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

Instructions For Use

CE IVD ***For In Vitro Diagnostic Use***

Store at -25°C to -15°C

REF RV3-RT100

100

geneMAP™ Respiratory Viral PCR Panel 3

(2019-nCoV, Inf A/B)

For Real-Time PCR

Multiplex Real-time PCR System for detection of 2019 Novel Corona Virus (2019-nCoV), Influenza A/B.

3-PLEX (FAM, VIC, CY5)

Validated on:

* Bio-Rad® CFX96, CFX384 Real-time PCR System (Bio-Rad)
* Life Technologies ABI Prism®- 7500
* Roche, LightCycler® 480 II

Contents

1. Intended Use

The geneMAP™ Resipratory Viral PCR Panel 3 is qualitative in vitro assay for the detection of 2019-nCoV, Influenza A/B, from Nasopharyngeal aspirate, Nasopharyngeal swab, Oropharyngeal swab, Bronchoalveolar lavage.

2. Safety Instructions & General Warnings

- This kit must be used strictly in accordance with the instructions provided in this manual, and only in combination with validated reagents and instruments. Any off-label use of this product, as well as any modification of its components, will nullify Genmark's liability.
- Obey proper laboratory safety protocols when working with chemicals and specimens.
- The protocol can be performed by only professional and trained personal.
- Perform the protocol in a well-ventilated and well-lit environment.
- Store the kit and its components within recommended temperature range in de-frost refrigerators. Do not use no-frost refrigerators.
- Avoid skin contact with any of the reagents in the kit
- Wipe workspace surfaces with 10% bleach followed by 70% alcohol.
- Instruments may exhibit performance variations due to differences in electrical currents and power outlets, as well as the effects of maintenance and calibration. It is the responsibility of the user to ensure that all instruments are properly maintained and regularly calibrated according to the manufacturer's guidelines. Genmark disclaims responsibility for any performance issues arising from improper maintenance, calibration, or variations in electrical supply.
- All biological specimens should be handled as potentially infectious, following standard precautions. For guidelines on specimen handling, refer to the World Health Organization.
- Consult environmental waste personnel for proper disposal of used plates, consumables, and reagents, considering federal and local hazardous waste regulations. Check local and national disposal requirements.

3. Principles and Procedure Overview

3.1 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.

Procedure Overview;

Samples

(Nasopharyngeal Swab etc.)

