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

1.3. PRINCIPLE OF THE KIT

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 : Tube - Genes- 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	50 µl
Negative Control	50 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.
- 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, Magnetic Beads Extraction, Automated RNA Extraction and phenol/chloroform RNA Extraction.

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, postive 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.**
- Agilent 3000P/3005P/4000, please use natural/transparent Real Time PCR tubes.

This system can be used with;

Bio-Rad CFX96

ABI Prism ® 7500/7500 Fast

Roche LightCycler® 96 System

Rotor Gene Q

Mic qPCR Cycler

Agilent 3000P/3005P/4000

LongGeneQ

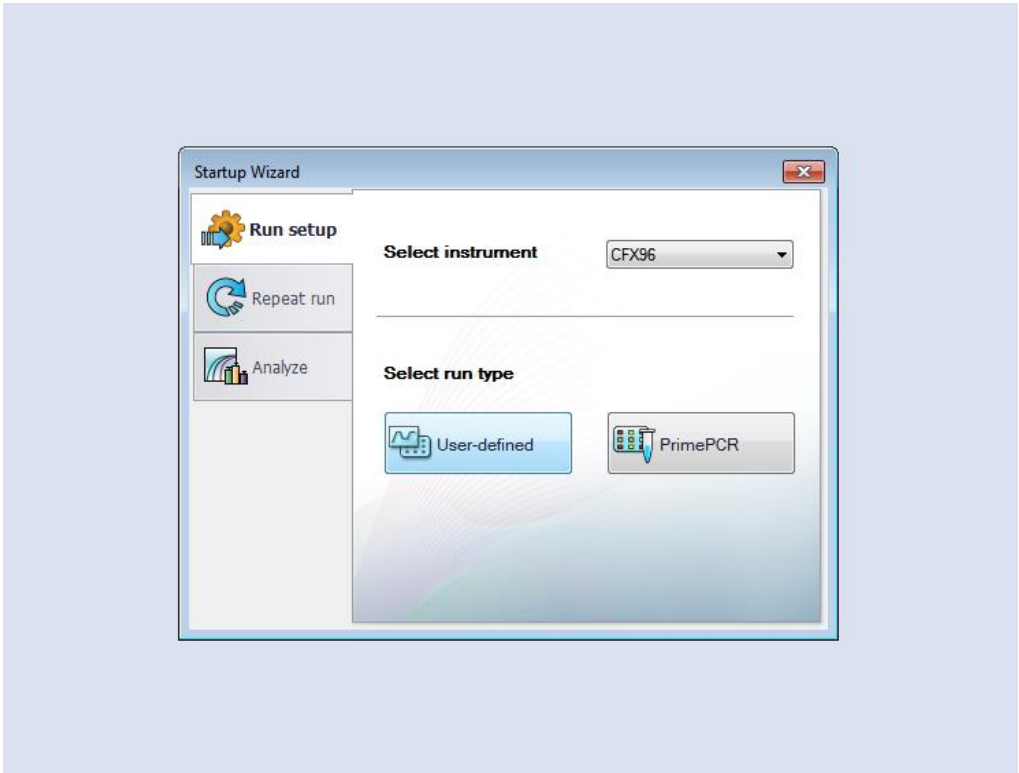

Figure 1: For the setup of PCR programme, choose **"User-Defined"**

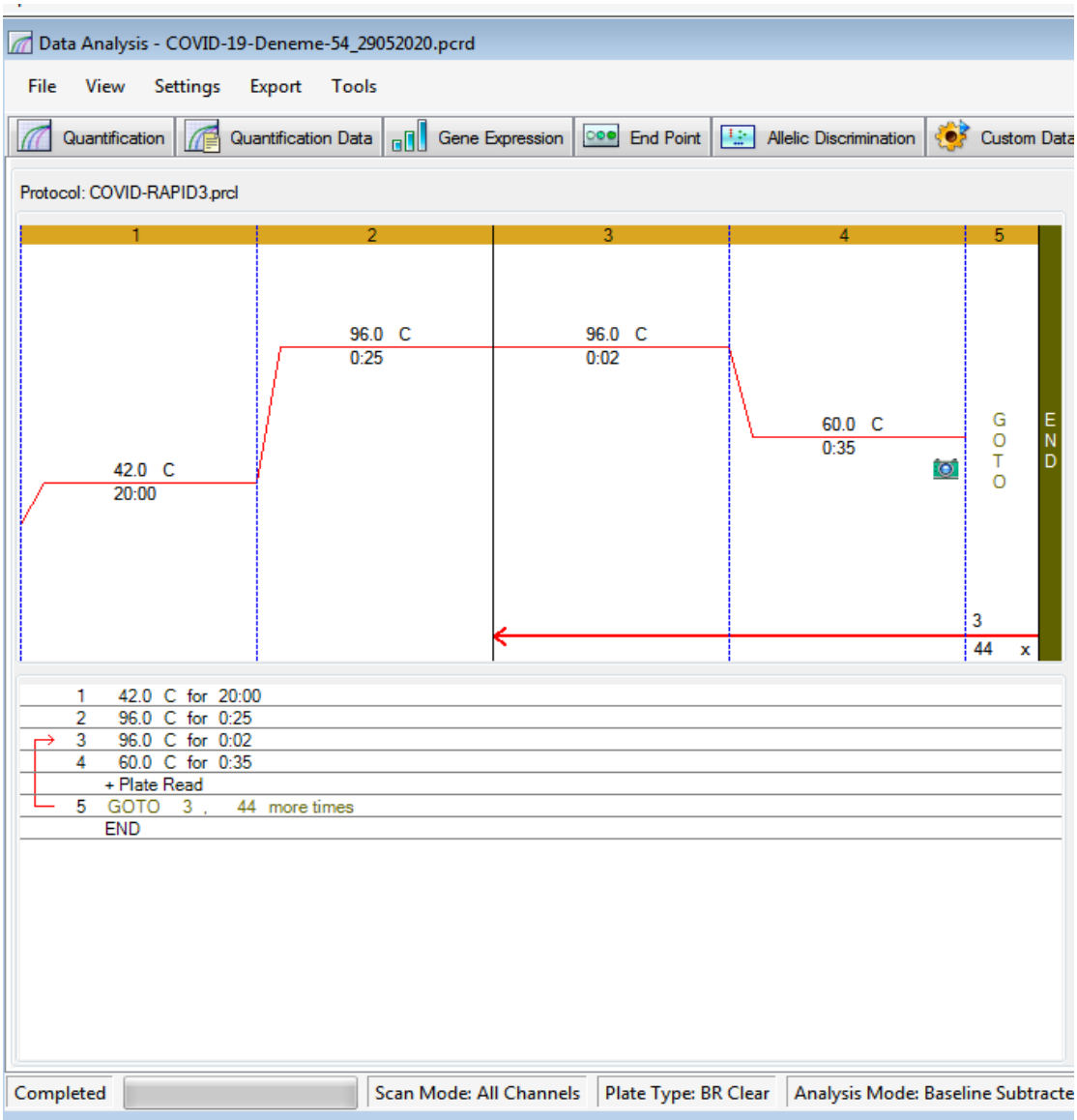

Figure 2: Set the run programme as seen table 2.

