SNP COVID-19

REAL TIME PCR KIT

Cat# 114R-10-01

CE IVD

For Emergency Use Authorization Only

For in vitro diagnostic use only

TABLE OF CONTENTS

1. KIT INFORMATION

1.1. INTRODUCTION

Coronaviruses are enveloped non-segmented positive-sense RNA viruses belonging to the family Coronaviridae and the order Nidovirales and broadly distributed in humans and other mammals. In December, 2019, a series of pneumonia cases of unknown cause emerged in Wuhan, Hubei, China, with clinical presentations greatly resembling viral pneumonia. Deep sequencing analysis from lower respiratory tract samples indicated a novel coronavirus, which was named 2019 novel coronavirus (SARS-CoV-2). The disease caused by this virus is called COVID-19. The System can detect **RNA-dependent RNA polymerase gene** (RdRp) and **Nucleocapsid protein gene** regions of **SARS-CoV-2** with % 100 sensitivity and % 100 specificity.

1.2. PRINCIPLE OF THE KIT

Reverse Transcripase component (M-MLV) of the kit is active at 45 or 50 °C, a half life of 230 minutes, and the activity of RNase H was reduced. HotStart Taq DNA Polymerase enzyme is a mixture of enzyme and Anti-Taq monoclonal antibodies to ensure specificity and thermostability. Buffer contains 0.2 mM of each dNTP and 3 mM $MgCl_2$. System can be used directly both with DNA and RNA samples. During the PCR reaction, the DNA polymerase cleaves the probe at the 5′ end and separates the reporter dye from the quencer dye only when the probe hybridizes perfectly to the target DNA. This cleavage results in the fluorescent signal which is monitored by Real-Time PCR detection system. An increase in the fluorescent signal (CT) is proportional to the amount of the specific PCR product.

1.3. PRODUCT SPECIFICATION

The kit provides reagents in a **"ready-to-use"** mastermix format which has been specifically adapted for 5' nuclease PCR. The test system is designed for use with sequence specific primers and probe. The fluorescence of COVID-19 analysis is FAM. Also each mastermix contains an internal control labelled with HEX dye. Diseases and related dyes can be seen in Table 1.

The limit of detection (LOD) in Covid-19 Real Time PCR Kit was determined between 1-10 Copies/Rxn.

Table 1 : Tubes- Viruses/Diseases- dyes.

Parameters	Dye
Internal Control	HEX
COVID-19	FAM

1.4. KIT CONTENTS

Reagents	150 rxns
Covid-19 Master Mix	1500 µl
Positive Control	50 µl
Negative Control	50 µl
User Manual	1

1.5. STORAGE

- All reagents should be stored at – 20 °C and dark.
- All reagents can be used until the expiration date on the box label.
- Repeated thawing and freezing (>4X) should be avoided, as this may reduce the sensitivity of the assay.

2. RNA EXTRACTION

- Nasopharyngeal samples should be collected in appropriate sterile swab and can be stored at +4°C up to one week.
- For more than one week specimen should be stored at -20°C.
- The system is optimized for any RNA Isolation System.

3. TEST SETUP*

3.1. PROCEDURE

- Before starting work, mix the mastermixes gently by pipetting.
- For each sample, pipet **10 µl mastermix** with micropipets of sterile filter tips to each optical well PCR tubes.
- Add **10 µl (~1-100 ng) RNA** into each tube.
- Mix gently by pipetting
- Run with the programme shown below.

3.2. PCR PROGRAMME SETUP

Table 2: PCR Programme**

42 °C	20 Min.	cDNA Syntesis
96 °C	25 Sec.	Holding
96 °C	2 Sec.	45 Cycles
60 °C	35 Sec.	

Fluorescent dyes are FAM and HEX.

* The following settings are valid for the Biorad CFX96 device. It may require different settings on different real time devices. For detailed information, please contact us; info@snp.com.tr

** Real Time PCR time is **83 minutes**. This time may differ slightly depending on the device.

If you use;

- ABI Prism® system, please choose **"none"** as passive reference and quencher.
- Mic qPCR Cycler, please adjust gain settings, **"Green Auto Gain"** to **20** and **"Yellow Auto Gain"** to **10.**

This system can be used with;

