydanı-Şişli/ İstanbul

Tel: +90212 288 74 92/93

Fax: +90212 288 74 53

Email: info@genmark.com.tr ; b.eratak@genmark.com.tr Web: www.genmark.com.tr