⇩ Nucleic acid extraction

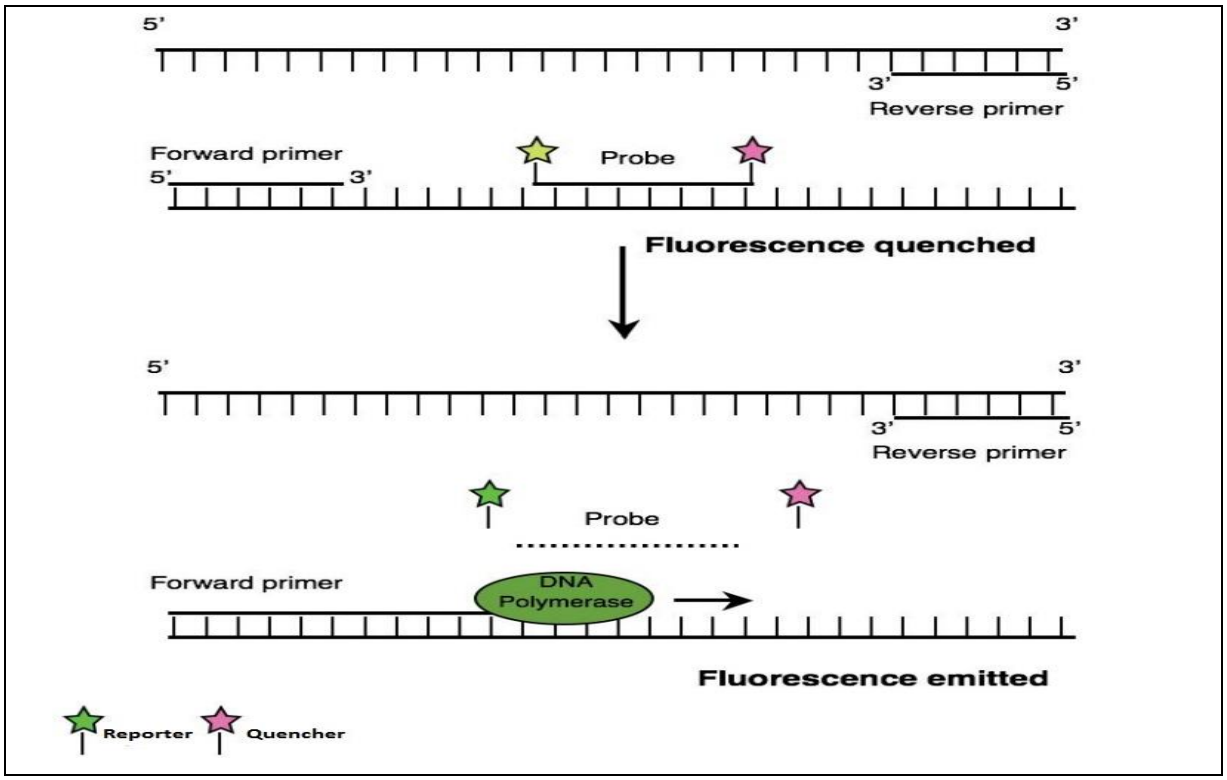
Nucleic acid

⇩ Amplification and detection using TaqMan Probe system

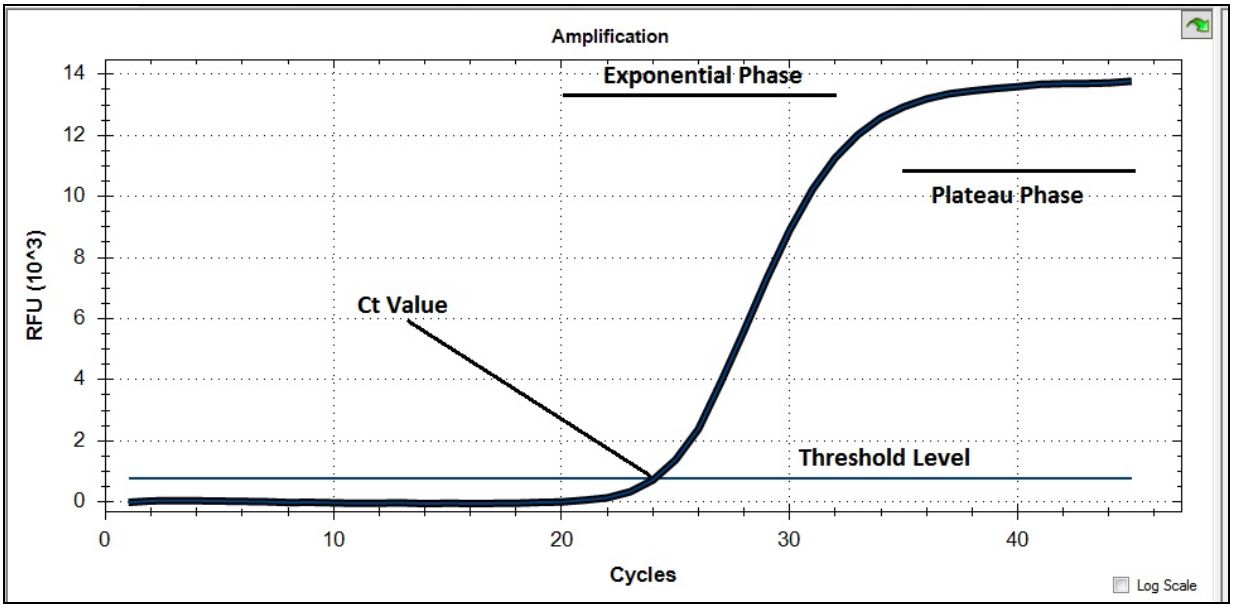
Analysis of results

3.2 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.

4. 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.

Influenza virus (family Orthomyxoviridae) is a single-stranded RNA genome located in 8 separate ribonucleoprotein segments. This segmentation of the genome is transmitted to the same cell. There are three types of influenza viruses: A, B and C. A and B viruses are the most common. In particular type A virus is not only known to humans, but also to birds and various mammalian species. Among influenza viruses invading humans, H1N1 and H3N2 become a problem for infecting children and elderly. Type B and C are limited to influencing people only. The onset of symptoms of influenza, nasal discharge, nasal congestion, sore throat, cough, fever, nasopharyngitis, or acute rhinitis are common symptoms of cold and muscle pain. Characteristically, influenza symptoms carry high fever and develop complications due to bacterial infections.

