



SARS-CoV-2 Major Variant Strains Multiplex RT-qPCR Screening Kit (SCVVS)

Catalog #RU7148
100 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. Among them, several major variant strains, including B.1.1.7 (also known as 501Y.V1), B.1.351 (also known as 501Y.V2), P.1, and P.2, have gained enormous attention. These variant strains show increased transmissibility and have become major strains spreading globally.

Several mutations identified in these strains are thought responsible for the higher infection rates and immune evasion. These mutations include spike-HV 69-70 deletion, the spike-N501Y mutation, and the spike-E484K mutation. The spike-HV 69-70 deletion mutation may promote viral immune escape and is considered the hallmark of the B.1.1.7 lineage, as it is not present in other major circulating SARS-CoV-2 strains including 501Y.V2, P.1, and P.2. The spike-N501Y mutation may contribute to the higher viral binding affinity to the ACE2 receptor on the human cell membrane, and is present in some major circulating SARS-CoV-2 strains including B.1.1.7, 501Y.V2, and P.1. The spike-E484K mutation may contribute to the immune evasion of SARS-CoV-2 and dampen current vaccine efficacy. This mutation is present in some major circulating SARS-CoV-2 strains including 501Y.V2, P.1 and P.2 (Table 1).

Table 1. List of major SARS-CoV-2 variant strains and their key mutations

<i>B.1.1.7</i>	HV69-70 deletion	N501Y	
<i>B.1.351</i>		N501Y	E484K
<i>P.1</i>		N501Y	E484K
<i>P.2</i>			E484K

ScienCell's SARS-CoV-2 Major Variant Strains Multiplex RT-qPCR Screening Kit (SCVVS) is designed to screen for the SARS-CoV-2 variant strain presence by detecting these major mutations. Four multiplex primer/probe set components (Cat #7148-REF, #7118-H69V70, #7128-N501Y, and #7138-E484K) are included in the kit. The reference primer/probe set component (Cat #7148-REF) contains 3 primer/probe sets, N1-FAM, N2-FAM, and RP-HEX (Table 2). Among them, N1-FAM and N2-FAM target two regions on the coronavirus SARS-CoV-2 nucleocapsid (N) gene, and RP-HEX targets the exon 1 of human RPP30 gene and serves as a control to assess specimen quality. The HV 69-70 primer/probe set component (Cat #7118-H69V70) contains 2 primer/probe sets, H69V70-Del-FAM and H69V70-Present-HEX (Table 3),

which target the coronavirus SARS-CoV-2 spike (S) gene with HV 69-70 deleted and present, respectively. The N501Y primer/probe set component (Cat #7128-N501Y) contains 2 primer/probe sets, Y501-FAM and N501-HEX (Table 4), which target the coronavirus SARS-CoV-2 spike (S) gene with a tyrosine (Y) and an asparagine (N) at position 501, respectively. The E484K primer/probe set component (Cat #7138-E484K) contains 2 primer/probe sets, K484-FAM and E484-HEX (Table 5), which target the coronavirus SARS-CoV-2 spike (S) gene with a lysine (K) and a glutamic acid (E) at position 484, respectively. For more efficient screening, if the expected mutation rate is low, a pool of up to 10 RNA samples can be used as the template for one qPCR reaction. If results for H69V70-Del-FAM, Y501-FAM, or K484-FAM are negative, then all pooled samples do not contain the spike-HV 69-70 deletion mutation, N501Y, or E484K mutation, respectively. For H69V70-Del-FAM, Y501-FAM, or K484-FAM positive pooled samples, the pooled samples should be tested individually to identify the mutated one(s). Please refer to Tables 9 through 12 for results interpretation.

