Takara Bio USA

SmartChip® Real-Time RT-PCR SARS-CoV-2 Assay User Manual

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

Cat. No. 640256
(011822)

Takara Bio USA, Inc.
2560 Orchard Parkway, San Jose, CA 95131, USA
U.S. Technical Support: technical_support@takarabio.com

United States/Canada 800.662.2566 | Asia Pacific +1.650.919.7300 | Europe +33.(0)1.3904.6880 | Japan +81.(0)77.565.6999

Table of Contents

Table of Figures

Table of Tables

I. Introduction

This assay is for Research Use Only (RUO). Not for use in diagnostic procedures.

The SmartChip Real-Time RT-PCR SARS-CoV-2 assay is a real-time RT-qPCR assay intended for the qualitative detection of nucleic acids from SARS-CoV-2 in upper respiratory specimens, such as nasopharyngeal swabs. The assay results in the identification of SARS-CoV-2 RNA, generally detectable in upper respiratory specimens during infection by the virus.

The SmartChip Real-Time RT-PCR SARS-CoV-2 assay is based on widely used nucleic acid amplification technology. The kit uses oligonucleotide primers, dual-labeled hydrolysis probes, and control material used in RT-qPCR for the in vitro qualitative detection of SARS-CoV-2 RNA in respiratory samples.

The assay uses the oligonulceotide primers and probes designed for the viral nucleoprotein (N) gene by the CDC and the CDC's FDA-approved method for detection of SARS-CoV-2 (https://www.fda.gov/media/134922/download). The assay is designed for specific detection of the SARS-CoV-2 nucleoprotein (two primer/probe sets – N1 and N2). A primer/probe set to detect the human RNase P gene (RP) is also used as a control for the quality of input RNA.

RNA isolated and purified from upper respiratory samples is dispensed, reverse transcribed to cDNA, and amplified in 5,184-well chips using the SmartChip Real-Time PCR System. In the process, the probe anneals to a specific target sequence located between the forward and reverse primers for each gene target. During the extension phase of the PCR cycle, the 5' nuclease activity of the polymerase degrades the probe, causing the reporter dye to separate from the quencher dye, generating a fluorescent signal. Additional reporter dye molecules are cleaved from their respective probes during each cycle, increasing the fluorescence intensity. Fluorescence intensity is monitored at each PCR cycle by the SmartChip Real-Time PCR System and recorded in the data.

II. List of Components

The SmartChip Real-Time RT-PCR for SARS-CoV-2 workflow requires use of a SmartChip Real-Time PCR System (Cat. No. 640022) and the SmartChip Real-Time RT-PCR for SARS-CoV-2 Reagents (Cat. No. 640256). The reagent kit contains enough reagents for one chip run with 378 samples and 6 controls (negative control in triplicate, positive control in duplicate, and an extraction control).

Table 1. SmartChip Real-Time RT-PCR for SARS-CoV-2 components.

SmartChip Real-Time RT-PCR SARS-CoV-2 Reagents	640256
Box 1 (Store at –80°C*)	
SmartChip Probe qPCR Master Mix	922 µl
SMARTScribe™ Reverse Transcriptase	58 µl
Negative Control	1 ml
Positive Control RNA Mix	20 µl
EASY Dilution (for Real Time PCR)	1 ml
Box 2 (Store at room temperature)	
384-Well Source Plate	2 Each
384-Well Source Plate Seal	2 Each
SmartChip MyDesign Chip, 250nl	1 Each
Blotting Paper	2 Each
Intermediate Film	1 Each
Cycler Sealing and Pressure Film	1 Each

*Store at –80°C in a constant temperature freezer. Do not use if past the expiration date or if thawed when initially purchased.

III. Additional Materials Required (Not Included)

Required equipment

SmartChip Real-Time PCR System	Quantity	640222
SmartChip MultiSample NanoDispenser with SmartChip Dispenser Software (v4.8.10 or higher)	1	640001
SmartChip Real-Time PCR Cycler with SmartChip qPCR Software (v2.8.68 or higher)	1	640023

Required reagents

Assay primers and probes	Catalog No.	Vendor
nCOV_N1 Forward Primer Aliquot, 50 nmol	10006821	Integrated DNA Technologies
nCOV_N1 Reverse Primer Aliquot, 50 nmol	10006822	
nCOV_N1 Probe Aliquot, 25 nmol	10006823	
nCOV_N2 Forward Primer Aliquot, 50 nmol	10006824	Integrated DNA Technologies
nCOV_N2 Reverse Primer Aliquot, 50 nmol	10006825	
nCOV_N2 Probe Aliquot, 25 nmol	10006826	
RNase P Forward Primer Aliquot, 50 nmol	10006827	Integrated DNA Technologies
RNase P Reverse Primer Aliquot, 50 nmol	10006828	
RNase P Probe Aliquot, 25 nmol	10006829	

Required kits, equipment, and consumables for RNA extraction

	Size	Catalog No.	Vendor
NucleoMag Pathogen (or equivalent)	4 x 96 Preps	744210.4	Takara Bio
NucleoMag SEP*	1	744900	Takara Bio
Square-well Block	4 Plates	740481	Takara Bio
MN Tube Strips	4 Sets	740477	Takara Bio
Self-adhering PE Foil	50 Each	740676	Takara Bio

