SARS-CoV-2 SYBR® Green qPCR Quantification Kit (SVSGQ)

Catalog #RU7058
100 reactions

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 (previously known as 2019-nCoV), which has a high sequence similarity to SARS-CoV, was identified.

ScienCell's SARS-CoV-2 SYBR® Green qPCR Quantification Kit (SVSGQ) is designed to purify and quantify the SARS-CoV-2 coronavirus presence in samples. Four primer sets are included in the kit. Two of them (Cat #7058a and 7058b) target coronavirus SARS-CoV-2 nucleocapsid (N) gene and spike (S) gene, respectively. The human ACE2 gene primer set (Cat #7058c) targets human angiotensin-converting enzyme 2 (ACE2) gene, which serves as a cellular receptor for SARS-CoV-2. The human ACTB gene primer set (Cat #7058d) targets human β-actin (ACTB) housekeeping gene, which serves as a normalization control for cell number quantification. All primer sets are verified to possess high specificity and efficiency near 100%, with no primer dimer formation under recommended PCR conditions.

Additionally, a non-infectious positive control (Cat #7058-Pos) and nuclease-free water (Cat #GQ100-4) are included in the kit. The positive control (Cat #7058-Pos) consists of non-infectious RNA fragments spiked with human small airway epithelial cells and serves to ensure reagents and instruments are working properly.

Kit Components

Cat #	Component	Quantity	Storage
MB6018a-1	GoldNStart TaqGreen qPCR Master Mix	1 mL x 4	-20°C
7058a	SARS-CoV-2 N gene primer set, in solution	200 µL	-20°C
7058b	SARS-CoV-2 S gene primer set, in solution	200 µL	-20°C
7058c	Human ACE2 gene primer set, in solution	200 µL	-20°C
7058d	Human ACTB gene primer set, in solution	200 µL	-20°C
GQ100-4	Nuclease-free H_2O	4 mL	4°C
7058-Pos	Positive control (non-infectious; RNA: 500 – 1000 copies/µL, cells: 200 – 300 counts/µL)	200 µL	-80°C

Additional Materials Required (Materials Not Included in Kit)

Component	Recommended
RNA Isolation Kit	ScienCell Viral RNA Isolation Kit (ScienCell, Cat #MB891)
cDNA Synthesis Master Mix	First-Strand cDNA Synthesis Master Mix (ScienCell, Cat #MB6008)
qPCR plate or tube	

Quality Control
The primer sets are validated by qPCR using serially diluted templates. The positive control are validated by qPCR. The PCR products are analyzed by gel electrophoresis.

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

Shipping and Storage
The product is shipped on dry ice. Upon receipt, store the GoldNStart TaqGreen qPCR master mix (Cat #MB6018a-1) and primer sets (Cat #7058a, 7058b, 7058c, and 7058d) at -20°C in a manual defrost freezer, the positive control (Cat #7058-Pos) at -80°C, and nuclease-free H_2O (Cat #7058-H2O) at 4°C. Aliquot as needed. Avoid repeated freeze-and-thaw cycles.

Procedures

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

Note: *This master mix does not contain a ROX passive reference dye. If the qPCR instrument being used has a "ROX passive reference dye" option, please deselect this option.*

1. The positive control (Cat #7058-Pos) consists of non-infectious RNA fragments spiked with human small airway epithelial cells. Its RNA isolation and cDNA synthesis should be done concurrently with test samples. 30 ul of the positive control (Cat #7058-Pos) is recommended for one RNA extraction procedure using ScienCell Viral RNA Isolation Kit (ScienCell, Cat #MB891) to get isolated positive control RNA. The positive control (Cat #7058-Pos) included in the kit is enough for 6 extractions. 10 ul of isolated positive control RNA is recommended for each cDNA synthesis reaction using First-Strand cDNA Synthesis Master Mix (ScienCell, Cat #MB6008) to get reverse-transcribed positive control.

2. Prior to use, allow the primer sets (Cat #7058a, 7058b, 7058c, and 7058d) to thaw to room temperature. Vortex gently to mix well.

3. Centrifuge the vials at 1,500x g for 1 minute.

4. Aliquot each primer set as needed. Store at -20°C in a manual defrost freezer. Avoid repeated freeze-and-thaw cycles. Keep on ice when thawed.