Bio-Rad CFX96

ABI Prism ® 7500/7500 Fast

Roche LightCycler® 480 System

Rotor Gene Q

Mic qPCR Cycler

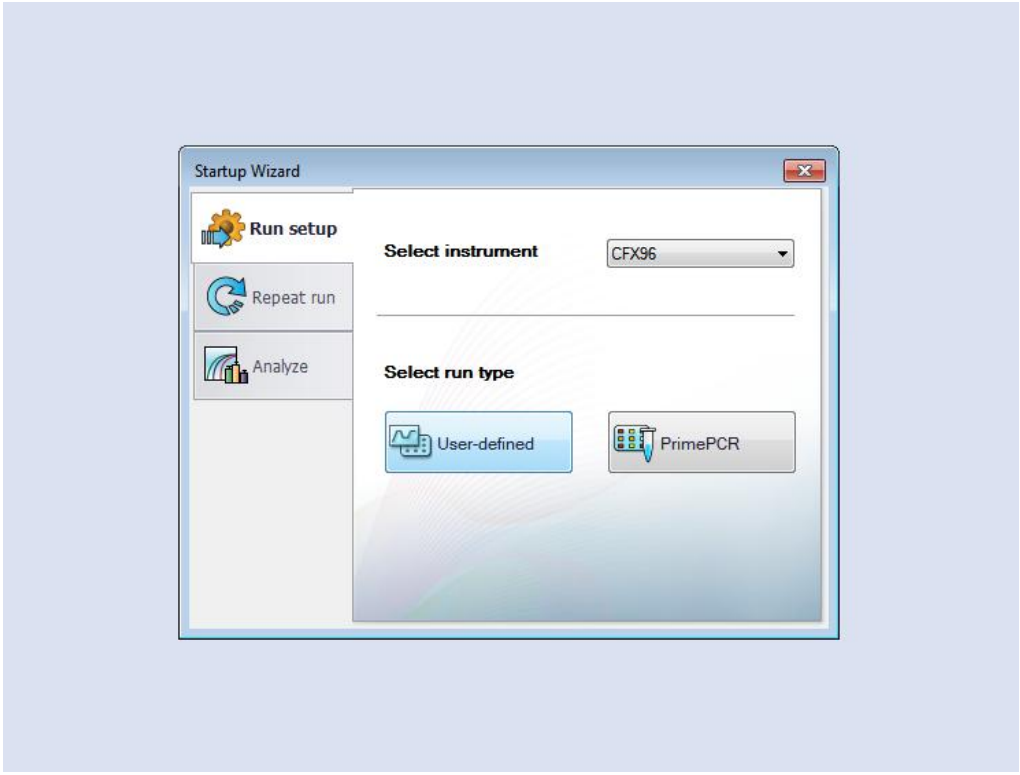

Figure 1: For the setup of PCR programme, choose **"User-Defined"**

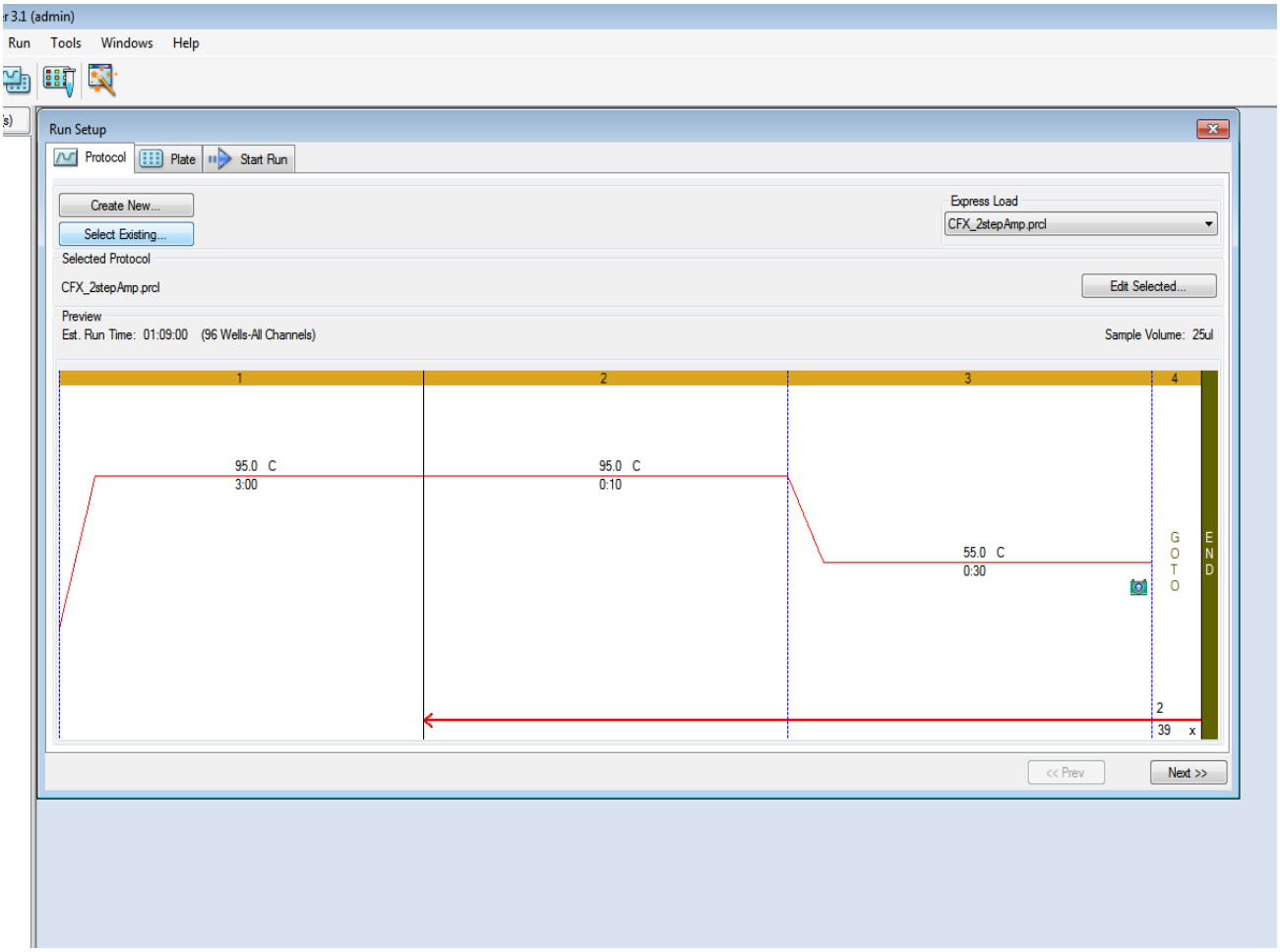

Figure 2: Set the run programme as seen table 2.