Reagents contained in a kit are sufficient for 100 reactions

Contents	Volume	Rxn. No.	Description
RV3 Reaction Mix	1500 µL	100	Primer Probe Mix (PPM): Buffer containing dNTPs), ddH2O
OneStep Enzyme Mix	165 µL	100	Taq Polymerase, Reverse Transcriptase and Uracil-DNA Glycosylase (UDG)
RV PC	100 µL	10	Positive Control (PC): Mixture of pathogen clones and IC clones
Negative Control	100 µL	10	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.

6. Materials Required But Not Provided

- Disposable powder-free gloves (latex or nitrile)
- 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)

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™ Extraction Buffer

1- Transfer 50 µL Extraction Buffer into a new PCR tube (1,5 mL).
2- Add 50 µL patient sample (Nasopharyngeal swab, oropharyngeal swab medium.) 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 on ice.
* Set up all reactions on ice to minimize the risk of RNA degradation.
* Briefly centrifuge the reagent tubes to remove drops from the inside of the cap.

RV2 Reaction Mix	13,5 µl
Enzyme Mix	1,5 µl
Total volume of PCR Master Mix	15µl

* 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
Influenza A/B	FAM
Internal Control	VIC
SARS nCoV-2019	CY5

For Biorad CFX96 (Duration 46 min.)

Temperature	Time	Cycles	Data Collection
50˚C	10 min	1	
95˚C	2 min	1	
95˚C	1 sec	42	
60˚C	1 sec		FAM, VIC, CY5

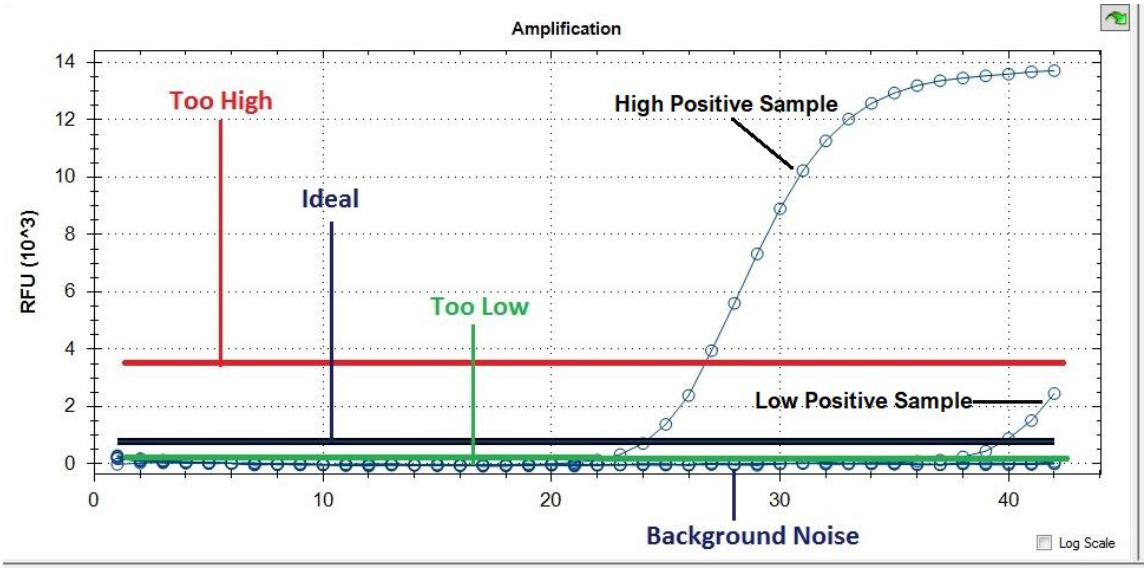
9. Results

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. Good indicators of improperly-set threshold values are false positives (Ct values obtained from negative control wells), known positive samples giving very late Cts or no Cts at all. Because of those reasons 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.