* Magnetic separator for use with 96-well plates

Human Sample Control (HSC)	Catalog No.	Vendor
SARS-CoV-2 External Control Kit	MP-32	ATCC

Small equipment and consumables

- 96-well plate shaker (Eppendorf MixMate or equivalent)
- Single-channel pipettes (2 or 10 µl, 200 µl, and 1,000 µl)
- Multichannel pipettes (20 µl, 200 µl, and 1,000 µl)
- Sterile reagent reservoirs
- Helium tank and regulator (purity: 99.9% or greater, capacity: approximately 223 standard cubic feet, pressure: 2,264 psi). Refer to the SmartChip MultiSample Dispenser and SmartChip Dispenser Software User Manual (SmartChip MSND UM) for details.
- Clean, dry air at 100–200 psi or compressed Nitrogen (N_2)
- Pipette tips (20 µl, 200 µl, and 1,000 µl)
- Refrigerated centrifuge with plate adapters (Eppendorf 5810 R or equivalent)
- Microcentrifuge (Eppendorf 5415 C or equivalent)
- 1.5-ml microcentrifuge tubes (DNase/RNase free)
- Racks for 1.5-ml microcentrifuge tubes
- 96-well cold block

- Plate Seal Applicator
- Molecular biology grade water, nuclease-free
- 10% bleach (1:10 dilution of commercial 5.25–6.0% hypochlorite bleach)
- Optional: DNAZap PCR DNA degradation solution (Thermo Fisher or equivalent)
- Optional: RNase Away (Thermo Fisher or equivalent)
- Disposable powder-free gloves

IV. General Considerations

A. Assay Limitations

- This assay is for **Research Use Only**. Not for use in diagnostic procedures. This assay **SHOULD NOT** be used for monitoring or treatment of SARS-CoV-2 infection.
- The performance of this assay has only been established in nasopharyngeal swabs. This assay **SHOULD NOT** be used for screening of blood or blood products for the presence of SARS-CoV-2.
- A false-negative result may occur if a specimen is improperly collected, transported, or handled. False-negative results may also occur if amplification inhibitors are present in the specimen or if inadequate numbers of viral particles are present.
- Use of any reagent past the expiration date may result in inconclusive and/or incorrect results.
- If the virus mutates in the RT-qPCR target region (N gene), the assay may not work or may be less predictable. Inhibitors or other types of interference may produce a false-negative result.
- This assay cannot rule out the presence of other bacterial or viral pathogens.

B. Calibration and Maintenance

Calibrate and maintain the SmartChip Real-Time PCR System according to the SmartChip MSND UM and SmartChip Real-Time PCR Cycler - SmartChip qPCR Software User Manual (SmartChip Cycler UM). A daily initialization protocol for the dispenser is described in the manual.

C. Reagent Storage, Handling, and Stability

- Always check the expiration date prior to use. DO NOT use expired reagents. DO NOT substitute or mix reagents from different kits, lots, or from other manufacturers unless specified in this manual.
- Once assay mixes (Assay Primer/Probe Stock solution, APPS) are formulated to a stock concentration for use (40X), aliquot and store at ≤ –15°C in a constant temperature (non-frost free) freezer and protected from light.
- Keep stocks of APPS (40X) and the enzyme master mix on a cold block (or ice) during preparation, assay plate setup, and use.
- Working stocks of controls (RNA mix) must be kept on a cold block (or ice) during preparation and use.

D. Biosafety Precautions

- Handle all specimens as if infectious, using safe laboratory procedures. Refer to Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with Coronavirus Disease 2019 (https://www.cdc.gov/coronavirus/2019-ncov/lab/lab-biosafety-guidelines.html).
- Specimen processing should be performed in accordance with national biological safety regulations.
- Perform all manipulations of live virus samples with a Class II (or higher) biological safety cabinet (BSC).

- Use personal protective equipment (PPE) such as (but not limited to) gloves, eye protection, and lab coats when handling kit reagents while performing this assay and handling materials, including samples, reagents, pipettes, and other equipment and reagents.
- Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in areas where reagents and human specimens are handled.

E. General PCR Precautions

Amplification technologies are sensitive to the accidental introduction of PCR product from previous amplification reactions. Incorrect results could occur if either the sample or the real-time reagents used in the amplification step become contaminated by the accidental introduction of amplification product (amplicon).

- Maintain separate, dedicated equipment (e.g., pipettes, microcentrifuges) and supplies (e.g., microcentrifuge tubes, pipette tips) for assay setup and handling of extracted nucleic acids.
- Workflow in the laboratory should proceed in a unidirectional manner.
- Change aerosol barrier pipette tips between all manual liquid transfers.
- During the preparation of samples, compliance with good laboratory techniques is essential to minimize the risk of cross-contamination between samples and the accidental introduction of nucleases into samples during and after the extraction procedure.
- Always use proper aseptic techniques when working with nucleic acids.
    - Clean and decontaminate work surfaces, pipettes, and centrifuges with cleaning products such as 10% bleach, DNAZap, or RNase AWAY to minimize the risk of nucleic acid contamination. Remove residual bleach using 70% ethanol.
    - Wear a clean lab coat and powder-free disposable gloves (not previously worn) when setting up assays.
    - Change gloves between batches of samples being processed and/or whenever contamination is suspected.
    - Keep reagent and reaction tubes capped or covered as much as possible.
- Extracted nucleic acid samples should be maintained on a cold block or ice during preparation and use to ensure stability.
- Dispose of unused kit reagents and human specimens according to local, state, and federal regulations.