3.3. PLATE SETUP

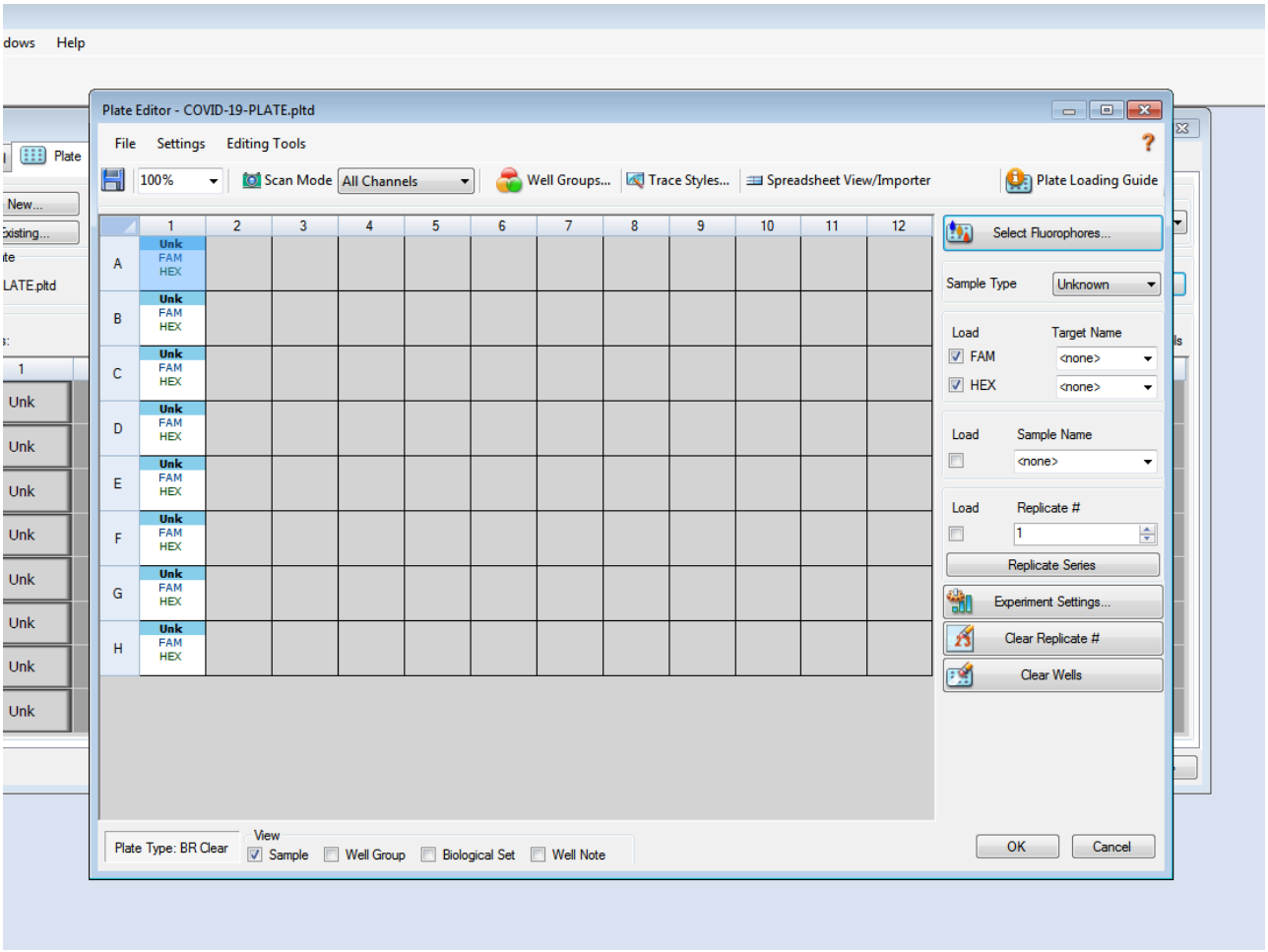

Figure 3: Select the type of wells to work as **unknown** and mark with **FAM** and **HEX.**

4. RESULTS ANALYSIS

After the run is completed data are analysed using the software with both dyes. **Internal control** amplification plots must be seen in all wells except NTC on HEX Dye. The CT value of internal controls should be X ≤ 40 according to RNA concentration (Figure 4). Any amplification plot in FAM dye should be evaluated as Positive (Figure 5). You can give **Negative** results to samples that are no amplification on FAM Dye (Figure 6).

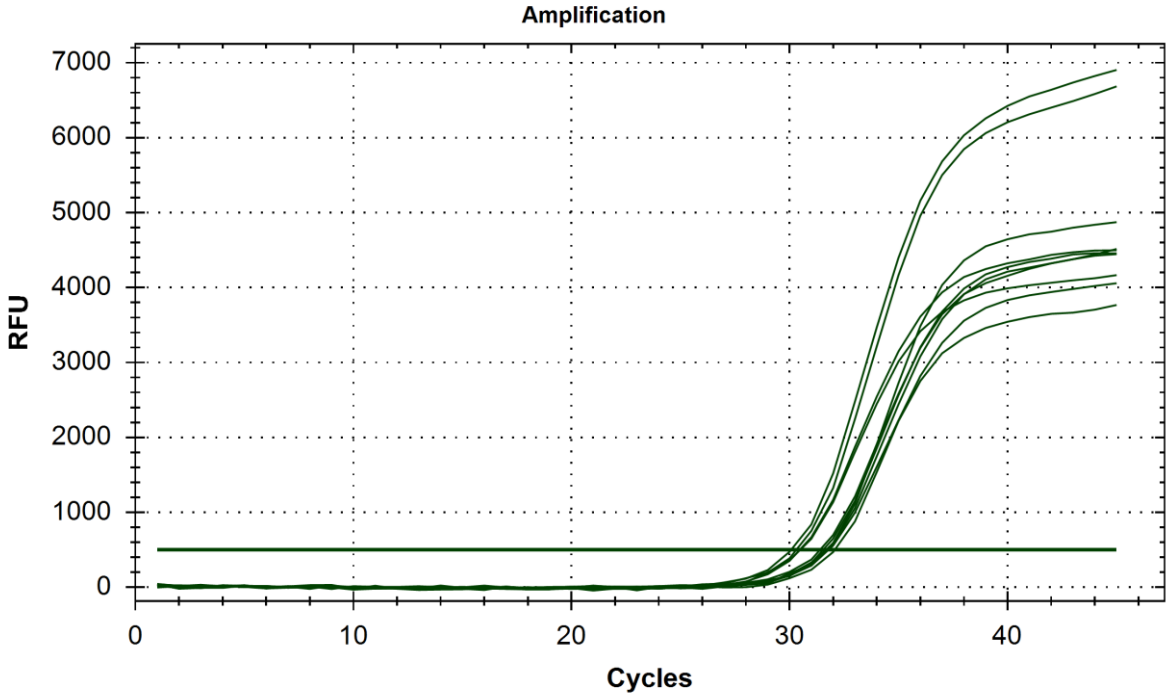

Figure 4: Internal Control Plots (HEX Dye)

