Pathogen Detection Using the Advanta RT-PCR Kit

Contents

About This Protocol

This protocol describes how to perform pathogen detection using the Advanta™ RT-PCR Kit and 192.24 Dynamic Array™ IFC (integrated fluidic circuit) on Biomark™ HD or Biomark using probe-based assays (for example, TaqMan® probes). For detailed instructions on instrument and software operation, see the Juno™ System User Guide (100-7070) or IFC Controller RX User Guide (100-3385) and the Biomark HD Data Collection User Guide (100-2451) or Biomark/EP1™ Data Collection User Guide (68000127).

IMPORTANT Before using this kit, read and understand the detailed instructions and safety guidelines in this document. For complete safety information, see Appendix G.

Safety Alert Conventions

Fluidigm documentation uses specific conventions for presenting information that may require your attention. Refer to the following safety alert conventions.

Safety Alerts for Chemicals

For hazards associated with chemicals, this document follows the United Nations Globally Harmonized System of Classification and Labelling of Chemicals (GHS) and uses indicators that include a pictogram and a signal word that indicates the severity level:

Indicator	Description
	Pictogram (see example) consisting of a symbol on a white background within a red diamond-shaped frame. Refer to the individual safety data sheet (SDS) for the applicable pictograms and hazards pertaining to the chemicals being used.
DANGER	Signal word that indicates more severe hazards.
WARNING	Signal word that indicates less severe hazards.

Safety Alerts for Instruments

For hazards associated with instruments, this document uses indicators that include a pictogram and signal words that indicate the severity level:

Indicator	Description
	Pictogram (see example) consisting of a symbol on a white background within a black triangle-shaped frame. Refer to the instrument user guide for the applicable pictograms and hazards pertaining to instrument usage.
DANGER	Signal word that indicates an imminent hazard that will result in severe injury or death if not avoided.
WARNING	Signal word that indicates a potentially hazardous situation that could result in serious injury or death if not avoided.
CAUTION	Signal word that indicates a potentially hazardous situation that could result in minor or moderate personal injury if not avoided.
IMPORTANT	Signal word that indicates information necessary for proper use of products or successful outcome of experiments.

Safety Data Sheets

Read and understand the SDSs before handling chemicals. To obtain SDSs for chemicals ordered from Fluidigm, either alone or as part of this system, go to fluidigm.com/sds and search for the SDS using either the product name or the part number.

Some chemicals referred to in this protocol may not have been provided with your system. Obtain the SDSs for chemicals provided by other manufacturers from those manufacturers.

Product Overview

The Advanta RT-PCR Kit can be used with purified nucleic acid samples (RNA or both DNA and RNA) or saliva specimens and allows detection of up to 24 pathogens (viral RNA) per sample using unique assays. Reverse transcription (RT) and preamplification of the samples is performed in plates. The samples and assays are transferred to the 192.24 IFC for loading on Juno or the IFC Controller RX and then thermal-cycling and data collection on Biomark HD or Biomark.

Workflow Overview

	Workflow Step		Run Time*
1	Prepare the reverse transcription (RT) and preamplification reactions.		—
2	Perform the RT and preamplification reactions.		70 min
3	Prepare the final assay mixes and final sample mixes for real-time PCR.		—
4	Prepare the 192.24 IFC (integrated fluidic circuit) by injecting control line fluid		—
5	Pipet each final sample and assay mix, Actuation Fluid and Pressure Fluid into the IFC, then load the IFC on Juno™ or IFC Controller RX.		35 min
6	Thermal-cycle and collect data on Biomark HD or Biomark.	Biomark HD: Biomark:	35 min 45 min

* Does not include hands-on time

Materials for the Advanta RT-PCR Kit

Kits and Reagents

For a list of the available kit bundles, see Appendix B. For a list of the reagent kit components, see Appendix C.

IMPORTANT Store reagents as soon as they are received, according to manufacturer's storage recommendations.

