Protocol for sanger full length sequencing of the SARS-CoV-2 spike(S) gene

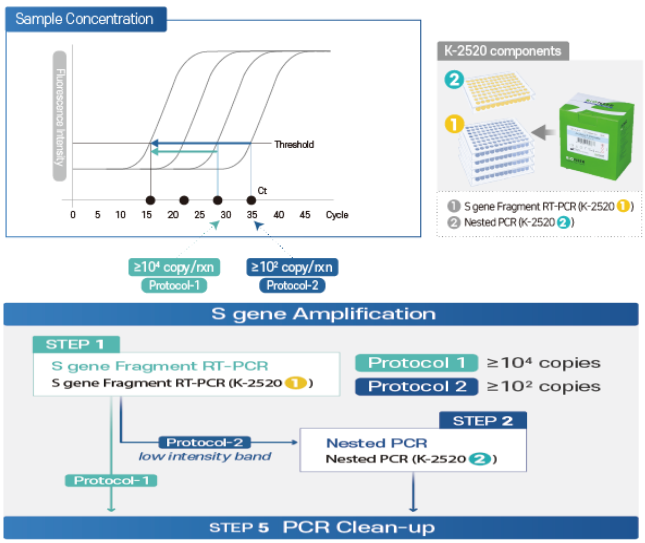
Introduction

COVID-19 virus is continuously evolving to new variants with different shedding capacity and susceptibility to the vaccine, mainly caused by the change of spike protein. The S protein should be sequenced more quickly and efficiently to prevent the super-spreading of the variants, *AccuPower*® SARS-CoV-2 S gene Amplification Kit provides a faster and convenient solution for S gene sequencing. The kit contains every RT-PCR component, including primers and enzymes. Just add your RNA sample into the four tubes provided in the kit. You can amplify the whole S gene for direct sanger sequencing. You can also purchase an additional PCR kit to analyze the S-Protein gene sequence of fewer positive samples, such as 100 copies or less. Direct Sanger Sequencing is possible after PCR purification without cloning.

Note: This protocol and the kit are for Research Use Only. Not for diagnostic procedures.

IMPORTANT: Be cautious with the experiment as it can cause air-contamination or cross-contamination.

Select a protocol according to the concentration of virus in the sample.



Ordering Information

Product Description		Specification	Cat. No.
AccuPower® SARS-CoV-2 S gene Amplification Kit	1 S gene Fragment RT-PCR (24 tests X 4 plate) 2 S gene Nested-PCR (24 tests X 1 plate)	96 rxns	K-2520
Related Products			**Cat. No.**
Sequencing Primer set for SARS-CoV-2 S gene Analysis, 8 Primers			N-8250

Materials needed

	Products	Supplier	Cat. No.
■	**Equipment**		
	AllInOneCycler™ 96 PCR system or similar thermal cycler	BIONEER	A-2041
	ExiSpin™ or similar centrifuge and vortex		A-7040
	Agaro-Power™ System and DUALED Blue/White Transilluminator (or similar instrument)		A-7020 A-6020
	Single-channel and multichannel micropipettes of various sizes capable of pipetting volumes form 1 µl to 1,000 µl	Any	Any
■	**Reagents, kits and consumables**		
	AccuPower® SARS-CoV-2 S gene Amplification Kit	BIONEER	K-2520
	Sequencing Primer set for SARS-CoV-2 S gene Analysis (or request a sanger sequencing service from Bioneer)		N-8250
	Nuclease-Free Water		C-9030
	Microtube	Any	Any
	Filter pipette tips		

Notice

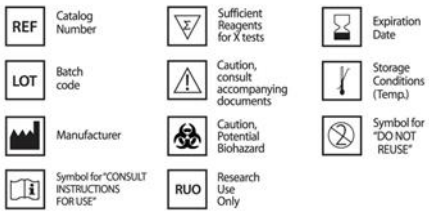
Bioneer corporation reserves the right to make corrections, modifications, improvements and other changes to its products, services, specifications or product descriptions at any time without notice.

Online Resources

Korean

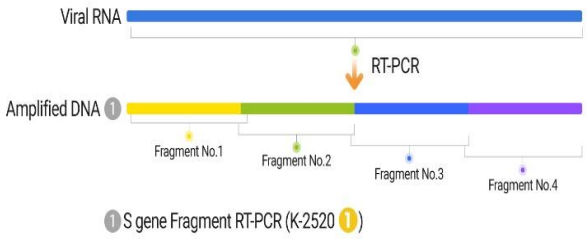
English

Explanation of Symbols



Protocol – 1: One-Step RT-PCR method
(If the concentration of virus is at least 10^4 copies/reaction)

1.1. The one-step RT-PCR is performed for sequencing analysis as follows.



1.2. Prepare a mixture for reaction as follows.

[Mixture composition]

Reagent	Final volume
	100 µl
Sample	1~100 µl (Final concentration >10^4 copies/rxn)
Water	To final 100 µl

1.3. Dispense the mixture into the tube.

1.3.1. Four tubes are in set.
(Tube pellet color. Yellow to Purple)

1.3.2. Dispense 20 µl of mixture in each tube (Figure 1.) and mix well.
Note: Please check the number on the tube before use.

1.3.3. Centrifuge the mixture for 5-10 seconds at 1,000 g, then vortex for 5-10 seconds.

1.3.4. Centrifuge the mixture for 5-10 seconds at 1,000 g.
Note: If the mixture does not dissolve well, please repeat the procedure in section 1.3.3 and 1.3.4.

