SNP COVID-19

REAL TIME PCR KIT v1

Cat# 114R-10-01

CE IVD

For Emergency Use Authorization Only

For in vitro diagnostic use only

TABLE OF CONTENTS

1. KIT INFORMATION

1.2. 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.

1.3. PRINCIPLE OF THE KIT

The System can detect **RNA-dependent RNA polymerase gene** (RdRp) and **Nucleocapsid protein gene (N)** regions of **SARS-CoV-2** with high sensitivity and specificity.

Reverse Transcriptase component (M-MLV) of the kit is active at 42 °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 quencher 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 is proportional to the amount of the specific PCR product.

1.4. PRODUCT SPECIFICATION

The kit provides reagents in a "ready-to-use" format for one step RT-PCR master mix which has been specifically adapted for cDNA and 5' nuclease PCR. The test system is designed for use with sequence specific primers and probes. The fluorescence of RdRp Gene is FAM dye and Nucleocapsid Gene is Texas RED dye. Also master mix contains an internal control labelled with HEX dye. Genes and related dyes can be seen in Table 1.

The limit of detection (LoD) in Covid-19 Real-Time PCR Kit was determined 1-10 copies/rxn.

Table 1 : Genes and Related Dyes.

Tube	Genes	Dyes
Covid-19 Master Mix	RdRp Gene	FAM
	Nucleocapsid Gene	Texas RED
	Internal Control	HEX

1.5. KIT CONTENTS

Reagents	150 rxns
Covid-19 Master Mix	1500 µl
Positive Control	60 µl
Negative Control	60 µl
User Manual	1

1.6. 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 (>5X) should be avoided, as this may reduce the sensitivity of the assay.

2. RNA EXTRACTION

- Human nasopharyngeal, oropharyngeal, anterior nasal and mid-turbinate nasal swab as well as nasopharyngeal wash/aspirate or nasal aspirate specimens and sputum samples should be collected with appropriate sterile swab into viral transport fluid. Inactivation & Transport Fluid Kit (Cat# 23S-04-01) for safe transport of specimens is a recommended product.
- Sample can be stored at +4°C up to one week in viral transport fluid.
- Sample can be transported at RT.
- For more than one week sample should be stored at -20°C.

- The system is optimized for any RNA Isolation System such as Spin Column RNA Extraction, Automated RNA Extraction, salt extraction and phenol/chloroform RNA Extraction. We advise the SNP Viral Extraction Kit (Cat# 21S-04) especially developed for the SNP Covid-19 Real Time PCR Kit.

3. TEST SETUP

3.2. PROCEDURE

- Remove the reagents from -20°C storage and thaw it completely.
- Before starting work, mix the master mix gently by pipetting.
- Spin down each tube of master mix, positive control and negative control by short centrifuge (~5 sec.).
- For each sample, pipet **10 µl master mix** with micropipets of sterile filter tips to each optical well PCR tubes.
- Add **10 µl RNA** into each tube.
- Mix gently by pipetting / Short centrifuge (~5 sec.) the tubes in minicentrifuge.
- Run with the programme shown below.

3.3. PCR PROGRAMME SETUP

Table 2: PCR Programme*

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

Fluorescent dyes are FAM, Texas RED and HEX.

* Real Time PCR time is **83 minutes for Bio-Rad CFX96**. This time may differ slightly depending on the device.

