



## SARS-CoV-2 detection using ScienCell™ RT-qPCR kit.

Created: Sep 17, 2021; Last modified: Sep 17, 2021 Version: 1.0

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causes the respiratory illness responsible for the COVID-19 (coronavirus disease 2019) pandemic. It was previously referred to as 2019 novel coronavirus (2019-nCoV, human coronavirus 2019 (HCoV-19 or hCoV-19) and Wuhan Coronavirus. On January 30, 2020, the World Health Organization declared the outbreak a Public Health Emergency of International Concern and a pandemic on 11 March 2020. ScienCell™ SARS-CoV-2 Coronavirus Real-time RT-PCR (RT-qPCR) Detection Kit is a three reaction, TaqMan qualitative one-step reverse transcription real-time Polymerase Chain Reaction (RT-qPCR) approach using viral RNA extracted from respiratory specimens such as nasal, nasopharyngeal, oropharyngeal swab specimens, and bronchoalveolar lavage from individuals suspected of COVID-19. This product targets the viral N1 and N2 genes as well as the human RPP30 gene which serves as an internal control to assess specimen quality (<https://www.sciencellonline.com/media/pdf/SCell-SARS-ifu.pdf>).

### Primer/probe preparation

RX7038-N1 primer/probe set	<b>Forward:</b> GAC CCC AAA ATC AGC GAA AT <b>Reverse:</b> TCT GGT TAC TGC CAG TTG AAT CTG <b>Probe:</b> FAM-ACC CCG CAT-ZEN-TAC GTT TGG TGG ACC-3IABkFQ
RX7038-N2 primer/probe set	<b>Forward:</b> TTA CAA ACA TTG GCC GCA AA <b>Reverse:</b> GCG CGA CAT TCC GAA GAA <b>Probe:</b> FAM-ACA ATT TGC-ZEN-CCC CAG CGC TTC AG-3IABkFQ
RX7038-RP primer/probe set	<b>Forward:</b> AGA TTT GGA CCT GCG AGC G <b>Reverse:</b> GAG CGG CTG TCT CCA CAA GT <b>Probe:</b> FAM-TTC TGA CCT-ZEN-GAA GGC TCT GCG CG-3IABkFQ

1. Upon receipt, store lyophilized primer/probe sets at -20 °C.
2. Prior to use, allow the primer/probe sets to warm to room temperature in the dark.
3. Centrifuge the vials at 1500 G for 1 minute.
4. Add 200 µl nuclease-free H<sub>2</sub>O to N gene primer/probe set 1 and allow to rehydrate for 15 min at room temperature to make N1 primer/probe stock solution.
5. Aliquot N1 primer/probe stock solution as needed and store at -20°C in a manual defrost freezer.
6. Add 200 µl nuclease-free H<sub>2</sub>O to N gene primer/probe set 2 and allow to rehydrate for 15 min at room temperature to make N2 primer/probe stock solution.





7. Aliquot N2 primer/probe stock solution as needed and store at  $-20^{\circ}\text{C}$  in a manual defrost freezer. cold and in the dark when thawed.
8. Add 200  $\mu\text{l}$  nuclease-free  $\text{H}_2\text{O}$  to Human RPP30 gene primer/probe set and allow to rehydrate for 15 min at room temperature to make RP primer/probe stock solution.
9. Aliquot RP primer/probe stock solution as needed and store at  $-20^{\circ}\text{C}$  in a manual defrost freezer. cold and in the dark when thawed.

### Positive template control, negative template control and negative control preparation

1. Positive control (catalog #RX7038-POS) is non-infectious RNA spiked into human small airway epithelial cells. Aliquot in small volumes (approximately 18  $\mu\text{L}$  each), and store at  $-80^{\circ}\text{C}$ . Do not freeze-and-thaw for more than once.
2. Negative Template Control (NTC) is a common control specimen used at our lab and corresponds to a SARS-CoV-2 NEGATIVE (previously tested) RNA extracted from human respiratory specimens. The NTC should be kept at  $-80^{\circ}\text{C}$  and care should be taken to avoid repeat freeze-and-thaw cycles.
3. Negative control (NC) is nuclease-free  $\text{H}_2\text{O}$  and is standard issue with the kit.

### Procedure

1. Clean and decontaminate all work surfaces, pipettes, centrifuges, and other equipment prior to use to minimize the risk of nucleic acid cross-contamination.
2. In the RT-PCR Area clean hood and thaw primer/probe sets, one-step RT-qPCR master mix and place them on ice or in a cold-block. Mix well by inversion 5 times. **Do not vortex!**
3. Briefly spin primer/probe sets and one-step RT-qPCR master mix for 5 seconds, place and keep them on ice or in a cold-block during preparation and use.
4. Label one 1.5 mL microcentrifuge tube for each primer/probe set (three in total)
5. Determine the number of reactions (N) to set up per test run. It is necessary to include excess reaction mix for the three control samples (PTC, NTC and NC) in these calculations.
  - a. If number of samples (n) including controls equals 3 to 12, then add an additional reaction.