Table 2. Primer/probe set list of the reference primer/probe set component (Cat #7148-REF)

Primer/Probe Set	Primer/Probe Target	Probe Reporter Dye
N1-FAM	SARS-CoV-2 nucleocapsid (N) gene, region 1	FAM
N2-FAM	SARS-CoV-2 nucleocapsid (N) gene, region 2	FAM
RP-HEX	Human RPP30 gene	HEX

Table 3. Primer/probe set list of the HV 69-70 primer/probe set component (Cat #7118-H69V70)

Primer/Probe set	Primer/Probe Target	Probe Reporter Dye
H69V70-Del-FAM	SARS-CoV-2 spike (S) gene, HV 69-70 deleted	FAM
H69V70-Present-HEX	SARS-CoV-2 spike (S) gene, HV 69-70 present	HEX

Table 4. Primer/probe set list of the N501Y primer/probe set component (Cat #7128-N501Y)

Primer/Probe set	Primer/Probe Target	Probe Reporter Dye
Y501-FAM	SARS-CoV-2 spike (S) gene, N501Y mutation	FAM
N501-HEX	SARS-CoV-2 spike (S) gene, N501 not mutated	HEX

Table 5. Primer/probe set list of the E484K primer/probe set component (Cat #7138-E484K)

Primer/Probe set	Primer/Probe Target	Probe Reporter Dye
K484-FAM	SARS-CoV-2 spike (S) gene, E484K mutation	FAM
E484-HEX	SARS-CoV-2 spike (S) gene, E484 not mutated	HEX

In addition, ScienCell One-Step TaqProbe RT-qPCR master mix (Cat #MB802a), a non-infectious positive control (Cat #7148-Pos), and nuclease-free water (Cat #7148-H2O) are included in the kit. The positive control (Cat #7148-Pos) consists of non-infectious viral RNA fragments of the original strain, the B.1.1.7 lineage, the B.1.351 lineage, the P.1 lineage, and the P.2 lineage of SARS-CoV-2 spiked into human small airway epithelial cells.

Kit Components

Cat #	Component	Quantity	Storage
MB802a	One-Step TaqProbe RT-qPCR master mix, 4x	2 x 1.5 mL	-20°C
7148-REF	Reference multiplex primer/probe sets, in solution	600 µL	-20°C
7118-H69V70	HV 69-70 multiplex primer/probe sets, in solution	600 µL	-20°C
7128-N501Y	N501Y multiplex primer/probe sets, in solution	600 µL	-20°C
7138-E484K	E484K multiplex primer/probe sets, in solution	600 µL	-20°C
7148-H2O	Nuclease-free H ₂ O	4 mL	4°C
7148-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 samples	Customers' samples
Viral RNA isolation kit	ScienCell Viral RNA Isolation Kit (ScienCell, Cat #MB891)
qPCR plate or tube	

Quality Control

The primer/probe sets and the positive control are validated by RT-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 One-Step TaqProbe RT-qPCR master mix (Cat #MB802a) and the primer/probe sets (Cat #7148-REF, #7118-H69V70, #7128-N501Y, and #7138-E484K) at -20°C in a manual defrost freezer, the positive control (Cat #7148-Pos) at -80°C, and nuclease-free H₂O (Cat #7148-H2O) at 4°C.

Procedures

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

1. Prior to use, allow the multiplex primer/probe sets (Cat #7148-REF, #7118-H69V70, #7128-N501Y, and #7138-E484K) to thaw to room temperature in the dark. Shake gently to mix well.
2. Centrifuge the vials at 1,500x g for 1 minute.
3. Aliquot multiplex primer/probe sets as needed. Store at -20°C in a manual defrost freezer. Avoid repeated freeze-and-thaw cycles. Maintain cold and in the dark when thawed.
4. For each test run, two control samples should be included, the non-infectious positive control (Cat #7148-Pos), and H₂O (Cat #7148-H2O) as the No Template Control (NTC). Prepare four 20 µl RT-qPCR reactions as shown in Table 6 for each control sample, one with the reference primer/probe set component (Cat #7148-REF), one with the HV 69-70 primer/probe set component (Cat #7118-H69V70), one with the N501Y primer/probe set component (Cat #7148- N501Y), and one with the E484K primer/probe set component (Cat #7148- E484K).

Table 6.