F. Quality Control

- Quality control procedures are intended to monitor reagent and assay performance.
- Test all positive controls prior to running samples with each new kit lot to ensure all reagents and kit components are working properly.
- The Human Sample Control (HSC) (ATCC, Cat. No. MP-32) must proceed through nucleic acid isolation for each batch of specimens to be assayed. This serves as the extraction chemistry control as well as demonstrating positive amplification of the RNase P assay (both RNA and genomic DNA).
- A total of three negative or non-template control (NTC) assays are included in each chip run to test for possible contamination of the amplification reagents. No amplification (i.e., fluorescence growth curves that cross the threshold line below Ct 33.5) should be observed in the N1, N2, and RP wells for the NTCs.
- A control RNA mixture is included as two positive control assays in each amplification run to demonstrate the functional performance of the reverse transcriptase as well as the N1, N2, and RP assays. Amplification should be observed in at least 4/5 replicate wells for each of the N1 and N2 assays and in both replicate wells (2/2) for the RP assay.

V. Protocol

IMPORTANT: Before you begin, carefully read the SmartChip MSND UM. Personnel must be trained to use the SmartChip system before starting this assay.

A. Protocol: Preparation of Reagents and Controls

1. Human Specimen Control (HSC)

With each specimen extraction run, extract and process an HSC using the NucleoMag Pathogen kit or equivalent, following the manufacturer's protocol. HSC serves as a positive control for the RP target.

2. Assay Primer/Probe Stock Solution (APPS) Preparation (40X)

PRECAUTIONS:

- Handle all reagents in a clean pre-PCR area and store them in the dark and at appropriate temperatures (as mentioned in the List of Components section).
- Avoid repeated freeze-thaw cycles.
- Maintain cold after thawing.

a. Prepare three separate 40X APPS solutions, one for each primer set (N1, N2, and RP) by combining the corresponding primers (to a final concentration of 20 µM) and probes (to a final concentration of 5 µM) using the formula in Table 2 below as an example. This example formula is sufficient to prepare aliquots for 20 SmartChip qPCR runs and can be adjusted for larger batch sizes as desired. The primer and probe concentrated stocks (100 µM) are sufficient to prepare 100 aliquots (25 µl each) of APPS solution.

Table 2. 40X APPS solution assembly.

Volume	Component	Final Conc.
100 µl	Forward Primer (100 µM)	20 µM
100 µl	Reverse Primer (100 µM)	20 µM
25 µl	Probe (100 µM)	5 µM
275 µl	1X Low TE buffer	–
500 µl	Total volume	

b. Mix gently and divide each of the 40X APPS solutions into 25-µl aliquots in pre-labeled tubes. Each aliquot of 40X APPS solutions of the three primers (N1, N2, and RP) is sufficient for one SmartChip run. Store the extra aliquots and any remaining 100 µM stocks in a sealed container at or below –15°C in a constant temperature (non-frost-free) freezer.

3. Positive Control RNA Mix (nCoVPC) Preparation

PRECAUTIONS:

- To prevent contamination, handle this high-copy number RNA mix in a dedicated nucleic acid handling area away from PCR primers and enzyme mixes.
- The Positive Control RNA Mix (nCoVPC) is shipped at –80°C; keep frozen until ready for use. It is sufficient for a single SmartChip run.
- Avoid repeated freeze-thaw cycles.

a. Prepare the sample plate by thawing the nCoVPC mix on ice.
b. Dilute it to a working concentration of 64 copies/µl using the EASY Dilution buffer.

4. No Template Control (NTC)

- The NTC is used to check for assay reagent contamination.
- Use the Negative Control provided in the kit as the NTC.

B. Protocol: General Preparation

NOTE: Clean and decontaminate all work surfaces, pipettes, centrifuges, and other equipment before use. Decontamination agents, such as 5% bleach, 70% ethanol, and DNAZap or RNase AWAY should be used to minimize the risk of nucleic acid contamination.

1. Nucleic Acid Extraction

- The NucleoMag Pathogen kit is designed to isolate high-quality RNA and DNA from different kinds of samples, including swabs. Be sure to include an aliquot of HSC as a control when extracting your samples.
- The protocol can be performed by manual extraction or run on automated systems, including instruments from Beckman, Eppendorf, Hamilton, Thermo Fisher Scientific, PerkinElmer, or Tecan; please contact Takara Bio technical support for specific inquiries related to the automated systems that can be used.
- Sample input volume for extraction is 200 µl. Elute purified nucleic acids in 60 µl of Elution Buffer.
- Once the extracts are isolated, proceed with dispensing samples on the SmartChip MultiSample NanoDispenser (Section 5 under Assay Setup, below), or store the samples in a 96-well plate at or below –15°C in a constant temperature (non-frost-free) freezer for later use.