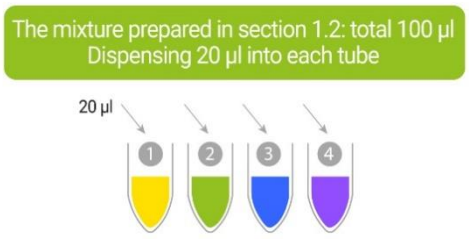


Figure 1. *AccuPower*® SARS-CoV-2 S gene Amplification Kit (S gene fragment RT-PCR)

1.4. Perform one-step RT-PCR

1.4.1. One-step RT-PCR condition

Step	Temp.	Time	Cycles
Reverse transcription	50℃	30 min	1 cycle
Pre-denaturation	95℃	5 min	1 cycle
Denaturation	96℃	10 sec	35 cycles
Annealing	62℃	15 sec	
Extension	68℃	2 min	
Final extension	72℃	5 min	1 cycle

1.4.2. Put samples in the thermal cycler and run the conditions in the table above.

1.4.3. Run agarose gel electrophoresis with 5 µl of PCR product to check whether the PCR has progressed well.

1.4.4. Each Fragment of PCR product size
Fragment 1(Yellow color): 1,707 bp
Fragment 2(Green color): 2,101 bp
Fragment 3(Blue color): 2,017 bp
Fragment 4(Purple color): 1,695 bp

Note: There is no need to add the loading dye because it is already included in the premix. It is recommended to freeze the PCR product if it is not being used immediately for sequencing analysis.

1.5. Prepare for sequencing analysis.

1.5.1. Purify the PCR product using PCR purification kit or PCR Clean-Up Kit.

1.6. Perform sequencing analysis.

1.6.1. Perform sequencing analysis for S gene with ‘Sequencing Primer set for SARS-CoV-2 S gene Analysis (Cat. No. N-8250)’, which include sequencing primers as shown in the table below.

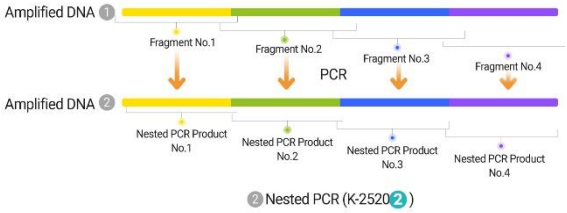
Note: If you request analysis from BIONEER, you do not need to purchase a Sanger Sequencing Primer set separately.

Note:
If the PCR product is not detected after executing protocol 1 or the band intensity is low, perform nested PCR with the protocol 2 method.

Protocol – 2: Nested PCR method for high sensitivity (If the concentration of virus is at least 10^2 copies/reaction)

2.1. To increase the sensitivity, Nested PCR are performed for sequencing analysis as follows.

(Amplified DNA 1 is PCR product using fragment RT-PCR PreMix)



2.2. Prepare a mixture for reaction as follows.

Using the *AccuPower*® SARS-CoV-2 S gene Amplification Kit (S gene Nested PCR), the tube color is yellow.

[Mixture composition]

Reagent	Final volume
	Each tube20 µl
Sample	2 µl (S gene Fragment RT-PCR Product)
Water	To final 18 µl

2.3. Dispense the mixture into the tube.

Using the *AccuPower*® SARS-CoV-2 S gene Amplification Kit (S gene Nested PCR).
Note: Please check the pellet color on the tube before use. (tube color is yellow)

2.3.1. Four tubes are in 1 set. (Tube pellet color. Yellow to Purple)
2.3.2. Add the fragment RT-PCR product to the nested PCR tube (yellow tube) of the same pellet color.
2.3.3. Centrifuge the mixture for 5-10 seconds at 1,000 g, then vortex for 5-10 seconds.
2.3.4. Centrifuge the mixture for 5-10 seconds at 1,000 g.

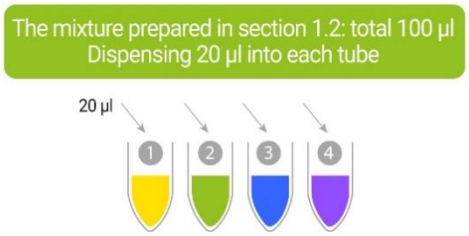


Figure 1. *AccuPower*® SARS-CoV-2 S gene Amplification Kit (S gene fragment RT-PCR)

2.4. Perform one-step RT-PCR

2.4.1. Nested-PCR condition

Step	Temp.	Time	Cycles
Pre-denaturation	95℃	5 min	1 cycle
Denaturation	96℃	10 sec	30 cycles
Annealing	62℃	15 sec	
Extension	68℃	30 sec	
Final extension	72℃	5 min	1 cycle

2.4.2. Put samples in the thermal cycler and run the conditions in the table above.
2.4.3. Run agarose gel electrophoresis with 5 µl of PCR product to check whether the PCR has progressed well.
2.4.4. Each Fragment of PCR product size
Fragment 1(Yellow color): 924 bp
Fragment 2(Green color): 1,316 bp
Fragment 3(Blue color): 1,249 bp
Fragment 4(Purple color): 1,297 bp

Note: There is no need to add the loading dye because it is already included in the premix. It is recommended to freeze the PCR product if it is not being used immediately for sequencing analysis.

2.5. Prepare for sequencing analysis.

2.5.1. Purify the PCR product using PCR purification kit or PCR Clean-Up Kit.

2.6. Perform sequencing analysis.

2.6.1. Perform sequencing analysis for S gene with 'Sequencing Primer set for SARS-CoV-2 S gene Analysis (Cat. No. N-8250)', which include sequencing primers as shown in the table below.

Note: If you request analysis from BIONEER, you do not need to purchase a Sanger Sequencing Primer set separately.