5. With test samples, two control samples should be run concurrently: the reverse-transcribed positive control and H_2O (Cat #GQ100-4) as the No Template Control (NTC). Prepare four qPCR reactions for each control sample, one with #7058a primer set, one with #7058b primer set, one with #7058c primer set, and one with #7058d primer set. Prepare a 20 µl qPCR reaction for one well as shown in Table 1.

Table 1.

Control sample (reverse-transcribed positive control or H_2O)	5 µl
Primer set (Cat #7058a, 7058b, 7058c, or 7058d)	2 µl
GoldNStart TaqGreen qPCR master mix (Cat # MB6018a-1)	10 µl
Nuclease-free H_2O (Cat #GQ100-4)	3 µl
Total volume	***20 µl***

6. For each reverse-transcribed cDNA test sample, prepare four qPCR reactions, one with #7058a primer set, one with #7058b primer set, one with #7058c primer set, and one with #7058d primer set. Prepare a 20 µl qPCR reaction for one well as shown in Table 2.

Table 2.

Reverse-transcribed cDNA test sample (concentration varies)	5 µl
Primer set (Cat #7058a, 7058b, 7058c, or 7058d)	2 µl
GoldNStart TaqGreen qPCR master mix (Cat # MB6018a-1)	10 µl
Nuclease-free H_2O (Cat #GQ100-4)	3 µl
Total volume	***20 µl***

7. Seal the qPCR reaction wells. Centrifuge the plates or tubes at 1,500x g for 15 seconds.
8. Setup qPCR reactions as shown in Table 3.

Table 3. Instrument settings for qPCR reactions.

Step	Temperature	Time	Number of cycles
Taq DNA polymerase activation	95°C	10 min	1
Denaturation	95°C	20 sec	40
Annealing	65°C	20 sec	
Extension	72°C	20 sec	
Data acquisition	Plate read, detector (SYBR® Green)		
Optional	Melting curve analysis		1
Hold	20°C	Indefinite	1

Figure 1. A typical amplification curve showing the amplification of a qPCR product.

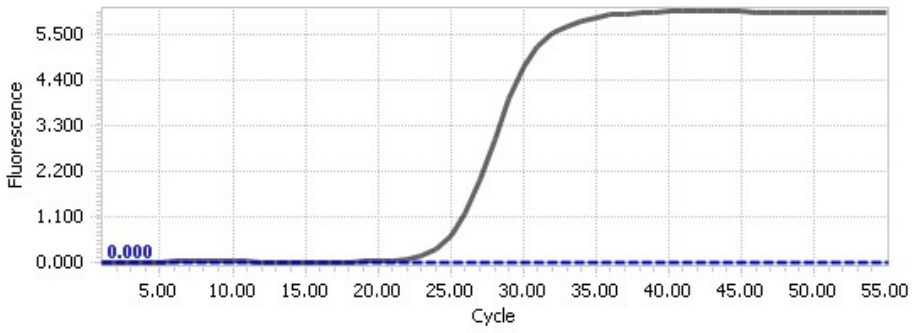
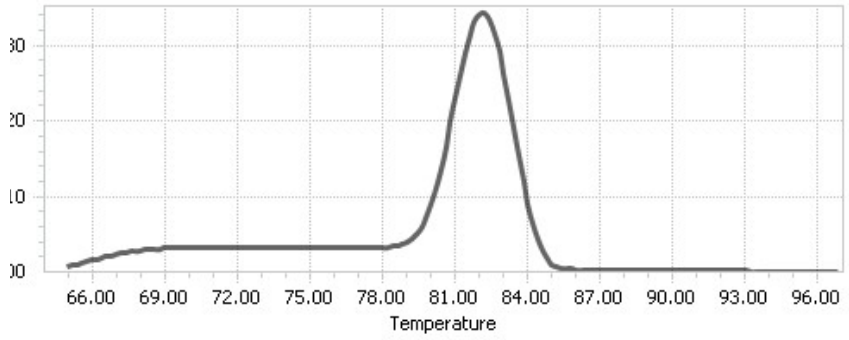


Figure 2. A typical melting peak of a qPCR product.



Results Interpretation

Table 4. SVSGQ kit control sample test results interpretation.

Sample	N gene	S gene	ACE2	ACTB	Results Interpretation
7058-Pos	+	+	+	+	Expected
	-	-	-	-	Reverse transcription and/or PCR failed
GQ100-4 (H_2O)	-	-	-	-	Expected
	If anyone of three targets is positive				Reagent(s) contaminated

Note: Cq values less than 35 are considered positive. Cq values equal to or greater than 35 are considered negative.