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

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® 96 System

Rotor Gene Q

Mic qPCR Cycler

LongGeneQ

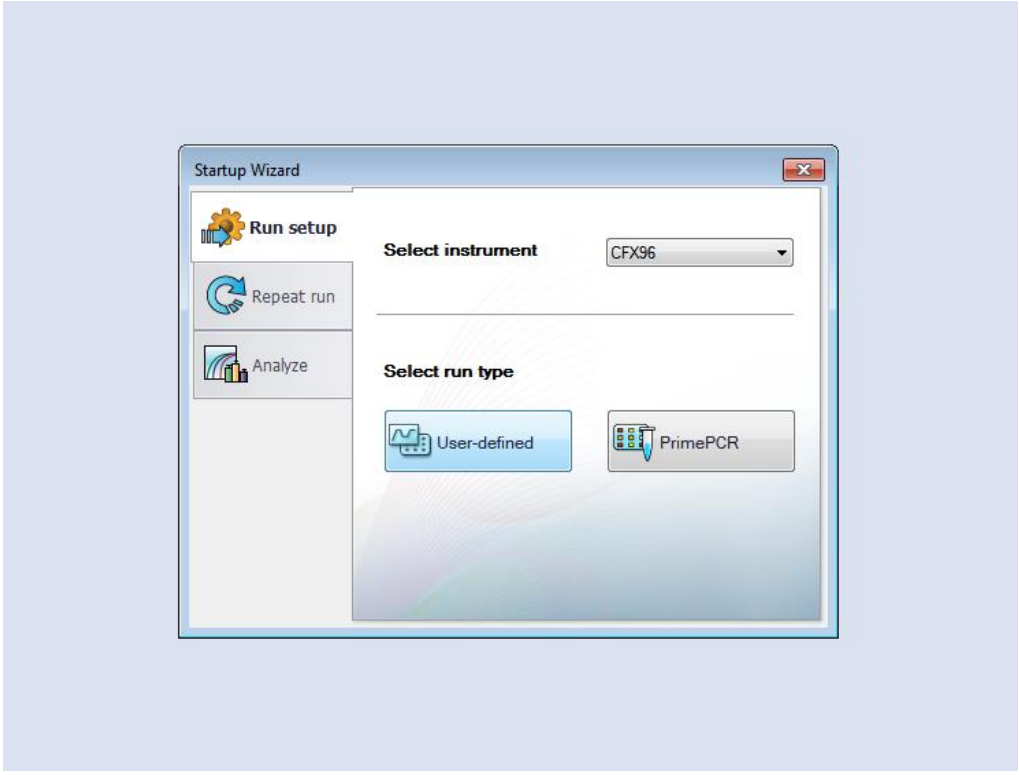

Figure 1: For the setup of PCR programme, choose **"User-Defined"**

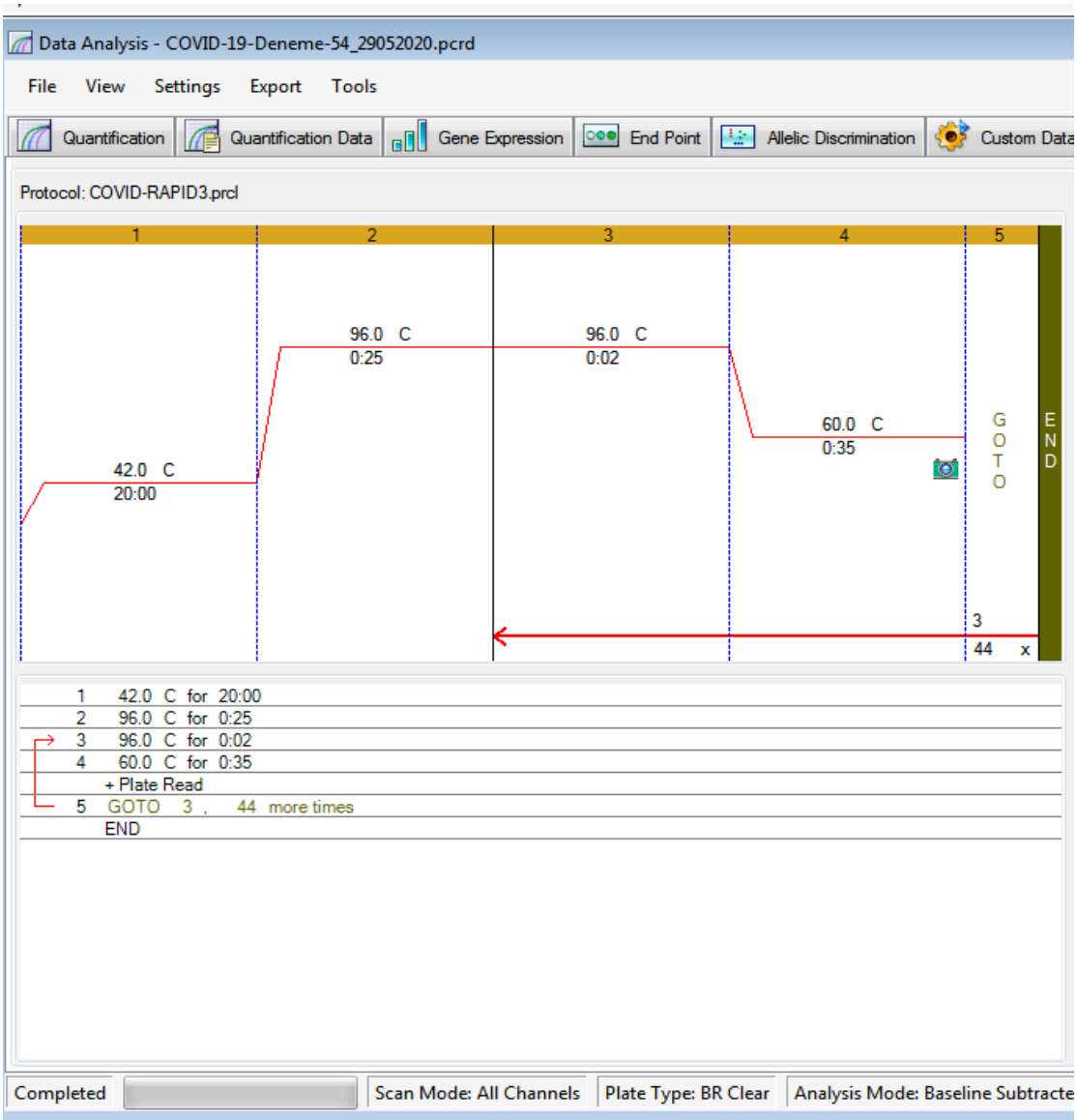

Figure 2: Set the run programme as seen table 2.

3.4. PLATE SETUP

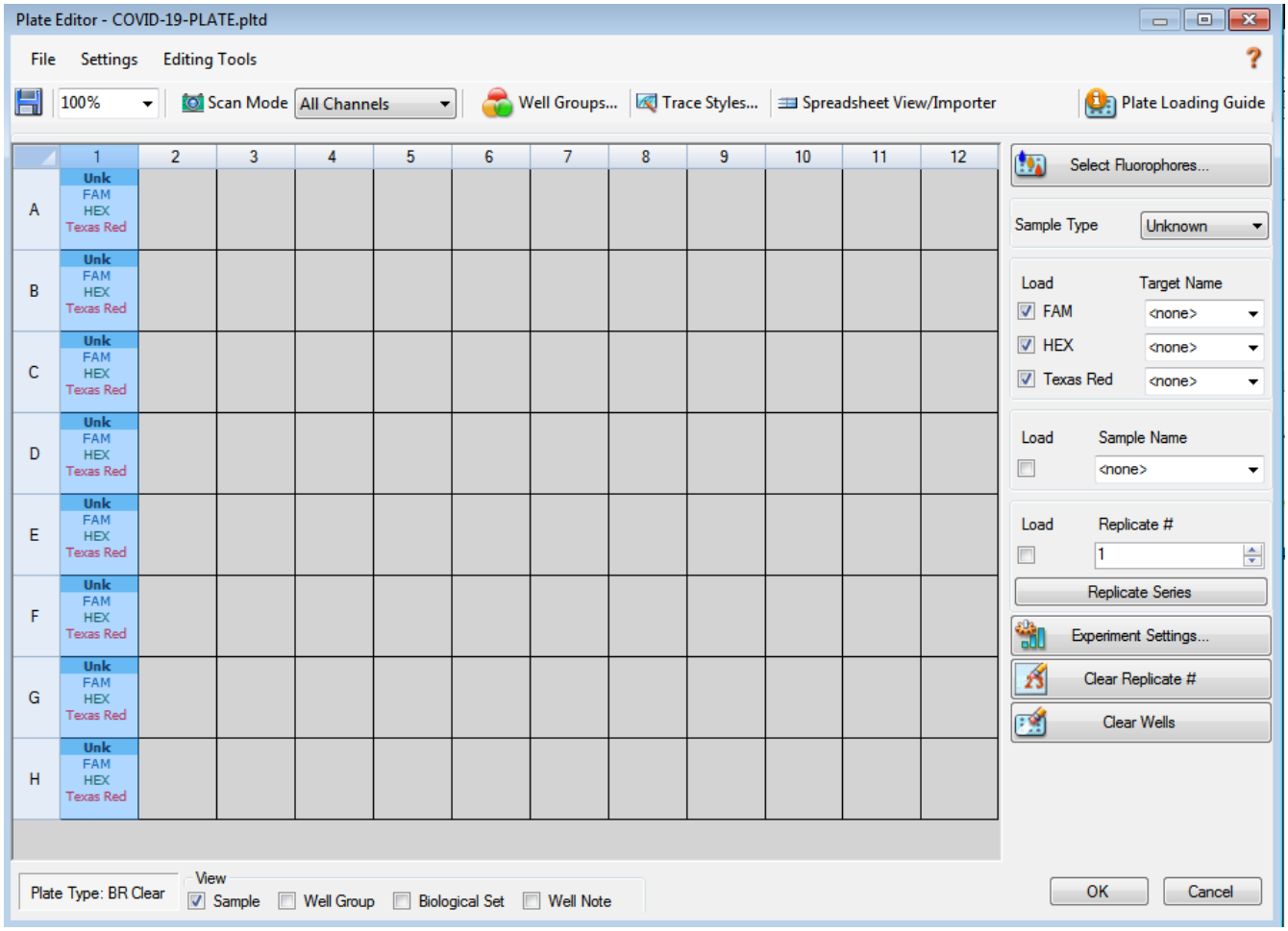

Figure 3: Select the type of wells to work as **unknown** and mark with **FAM, Texas RED** and **HEX.**

4. RESULTS ANALYSIS

After the run is completed data are analysed using the software with all dyes (Table 3). The below results were studied with Bio-Rad CFX96. The CT value of internal controls should be X ≤ 35 according to RNA concentration (Figure 4). Amplification plots in FAM and/or Texas RED dyes should be accepted as **"Positive"** for COVID-19 (Figure 5,6 and 7). You can give **"Negative"** results to samples that are no amplification plots in FAM and TEXAS RED dyes for COVID-19 (Figure 8-9).