2. Assay Setup

IMPORTANT: Refer to the SmartChip MSND UM for general operating instructions. Personnel handling the SmartChip system must be fully trained to use the system. Instructions specific to the operation of the SmartChip Real-Time RT-PCR SARS CoV-2 assay are outlined below.

a) Preparation of the 384-Well Sample Source Plate

1. Ensure that all the samples that will be assayed are resuspended well. If necessary, vortex each 96-well plate and briefly centrifuge.
2. Label one of the 384-Well Source Plates supplied with the kit as “Sample Source Plate” using a marker.

   NOTE: Do not use 384-well plates from other vendors. The SmartChip system requires specific plate types and dimensions for accurate dispensing.

3. Transfer 12 µl of Negative Control (NTC1-3) from the kit into well positions A1, B1, and I1 (i 1). Add 12 µl of the freshly diluted nCoVPC RNA Mix (POS1 and POS2) to wells E1 and F1. Add 12 µl of extracted HSC (HSC) to well J1 (Figure 1).

   NOTE: When transferring a large number of samples from a 96-well intermediate plate, we highly recommend using either an 8- or 12-tip multichannel pipette.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	NTC1																							
B	NTC2																							
C																								
D																								
E	POS1																							
F	POS2																							
G																								
H																								
I	NTC3																							
J	HSC																							
K																								
L																								
M																								
N																								
O																								
P																								

Figure 1. 384-Well Source Plate layout demonstrating locations of the three NTCs, two replicates of the nCoVPC mix (POS), and one Human Specimen Control (HSC) reaction.

4. Using a multichannel pipette, transfer 12 µl of each sample into individual wells of the 384-Well Source Plate.
   a. The proposed layout accommodates a total of 378 unique samples plus six controls: three of NTC, two of nCoVPC RNA mix, and one of the HSC.
   b. Array the first set of 90 samples to a 96-well plate as shown below (top half of Figure 2). Then, transfer 12 µl of each sample from the first 96-well plate to the first 96 wells of the 384-Well Source Plate, as shown below (bottom half of Figure 2).

	1	2	3	4	5	6	7	8	9	10	11	12
A			S11	S19	S27	S35	S43	S51	S59	S67	S75	S83
B	S1	S6	S12	S20	S28	S36	S44	S52	S60	S68	S76	S84
C			S13	S21	S29	S37	S45	S53	S61	S69	S77	S85
D	S2	S7	S14	S22	S30	S38	S46	S54	S62	S70	S78	S86
E			S15	S23	S31	S39	S47	S55	S63	S71	S79	S87
F	S3	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80	S88
G	S4	S9	S17	S25	S33	S41	S49	S57	S65	S73	S81	S89
H	S5	S10	S18	S26	S34	S42	S50	S58	S66	S74	S82	S90

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	NTC1	S11	S27	S43	S59	S75																		
B	NTC2	S19	S35	S51	S67	S83																		
C	S1	S12	S28	S44	S60	S76																		
D	S6	S20	S36	S52	S68	S84																		
E	POS1	S13	S29	S45	S61	S77																		
F	POS2	S21	S37	S53	S69	S85																		
G	S2	S14	S30	S46	S62	S78																		
H	S7	S22	S38	S54	S70	S86																		
I	NTC3	S15	S31	S47	S63	S79																		
J	HSC	S23	S39	S55	S71	S87																		
K	S3	S16	S32	S48	S64	S80																		
L	S8	S24	S40	S56	S72	S88																		
M	S4	S17	S33	S49	S65	S81																		
N	S9	S25	S41	S57	S73	S89																		
O	S5	S18	S34	S50	S66	S82																		
P	S10	S26	S42	S58	S74	S90																		

Figure 2. Plating of the first 90 samples with 6 controls in the 384-Well Source Plate.

   c. Dispense samples from up to three additional 96-well sample plates in the remaining wells (A7–P24) of the 384-Well Source Plate.

5. When all samples have been transferred to the Sample Source Plate, cover the plate with the 384-Well Source Plate Seal. Apply pressure to ensure an even seal across all wells of the plate with the Plate Seal Applicator.
6. Spin at high speed (3,220*g*) for 5 minutes in a prechilled centrifuge set at 4°C.

SAFE STOPPING POINT: The plate may be stored at 4°C for up to four hours until ready to dispense.

Overnight storage before dispensing is NOT recommended.

b) Preparation of the 384-Well Assay Source Plate

NOTE: The Assay Source Plate set-up configuration will be the same regardless of the number of samples on the Sample Source Plate.

1. In the pre-PCR reagent set-up area, place the SmartChip Probe qPCR Master Mix, SMARTScribe Reverse Transcriptase, and the three tubes (one each of N1, N2, and RP) of 40X APPS aliquots on ice or an assay cold block; keep cold during preparation and use.
2. Vortex and briefly spin all components.
3. Obtain three 1.5-ml tubes and label them N1, N2, and RP.
4. Prepare the mixes in each of the three 1.5-ml tubes as shown in Table 3.