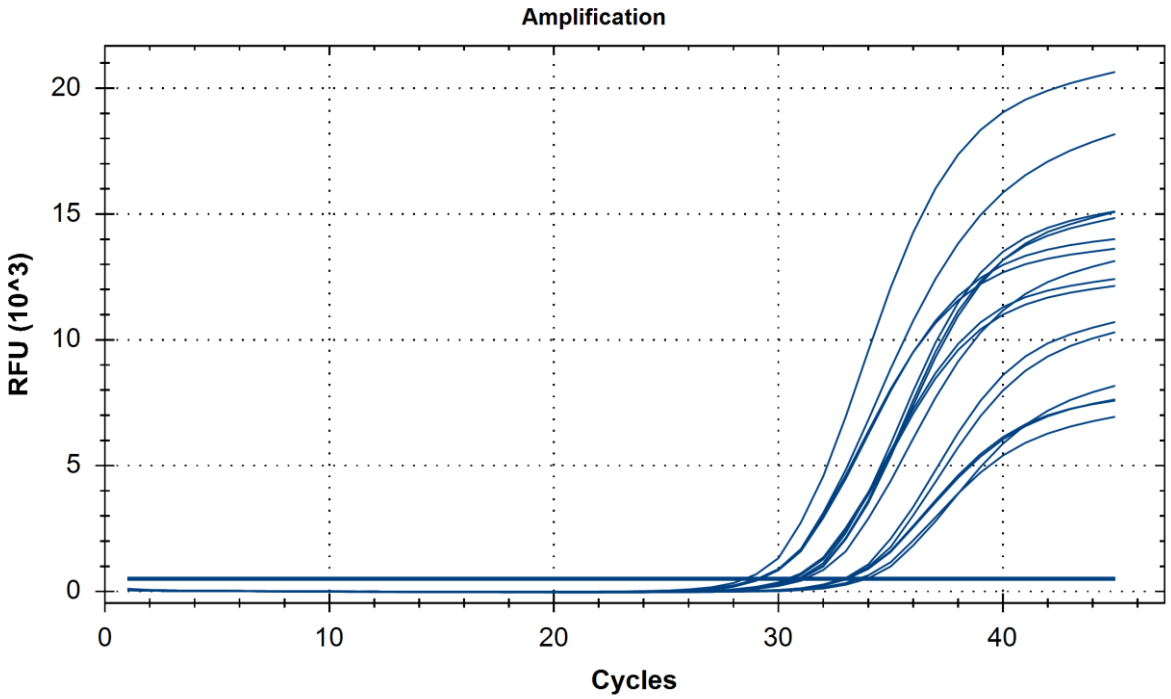

Figure 5: Positive Control Plots (FAM Dye)

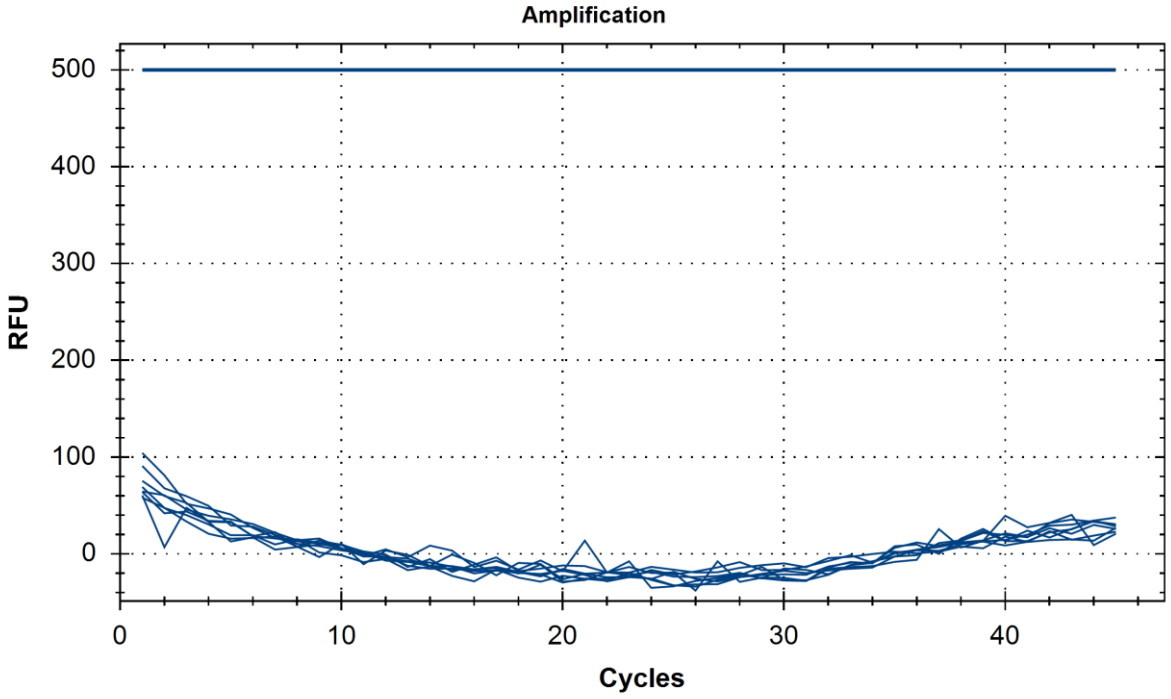

Figure 6: Negative Control Plots (FAM Dye)

5. LIMITATIONS

1. The use of this assay as an in vitro diagnostic under FDA Emergency Use Authorization (EUA) is limited to laboratories that are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. § 263a, to perform high complexity tests.

2. This kit is used for qualitative detection of SARS-CoV-2 RNA from human nasopharyngeal, oropharyngeal, anterior nasal and mid-turbinate nasal swab as well as nasopharyngeal wash/aspirate or nasal aspirate specimens and sputum specimens. The results do not reflect the viral load in the original specimens.

3. The performance of the SNP COVID-19 Real-Time PCR Kit was established using contrived nasopharyngeal swab and sputum specimens. Anterior nasal swabs, mid-turbinate nasal swabs, nasal washes, nasal aspirates and bronchoalveolar lavage (BAL) fluid are also considered acceptable specimen types for use with the SNP COVID-19 Real-Time PCR Kit. Testing of nasal and mid-turbinate nasal swabs (self-collected or collected by a healthcare provider) is limited to patients with symptoms of COVID-19. Please refer to FDA's FAQs on Diagnostic Testing for SARS-CoV-2 for additional information. http://www.snp.com.tr/EN,38/covid-19.html

4. SNP COVID-19 Real-Time PCR Kit performance has only been established with nasopharyngeal swabs and sputum specimens.

5. The specimens to be tested shall be collected, processed, stored and transported in accordance with the conditions specified in the instructions. Inappropriate specimen preparation and operation may lead to inaccurate results.