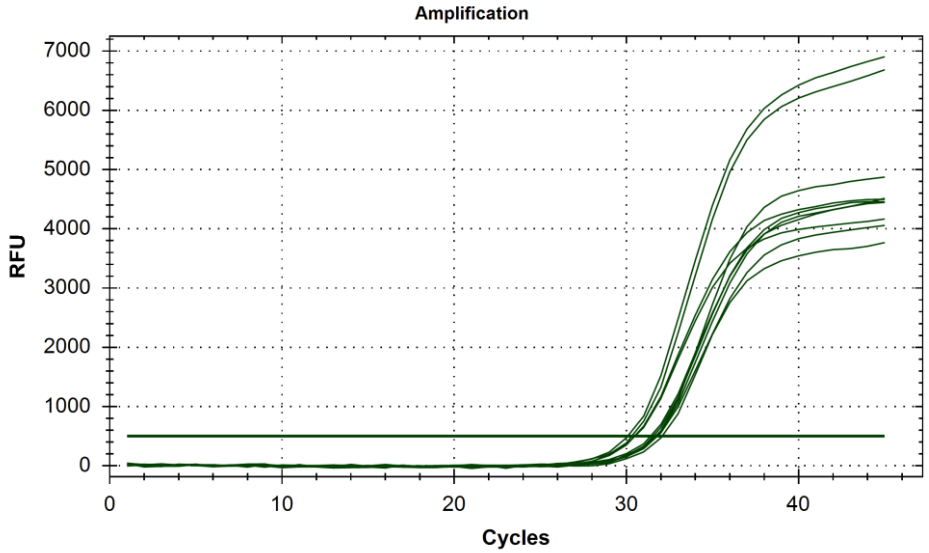

Figure 4: Internal Control Plots (HEX Dye)

Table 3: Results interpretation and evaluation.

Sample	Internal Control (HEX)	RdRp Gene (FAM)	Nucleocapsid Gene (Texas RED)	Results	Interpretation
Case 1	+	+	+	SARS-CoV-2 **Positive**	All results are valid SARS-CoV-2 RNA is detected.
Case 2	+	-	+		
Case 3	+	+	-		
Case 4	+	-	-	SARS-CoV-2 **Negative**	All results are valid SARS-CoV-2 RNA is not detected.
Case 5	+	-	-		
Case 6	-	-	-	Invalid	Dilute to extracted RNA or re-extract clinic sample.
Case 7	-	-	-		

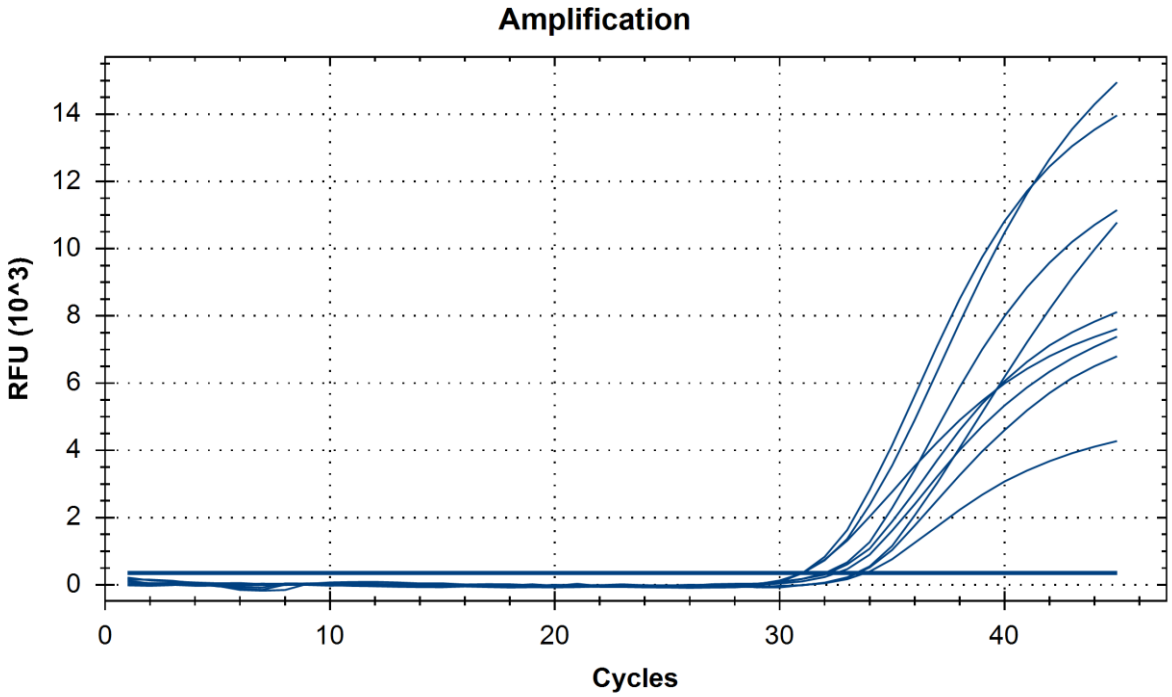

Figure 5: Positive Sample Plots-RdRp Gene (FAM Dye)

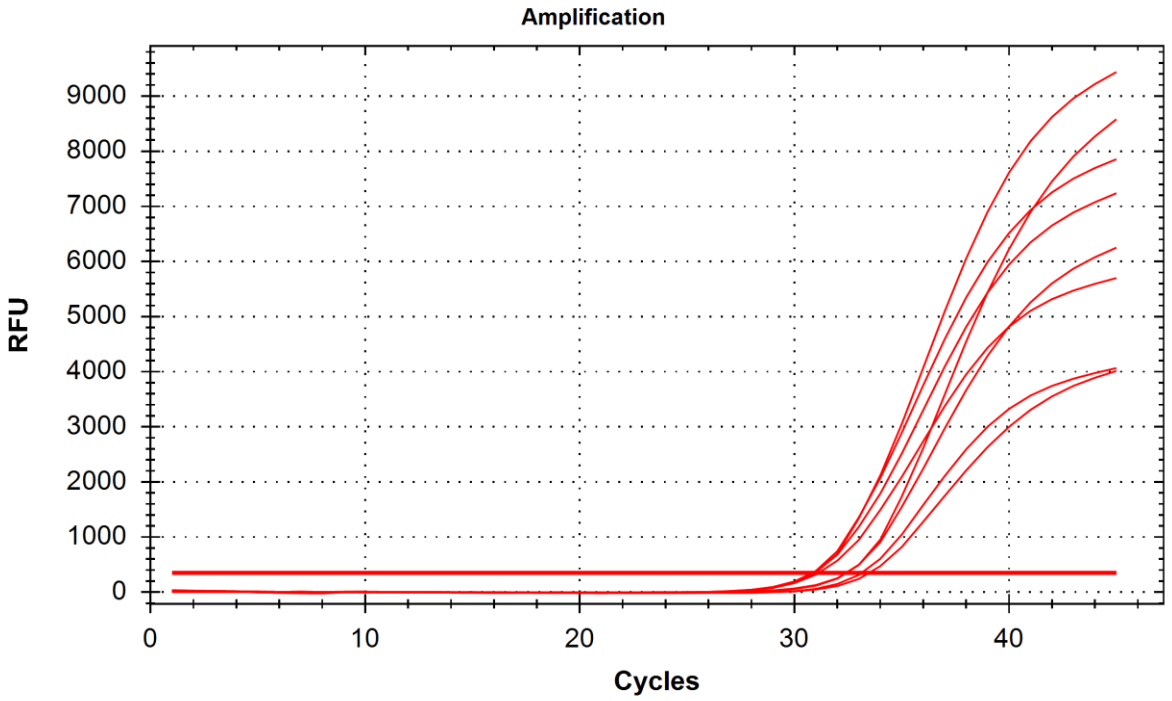

Figure 6: Positive Samples Plots – Nucleocapsid Gene (Texas RED Dye)