Table 3. Setup for the three assay APPS mixes (N1, N2, and RP).

Components	Assay mix	N1	N2	RP
	Per rxn	20 rxns	20 rxns	8 rxns
SmartChip Probe qPCR Master Mix	16 µl	384 µl	384 µl	153.6 µl
SMARTScribe Reverse Transcriptase	1 µl	24 µl	24 µl	9.6 µl
40X APPS (N1, N2, or RP)	1 µl	24 µl	24 µl	9.6 µl
Total	**18 µl**	**432 µl**	**432 µl**	**172.8 µl**

5. Briefly centrifuge the assembled mixes, then place the 1.5-ml tubes in a cold rack.
6. Aliquot 85 µl of each assembled assay mixture to 5 wells of a 96-well plate (N1 and N2 assays) and 2 wells of the 96-well plate (RP assay, RNaseP), as shown in Figure 3.

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

Figure 3. Arrangement of assay mix aliquots in a 96-well plate.

7. Label the second supplied 384-Well Source Plate as "Assay Source Plate" using a marker.
8. Using a multichannel pipette, pipette 18 µl of each assay master mix from the 96-well plate into the designated wells of the Assay Source Plate according to the layout

shown in Figure 4. Each well of the 96-well plate can provide enough reagent for four wells of the 384-Well Source Plate.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	N1	N1	N1	N1																				
B	N1	N1	N1	N1																				
C	N2	N2	N2	N2																				
D	N2	N2	N2	N2																				
E	N1	N1	RNaseP	RNaseP																				
F	N1	N1	RNaseP	RNaseP																				
G	N2	N2	RNaseP	RNaseP																				
H	N2	N2	RNaseP	RNaseP																				
I	N1	N1																						
J	N1	N1																						
K	N2	N2																						
L	N2	N2																						
M	N1	N1																						
N	N1	N1																						
O	N2	N2																						
P	N2	N2																						

Figure 4. Arrangement of assay mixes transferred from the 96-well plate to the Assay Source Plate.

9. Seal the plate with the second 384-Well Source Plate Seal, and spin at high speed (3,220*g*) for 5 min in a centrifuge prechilled to 4°C.

SAFE STOPPING POINT: The plate may be stored at 4°C for up to four hours until ready to dispense.

Overnight storage before dispensing is NOT recommended.

C. Protocol: SmartChip MSND Setup

NOTE: Refer to the SmartChip MSND UM for general instructions on using the SmartChip MSND.

1. Make sure the helium tank is turned on and check for the appropriate inlet and outlet pressure reading in the regulator (refer to the SmartChip MSND UM, Section V).
2. Launch the SmartChip Dispenser Software (v4.8.10 or later) on the computer attached to the MSND.
3. Ensure that the proper chip size has been selected in the software by following the next steps:
   a. Go to **Utilities > Select chip type** in the top menu bar.
   b. From the "Chip type" dropdown menu, select '72 rows x 72 columns 1 zone non-SBS 250nl' for 250-nl chips (Figure 5).

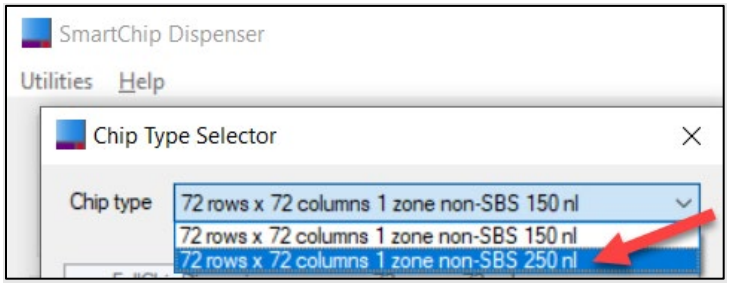

Figure 5. Selection of chip size on the SmartChip Dispenser Software GUI.

4. Perform the necessary MSND instrument daily maintenance procedures as described in the SmartChip MSND user manual.
5. Under the *Setup* tab, perform the following:
   a. Set "Mode" to 'Genotyping'
   b. Enter the SmartChip serial number in the "SmartChip number" field.

NOTE: You can either type the number (found on the back of the SmartChip MyDesign Chip) or place your cursor in the "SmartChip number" field and use the barcode reader to scan the 2-D

barcode on the back of the chip. The SmartChip number can be used later to identify the SmartChip Layout file for this chip.

c. (Optional) Enter a Customer Name and/or User Name in the appropriate fields.

1. Dispense Samples from the Sample Source Plate

IMPORTANT: Refer to the MSND UM (Section VIII) for general instructions on loading the Sample Source Plate into the instrument. Figure 6 below shows a general workflow of the SmartChip system.