Required Kits and Reagents from Fluidigm

Product Name	Part Number	Storage
Advanta™ RT-PCR Kit—192.24	102-0525 (2 IFCs) 102-0424 (10 IFCs)	–15 °C to –25 °C

Other Kits and Reagents

Product Name	Source	Part Number
2019-nCoV Probe & Primer RUO Kit*,† or	Fluidigm	102-0690
2019-nCoV RUO Kit, 500 rxn*,‡ or	Fluidigm§	10006713
Probe-based assays (FAM Quencher)	Major oligo supplier	—

* These targets may only be used for public health surveillance purposes and a diagnostic result may not be reported.
† 2019-nCoV Probe & Primer RUO Kit (102-0690) is sufficient for 10 IFCs.
‡ 2019-nCoV RUO Kit, 500 rxn (10006713) is sufficient for up to 24 IFCs.
§ Manufactured by IDT and distributed by Fluidigm

NOTE For SARS-CoV-2 research, see Appendix D for the viral sequences from the Centers for Disease Control and Prevention (CDC)

Consumables

Product Name	Source	Part Number
Disposable microcentrifuge tubes, polypropylene, 1.5 mL, 2 mL, and 5 mL	Major laboratory supplier (MLS)	—
25 mL reagent reservoir		—
96-well PCR plates		—
8-well PCR tube strips with caps*		—
Clear adhesive film for 96-well plates		—

* Recommended: MicroAmp® Clear Adhesive Film (Thermo Fisher Scientific, 4306311)

Equipment

Product Name	Source	Part Number
Biomark™ HD system or Biomark	Fluidigm	BMKHD-BMKHD BMK-BMK
Juno™ system or IFC Controller RX		101-6455 IFC-RX
RX Interface Plate, if using Juno		101-6114
2 centrifuges: 1 for microtubes, 1 for 96-well PCR plates	MLS	—
Pipettes (P2–P1000) and appropriate filtered, low-retention tips*		—
8-channel pipettes and appropriate filtered, low-retention tips*		—
Vortexer		—
Thermal cycler for 96-well plates (for example, Applied Biosystems® Veriti™ 96-Well Thermal Cycler)		—

* Recommended: Rainin® pipettes

Sample Requirements

This protocol supports using purified nucleic acid samples (RNA or both DNA and RNA) or saliva specimens (see Appendix A). Each reaction requires 5 µL of nucleic acid template.

For better traceability, assemble your samples in a 96-well PCR plate and record in a sample map.

Best Practices

IMPORTANT Read and understand the safety information in Appendix G.

For the overall success of the protocol, we recommend the following best practices.

IFC and Control Line Fluid Handling

- Use the IFC within 24 hr of opening the package.
- Inspect the IFC for any signs of visible damage before use. Ensure that the barcode label is intact and the IFC surfaces are clear of particulates.
- Do not evacuate air from syringes prior to injecting control line fluid.
- Avoid bending the control line fluid syringe tip.
- Be careful when removing the control line fluid syringe cap to prevent drips.
- Before removing the syringe from the accumulator, ensure that all of the control line fluid and air are purged from the syringe to avoid dripping fluid on the surface of the IFC.
- Avoid getting control line fluid on the exterior of the IFC or in the inlets because this makes the IFC unusable. If this occurs, use a new IFC.
- During use, take care to avoid the introduction of particulates, reagents, and fluids to the surface of the IFC.

Sample Handling

- To prevent cross-contamination of samples and controls with preamplified amplicons:
  - Designate space for the preparation of controls, saliva specimens and the 1-step RT and preamplification reactions that is separate from the remaining processes.
  - Use a separate set of pipettes, filter tips, racks, vortexers, centrifuges, generic lab reagents and supplies at their respective areas.
  - Clean the work areas and pipettes with DNA-destroying surface decontaminants.
  - Change gloves between tasks.
- To prevent cross-contamination in 96-well sample preparation:
  - Always change the pipette tip after each sample. Use new, unopened pipette tips.
  - Do not reuse plate seals.
  - Centrifuge the plate to collect contents before removing a plate seal.
  - Press the plate firmly down on a flat surface when removing a plate seal.

- Ensure a secure uniform seal around all wells when sealing the plate with a plate seal.
- Ensure that all samples in the 96-well plates are mixed thoroughly at every step.

Reagent Handling

- Use good laboratory practices to minimize contamination of samples:
  - Use a new pipette tip for every new sample.
  - Whenever possible, separate RT and preamplification activities and IFC setup from sample preparation activities. Dedicate laboratory materials to designated areas.
- Ensure that lab consumables (tubes, tips, plates) used for the RNA handling steps are RNase-free.
- Use separate bottles of Dilution Reagent (100-8730) at their respective areas.
- Retrieve only the reagents required from each kit based on the number of IFCs that you will run.
- Use only the reagents provided in the required kit and specified in the protocol.
- Do not swap reagents between kit lots.
- Unless otherwise specified, thaw reagents at room temperature (+15 °C to +30 °C), and then use them at room temperature.
- Mix and centrifuge reagents as directed.
- Before use, briefly vortex reagents at medium speed for at least 5 sec, then centrifuge for at least 2 sec to ensure that all reagents are homogeneous.
- Place the sample mixes on ice when not in use.
- To reduce the number of pipetting steps, we recommend first transferring reagents into an 8-well PCR tube strip to enable transfer into a 96-well plate using an 8-channel pipette.