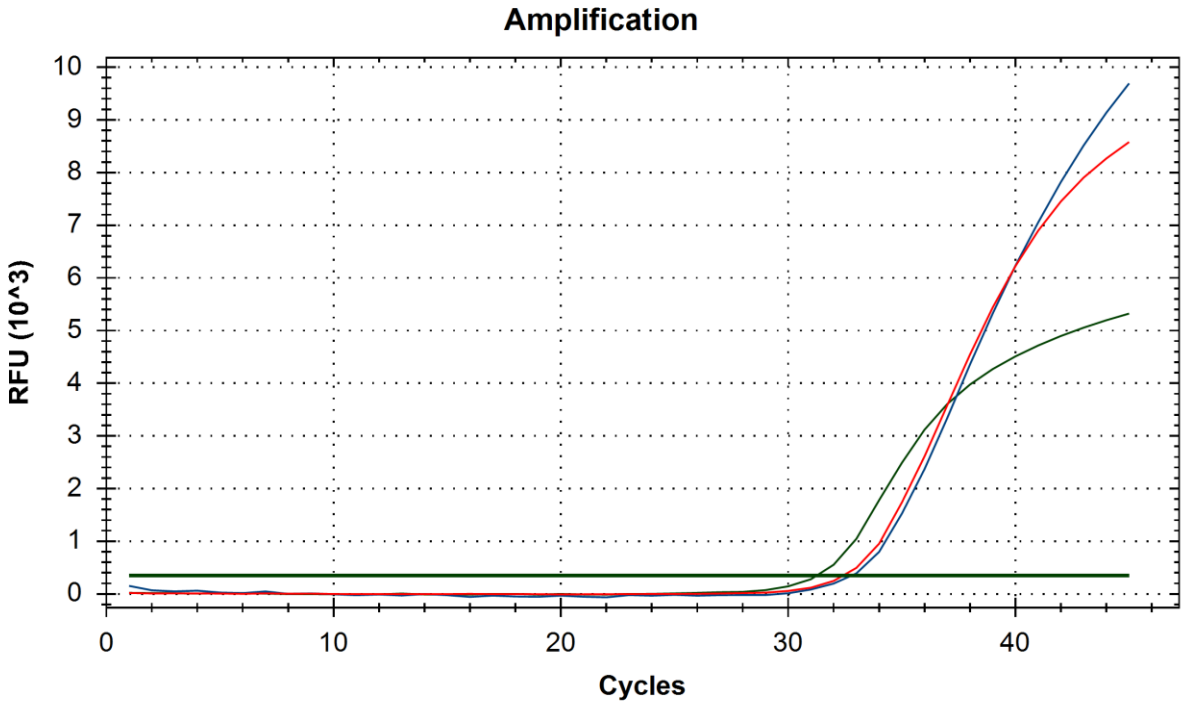

Figure 7: Positive Sample Plots (All Dyes)

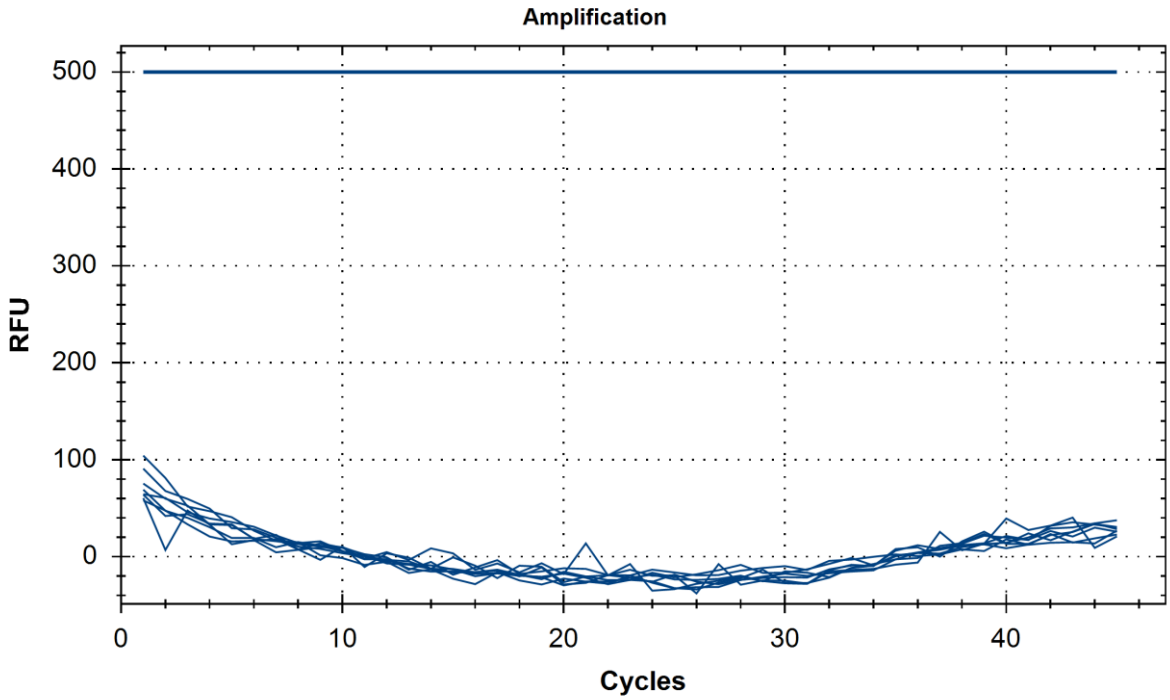

Figure 8: Negative Samples Plots (FAM Dye)