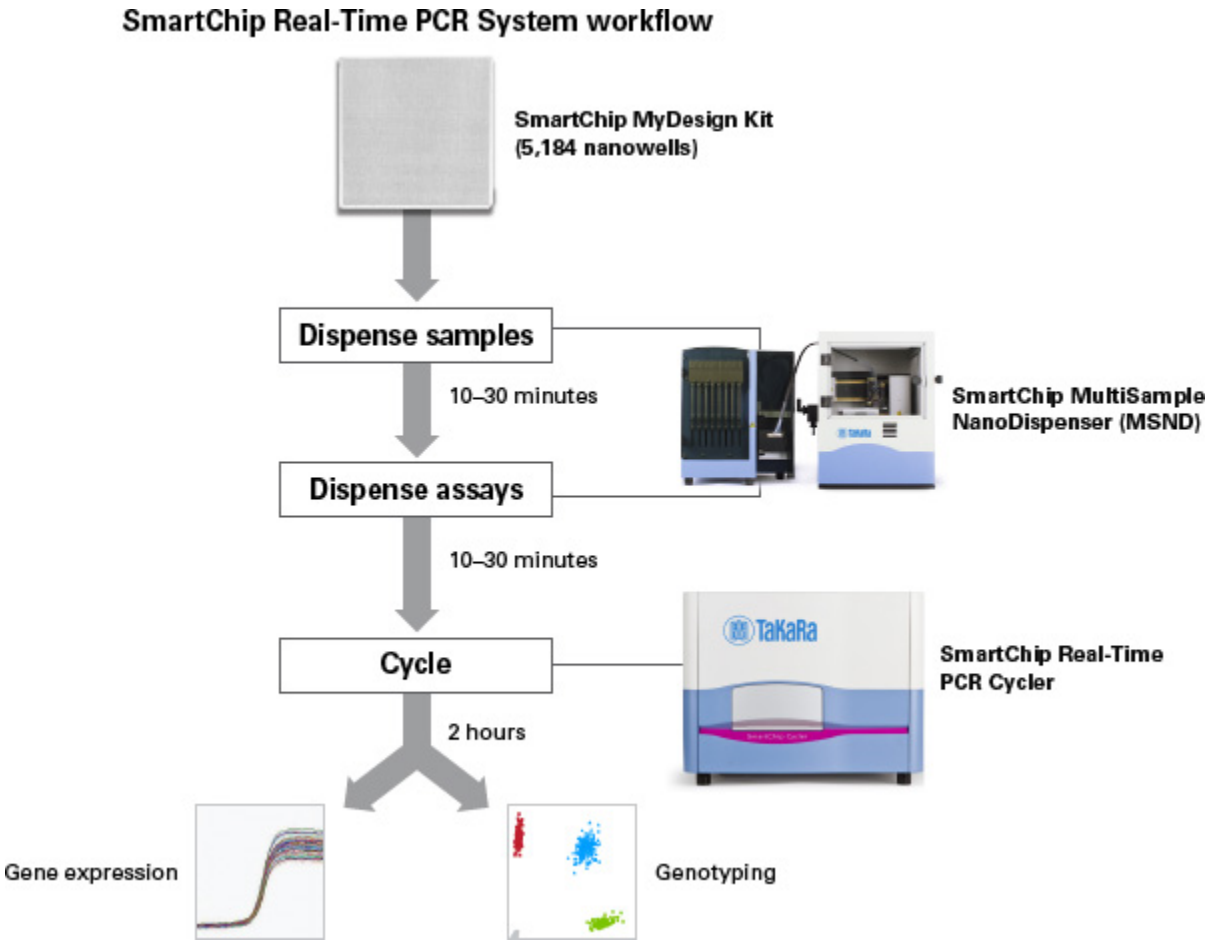

Figure 6. SmartChip Real-Time PCR System workflow. Add your samples and assays to the SmartChip MyDesign chip and then analyze using the SmartChip system.

a. Dispense samples into the MyDesign Chip, followed by a brief centrifuge step, then dispense the reagents from the assay source plate. Reversing this order is not recommended.
b. Refer to the SmartChip MSND UM (Section VIII) for general instructions on loading the Sample Source Plate into the plate nest of the instrument; make sure well A1 is positioned correctly in the back right corner of the platform.
c. Make sure to load the SmartChip MyDesign Chip, 250nl into the chip nest. Ensure the chip lies completely flat on the black chuck, then click the [Vacuum] button in the *Run* tab to verify proper seating. If the chip is seated properly, the button will change from red to green.
d. In the SmartChip Dispenser software, begin dispensing samples by pressing the [Dispense Samples] button in the *Run* tab. If dispensing a full set of samples from a 384-well plate, dispensing will take approximately 60 minutes.

NOTE: Additional details about the sample layout can be entered by following Sections VII.C, D, and E in the SmartChip MSND UM.

2. Dispense Reagents from the Assay Source Plate

a. Refer to the SmartChip MSND UM (Section VIII) for general instructions on loading the Assay Source Plate into the instrument plate nest; make sure well A1 is positioned correctly in the back right corner of the platform.
b. Make sure to load the SmartChip MyDesign Chip, 250nl back into the chip nest. Ensure the chip lies completely flat on the black chuck, then click the [Vacuum] button in the *Run* tab to verify the proper seating. If the chip is seated properly, the button will change from red to green.
c. Begin dispensing assays by pressing the [Dispense Assays] button in the *Run* tab. The dispense will take approximately 20 minutes to complete.
d. Generate and save your SmartChip layout file. Follow SmartChip MSND UM (Section VIII.E) for instructions.