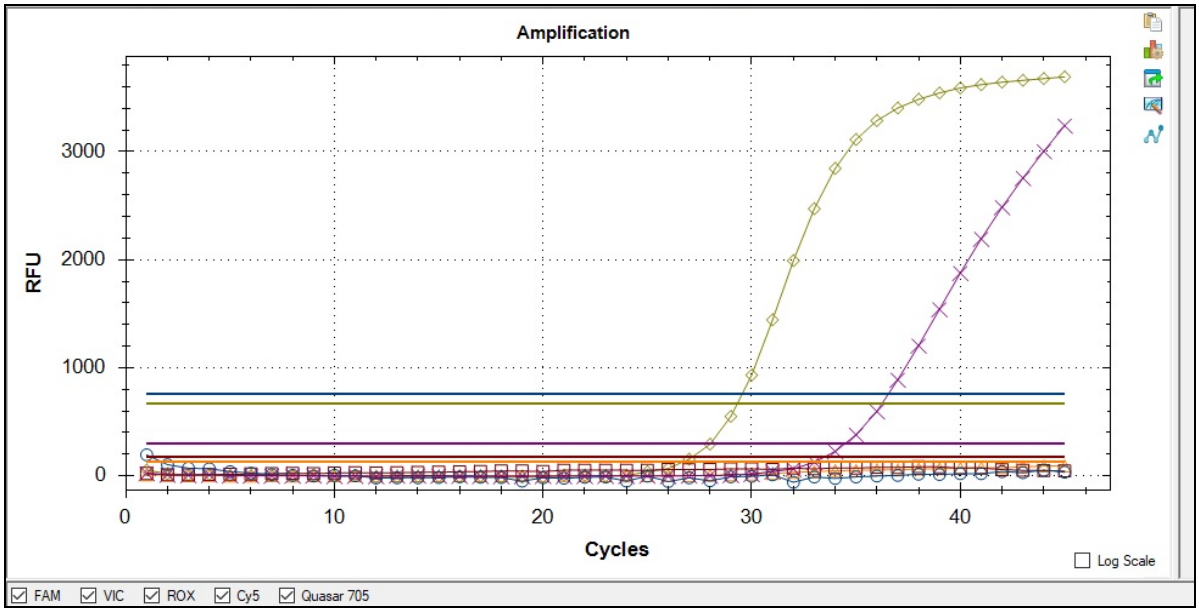
9.2 Interpretation of Results

Target	Reporter Fluorophore	Sample	
		Ct Value	Result
Influenza A/B	FAM	<41	Positive (+)
		≥41 or NA	Negative (-)
2019-nCoV	CY5	<40	Positive (+)
		≥41 or NA	Negative (-)
Internal Control	VIC	<35	Positive (+)
		≥35 or NA	Negative (-)

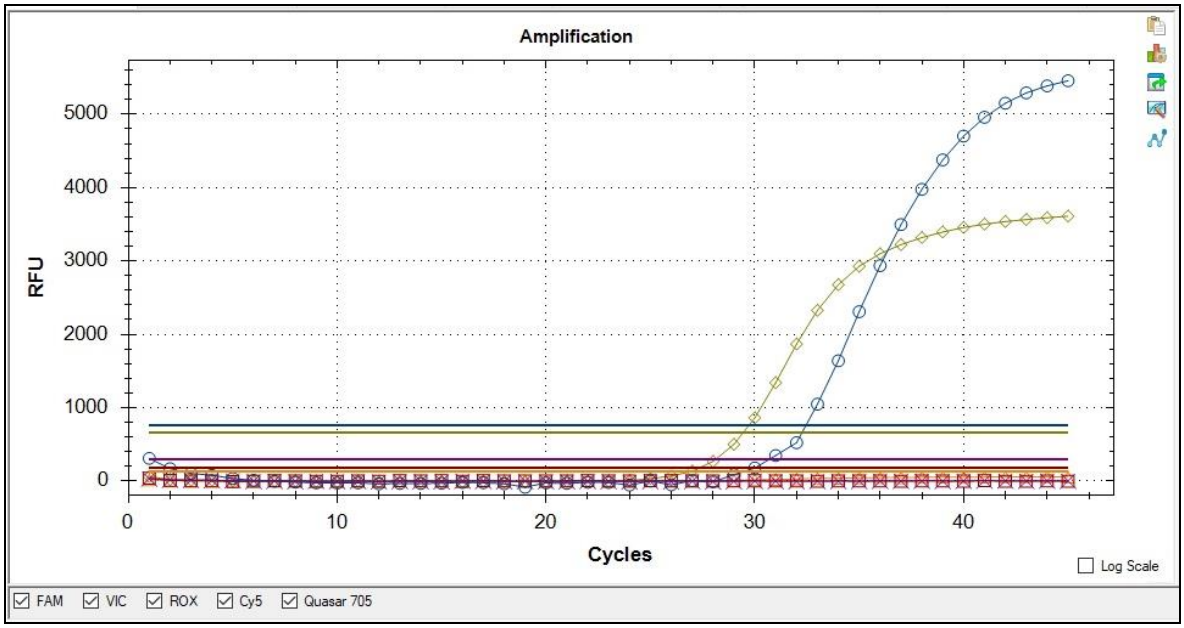
Interpretation	FAM	CY5	VIC	Reporting
Case 1	+	-	+/-	InfA/B positive
Case 2	-	+	+/-	2019-nCoV Positive
Case 3	-	-	+*	Negative
Case 4	-	-	-**	Invalid (Repeat the test)
Case 5	+	+	+/-	InfA/B & 2019-nCoV Positive (Co-Infection)