3.4. PLATE SETUP

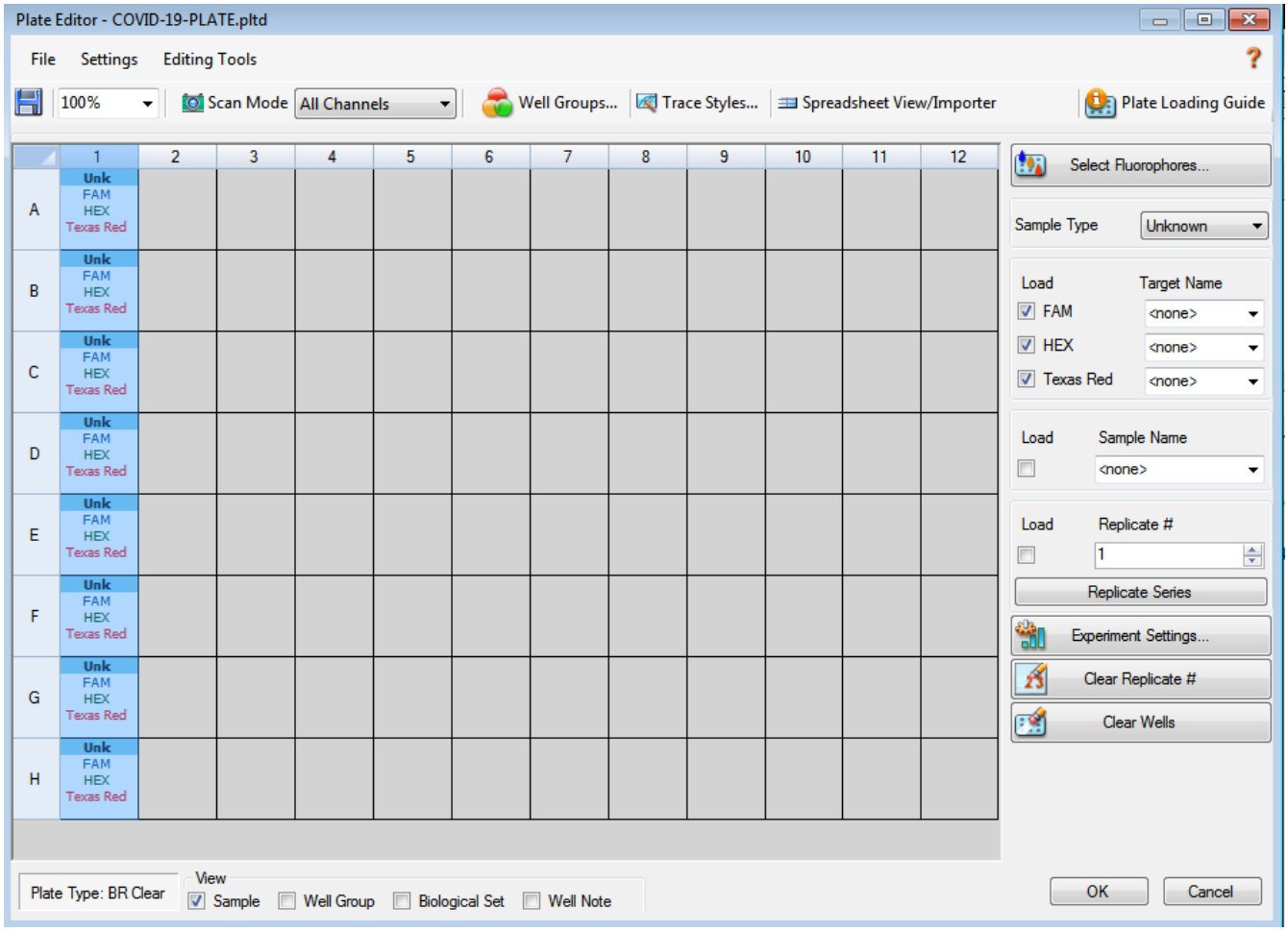

Figure 3: Select the type of wells to work as **unknown** and mark with **FAM, Texas RED** and **HEX.**

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). Ct value X ≤ 43 in FAM and/or Texas RED dyes should be evaluated as **"Positive"** for COVID-19 (Figure 5,6 and 7). You can give **"Negative"** results to samples that are no amplification on FAM and TEXAS RED dyes for COVID-19 (Figure 8-9).

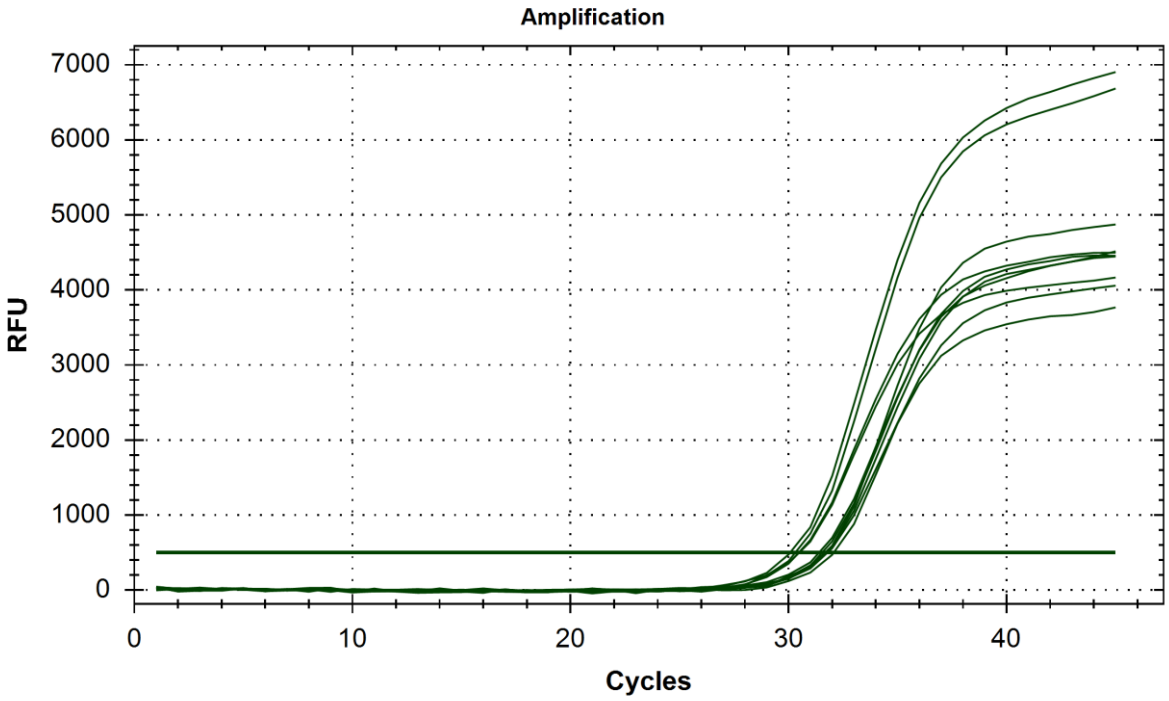

Figure 4: Internal Control Plots (HEX Dye)

Table 3: Results interpretation and evaluation.