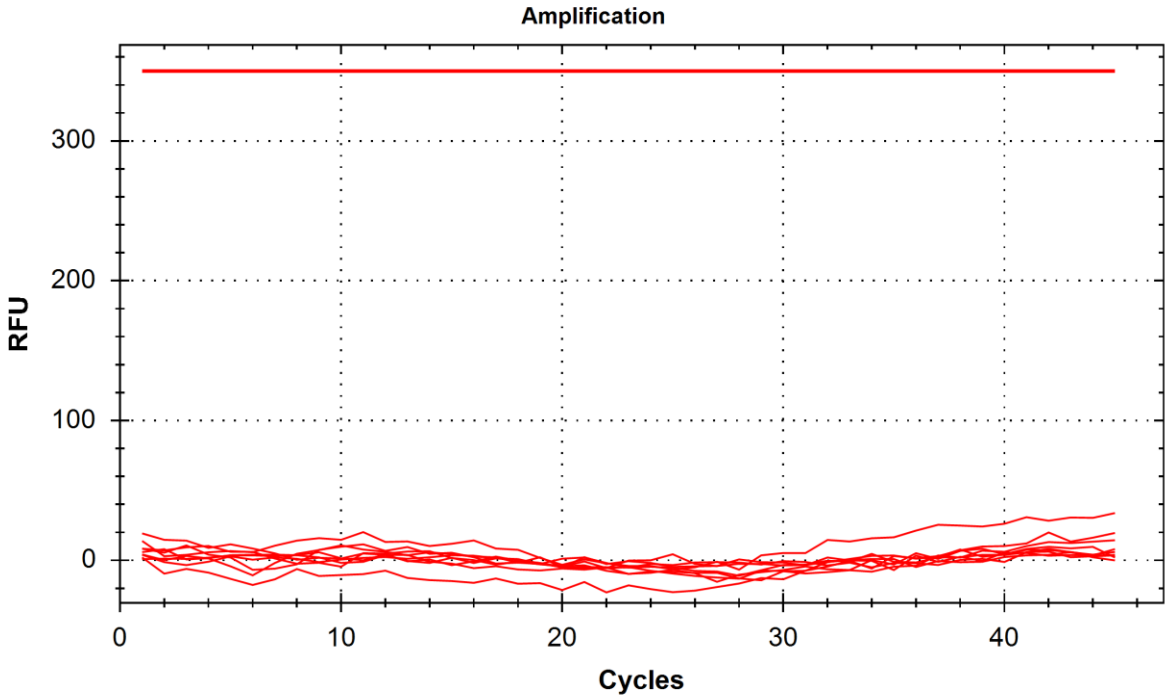

Figure 9: Negative Samples Plots (Texas Red Dye)

5. LIMITATIONS

1. 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.
2. SNP COVID-19 Real-Time PCR Kit performance has only been established with nasopharyngeal swabs and sputum specimens.
3. 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.
4. Extraction and amplification of nucleic acid from clinical samples must be performed according to the specified methods listed in this procedure.
5. 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.

6. 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.
7. 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.
8. Negative results do not preclude SARS-CoV-2 infections and should not be used as the sole basis for treatment or other management decisions.
9. Laboratories are required to report all positive results to the appropriate public health authorities.

6. ANALYTICAL VERIFICATION

6.1. VALIDATION

Table 4: Abbreviations

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

Table 5: Analytical verification formula

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

Table 6: Values of verification

Covid-19			
Method	Positive (Presence)	Negative (Absence)	Total
Positive	30,00	-	30,00
Negative	-	30,00	30,00
Total	30,00	30,00	60,00

Table 7: Results of Verification

Accuracy	(TP+TN)/(TP+TN+FP+FN)	(30+30)/60	1
Sensitivity	TP/(TP+FN)	30/30	1
Specificity	TN/(TN+FP)	30/30	1
False Positive	FP/(TN+FP)	0/30	0
False Negative	FN/(TP+FN)	0/30	0
Efficiency	(TP+TN)/(TP+TN+FP+FN)	(30+30)/60	1

6.2. CROSS-REACTIVITY AND STABILITY

6.2.1. 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 lists of organisms to be analyzed in silico and wet testing are provided in the table 8 and 9.

Table 8: List of organisms to be analyzed in silico.

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	

Table 9: List of organisms in wet testing. For wet testing, concentrations of $1x10^5$ pfu/ml or higher for viruses and $1x10^6$ 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
Respiratory syncytial virus	MERS-coronavirus
Rhinovirus	
Haemophilus influenzae	
Mycobacterium tuberculosis	
Streptococcus pneumoniae	
Streptococcus pyogenes	
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	

6.2.2. Microbial Interference Studies:

The primers and probes used within the kit were designed and 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 the pathogens in Table 8 and 9 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.

6.2.3. Stability

To evaluate the stability, the kit was freezed and 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. The shelf life of the kit under -20°C condition is 12 months.

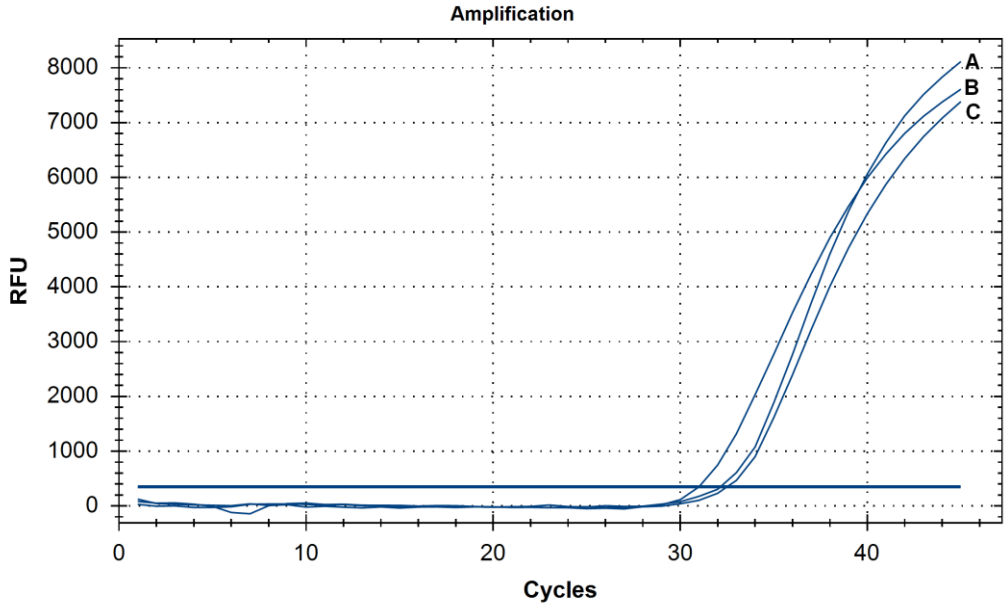

Figure 10: Amplification plots of the same sample freezed and thawed for RdRp gene. **A:** First thaw **B:** Third thaw **C:** Fifth thaw.