* If amplification curve Ct is ≥36

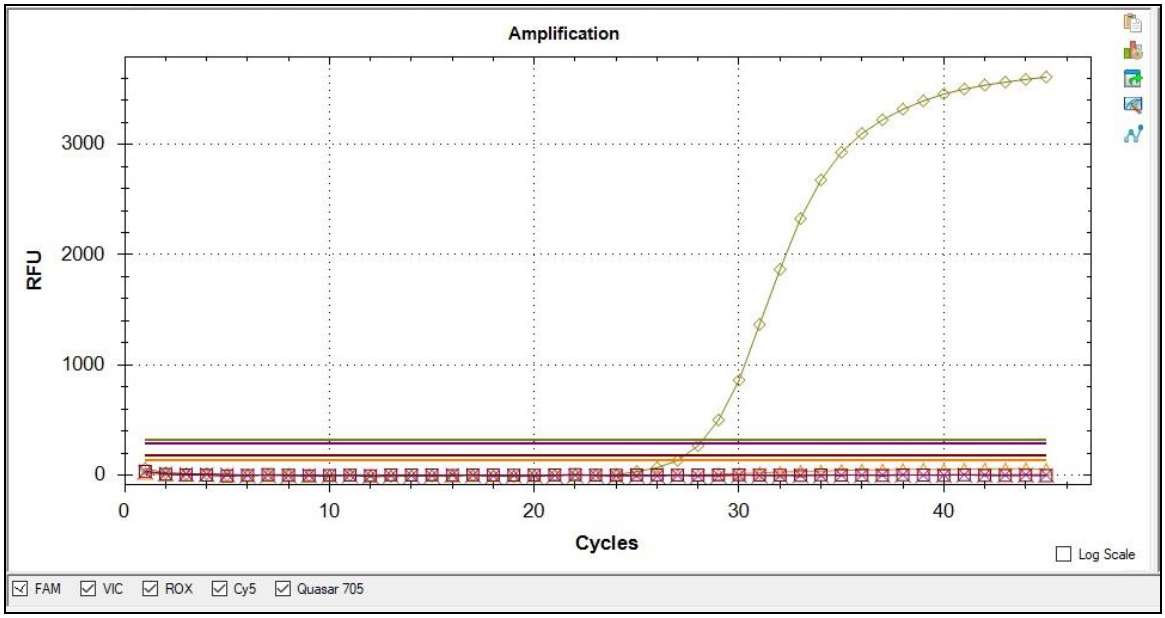
** Repeat the test if the result is the same, confirm the result alternative tests



2019-nCoV Positive: CY5 and VIC amplification curves are obsereved.



Influenza A/B Positive: FAM and VIC amplification curves are observed.



RV Negative: VIC (Internal Control) amplification curve are obsereved.

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:s
1	Human respiratory syncytial virüs A/B	No aligment found
2	Influenza A virus	100% Match
3	Influenza B virus	100% Match
4	Human coronavirus HKU1	No alignment found
5	Human adenovirus	No alignment found
6	Human rhinovirus	No alignment found
7	Human metapneumovirus	No alignment found
8	Human parainfluenza virus	No alignment found
9	Human bocavirus	No alignment found
10	*Streptococcus* (Taxid: 1301)	No alignment found
11	*Mycobacteria* (Taxid:85007)	No alignment found
12	*Mycoplasma* (Taxid:2085)	No alignment found
13	*Legionella* (Taxid: 445)	No alignment found
14	*Bordetella pertussis*	No alignment found