Table 5. SVSGQ kit target sample test results interpretation when control results are as expected.

N gene	S gene	ACE2	ACTB	Results Interpretation
If one or two targets are positive		±	±	SARS-CoV-2 detected
-	-	If one or two targets are positive		SARS-CoV-2 not detected
-	-	-	-	Invalid result

Note: Cq values less than 35 are considered positive. Cq values equal to or greater than 35 are considered negative.

Quantification Method: Comparative ΔΔCq (Quantification Cycle Value) Method

Note: *When quantifying SARS-CoV-2 level in a sample, ONLY proceed to quantification calculations when three Cq values (N gene, S gene, and ACTB) for that sample are less than 35. When quantifying ACE2 level in a sample, ONLY proceed to quantification calculations when both Cq values (ACE2 and ACTB) for that sample are less than 35.*

1. For SARS-CoV-2 virus (SCV2), Cq (SCV2) is the average of the two Cq values for N gene and S gene, and ΔCq (SCV2) is the quantification cycle number difference of SCV2 between the target and the reference samples.

   Cq (SCV2) = (Cq (N gene) + Cq (S gene))/2

   ΔCq (SCV2) = Cq (SCV2, target sample) - Cq (SCV2, reference sample)

   Note: the value of ΔCq (SCV2) can be positive, 0, or negative.

2. For human ACE2 gene (ACE2), ΔCq (ACE2) is the quantification cycle number difference of ACE2 between the target and the reference samples.

   ΔCq (ACE2) = Cq (ACE2, target sample) - Cq (ACE2, reference sample)

   Note: the value of ΔCq (ACE2) can be positive, 0, or negative.

3. For human β-actin housekeeping gene (ACTB), ΔCq (ACTB) is the quantification cycle number difference of ACTB between the target and the reference samples.

   ΔCq (ACTB) = Cq (ACTB, target sample) - Cq (ACTB, reference sample)

   Note: the value of ΔCq (ACTB) can be positive, 0, or negative.

4. ΔΔCq (SCV2) = ΔCq (SCV2) - ΔCq (ACTB)

5. ΔΔCq (ACE2) = ΔCq (ACE2) - ΔCq (ACTB)

6. The ratio of SARS-CoV-2 level in target sample to reference sample on a per cell basis = $2^{-\Delta\Delta \text{ Cq (SCV2)}}$

7. The ratio of ACE2 expression level in target sample to reference sample = $2^{-\Delta\Delta Cq \text{ (ACE2)}}$

Example Calculations: Comparative ΔΔCq (Quantification Cycle Value) Method

Table 6. Cq values obtained for the samples.

Primer set	*Target sample*	*Reference sample*
N gene	15.33	18.54
S gene	15.17	18.72
ACE2	21.08	20.99
ACTB	25.64	20.80

Cq (SCV2, target sample) = (Cq (N gene, target sample) + Cq (S gene, target sample))/2

= (15.33 + 15.17)/2

= 15.25

Cq (SCV2, reference sample) = (Cq (N gene, reference sample) + Cq (S gene, reference sample))/2

= (18.54 + 18.72)/2

= 18.63

ΔCq (SCV2) = Cq (SCV2, target sample) - Cq (SCV2, reference sample)

= 15.25 – 18.63

= -3.38

ΔCq (ACE2) = Cq (ACE2, target sample) - Cq (ACE2, reference sample)

= 21.08 - 20.99

= 0.09

ΔCq (ACTB) = Cq (ACTB, target sample) - Cq (ACTB, reference sample)

= 25.64 - 20.80

= 4.84

ΔΔCq (SCV2) = ΔCq (SCV2) - ΔCq (ACTB)

= -3.38- (4.84)

= -8.22

ΔΔCq (ACE2) = ΔCq (ACE2) - ΔCq (ACTB)

= 0.09- (4.84)

= -4.75

The ratio of SARS-CoV-2 level in target sample to reference sample on a per cell basis

$= 2^{8.22}$

= 298

The ratio of ACE2 expression level in target sample to reference sample

$= 2^{-\Delta\Delta Cq}$

$= 2^{4.75}$

= 26.9

Conclusions:

The ratio of SARS-CoV-2 level in target sample to reference sample on a per cell basis is 298.
The ratio of ACE2 expression level in target sample to reference sample is 26.9.