D. Protocol: SmartChip Cycler Setup

NOTES:

- Refer to the SmartChip Cycler UM for general instructions on using the SmartChip Cycler.
- Additional details about the Probe-Based Expression Analysis run can be found in Sections IX–XI of the SmartChip Cycler UM.

1. Launch the SmartChip Cycler Software (v2.8.68 or higher) on the computer attached to the SmartChip cycler.
2. Referring to the user manual, make sure the N_2 tank (or compressed dry air supply) connected to the Cycler is turned on and verify the appropriate inlet and outlet pressure on the regulator.
3. Wait until the Cycler has completed the initiation routines (1–2 min).
4. Go into the **Actions** menu and select **Probe-Based Expression Analysis**.
5. Click the [Setup] button (at the top right). Click the [...] button in the top center and import the COVID-19 SmartChip MyDesign run layout (.MD file) generated from the MSND in Section V.C, Step 2.d.
6. Select the "Thermal Profile" labeled 'Takara SARS CoV2', which has been preprogrammed with the following parameters:

Reverse transcription	50°C	5 min
Polymerase activation	95°C	1 min
45 cycles:		
Denaturation	95°C	5 sec
Extension/Imaging	55°C	30 sec

7. Click the [Run] button to enter Run-mode.
8. Click the [Open Drawer] icon. A chip nest will swivel out of the Cycler drawer.
9. Load the dispensed MyDesign Chip from the MSND into the Cycler chip nest. Ensure the chip is well-seated in the nest.

10. Initiate the rest of the run following the SmartChip Cycler UM. The run should take about 80 min to complete.

VI. Results Analysis

After the run is complete, proceed with the data analysis and export following the steps below.

1. Click the [Analysis] button in the upper right of the screen and select the 'Full data' view of the data.
2. Transfer the data out to a spreadsheet analysis program like Microsoft Excel, either by
   - Selecting all the data in the window (**[Ctrl][A]**) and copying and pasting it into the open spreadsheet, or
   - Exporting the well data as a tab-delimited text file under **File > Save File**.
3. Sort the data first by "Sample ID", then by "Assay ID". Each sample will have five replicates of N1, five replicates of N2, and two replicates of RP.

NOTE: Additional details about Expression Analysis analysis-mode can be found in Section XII of the SmartChip Cycler UM.

A. Interpretation of Control Results

See Table 4 for the expected performance of controls included in the SmartChip Real-Time RT-PCR SARS-CoV-2 assay panel.

Table 4. Interpretation of results.

Control type	External control name	Used to monitor	2019 nCoV_N1	2019 nCoV_N2	RP	Expected Ct values	Number of replicate wells
Extraction	HSC	Failure in lysis and extraction procedure, potential contamination during extraction	–	–	+	<33.5 Ct	<=3/5 for both N1 and N2 assays; 2/2 for RP assay
Positive	nCoVPC	Substantial reagent failure, including primer and probe integrity	+	+	+	<33.5 Ct	>=4/5 for N1 and N2 assays; 2/2 for RP assay
Negative	NTC	Reagent and/or environmental contamination	–	–	–	None detected below 33.5	0/5 for N1 and N2, 0/2 for RP

How to read Table 4

1. HSC (Extraction Control)

- All samples containing nucleic acids of human origin (cultured cells or samples) should exhibit fluorescence growth curves in the RNase P control reaction that cross the threshold line (Ct <33.5), indicating the presence of the human RNase P gene. Failure to detect RNase P in any sample may indicate:
  - Improper extraction of nucleic acid resulting in loss of RNA or DNA, and/or RNA degradation

  - Absence of sufficient human cellular material or loss of specimen integrity
  - Improper assay setup and execution
  - Reagent or equipment malfunction

- **Assay successful:** If the RP assay for the HSC produces a positive result (2/2 reps below Ct 33.5), then the RNA extraction is considered to have worked successfully.
- **Assay failure:** If the RP assay for the HSC does not produce a positive signal, the run is considered to have failed.
    - Recommendation: Repeat RNA extraction and the assay.
- **Contamination:** If the HSC sample is positive for N1 and/or N2 (³4/5 reps each below Ct 33.5), contamination may have occurred during extraction or sample processing.
    - Recommendation: Review the assay procedure for possible sources of cross-contamination. Repeat RNA extraction and the assay.

2. nCoVPC (Positive Control RNA Mix for N1, N2, and RP)

- Reaction wells containing the nCoVPC mix should exhibit fluorescence growth curves in all three assays (N1, N2, and RP) that cross the threshold line within 33 cycles (Ct <33.5). Failure to detect all three targets may indicate:
  - Improper assay setup and execution
  - Reagent or equipment malfunction
  - Presence of contaminating RNase activity
- **Successful:** when at least 4/5 replicates for each of the N1 **and** N2 assays cross the threshold line (Ct < 33.5) **and** both of the RNase P growth curves cross the threshold line (Ct <33.5), indicating that the reagents are performing as expected.
- **Unsuccessful:** when any of the three assays fail to generate a positive result (i.e., ≥4/5 for both N1 and N2 assays and 2/2 for the RP assay).
    - Recommendation: Reverify reagent performance separately (assay primers and probes, and enzyme master mix), then rerun the assay.