10.1.2 Clinical Studies

Cross-reactivity of geneMAP™ Respiratory Viral Panel 3 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	Detected
4	Influenza A virus (H1N1)	TURKEY ISOLATE	Detected
5	Influenza B virus	TURKEY ISOLATE	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

10.2.1 Influenza A and B

Sensitivity study has been performed with synthetic oligonucleotids;

5 copies/reaction for Influenza A (95% CI)

20 copies/reaction for Influenza B (95% CI)

8.2.2 2019-nCoV

Analytical Sensitivity/Limit of Detection summarized table below. Study has been performed with Recombinant Viral Partical in Viral Transport Media (VTM) that consists of Tris-buffered saline, with added glycerol, anti-microbial agents and human proteins.

The Run performed with CFX96 PCR Insturment/BioRad Inc.

		N Gene (CY5)	
*Reference Material copies/mL	Interpratation	% Detection (# Detected / # Tested)	Mean Ct
5000	100% (25/25) Positive	100% (25/25)	33,1
2000	100% (25/25) Positive	100% (25/25)	34.6
1000	100% (25/25) Positive	100% (25/25)	35.5
500	100% (25/25) Positive	100% (25/25)	36.3

*AccuPlex™ SARS-CoV-2 Reference Material Kit (SeraCare Life Science Inc. USA)

10.3 Clinical Evaulation

The clinical performance of the geneMAP™ Respiratory Viral Panel 3 assays was established in one site clinical evaluation. Fresh or freze-thaw clinical Nasopharyhngeal Swab (NPS) and Orapharyngeal Swab (OPS) specimens were tested with geneMAP™ Respiratory Viral Panel 3 PCR Kit and one commercial CE-IVD marked kit has chosen as comparator. Results are summarized below.

Influenza A (H1N1 & H3N2)

Test Name		Comparator 1		Total
		Positive	Negative	
geneMAP™ Respiratory Viral PCR Panel 3	Positive	13	0	13
	Negative	0	25	25
Total		13	25	38

Positive Agreement Rate: 13/13x100%= 100%
Negative Agreement Rate: 25/25x100%= 100%

Influenza B

Test Name		Comparator 1		Total
		Positive	Negative	
geneMAP™ Respiratory Viral PCR Panel 3	Positive	8	0	8
	Negative	0	25	25
Total		8	25	33

Positive Agreement Rate: 8/8x100%= 100%
Negative Agreement Rate: 25/25x100%= 100%

2019-nCoV;

Test Name		Comparator 1		Comparator 2		Total
		Positive	Negative	Positive	Negative	
geneMAP™ Respiratory Viral PCR Panel 3	Positive	84	1	65	0	150
	Negative	2	213	3	212	430
Total		86	214	68	212	580

Positive Agreement Rate: 149/150x100%= 99%
Negative Agreement Rate: 425/430x100%= 98%

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 targeted genes were tested against the complete available viruses genome sequence. The analysis demonstrated that the regions recognized by the designed primers and probes have 100% homology with all available target pathogen sequences from the National Center for Biotechnology Information (NCBI).

Database	Identity		
	Influenza A Primers/Probe %	Influenza B Primers/Probe %	2019-nCoV Primers/Probe %
NCBI	100%	100%	100%

11. Limitations

Mutation in the target sequence of targeted pathogens 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 can not distinguish among Influenza A and Influenz B.

The kit is validated for specimens only from NPS and OPS in medium (VTM, UTM, PBS, Saline Solution and Steril Distilled Water) and BAL (bronchoalveolar lavage). 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.

12. Revision History

Date of Last Edit: July 2024		
Change	**Affected Section**	**Page**
Added reaction numbers for the kit	5.Reagents	5
Added ∑ (total numbers of tests) symbol	Cover Page	1
Added Safety Instructions and General Warnings	2. Safety Instructions and General Warnings	3

13. Troubleshooting

geneMAP™ Respiratory Viral PCR Panel 3		
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.

14. Symbols Used

Catalog Number

Lot/Batch Number


Expiration Date

Storage Conditions


Manufactured by

Intended Use

15. Contact Information

Genmark Sağlık Ürünleri

İthalat İhracat ve Ticaret Limited Şirketi

Halil Rıfat Paşa Mah. Güler Sok. GNM Plaza No:51-1 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