Sample Type	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	-	-	-	Invalid	Invalid Results The sample needs to be tested again.

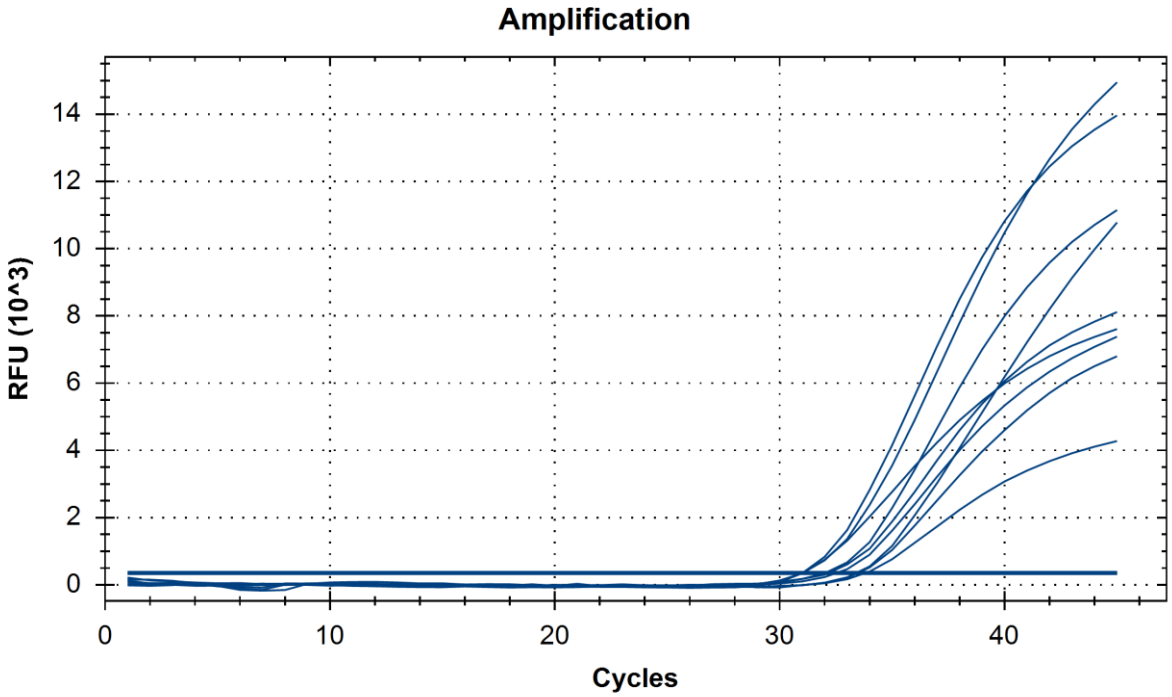

Figure 5: Positive Sample Plots-RdRp Gene (FAM Dye)

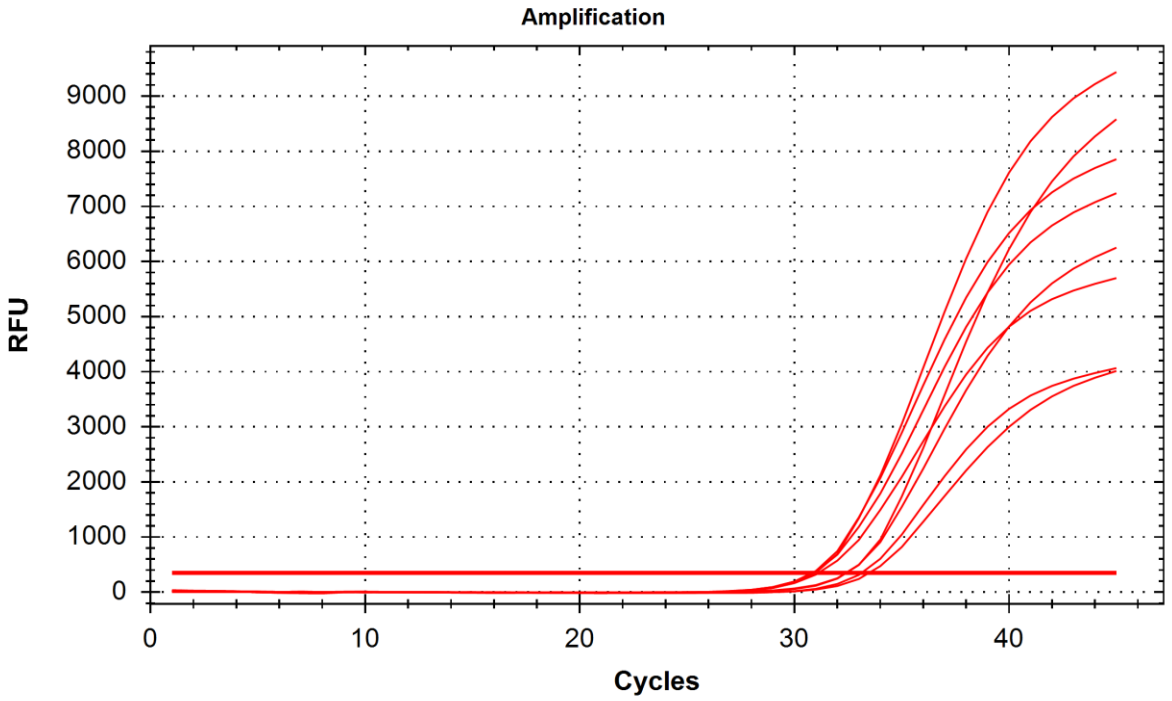

Figure 6: Positive Samples Plots – Nucleocapsid Gene (Texas RED Dye)