3. NTC (Negative Control)

- Reaction wells containing the Negative Control mixture should not exhibit fluorescence growth curves that cross the threshold line (Ct <33.5) for any of the three assays (N1, N2, and RP). Detection of any of the three targets may indicate:
  - Reagent and/or environmental contamination
  - Improper assay setup and execution
  - Reagent or equipment malfunction
- In case the NTC wells show fluorescence curves (Ct <33.5), the run should be considered to have failed. Review the assay procedure for possible sources of cross-contamination. Repeat the extraction procedure and repeat the assay.

B. Interpretation of Assay Results

Table 5 below lists the expected results for the SARS-CoV-2 RT-qPCR Assay Panel.

Table 5. Interpretation of assay results.

2019 nCoV_N1	2019 nCoV_N2	RP	Expected Ct value	Number of replicate wells	Result interpretation
+	+	±	<33.5 Ct	>=4/5 for N1 and N2 assays; >=1/2 for RP assay	Sample positive for SARS-CoV-2
–	–	+	<33.5 Ct	<4/5 for N1 and N2 assays; 2/2 for RP assay	Sample negative for SARS-CoV-2
+	+	–	<33.5 Ct	>=4/5 for N1 and N2 assays; 0/2 for RP assay	Assay failure
+	–	±	<33.5 Ct	>=4/5 for N1 <4/5 for N2; >=1/2 for RP assay	Inconclusive result
–	+	±	<33.5 Ct	<4/5 for N1 >=4/5 for N2; >=1/2 for RP assay	Inconclusive result
–	–	±	<33.5 Ct	<4/5 for N1 and N2 assays; 1/2 for RP assay	Inconclusive result
–	–	–	None detected below 33.5	0/5 for N1 and N2, 0/2 for RP	Assay failure

How to read Table 5

When all controls exhibit the expected performance, the following criteria can be used to interpret the sample results:

- **SARS-CoV-2 Positive:** A sample is considered positive for SARS-CoV-2 if at least 4/5 replicates for both SARS-CoV-2 assays (N1 **and** N2) show Ct values <33.5 **and** at least one of the RP replicates shows a Ct value <33.5.
- **SARS-CoV-2 Negative:** A sample is considered negative if fewer than 4/5 replicates for both SARS-CoV-2 assays show Ct values <33.5 **and** both of the RNase P replicates show Ct values <33.5.

The following cases represent failed or inconclusive results:

- When at least 4/5 replicates of the growth curves for both of the SARS-CoV-2 assays show Ct values <33.5 **and** 0/2 replicates of the RNase P assay show a Ct value <33.5, the assay is considered to have **failed**.
  - Recommendation: Re-extract RNA from residual sample and repeat the assay.

- When the cycle threshold growth curve for only one of the two SARS-CoV-2 assays show Ct values <33.5 for at least 4 of the 5 replicates (Ct <33.5), **and** at least one of the RP replicates shows a Ct value <33.5, the result is **inconclusive**.
  - Recommendation: Re-extract RNA from residual sample and repeat the assay.

- When less than 4/5 replicates of the growth curves for both SARS-CoV-2 assays show Ct values <33.5 **and** 1/2 replicates of the RNase P assay show a Ct value <33.5, the result is **inconclusive**.
  - Recommendation: Re-extract RNA from residual sample and repeat the assay.

- When none of the replicate wells for any assay (N1, N2, and RP) show growth curves that cross the threshold line within 33 cycles (Ct <33.5), the assay is considered to have failed.
  - Recommendation: Re-extract RNA from residual sample and repeat the assay. If the newly assayed sample is not positive for RNAse P (i.e., 0/2 of the RP replicates shows a Ct value <33.5), the result is invalid, and a new collection of the sample should be considered.

VII. References

- Centers for Disease Control and Prevention Division of Viral Diseases, "CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel", Document # CDC-006-00019, Revision: 01: https://www.fda.gov/media/134922/download

Contact Us	
Customer Service/Ordering	**Technical Support**
tel: 800.662.2566 (toll-free)	tel: 800.662.2566 (toll-free)
fax: 800.424.1350 (toll-free)	fax: 800.424.1350 (toll-free)
web: takarabio.com/service	web: takarabio.com/support
e-mail: ordersUS@takarabio.com	e-mail: technical_support@takarabio.com

Notice to Purchaser

Our products are to be used for **Research Use Only**. They may not be used for any other purpose, including, but not limited to, use in humans, therapeutic or diagnostic use, or commercial use of any kind. Our products may not be transferred to third parties, resold, modified for resale, or used to manufacture commercial products or to provide a service to third parties without our prior written approval.

Your use of this product is also subject to compliance with any applicable licensing requirements described on the product's web page at takarabio.com. It is your responsibility to review, understand and adhere to any restrictions imposed by such statements

This document has been reviewed and approved by the Quality Department.