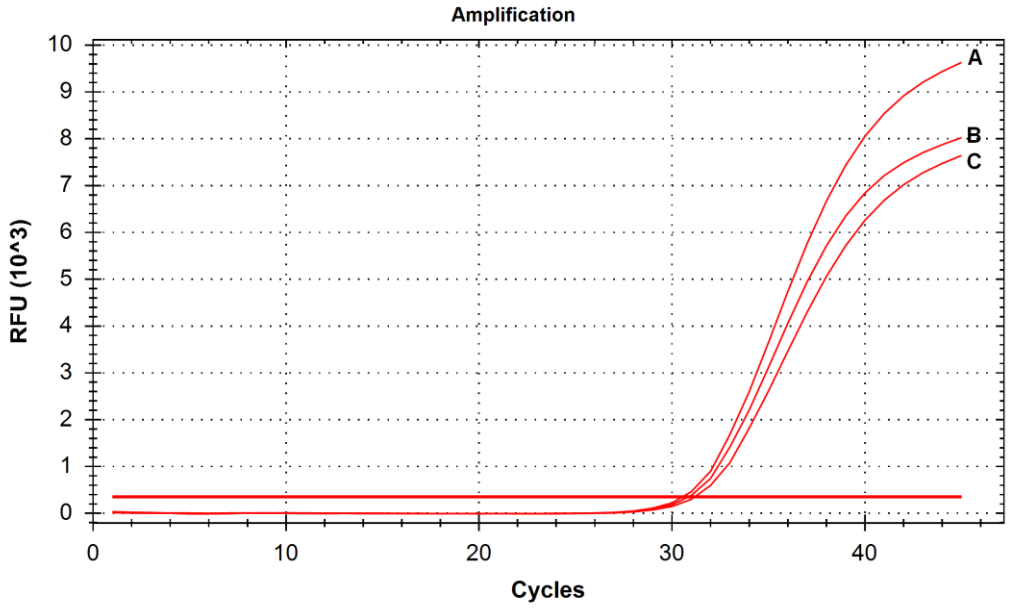

Figure 11: Amplification plots of the same sample freezed and thawed for Nucleocapsid gene. **A:** First thaw **B:** Third thaw **C:** Fifth thaw.

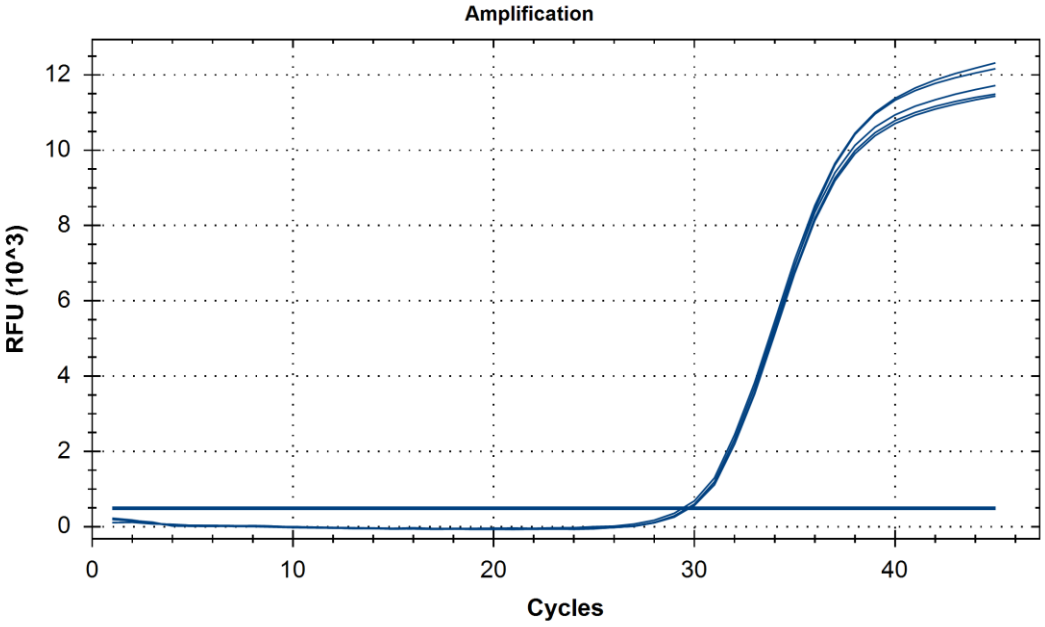

Figure 12: Amplification plots of the positive samples were repeated 5 times in FAM dye.

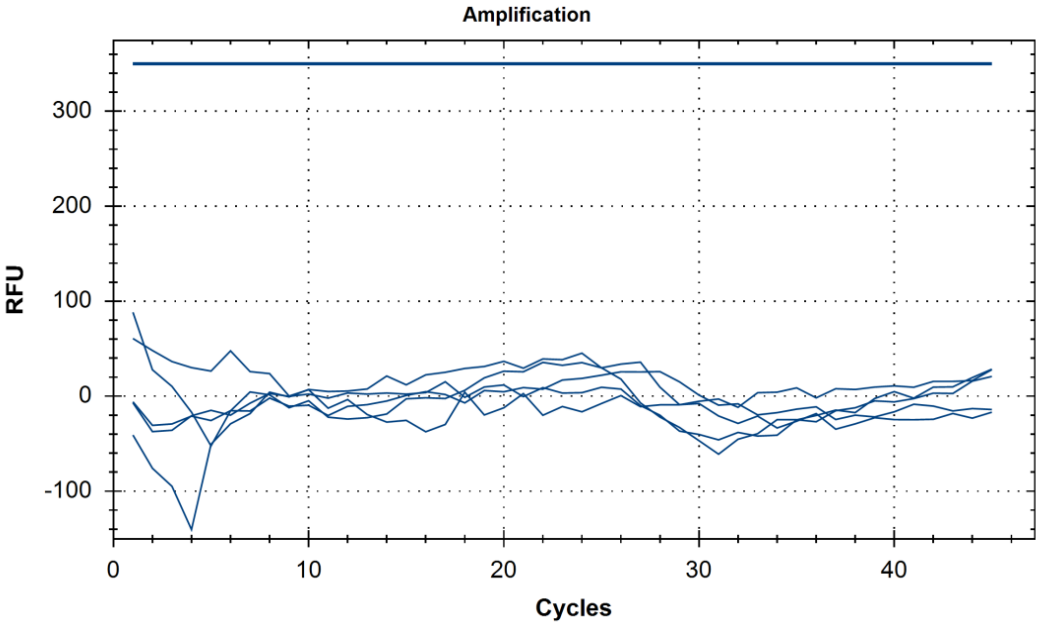

Figure 13: Amplification plots of the negative samples were repeated 5 times in FAM dye.