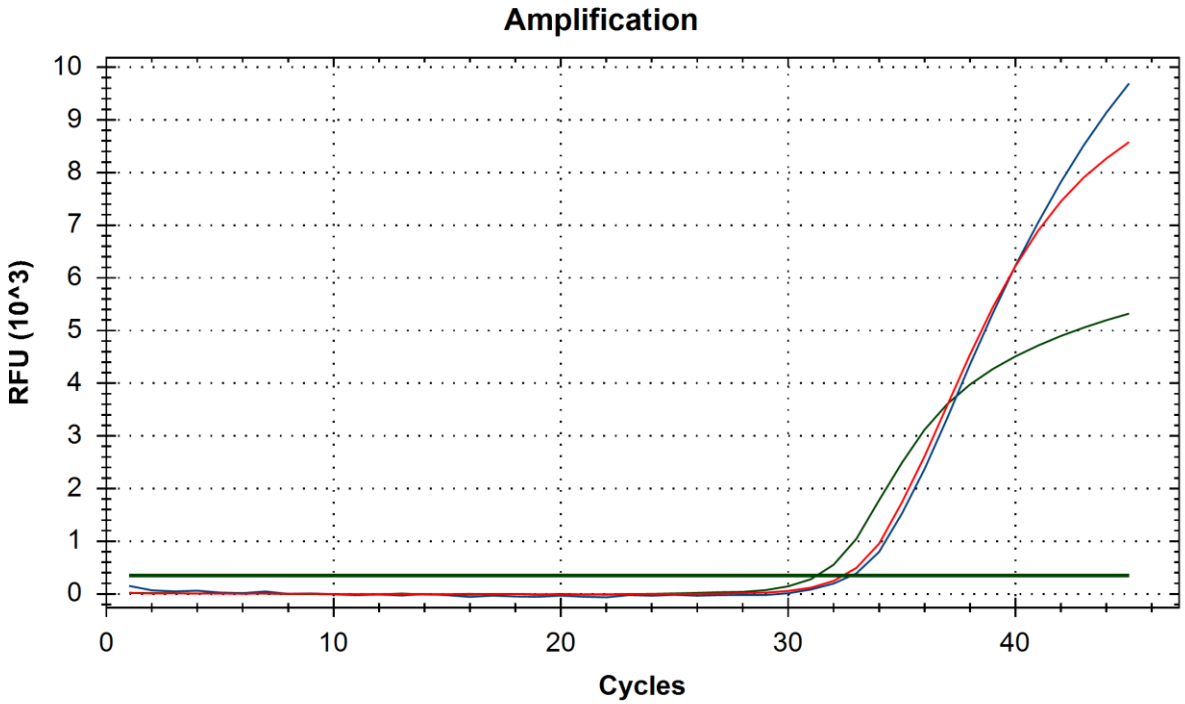

Figure 7: Positive Sample Plots (All Dyes)

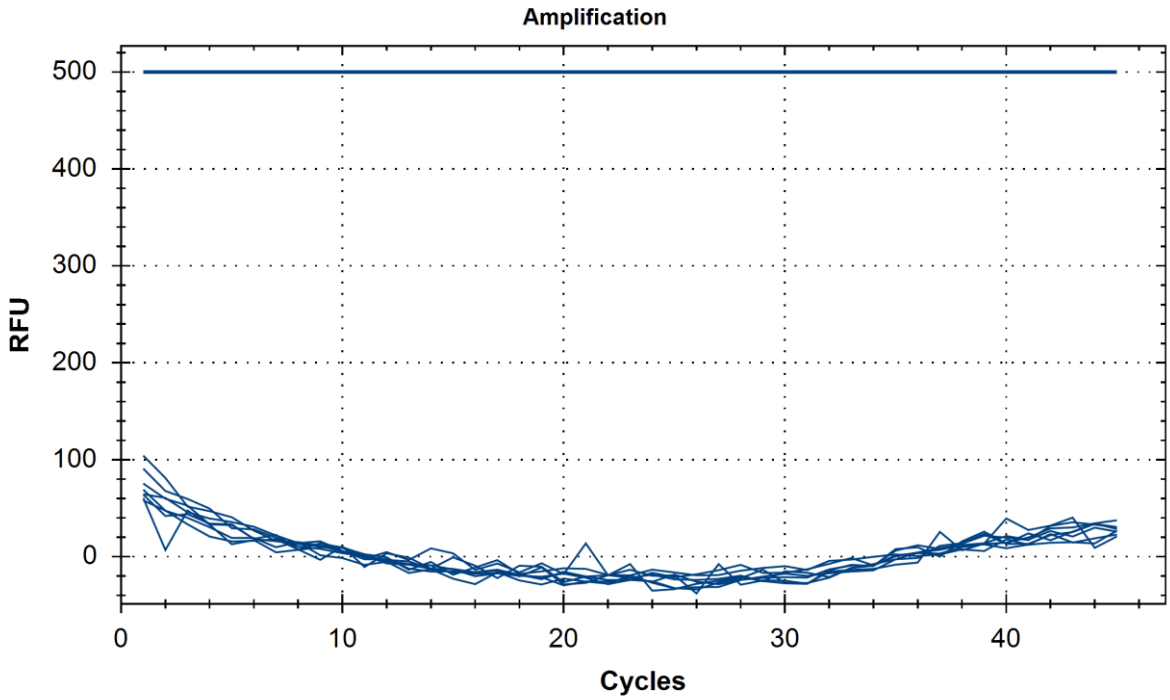

Figure 8: Negative Samples Plots (FAM Dye)