6. Extraction and amplification of nucleic acid from clinical samples must be performed according to the specified methods listed in this procedure. Other extraction approaches and processing systems have not been evaluated.

7. Amplification and detection of SARS-CoV-2 with the SNP COVID-19 Real-Time PCR Kit has been validated with the Bio-Rad CFX96 Real-Time PCR and Applied Biosystem 7500 Real Time PCR instruments. Use of other instrument systems may cause inaccurate results.

8. The limit of detection (LoD) is determined based on a 99% confidence of detection. When SARS-CoV-2 presents at the LoD concentration in the test specimen, there will be a low probability that SARS-CoV-2 is not detected. When SARS-CoV-2 presents below the LoD concentration in the test specimen, there will also be a certain probability that SARSCoV- 2 can be detected.

9. Primers and probes for this kit target highly conserved regions within the genome of SARS- CoV-2. Mutations occurred in these highly conserved regions (although rare) may result in RNA being undetectable.

10. Negative results do not preclude SARS-CoV-2 infections and should not be used as the sole basis for treatment or other management decisions.

11. Laboratories are required to report all positive results to the appropriate public health authorities.

6. CONDITIONS OF AUTHORIZATION FOR THE LABORATORY

The SNP COVID-19 Real-Time PCR Kit's Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients, and authorized labeling are available on the FDA website: https://www.fda.gov/medicaldevices/emergency-situations-medical-devices/emergency-use-authorizations#covid19ivd.
However, to assist clinical laboratories using the SNP COVID-19 Real-Time PCR Kit, the relevant Conditions of Authorization are listed below:

A. Authorized laboratories[1] using your product will include with result reports of your product, all authorized Fact Sheets. Under exigent circumstances, other appropriatemethods for disseminating these Fact Sheets may be used, which may include mass media.

B. Authorized laboratories using your product will use your product as outlined in theInstructions for Use. Deviations from the authorized procedures, including theauthorized instruments, authorized extraction methods, authorized clinical specimen
types, authorized control materials, authorized other ancillary reagents and authorized materials required to use your product are not permitted.

C. Authorized laboratories that receive your product will notify the relevant public health http://www.snp.com.tr/EN,31/contact.html authorities of their intent to run your product prior to initiating testing.

D. Authorized laboratories using your product will have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.

E. Authorized laboratories will collect information on the performance of your product and report to DMD/OHT7-IR/OPEQ/CDRH (via email: CDRH-14-EUAreporting@ fda.hhs.gov) and You (via email: info@snp.com.tr) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of your product of which they become aware.

F. All laboratory personnel using your product must be appropriately trained in RT-PCR techniques and use appropriate laboratory and personal protective equipment when handling this kit, and use your product in accordance with the authorized labeling.

G. You, authorized distributors, and authorized laboratories using your product will ensure that any records associated with this EUA are maintained until otherwisenotified by FDA. Such records will be made available to FDA for inspection upon request.

[1] The letter of authorization refers to, "Laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high complexity tests" as "authorized laboratories."

7. ANALYTICAL VERIFICATION

7.1.VALIDATION

Table 3: Abbreviations

TP	True Positive
FP	False Positive
TN	True Negative
FN	False Negative

Table 4: Analytical verification formula

Sample			
Method	**Positive (Presence)**	**Negative (Absence)**	**Total**
Positive	TP	FP	TP+FN
Negative	FN	TN	FN+TN
Total	TP+FN	FP+TN	TP+TF+FP+FN

Table 5: Values of verification

Covid-19			
Method	**Positive (Presence)**	**Negative (Absence)**	**Total**
Positive	30,00	-	30,00
Negative	-	50,00	50,00
Total	30,00	50,00	80,00

Table 6: Results of Verification

Accuracy	(TP+TN)/(TP+TN+FP+FN)	(30+50)/ 80	1
Sensivity	TP/(TP+FN)	30/30	1
Specificity	TN/(TN+FP)	50/50	1
False Positivite	FP/(TN+FP)	0/50	0
False Negativite	FN/(TP+FN)	0/30	0
Efficiency	(TP+TN)/(TP+TN+FP+FN)	(30+50)/ 80	1

7.2. CROSS ACTIVITY AND STABILITY

7.2.1. Cross Reactivity

Cross-reactivity studies are performed to demonstrate that the test does not react with related pathogens, high prevalence disease agents and normal or pathogenic flora that are reasonably likely to be encountered in the clinical specimen. The recommended list of organisms to be analyzed in silico is provided in the table below. In addition, we recommend that the organisms in the table below are wet-tested.

Table 7: List of Organisms to be analyzed in silico and by Wet Testing. For wet testing, concentrations of 10^5 pfu/ml or higher for viruses and 10^6 CFU/ml for organisms.

High priority organisms likely in the circulating area	**Other high priority pathogens from the same genetic family**
Adenovirus (e.g. C1 Ad. 71)	Human coronavirus OC43
Human Metapneumovirus (hMPV)	Human coronavirus HKU1
Parainfluenza virus 1-4	Human coronavirus NL63
Influenza A & B	SARS-coronavirus
Enterovirus (e.g. EV68)	MERS-coronavirus
Respiratory syncytial virus	
Rhinovirus	
Chlamydia pneumoniae	
Haemophilus influenzae	
Legionella pneumophila	
Mycobacterium tuberculosis	
Streptococcus pneumoniae	
Streptococcus pyogenes	
Bordetella pertussis	
Mycoplasma pneumoniae	
Pneumocystis jirovecii (PJP)	
Pooled human nasal wash - to represent diverse microbial flora in the human respiratory tract	
Candida albicans	
Pseudomonas aeruginosa	
Staphylococcus epidermis	
High priority organisms likely in the circulating area	

7.2.2. Microbial Interference Studies:

The primary probes used for the kit were controlled via special software (OligoYap 8.0) in our laboratory and were found to be 100% homologous of SARS-CoV-2. Cross-reaction studies were checked using by the pathogens in Table 7 to detect cross reaction. And no cross-reaction was detected. In addition, with the limit of detection (LoD) study, the lowest amount that the kit can detect was determined. During all these studies, each sample was repeated at least three times during the same study.