Control sample (Cat #7148-Pos or 7148-H2O)	5 µl
Multiplex primer/probe sets (Cat #7148-REF, #7118-H69V70, #7128-N501Y, or #7138-E484K)	6 µl
1-step RT-qPCR Master mix, 4x (Cat #MB802a)	5 µl
Nuclease-free H ₂ O (Cat #7148-H2O)	4 µl
Total volume	20 µl

1. For each extracted RNA test sample (individual or pooled of up to 10 samples), prepare four 20 µl RT-qPCR reactions as shown in Table 7, one with the reference primer/probe set component (Cat #7148-REF), one with the HV 69-70 primer/probe set component (Cat #7118-H69V70), one with the N501Y primer/probe set component (Cat #7148-N501Y), and one with the E484K primer/probe set component (Cat #7148- E484K).

2. Table 7.

RNA test sample (individual or pooled, concentration varies)	5 µl
Multiplex primer/probe sets (Cat #7148-REF, #7118-H69V70, #7128-N501Y, or #7138-E484K)	6 µl
1-step RT-qPCR Master mix, 4x (Cat #MB802a)	5 µl
Nuclease-free H ₂ O (Cat #7148-H2O)	4 µl
Total volume	20 µl

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

Table 8. Instrument settings for RT-qPCR reactions. Fluorescence data for both FAM and HEX channels should be collected during the data acquisition step.

Step	Temperature	Time	Number of cycles
UNG incubation	25°C	2 min	1
Reverse transcription	50°C	15 min	1
Enzyme activation	95°C	2 min	1
Denaturation	95°C	3 sec	45
Annealing and extension	66°C	30 sec	
Data acquisition	Plate read, detector (both FAM and HEX)		

Results Interpretation

Table 9. SCVVS kit control sample test results interpretation. A Cq value lower than 40 is considered positive.

Sample	Primer/probe Set	FAM	HEX	Results Interpretation
7148-Pos	7148-REF	+	+	Expected
		-	-	Reverse transcription and/or PCR failed
	7118-H69V70	+	+	Expected
		-	-	Reverse transcription and/or PCR failed
7148-H2O	7148-REF	-	-	Expected
		If either or both are positive		Reagent(s) contaminated
	7118-H69V70	-	-	Expected
		If either or both are positive		Reagent(s) contaminated

Table 10. SCVVS kit target sample test results interpretation for spike-HV 69-70 deletion when control results are as expected. A Cq value lower than 40 is considered positive.

Primer/probe Set	7148-REF		7118-H69V70		Results Interpretation
	FAM	HEX	FAM	HEX	
Results	+	±	+	-	SARS-CoV-2 detected, B.1.1.7 lineage implied
	+	±	-	+	SARS-CoV-2 detected, NOT B.1.1.7 lineage
	+	±	+	+	SARS-CoV-2 detected, mix of lineages with B.1.1.7 present implied
	+	±	-	-	SARS-CoV-2 detected, possibly a novel lineage
	-	+	-	-	SARS-CoV-2 NOT detected
	All other combinations				Invalid result

Table 11. SCVVS kit target sample test results interpretation for N501Y mutation when control results are as expected. A Cq value lower than 40 is considered positive.

Primer/probe Set	7148-REF		7128-N501Y		Results Interpretation
Detection channel	FAM	HEX	FAM	HEX	
Results	+	±	+	-	SARS-CoV-2 detected, N501Y mutation present
	+	±	-	+	SARS-CoV-2 detected, N501 not mutated
	+	±	+	+	SARS-CoV-2 detected, mix of N501 and Y501
	+	±	-	-	SARS-CoV-2 detected, possibly a novel lineage
	-	+	-	-	SARS-CoV-2 NOT detected
	All other combinations				Invalid result

Table 12. SCVVS kit target sample test results interpretation for E484K mutation when control results are as expected. A Cq value lower than 40 is considered positive.

Primer/probe Set	7148-REF		7138-E484K		Results Interpretation
Detection channel	FAM	HEX	FAM	HEX	
Results	+	±	+	-	SARS-CoV-2 detected, E484K mutation present
	+	±	-	+	SARS-CoV-2 detected, E484 not mutated
	+	±	+	+	SARS-CoV-2 detected, mix of E484 and K484
	+	±	-	-	SARS-CoV-2 detected, possibly a novel lineage
	-	+	-	-	SARS-CoV-2 NOT detected
	All other combinations				Invalid result