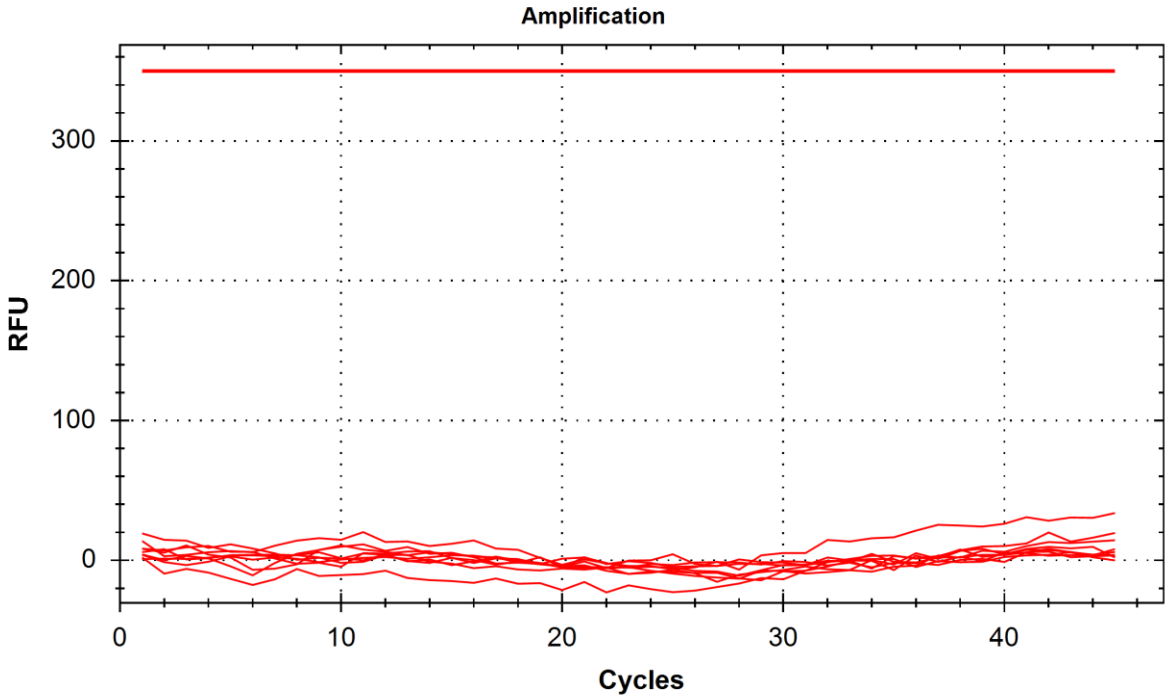

Figure 9: Negative Samples Plots (Texas Red Dye)

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	-	50,00	50,00
Total	30,00	50,00	80,00

Table 7: Results of Verification

Accuracy	(TP+TN)/(TP+TN+FP+FN)	(30+50)/80	1
Sensitivity	TP/(TP+FN)	30/30	1
Specificity	TN/(TN+FP)	50/50	1
False Positive	FP/(TN+FP)	0/50	0
False Negative	FN/(TP+FN)	0/30	0
Efficiency	(TP+TN)/(TP+TN+FP+FN)	(30+50)/80	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.

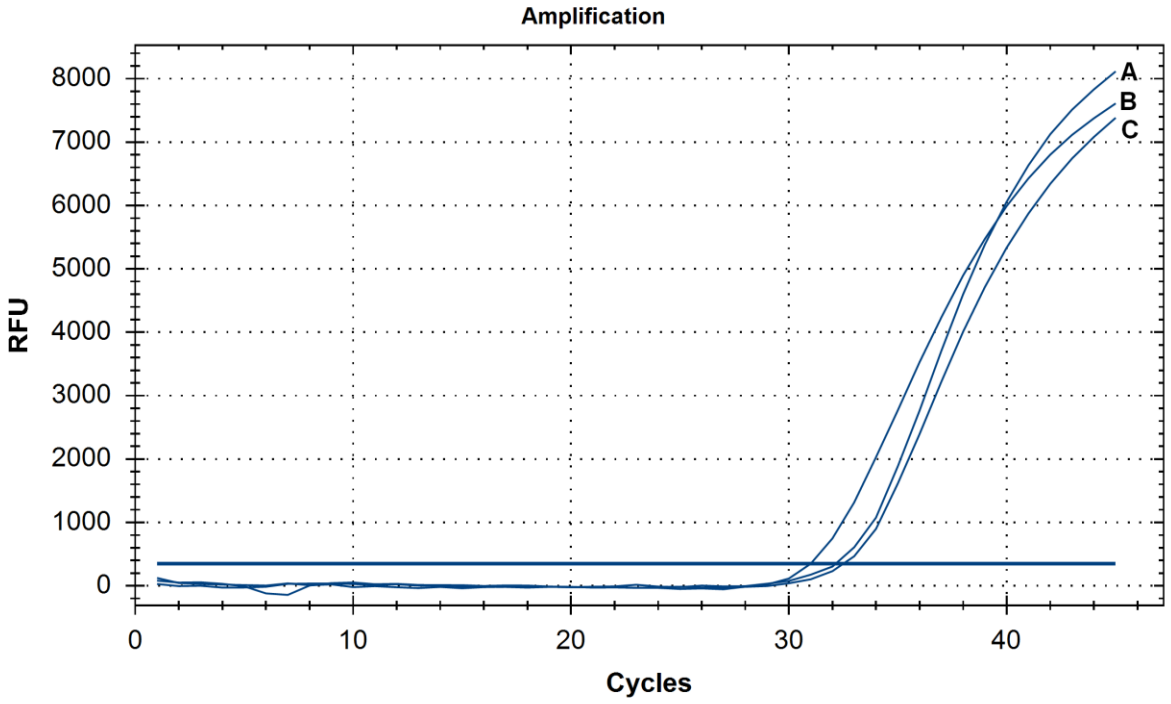

Figure 10: Amplification plots of the same sample freezed and thawed for RdRp gene. **A:** First thaw, **B:** Third thaw **C:** Fifth thaw.