7.2.3. Stability

To evaluate the stability, the kit was freeze-thawed in different numbers and studied with the same RNA sample. In addition, both positive and negative samples were repeated 5 times in the same study.

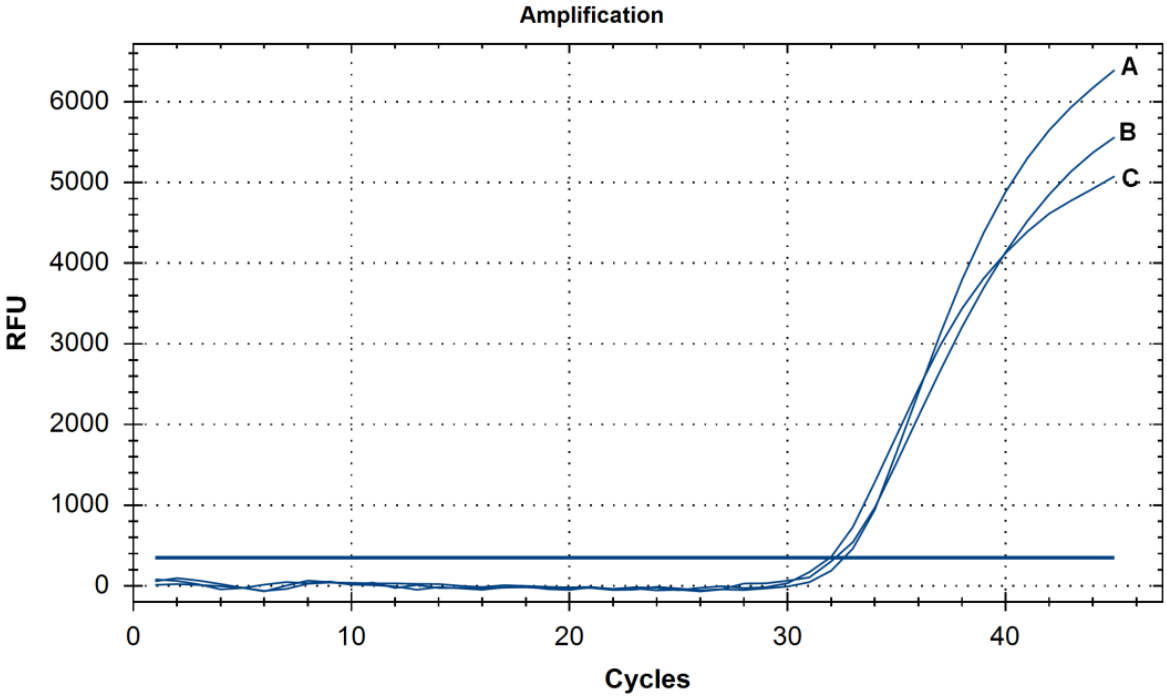

Figure 7: Amplification plots of the same sample freeze-thawed. **A:** First thawed, **B:** Third thawed **C:** Fifth thawed.

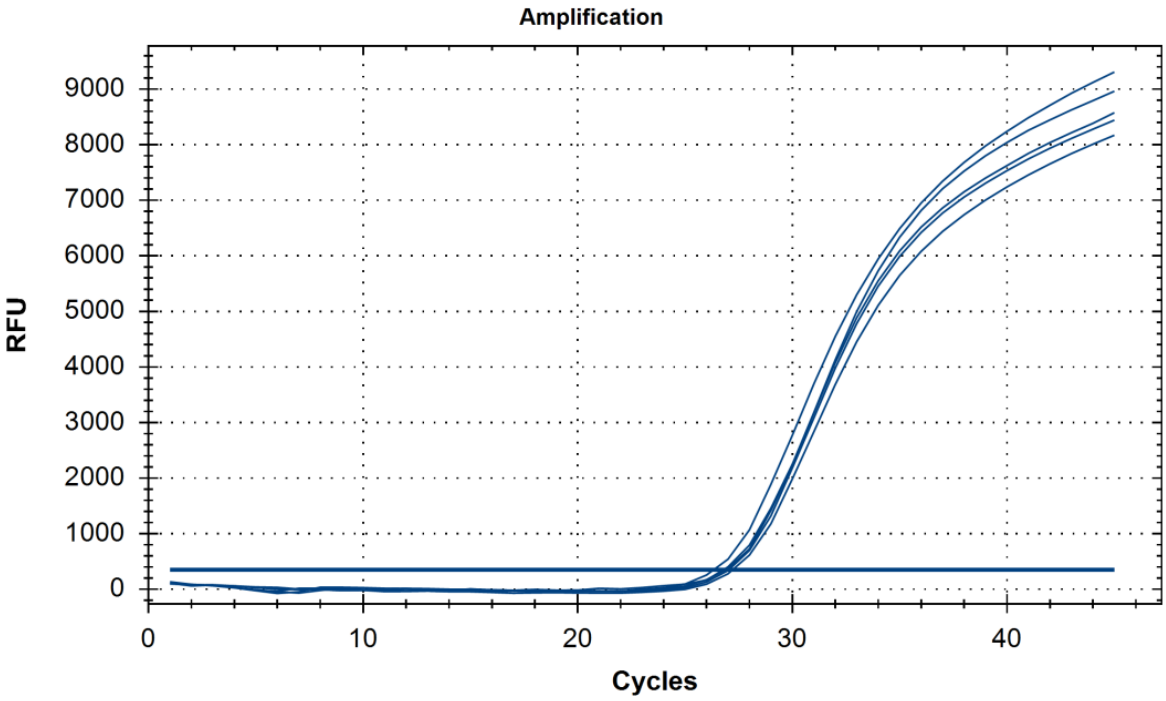

Figure 8: Amplification plots of the positive control were repeated 5 times.

