



SARS-CoV-2 S Gene PCR+Sanger Sequencing Kit (SSGPS)

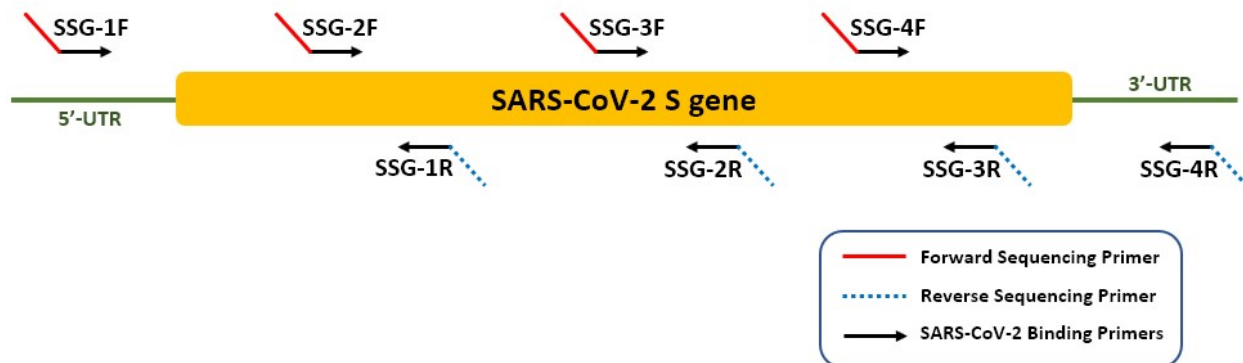
Catalog #RU7158
40 samples

Product Description

Coronaviruses are a family of large RNA viruses with size ranging from 26 to 32 kb. These viruses are zoonotic and in humans can cause respiratory infections. As the coronavirus is an RNA virus, it has a relatively high mutation rate resulting in rapid evolution. In December 2019, a new deadly coronavirus known as SARS-CoV-2, which has a high sequence similarity to SARS-CoV, was identified as the cause of the Covid-19 outbreak. Since then, numerous variants of SARS-CoV-2 were reported around the world. Evidence has shown that some S gene mutations have the potential to increase transmissibility by altering the interactions with human angiotensin-converting enzyme 2 (ACE2) gene, which serves as a cellular receptor for SARS-CoV-2. It is critical, therefore, to monitor the mutations in the S gene of SARS-CoV-2 viruses.

ScienCell's SARS-CoV-2 S Gene PCR+Sanger Sequencing Kit (SSGPS) provides a convenient and fast way to sequence the entire S gene of SARS-CoV-2 coronavirus in samples. Included in the kit are four amplification primer sets (SSG-1F/1R, 2F/2R, 3F/3R, and 4F/4R, see Figure 1), which can amplify the four regions that together cover the entire SARS-CoV-2 S gene. Linked to each of the amplification primers is a sequencing primer (forward or reverse, see Figure 1), therefore, all four PCR products can be conveniently sequenced by the forward and reverse sequencing primers that are included in the kit.

Figure 1. Primer binding locations on the SARS-CoV-2 S gene.



Kit Components

Cat #	Component	Quantity	Storage
RU7158a	SSG-1 primer set (SSG-1F and SSG-1R, 10 μ M each)	200 μ L	-20°C
RU7158b	SSG-2 primer set (SSG-2F and SSG-2R, 10 μ M each)	200 μ L	-20°C
RU7158c	SSG-3 primer set (SSG-3F and SSG-3R, 10 μ M each)	200 μ L	-20°C
RU7158d	SSG-4 primer set (SSG-4F and SSG-4R, 10 μ M each)	200 μ L	-20°C
RU7158e	SSG Forward sequencing primer, 10 μ M	400 μ L	-20°C
RU7158f	SSG Reverse sequencing primer, 10 μ M	400 μ L	-20°C
MB6058a	RubyNStart One-step RT-PCR master mix	500 μ L	-20°C
MB6058b	RubyNStart One-step RT-PCR enhancer	1 mL	-20°C

Additional Materials Required (Materials Not Included in Kit)

Component	Recommended
Viral RNA extraction kit	ScienCell Viral RNA Isolation Kit (ScienCell, Cat #MB891)
PCR purification kit	SpeedDNA PCR Purification Kit (ScienCell, Cat #MB6948)
Sanger sequencing kit	Customer's choice
PCR plates or tubes	

Quality Control

The primers are validated by PCR, followed by gel electrophoresis and Sanger sequencing.

Product Use

For Research Use Only. Not for use in diagnostic procedures.

Shipping and Storage

SSGPS is shipped on dry ice. Upon receipt, store the primers, RubyNStart One-step RT-PCR master mix, and RubyNStart One-step RT-PCR enhancer at -20°C in a manual defrost freezer.

Procedures

Important: *Only use nuclease-free reagents in PCR amplification.*

1. Thaw the four amplification primer sets, SSG-1F/1R, 2F/2R, 3F/3R, and 4F/4R (Cat #RU7158a-d). Aliquot as needed. Store at -20°C in a manual defrost freezer. Avoid repeated freeze-and-thaw cycles.
2. For each purified SARS-CoV-2 RNA sample, prepare four 40 µl PCR reactions as shown in Table 2.

Table 2.

Purified SARS-CoV-2 RNA	5 µl
Amplification primer set (Cat #RU7158a, b, c or d)	5 µl
4X RubyNStart One-step RT-PCR master mix	10 µl
2X RubyNStart One-step RT-PCR enhancer	20 µl
Total volume	40 µl

3. Seal the PCR reaction tubes. Centrifuge the tubes at 1,500x g for 15 seconds.
4. Refer to Table 3 for PCR program setup.

Table 3.

Step	Temperature	Time	Number of cycles
Reverse transcription	55°C	20 min	1
Initial denaturation	95°C	10 min	1
Denaturation	95°C	10 sec	40
Annealing	66°C	10 sec	
Extension	72°C	1 min	
Final extension	72°C	5 min	1

5. Refer to the Instructions for Use of the PCR purification kit to be used for the purification of the PCR products. This step is necessary to remove primer dimers formed during the PCR amplification. In the final DNA elution step, to maximize the product concentration, use the minimum amount of H₂O or elution buffer required by the PCR purification kit to elute. Purified PCR products can be used in the following Sanger sequencing analyses.
6. Refer to the Instructions for Use of the Sanger sequencing kit to be used for the sequencing of the PCR products. For each of the four purified PCR products, two sequencing reactions should be prepared with each of the two sequencing primers included in the kit.