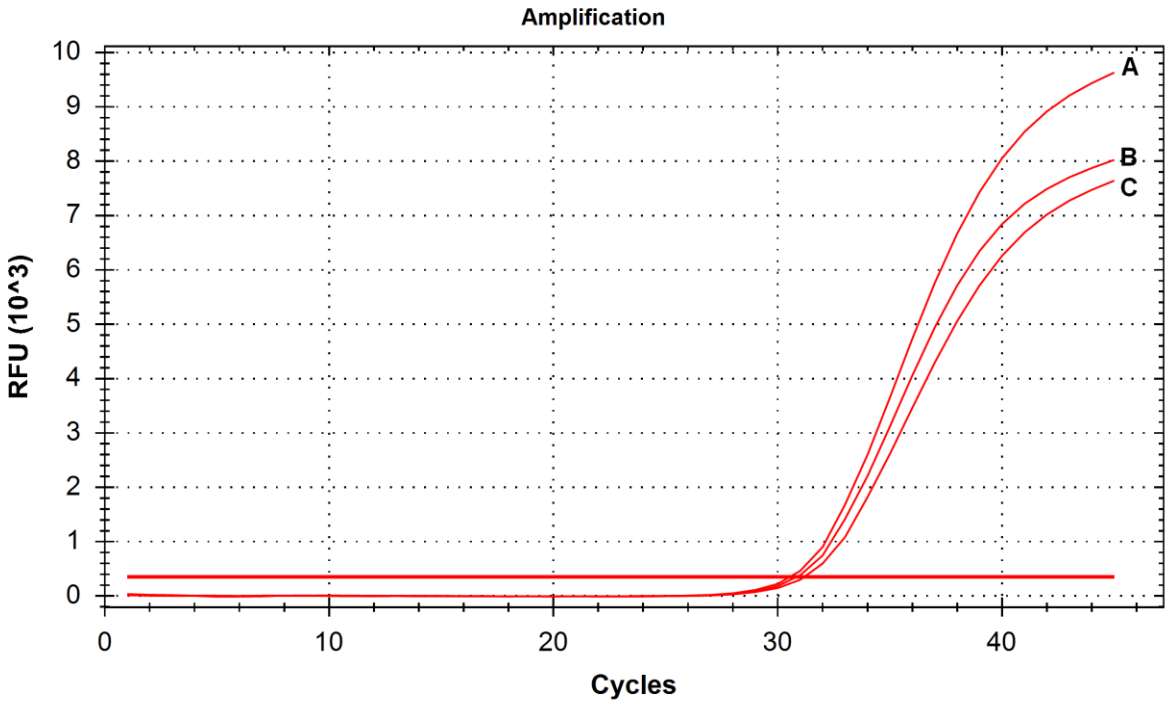

Figure 11: Amplification plots of the same sample freezed and thawed for Nucleocapsid gene. **A:** First thaw, **B:** Third thaw **C:** Fifth thaw.

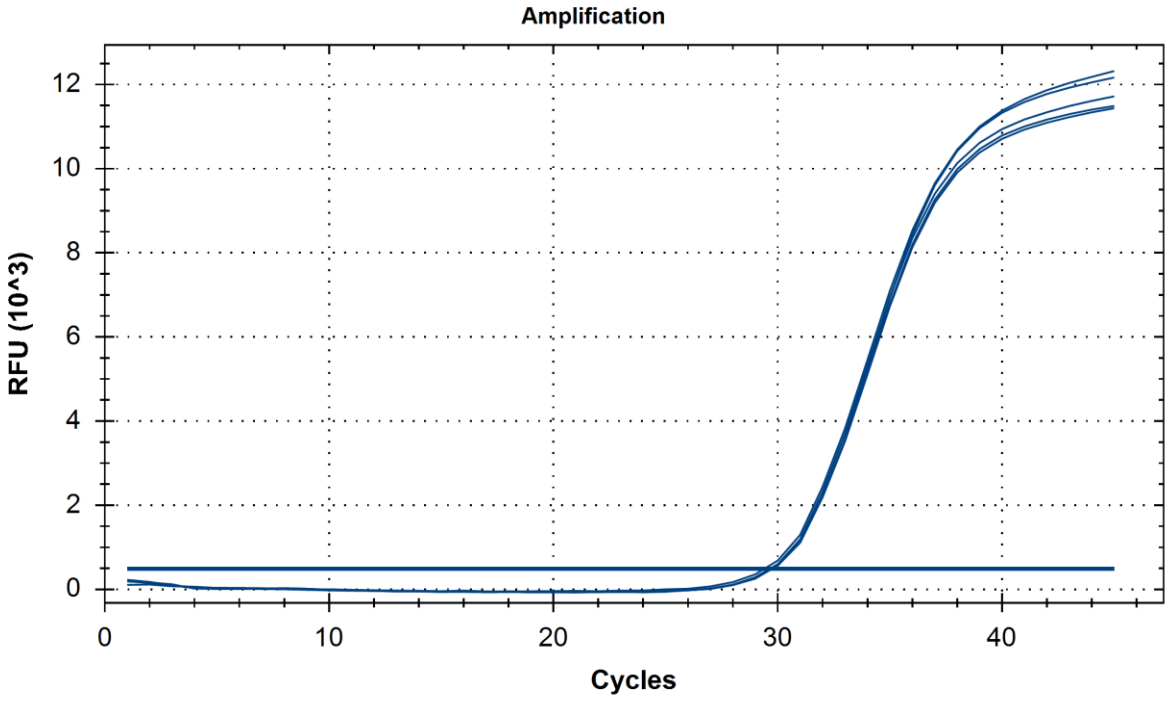

Figure 12: Amplification plots of the positive samples were repeated 5 times in FAM dye.

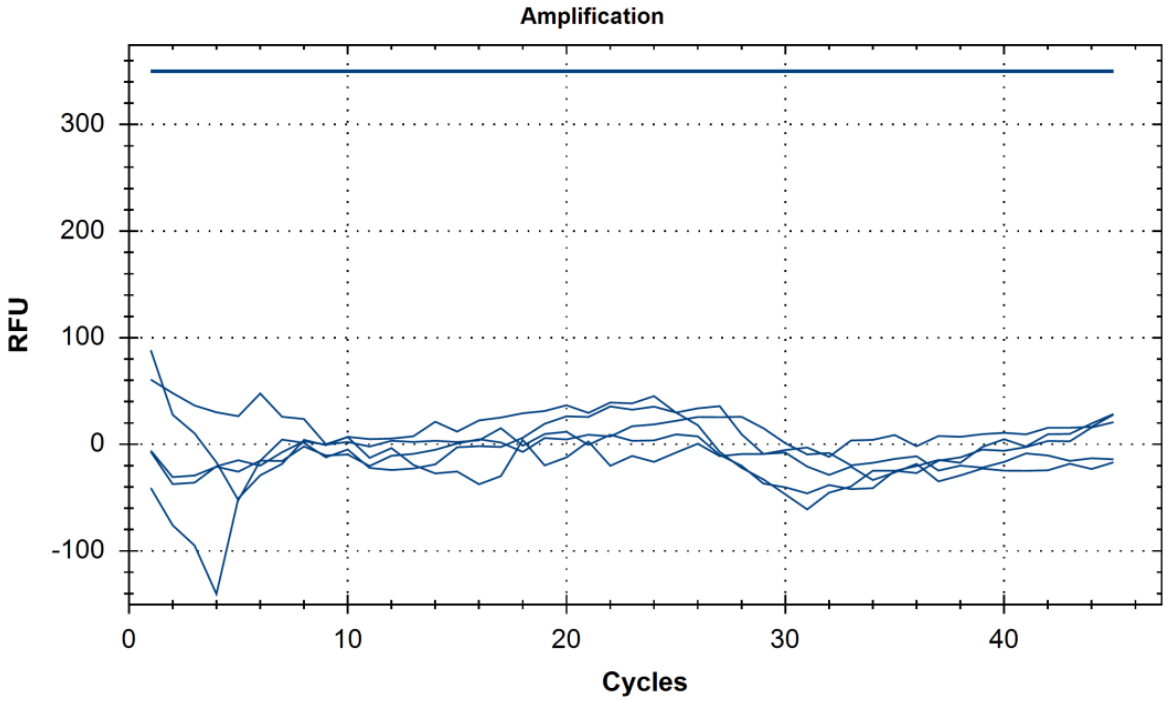

Figure 13: Amplification plots of the negative samples were repeated 5 times in FAM dye.