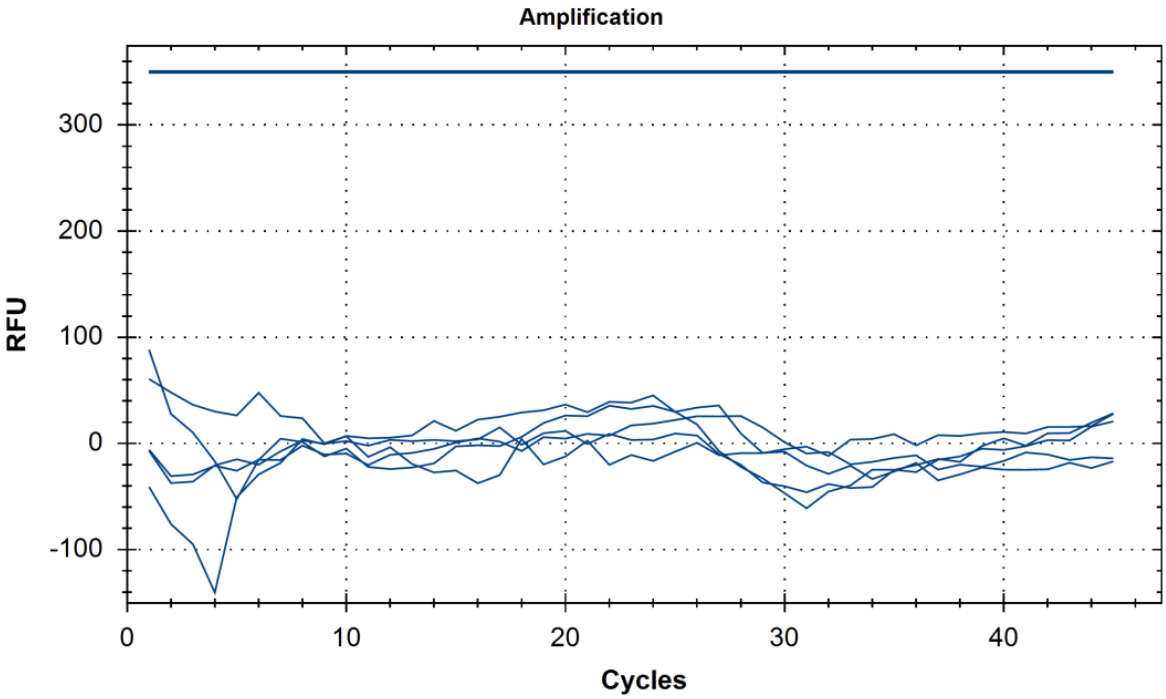

Figure 9: Amplification plots of the negative control were repeated 5 times.

7.2.4. Limit of Detection:

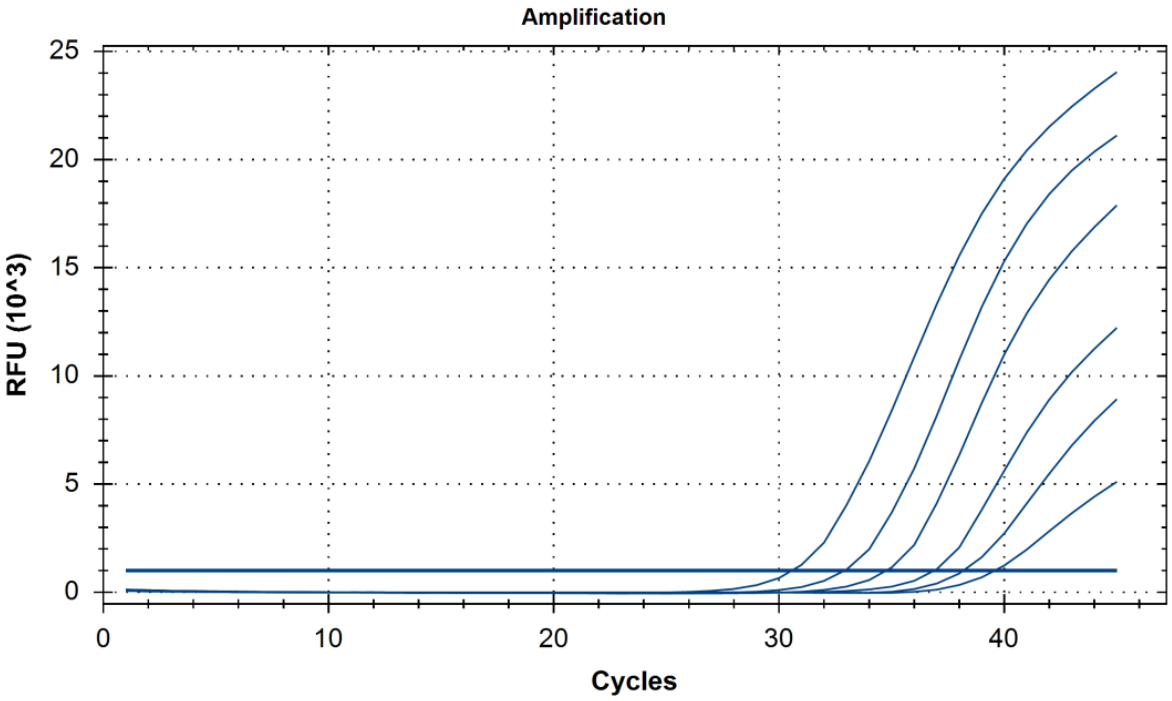

Figure 10: Limit of Detection

The virus dilutions were tried at different concentrations ($1x10^4$, $5x10^3$, $1x10^3$, $5x10^2$, $1x10^2$ and $1x10^1$ respectively) and the LOD of the test is under 1 copy/rxn and detected ≤ Ct 44 value for synthetic oligonucleotide of virus genome according to J.M. Gallup method. The limit of detection (LOD) in Covid-19 Real Time PCR Kit was determined between 1-10 Copies/Rxn.

7.2.5. Inclusivity:

"Turkish Ministry of Health, General Directorate of Public Health, Department of Microbiology Reference Laboratories and Biological Products (HSGM)" has tested and confirmed the inclusivity of the COVID-19 Real Time PCR Kit with 190 different clinical specimens that were previously studied.