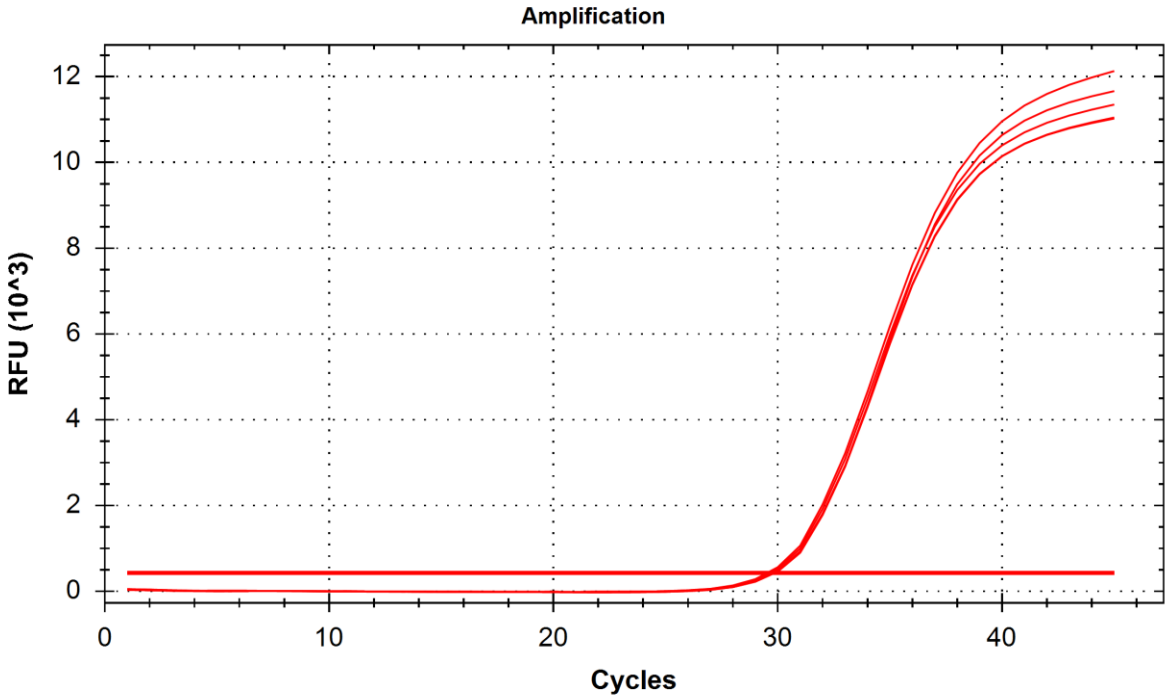

Figure 14: Amplification plots of the positive samples were repeated 5 times in Texas RED dye.

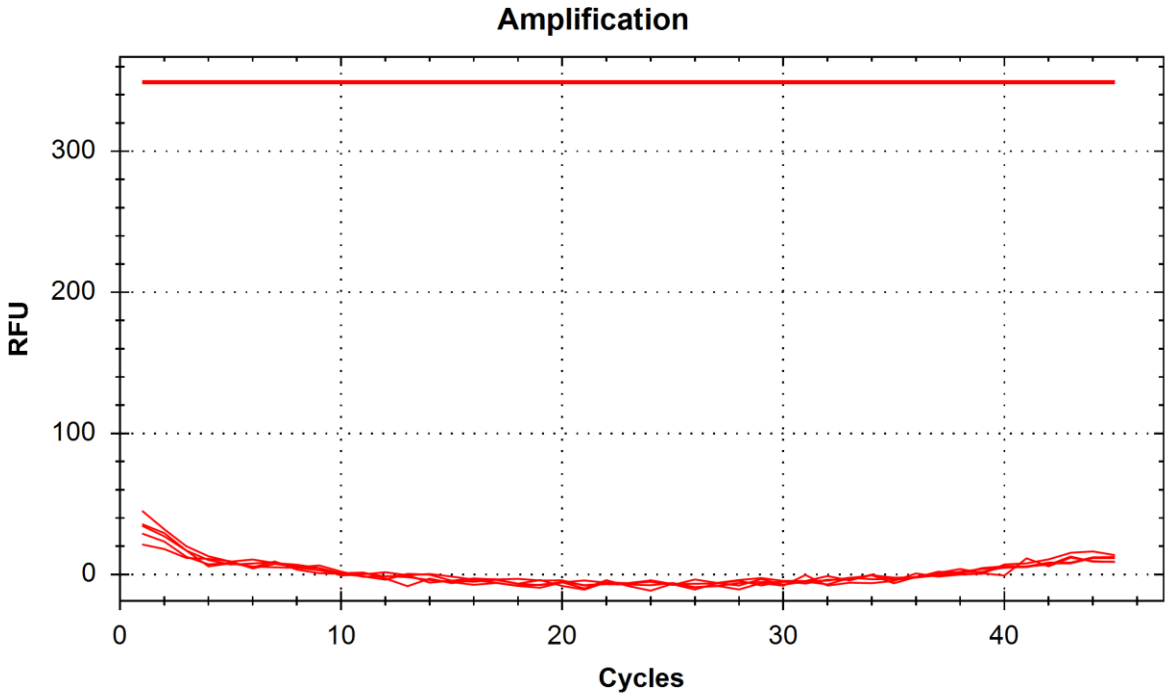

Figure 15: Amplification plots of the negative samples were repeated 5 times in Texas RED dye.

6.2.4. Limit of Detection:

In the LoD determination study, 5 fold serial dilutions were prepared and then tested in three replicates. The tentative LoD determined was 100 copies/mL (Table 10). For confirmation of LoD, 20 replicates of 100 copies/mL and 20 copies/mL were tested. The final LoD was determined as 100 copies/mL (1 copy/rxn.) with 20/20 positive results (Table 11).

Table 10 : Tentative LoD Evaluation

Sample	Target	Target Ct Value 1	Target Ct Value 2	Target Ct Value 3	Target Mean Ct	Copies / Rxn	Copies / mL
1.25 x 10^2	**RdRp Gene**	31,75	31,86	31,88	31,83	125,00	12.500,00
	N Gene	30,02	30,15	30,21	30,16		
2.5 x 10^1	**RdRp Gene**	33,05	33,11	33,09	33,08	25,00	2.500,00
	N Gene	31,92	32,01	32,12	32,02		
5 x 10^0	**RdRp Gene**	35,01	34,99	35,15	35,05	5,00	500,00
	N Gene	33,90	34,01	34,12	34,01		
1 x 10^0	**RdRp Gene**	36,53	36,42	36,32	36,42	1,00	100,00
	N Gene	35,42	35,18	35,31	35,30		
2 x 10^{-1}	**RdRp Gene**	-	38,11	-	38,11	0,20	20,00
	N Gene	-	37,35	37,42	37,39		

Table 11 : Final LoD Evaluation

Sars-CoV-2 Confirmatory LoD BIO-RAD CFX 96							
Sample	**Genes**	**Target Copy Number / Rxn**	**Target Copy Number / mL**	**Valid Results**	**Positive**	**Mean Ct**	**Detection Rate**
1 x 10^0	**RdRp Gene**	1	100	20	17	36,33	85%
	N Gene	1	100	20	20	35,47	100%
2 x 10^{-1}	**RdRp Gene**	0,2	20	20	6	38,42	30%
	N Gene	0,2	20	20	12	37,90	60%

6.2.5. 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.

6.2.6. Clinical Evaluation:

Clinical evaluation of SNP COVID-19 Real Time PCR Kit was performed by comparing 30 positive and 30 negative samples with FDA-EUA approved DiaPlex Q Novel Coronavirus (2019-nCoV) Detection Kit. According to the comparison results, positive and negative percent agreement of SNP COVID-19 Real Time PCR Kit is evaluated as 100% (95% CI: 88,4%-100.0%). The results are shown at Table 12 and Table 13.

Table 12 : Clinical Evaluation ct values (Positive Samples).