- b. If number of samples including controls is between 13 and 24, then add two additional reactions.
  - c. If number of samples including controls is 25 to 32, then add three additional reactions
  - d. **32 is the maximum amount** of individual patient (and control) samples that can be run in a standard 96-well plate.
6. For each primer/probe set, calculate the amount of each reagent to be added for each reaction mixture as shown in the table below.

Reagent	Volume of Reagent Added
Primer/probe stock solution (N1, N2, or RP)	N x 2 $\mu$ l
One-step RT-qPCR Master mix, 4x (catalog #MB802a)	N x 5 $\mu$ l
Nuclease-free H <sub>2</sub> O (catalog #RX7038-H2O)	N x 8 $\mu$ l
<b>Total volume</b>	<b>N x 15 <math>\mu</math>l</b>

7. Add reagents into each respectively labelled 1.5 mL microcentrifuge tube.
8. Mix reaction mixtures by pipetting up and down. **Do not vortex!**
9. Centrifuge for 5 seconds to collect contents at the bottom of the tube, and then place the tubes in a cold rack.
10. For each control sample and test specimen, three RT-qPCR reactions need to be prepared, one with N1 primer/probe stock solution, one with N2 primer/probe stock solution, and one with RP primer/probe stock solution.
11. Dispense 15  $\mu$ L of each reaction mixture into the appropriate wells going across columns as shown below.

	1	2	3	4	5	6	7	8	9	10	11	12
A	N1	N2	RP	N1	N2	RP	N1	N2	RP	N1	N2	RP
B	N1	N2	RP	N1	N2	RP	N1	N2	RP	N1	N2	RP
C	N1	N2	RP	N1	N2	RP	N1	N2	RP	N1	N2	RP
D	N1	N2	RP	N1	N2	RP	N1	N2	RP	N1	N2	RP
E	N1	N2	RP	N1	N2	RP	N1	N2	RP	N1	N2	RP
F	N1	N2	RP	N1	N2	RP	N1	N2	RP	N1	N2	RP
G	N1	N2	RP	N1	N2	RP	N1	N2	RP	N1	N2	RP
H	N1	N2	RP	N1	N2	RP	N1	N2	RP	N1	N2	RP

12. Gently vortex (3 seconds) the NTC, NC and patient samples.
13. Within the pre-RT-PCR workstation of the RT-PCR/RNA area of our lab, load 5  $\mu$ L of NTC into tubes A1-3 shown in dark green highlight in the table above. Securely cap NTC wells before proceeding.





14. Within the pre-RT-PCR workstation of the RT-PCR/RNA area of our lab, load 5  $\mu$ L of NC into tubes B1-3 shown in light green highlight in the table above. Securely cap NC wells before proceeding.
15. Within the pre-RT-PCR workstation of the RT-PCR/RNA area of our lab, load 5  $\mu$ L of the patient samples into their corresponding tubes, capping each of the three reaction tubes (i.e., wells C1-3 highlighted in light grey in the table above) before moving to the next patient samples (i.e., wells D1-3 shown in dark grey highlight in the table above).
16. Once all NTC, NC and patient samples have been added, verify that all tubes are securely capped and move into the PTC-workstation located in the main lab (peninsular table).
17. Within the PTC workstation gently vortex (3 seconds) PTC.
18. Pipette 5  $\mu$ L of the PTC control to the last sample wells (tubes H10-12 shown in red highlight in the table above. Securely cap wells after addition of the positive control.
19. Centrifuge plate or tubes for 30 seconds to collect contents at the bottom of the tube.
20. After centrifugation, in the cold rack once again before loading into instrument.
21. Change gloves often and as deemed necessary to avoid contamination.

### Real time instrument RT-qPCR conditions

1. Fluorescence data (FAM) 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	55°C	30 sec	
Data acquisition	Plate read, detector (FAM)		





## Result interpretation

1. For the assay to be considered valid:
  - a. PTC should exhibit amplification curves <40 Ct for the three gene targets (N1, N2 and RP).
  - b. NC should NOT exhibit amplification curves <40 Ct for ANY of the three gene targets.
  - c. NTC should only exhibit amplification curve <40 for RP gene.
2. For patient samples to be considered **SARS-CoV-2 POSITIVE** (after validating assay), either N1 or N2 or both N1 and N2 should exhibit amplification before 40 Ct with or without RP gene amplification.
3. For patient samples to be considered **SARS-CoV-2 NEGATIVE** (after validating assay), neither N1 nor N2 should exhibit amplification but RP gene should exhibit amplification before 40 Ct.
4. If patient samples do not exhibit neither N1 nor N2 nor RP amplification curves < 40 Ct, the test result should be considered invalid and should be repeated.

## References

1. Instructions for use ScienCell™ SARS-CoV-2 Coronavirus Real-time RT-PCR (RT-qPCR) Detection Kit Cat Num. RX7038 Emergency Use Authorization (EUA) ScienCell Research Laboratories 1610 Faraday Ave Carlsbad, CA 92008 USA

## Revision history

- 1.0 Original document.