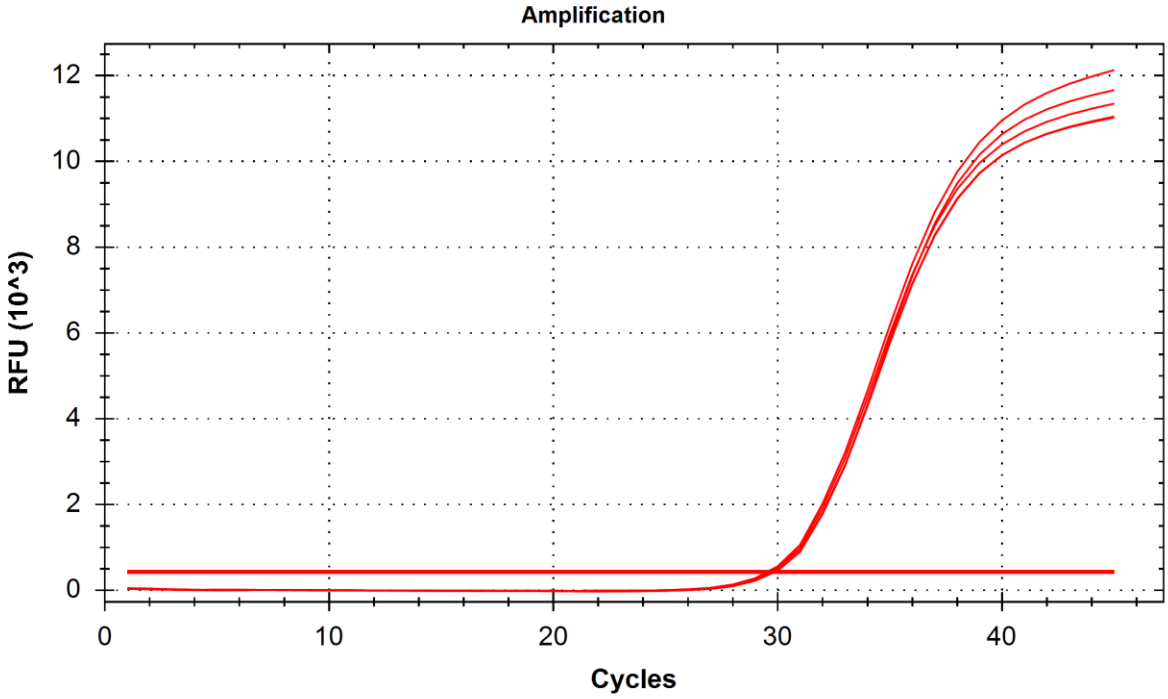

Figure 14: Amplification plots of the positive samples were repeated 5 times in Texas RED dye.

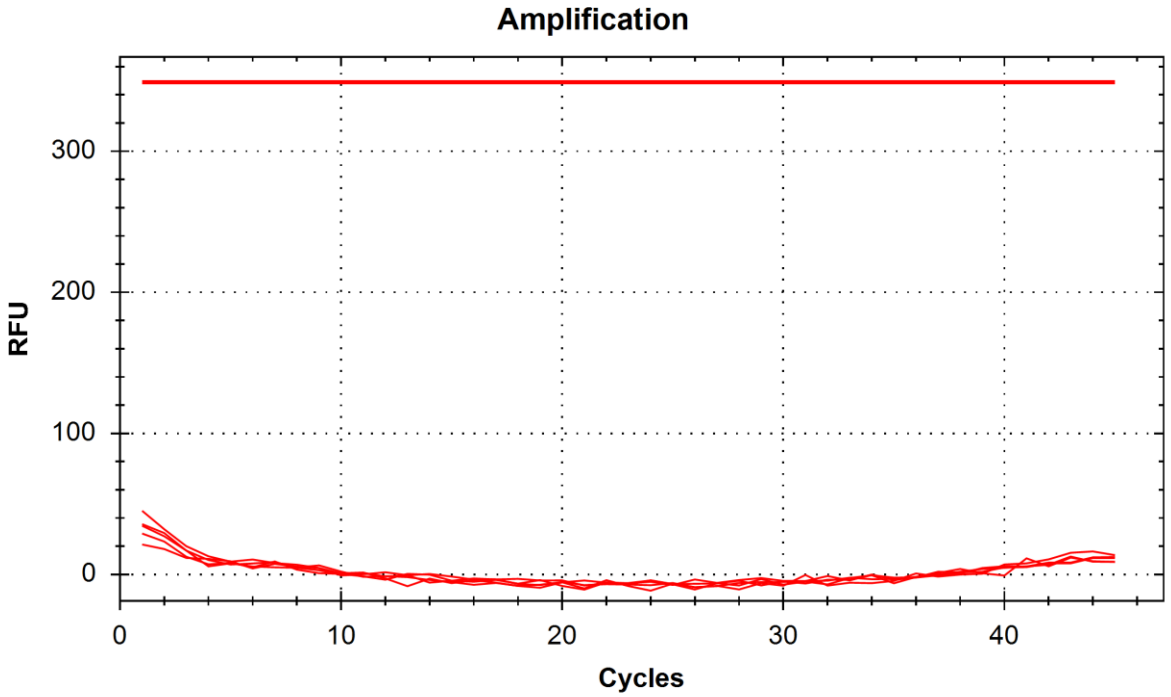

Figure 15: Amplification plots of the negative samples were repeated 5 times in Texas RED dye.

