



SARS-CoV-2 E484K Mutation Multiplex RT-qPCR Screening Kit (SCVEK)

Catalog #RU7138

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, one mutation was detected within the Spike protein of SARS-CoV-2 and is known as the spike-E484K mutation. This variant causes the amino acid glutamic acid at position 484 (E484) mutate to a lysine (K484), and it 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.

ScienCell's SARS-CoV-2 E484K Mutation Multiplex RT-qPCR Screening Kit (SCVEK) is designed to screen for the presence of the SARS-CoV-2 spike-E484K mutation. Two multiplex primer/probe set components (Cat #7138-REF and #7138-E484K) are included in the kit. The reference primer/probe set component (Cat #7138-REF) contains 3 primer/probe sets, N1-FAM, N2-FAM, and RP-HEX (Table 1). Among them, N1-FAM and N2-FAM target two regions on the coronavirus SARS-CoV-2 nucleocapsid (N) gene. RP-HEX targets the exon 1 of human RPP30 gene and serves as a control to assess specimen quality. The E484K primer/probe set component (Cat #7138-E484K) contains 2 primer/probe sets, K484-FAM and E484-HEX (Table 2), 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 E484K 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 K484-FAM are negative, then all pooled samples do not contain the E484K mutation. For K484-FAM positive pooled samples, the pooled samples should be tested individually to identify the E484K mutated one(s). Please refer to Tables 6 and 7 for results interpretation.

Table 1. Primer/probe set list of the reference primer/probe set component (Cat #7138-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 2. 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 #7138-Pos), and nuclease-free water (Cat #7138-H2O) are included in the kit. The positive control (Cat #7138-Pos) consists of non-infectious viral RNA fragments of both the E484 non-mutated lineage and the E484K mutated lineage of SARS-CoV-2 spiked into human small airway epithelial cells. It serves to ensure reagents and instruments are working properly.

Kit Components

Cat #	Component	Quantity	Storage
MB802a	One-Step TaqProbe RT-qPCR master mix, 4x	1.5 mL	-20°C
7138-REF	Reference multiplex primer/probe sets, in solution	600 µL	-20°C
7138-E484K	E484K multiplex primer/probe sets, in solution	600 µL	-20°C
7138-H2O	Nuclease-free H ₂ O	4 mL	4°C
7138-Pos	Positive control (non-infectious; RNA: 500 – 1000 copies/µL, cells: 200 – 300 counts/µL)	50 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 #7138-REF and #7138-E484K) at -20°C in a manual defrost freezer, the positive control (Cat #7138-Pos) at -80°C, and nuclease-free H₂O (Cat #7138-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 #7138-REF 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 #7138-Pos), and H₂O (Cat #7138-H2O) as the No Template Control (NTC). Prepare two 20 µl RT-qPCR reactions as shown in Table 3 for each control sample, one with the reference primer/probe set component (Cat #7138-REF), and one with the E484K primer/probe set component (Cat #7138-E484K).

Table 3.

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

5. For each extracted RNA test sample (individual or pooled of up to 10 samples), prepare two 20 µl RT-qPCR reactions as shown in Table 4, one with the reference primer/probe set component (Cat #7138-REF), and one with the E484K primer/probe set component (Cat #7138-E484K).

Table 4.

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

6. Seal the RT-qPCR reaction wells. Centrifuge the plates or tubes at 1,500x g for 15 seconds.
7. Setup RT-qPCR reactions as shown in Table 5.

Table 5. 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 6. SCVEK kit control sample test results interpretation. A Cq value lower than 40 is considered positive.

Sample	Primer/probe Set	FAM	HEX	Results Interpretation
7138-Pos	7138-REF	+	+	Expected
		-	-	Reverse transcription and/or PCR failed
	7138-E484K	+	+	Expected
		-	-	Reverse transcription and/or PCR failed
7138-H2O	7138-REF	-	-	Expected
		If anyone is positive		Reagent(s) contaminated
	7138-E484K	-	-	Expected
		If anyone is positive		Reagent(s) contaminated

Table 7. SCVEK kit target sample test results interpretation when control results are as expected. A Cq value lower than 40 is considered positive.

Primer/probe Set	7138-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