Clinical Study of Sars-CoV-2 - Nasal and Nasopharyngeal Swabs- BIO-RAD CFX96						
POSITIVE SAMPLES						
	SNP COVID-19 Real Time PCR Kit v1			**DiaPlex Q Novel Coronavirus (2019-nCoV) Detection Kit**		
	Ct Values					
Sars-CoV-2 Samples	**RdRp Gene**	**N Gene**	**Internal Control**	**ORF1a**	**N gene**	**Internal Control**
Positive 1	31,69	32,18	34,14	37,06	N/A	21,10
Positive 2	31,54	31,65	34,25	37,14	N/A	20,49
Positive 3	34,92	34,67	31,51	34,15	N/A	20,22
Positive 4	30,99	30,71	34,93	38,70	N/A	20,21
Positive 5	31,98	31,31	32,61	39,06	37,17	20,48
Positive 6	33,58	34,06	27,02	36,20	N/A	20,29
Positive 7	33,09	33,19	32,61	35,77	N/A	20,63
Positive 8	32,13	32,64	29,50	38,66	N/A	20,50
Positive 9	30,69	30,48	28,82	38,79	37,89	20,40
Positive 10	N/A	35,81	32,45	32,07	30,17	25,93
Positive 11	34,22	33,24	29,09	38,32	37,02	30,84
Positive 12	34,24	32,17	25,58	33,37	31,73	39,23
Positive 13	34,31	34,35	29,16	30,44	28,60	21,68
Positive 14	30,15	28,79	29,71	29,29	28,15	27,09
Positive 15	38,94	36,08	27,49	33,25	31,57	24,56
Positive 16	35,50	31,40	26,60	34,35	33,88	21,84
Positive 17	32,00	31,93	28,92	29,67	28,70	20,58
Positive 18	35,86	34,38	28,98	32,72	29,89	25,65
Positive 19	35,07	34,13	32,88	35,23	33,23	23,71
Positive 20	N/A	35,74	28,02	30,18	28,52	24,37
Positive 21	N/A	33,25	27,78	32,18	30,33	25,85
Positive 22	38,48	34,55	32,03	33,10	43,85	23,42
Positive 23	32,53	31,03	26,14	28,87	28,26	24,38
Positive 24	31,01	39,22	30,99	35,24	32,71	23,59
Positive 25	33,84	32,23	26,09	30,56	29,88	26,92
Positive 26	N/A	37,36	31,32	34,74	29,42	23,41
Positive 27	N/A	35,95	26,56	34,00	33,30	24,57
Positive 28	28,51	27,19	29,27	25,29	25,16	29,65
Positive 29	35,52	34,09	28,85	36,05	35,33	26,48
Positive 30	34,76	31,95	31,92	38,33	37,52	24,14
TPC	31,42	30,37	27,30	31,07	32,08	24,40
NTC	N/A	N/A	N/A	N/A	N/A	27,84

Table 13 : Clinical Evaluation ct values (Negative Samples).

Clinical Study of Sars-CoV-2 - Nasal and Nasopharyngeal Swabs- BIO-RAD CFX96						
NEGATIVE SAMPLES						
	SNP COVID-19 Real Time PCR Kit v1			**DiaPlex Q Novel Coronavirus (2019-nCoV) Detection Kit**		
	Ct Values					
Sars-CoV-2 Samples	**RdRp Gene**	**N Gene**	**Internal Control**	**ORF1a**	**N gene**	**Internal Control**
Negative 1	N/A	N/A	26,54	N/A	N/A	20,12
Negative 2	N/A	N/A	28,64	N/A	N/A	20,56
Negative 3	N/A	N/A	26,54	N/A	N/A	20,38
Negative 4	N/A	N/A	25,97	N/A	N/A	20,24
Negative 5	N/A	N/A	27,49	N/A	N/A	20,33
Negative 6	N/A	N/A	24,29	N/A	N/A	20,39
Negative 7	N/A	N/A	26,00	N/A	N/A	20,40
Negative 8	N/A	N/A	26,03	N/A	N/A	20,25
Negative 9	N/A	N/A	26,24	N/A	N/A	20,31
Negative 10	N/A	N/A	24,08	N/A	N/A	19,90
Negative 11	N/A	N/A	21,06	N/A	N/A	20,32
Negative 12	N/A	N/A	26,41	N/A	N/A	20,40
Negative 13	N/A	N/A	28,38	N/A	N/A	20,49
Negative 14	N/A	N/A	27,34	N/A	N/A	20,39
Negative 15	N/A	N/A	22,72	N/A	N/A	20,27
Negative 16	N/A	N/A	25,97	N/A	N/A	19,93
Negative 17	N/A	N/A	25,48	N/A	N/A	20,20
Negative 18	N/A	N/A	27,69	N/A	N/A	20,02
Negative 19	N/A	N/A	23,88	N/A	N/A	20,12
Negative 20	N/A	N/A	25,03	N/A	N/A	20,12
Negative 21	N/A	N/A	25,56	N/A	N/A	20,32
Negative 22	N/A	N/A	29,09	N/A	N/A	20,22
Negative 23	N/A	N/A	22,37	N/A	N/A	20,28
Negative 24	N/A	N/A	25,81	N/A	N/A	19,83
Negative 25	N/A	N/A	29,26	N/A	N/A	19,87
Negative 26	N/A	N/A	25,43	N/A	N/A	20,27
Negative 27	N/A	N/A	27,58	N/A	N/A	20,35
Negative 28	N/A	N/A	26,53	N/A	N/A	20,05
Negative 29	N/A	N/A	23,07	N/A	N/A	20,26
Negative 30	N/A	N/A	26,92	N/A	N/A	19,97
TNC	N/A	N/A	25,59	-	-	-

Table 14: The Comparison of Two Kits for Detection of SARS-CoV-2.

Clinical Study of Sars-CoV-2 - Nasal and Nasopharyngeal Swabs BIO-RAD CFX96			
POSITIVE SAMPLES			
	Valid Result (n)	**Positive Result**	**Negative Result**
SNP Covid-19 Real Time PCR Kit v1	30	30	0
DiaPlex Q Novel Coronavirus (2019-nCoV) Detection Kit	30	30	0
Positive Percent Agreement: 30/30 = 100% (95% CI: 88,4%-100.0%)			
NEGATIVE SAMPLES			
	Valid Result (n)	**Positive Result**	**Negative Result**
SNP Covid-19 Real Time PCR Kit v1	30	0	30
DiaPlex Q Novel Coronavirus (2019-nCoV) Detection Kit	30	0	30
Negative Percent Agreement: 30/30 = 100% (95% CI: 88,4%-100.0%)			

Sensitivity=%100

Specifity=%100

Positive predictive value=%100

Negative predictive value=%100

6.3. QUALITY CONTROL RESULTS

Table 15: Quality Control Results for Master Mix and Control RNAs

TEST	SPECIFICATION	RESULT
Sterility	DNAse-RNAse Free	Pass*
Appearance	Clear, Solution	Pass
Amplification Performance on Control RNAs	Figure 4-5-6-7-8 and 9 Table 3	Pass

*All reagents tested with Real Time PCR for DNAse-RNAse free specification.

7. TROUBLESHOOTING AND CAUTIONS

7.1. TROUBLESHOOTING

If internal control doesn't work;

- Unloaded well.
- Sample is containing PCR inhibitor(s).

If target plots start late;

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

- The amount of target RNA may be low.
- Target RNA quality is not good. Please dilute RNA by adding 1 to 1 PCR grade water.

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

7.2. 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 defined conditions.
- Do not use the PCR master mix forgotten at room temperature.
- Thaw PCR master mix at room temperature and slowly mix by pipetting before use.
- Shelf-life of PCR master mix is 12 months. Please check the manufacturing date before use.
- For in vitro diagnostic use.

8. SYMBOLS

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

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