6.2.4. Limit of Detection:

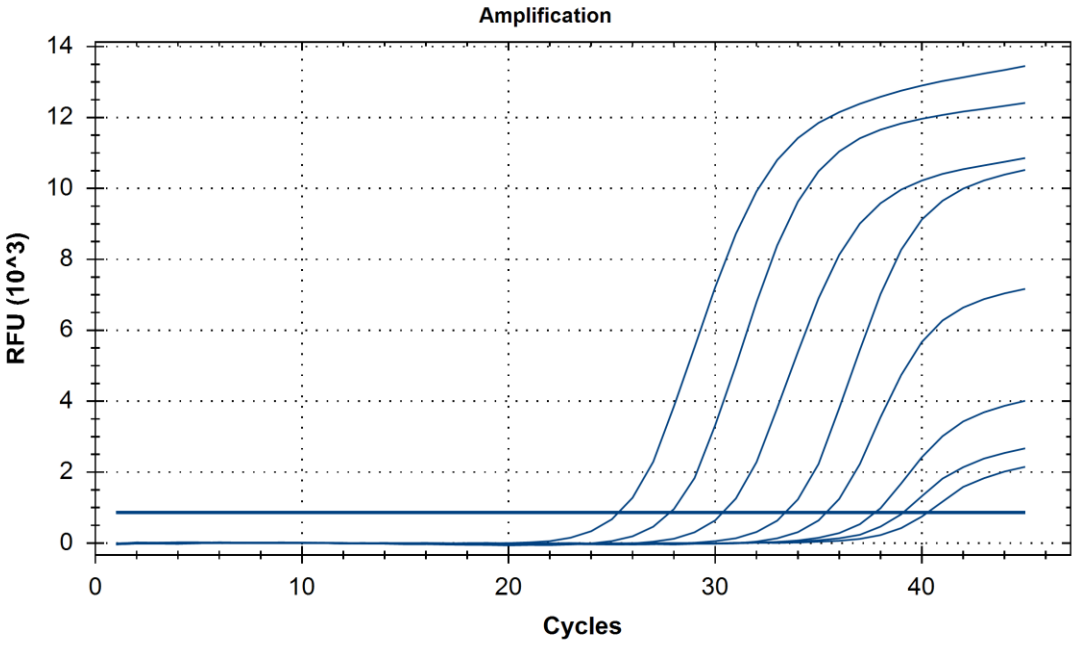

Figure 16: Limit of Detection for RdRp Gene (FAM Dye)

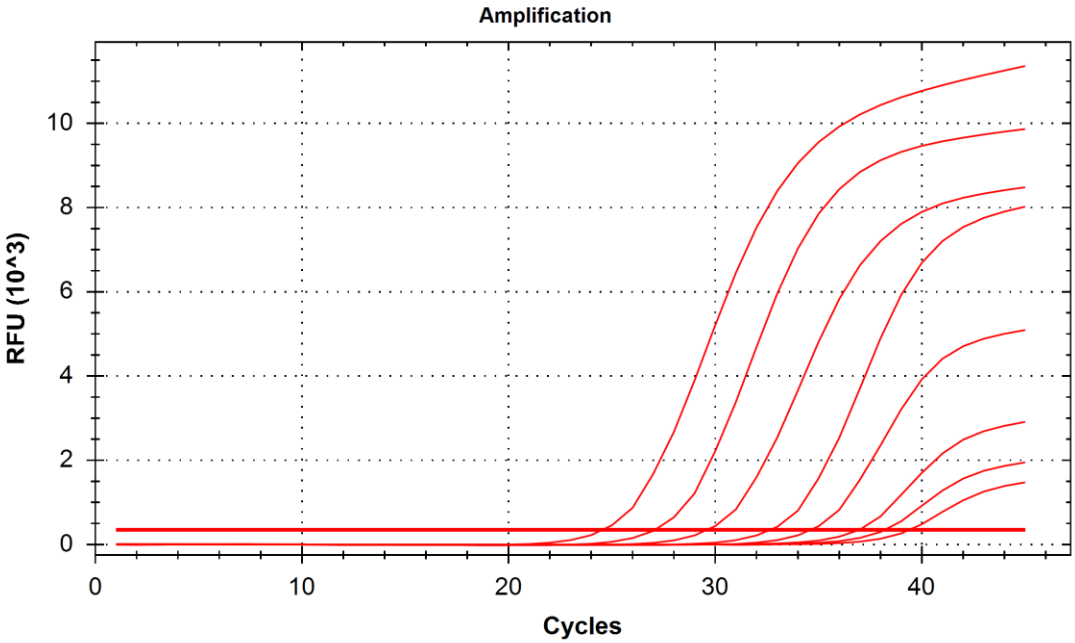

Figure 17: Limit of Detection for Nucleocapsid Gene (Texas RED Dye)

The virus dilutions were tried at different concentrations (Table 10) and the LoD of the test is 1-10 copies/rxn and detected ≤ Ct 43 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 1-10 copies/rxn.

Table 10 : LoD Evaluation

Sample No	Sample (copies/rxn)	FAM Dye (Ct Value)	T.RED Dye (Ct Value)
1	$1x10^7$	25,31	24,54
2	$1x10^6$	27,81	27,08
3	$1x10^5$	30,36	29,59
4	$1x10^4$	33,38	32,65
5	$5x10^3$	35,37	34,61
6	$5x10^2$	37,73	36,97
7	$1x10^2$	39,10	38,20
8	$1x10^1$	40,27	39,39

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:

The performance evaluation of COVID-19 Real Time PCR Kit was tested using known samples. 12 positive samples at 10^3 ~10^8 copies/rxn and additional 12 positive samples at ~ 1-10 copies/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 11.

Table 11: Clinical Evaluation with Known Samples *

Sample Type	Sample Number	FAM	Texas RED	HEX	Result
Nasal / Nasopharyngeal Positive Samples	1	28,35	29,12	24,21	Positive
	2	30,95	31,55	25,05	Positive
10^3 ~10^8 copies/rxn	3	25,63	26,63	23,79	Positive
	4	29,27	30,72	25,81	Positive
	5	20,11	21,45	23,25	Positive
	6	29,48	30,05	24,08	Positive
	7	28,99	29,11	25,91	Positive
	8	27,95	29,94	25,36	Positive
	9	23,45	24,55	23,90	Positive
	10	27,12	28,89	25,03	Positive
	11	29,53	30,75	26,18	Positive
	12	30,60	31,25	25,61	Positive
Nasal / Nasopharyngeal	13	39,44	40,41	24,45	Positive
	14	38,51	39,59	23,31	Positive
1-10 copies/rxn (LoD)	15	40,01	41,05	25,40	Positive
	16	39,25	40,95	24,25	Positive
	17	38,77	39,75	24,99	Positive
	18	39,90	40,88	23,84	Positive
	19	40,12	41,95	25,47	Positive
	20	40,19	41,87	23,75	Positive
	21	39,41	39,98	24,55	Positive
	22	38,27	39,15	25,40	Positive
	23	38,56	39,63	24,12	Positive
	24	40,38	41,84	24,05	Positive
Nasal / Nasopharyngeal Negative Samples	25	-	-	26,15	Negative
	26	-	-	25,89	Negative
	27	-	-	25,74	Negative
	28	-	-	23,48	Negative
	29	-	-	23,18	Negative
	30	-	-	25,62	Negative
	31	-	-	24,15	Negative
	32	-	-	24,75	Negative
	33	-	-	23,73	Negative
	34	-	-	24,54	Negative
	35	-	-	25,75	Negative
	36	-	-	23,89	Negative

*It can be estimated about 95% confident interval, CI, falls between ct18 and ct41.

6.3. QUALITY CONTROL RESULTS

Table 12: 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. TROUBLE SHOOTING AND CAUTIONS

7.1. TROUBLE SHOOTING

If internal control doesn't work,

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

If plots start late,

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

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

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

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

9. REFERENCES

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