7.2.6. Clinical Evaluation:

The performance evaluation of COVID-19 Real Time PCR Kit was tested using known samples. 12 positive samples at 10^3 ~10^8 copy / rxn and additional 12 positive samples at ~ 1-10 copy / rxn were prepared by diluting with SARS-CoV-2 RNA to negative samples. 12 negative samples were also included in this study. The results are shown at Table 8.

Table 8: Clinical Evaluation with Known Samples

Sample Type	Sample Number	FAM	HEX	Result
Nasal / Nasopharyngeal Positive Samples 10^3 ~10^8 copy / rxn	**1**	28,35	26,21	Positive
	2	30,95	28,05	Positive
	3	25,63	23,79	Positive
	4	29,27	26,81	Positive
	5	26,81	24,25	Positive
	6	29,48	27,08	Positive
	7	28,99	25,91	Positive
	8	27,95	25,36	Positive
	9	30,18	28,40	Positive
	10	27,12	25,03	Positive
	11	29,53	27,18	Positive
	12	30,60	28,61	Positive
Nasal / Nasopharyngeal ~ 1-10 copy / rxn (LoD)	**13**	39,44	35,45	Positive
	14	38,51	34,31	Positive
	15	40,01	37,40	Positive
	16	39,25	36,25	Positive
	17	38,77	35,99	Positive
	18	39,90	37,84	Positive
	19	40,12	38,47	Positive
	20	40,19	38,75	Positive
	21	39,41	36,55	Positive
	22	38,27	35,40	Positive
	23	38,56	35,12	Positive
	24	40,38	36,05	Positive
Nasal / Nasopharyngeal Negative Samples	**25**	-	36,15	Negative
	26	-	35,89	Negative
	27	-	34,74	Negative
	28	-	34,48	Negative
	29	-	35,18	Negative
	30	-	35,62	Negative
	31	-	36,15	Negative
	32	-	35,75	Negative
	33	-	36,73	Negative
	34	-	35,54	Negative
	35	-	34,75	Negative
	36	-	34,89	Negative

7.3. QUALITY CONTROL RESULTS

Table 9: Quality Control Results

TEST	SPECIFICATION	RESULT
Sterility	DNAse-RNAse Free	Pass
Appearance	Clear, Solution	Pass
Amplification Performance on Control RNAs	Figure 4-5-6	Pass

8. TROUBLE SHOOTING AND CAUTIONS

8.1. TROUBLE SHOOTING

If internal control doesn't work,

- Unloaded well
- Sample is containing RNA inhibitor(s)

If plots start late,

Compare positive control and sample. If there is no problem in positive control;

- Target RNA quality is not good.
- The amount of target RNA may be low.
- **"Elution Buffer",** used in obtaining target RNA, contains more than 5 mM Tris-HCl.

Please contact us for your questions. tech@snp.com.tr

8.2. CAUTIONS

- In case of contact, it may irritate skin.
- Do not use without gloves.
- In case of contact, immediately wash skin with copious amounts of water.
- All reagents should be stored at suitable conditions.
- Do not use the PCR mastermixes forgotten at room temperature.
- Thaw PCR mastermix at room temperature and slowly mix by inverting before use.
- Shelf-life of PCR mastermix is 12 months. Please check the manufacturing data before use.
- Only use in vitro diagnostics.

9. SYMBOLS

	Catalogue Number		Expiration Date
	Lot Number		Manufacturer
	In vitro Diagnostic Medical Device		Temperature Limitation (Storage temperature)
	Conformite European		

10. REFERENCES

1. Centers for Disease Control and Prevention (CDC) **" 2019-Novel Coronavirus (2019-nCoV) Real-time rRT-PCR Panel Primers and Probes"** DEPARTMENT OF HEALTH & HUMAN SERVICES. 24 Jan 2020.
2. Shannon L. Emery, Dean D. Erdman, Michael D. Bowen, Bruce R. Newton, Jonas M. Winchell, Richard F. Meyer, Suxiang Tong, Byron T. Cook, Brian P. Holloway, Karen A. McCaustland, Paul A. Rota, Bettina Bankamp, Luis E. Lowe, Tom G. Ksiazek, William J. Bellini, and Larry J. Anderson.**" Real-Time Reverse Transcription–Polymerase Chain Reaction Assay for SARS-associated Coronavirus"** Emerging Infectious Diseases, Vol. 10, No. 2, February 2004.
3. Victor Corman, Tobias Bleicker, Sebastian Brünink, Christian Drosten, Olfert Landt, Marion Koopmans, Maria Zambon, Malik Peiris. **"Diagnostic detection of 2019-nCoV by real-time RT-PCR".** Berlin, Jan 17th, 2020.
4. Chaolin Huang, Yeming Wang, Xingwang Li, Lili Ren, Jianping Zhao, Yi Hu, Li Zhang, Guohui Fan, Jiuyang Xu, Xiaoying Gu, Zhenshun Cheng, Ting Yu, Jiaan Xia, Yuan Wei, Wenjuan Wu, Xuelei Xie, Wen Yin, Hui Li, Min Liu, Yan Xiao, Hong Gao, Li Guo, Jungang Xie, Guangfa Wang, Rongmeng Jiang, Zhancheng Gao, Qi Jin, Jianwei Wang, Bin Cao. **"Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China"** January 24, 2020 https://doi.org/10.1016/S0140-6736(20)30183-5.
5. Naganori Nao, Kazuya Shirato, Shutoku Matsuyama and Makoto Takeda**."Detection of WN-Human1 sequence from clinical specimen"**. Laboratory of Acute Viral Respiratory Infections and Cytokines, Department of Virology III, National Institute of Infectious Diseases, 4-7-1 Gakuen, Musashimurayama, 208-0011 Tokyo, Japan.
6. **"Detection of 2019 novel coronavirus (2019-nCoV) in suspected human cases by RT-PCR"**. HKU Med. LKS Faculty of Medicine School of Public Health.
7. Cormac Sheridan.**"Coronavirus and the race to distribute reliable diagnostics"**. Nature Biot echnology VOL 38 April 2020 379–391.
8. Anthony R. Fehr and Stanley Perlman. **"Coronaviruses: An Overview of Their Replication and Pathogenesis"**. Methods Mol Biol. 2015 ; 1282: 1–23.