

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).

| B.1.1. 7 | HV69-70 deletion | N501Y | |
|-----------------|------------------|-------|-------|
| B.1.351 | | N501Y | E484K |
| P.1 | | N501Y | E484K |
| <i>P.2</i> | | | E484K |

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

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 t | the reference p | primer/probe s | et 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-H69V |
|--|
|--|

| 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 E484 | K primer/probe set comp | oonent (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 noninfectious 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.

| Nuclease-free H ₂ O (Cat #/148-H2O) | 4 μ1 |
|--|------|
| $N_{\rm eff} = (1 + 0.00 \pm 0.000)$ | 4 1 |
| 1-step RT-qPCR Master mix, 4x (Cat #MB802a) | 5 µl |
| Multiplex primer/probe sets (Cat #7148-REF, #7118-H69V70, #7128-N501Y, or #7138-E484K) | 6 µl |
| Control sample (Cat #7148-Pos or 7148-H2O) | 5 µl |

 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) | |
|--|-------|
| Multiplex primer/probe sets (Cat #7148-REF, #7118-H69V70, #7128-N501Y, or #7138-E484K) | |
| 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 | |
| Annealing and extension | 66°C | 30 sec | 45 |
| Data acquisition | Plate read, detected | | |

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 7148-Pos 7118-F | 7149 DEE | + | + | Expected |
| | /148-KEF | - | - | Reverse transcription and/or PCR failed |
| | | + | + | Expected |
| | /118-H09V/0 | - | - | Reverse transcription and/or PCR failed |
| | 7140 DEE | - | - | Expected |
| 7148-H2O | /148-REF | If either or both are positive | | Reagent(s) contaminated |
| | | - | - | Expected |
| | /118-H69V70 | 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 | | Deculto Internation | |
|------------------------|------|------|-------------|----------------|--|--|
| Detection channel | FAM | HEX | FAM | HEX | Kesuits Interpretation | |
| 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 | | |

| Primer/probe Set | 7148 | -REF | 7128-N501Y | | Desalts Intermetation |
|------------------------|------|------|------------|----------------|---|
| Detection channel | FAM | HEX | FAM | HEX | Results Interpretation |
| 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 | | | ombinatio | 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.

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 | | Deculte Internetation | |
|----------------------------|------------------------|------|------------|-----|---|--|
| Detection channel | FAM | HEX | FAM | HEX | Kesuits interpretation | |
| + + + + + - | + | ± | + | - | 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 | |