Bubble Prevention

- Vortex gently (low speed) but thoroughly (at least 5 sec) to ensure that all reagents and reagent mixes are homogeneous.
- After vortexing the assay and sample mixes, centrifuge them to collect all mixes at the bottoms of the wells before pipetting into the IFC inlets. Failure to do so may result in a decrease in data quality.
- Check the source plate or tube for bubbles before pipetting.
- Check pipette tips for air gaps while pipetting.
- Pipet reagents slowly and carefully to transfer entire volumes and to minimize bubbles.
- To avoid creating bubbles in the IFC inlets, pipet into the inlets at an angle and do not go past the first stop on the pipette. If a bubble is introduced, ensure that it floats to the top of the inlet.
- If necessary, remove bubbles from an IFC inlet by removing the contents of the inlet by pipette and then carefully re-pipetting the contents into the inlet.

Prepare and Perform the 1-Step Reverse Transcription and Preamplification Reactions

IMPORTANT Assemble the 1-step pre-mix, sample mixes, and 1-step reactions in the pre-PCR area of the facility.

Pool and Dilute the Primer Sets for Preamplification

IMPORTANT Prepare in the pre-PCR area of the facility.

1 Briefly vortex and centrifuge the reagents before use.

2 Pool and dilute the assays in a new 2 mL tube. The assay mix should be prepared and used immediately.

NOTE Volumes can be adjusted proportionally based on the number of samples to be amplified, up to 192 reactions.

- If using 2019-nCoV RUO assays (6.7 µM primers, forward and reverse; 1.7 µM probe), use components in Table 1.
- If using custom assays (100 µM primers, forward and reverse; 100 µM probe), use components in Table 2.
- If using 20X TaqMan® Gene Expression Assays (18 µM primers, forward and reverse; 5 µM probe), use components in Table 3.

NOTE Probes are not needed in the reverse transcription and preamplification reactions, but are often already combined as primers and probe sets from commercial vendors.

Table 1. Pooled and diluted 2019-nCoV RUO Kit primer mix

Component	Vol for 3 Assays/ 1 Reaction (µL)*	Vol for 3 Assays/ 192 Reactions (µL)†
2019-nCoV RUO assays (102-0690 or 10006713)		
• 2019 nCoV_N1	0.112	25.0
• 2019 nCoV_N2	0.112	25.0
• RNase P	0.022	5.0
Dilution Reagent (100-8730)	6.754	1,509
Total	7.0	**1,564**

Concentration of pooled 2019-nCoV RUO assays:

- 2019 nCoV_N1 and 2019 nCoV_N2 assays: 107.10 nM primers; 27.17 nM probe
- RNase P assays: 21.42 nM primers; 5.43 nM probe

* When preparing master mixes for less than 192 reactions, include an additional 10% to the volumes for overage.

† Includes overage for ease of pipetting

Table 2. Pooled and diluted custom primer mix

Component	Vol for *N* Assays/ 192 Reactions (µL)
Custom assays:	
• Forward primer	*N* x 1.7
• Reverse primer	*N* x 1.7
• Probe	*N* x (up to 0.42)
Dilution Reagent (100-8730)	1,564 – (volume of custom assays)
Total	**1,564**

Concentration of each assay: 108.70 nM primers; up to 26.85 nM probe

Table 3. Pooled and diluted TaqMan Gene Expression Assay primer mix

Component	Vol for *N* Assays/ 192 Reactions (µL)
20X TaqMan Gene Expression Assays	*N* x 9.3
Dilution Reagent (100-8730)	1,564 – (*N* x 9.3)
Total	**1,564**

Concentration of each assay: 107.03 nM primers; 29.73 nM probe

NOTE Volume can be adjusted proportionally based on the number of samples to be amplified.

Prepare the 1-Step Reverse Transcription and Preamplification Reactions

IMPORTANT Prepare in the pre-PCR area of the facility.

1 Thaw the Advanta RT-Preamp Master Mix and keep on ice. Briefly vortex and centrifuge the reagents before use.

2 In a DNA-free hood, combine the components shown in Table 4 in a new 5 mL tube to make the 1-step pre-mix and place on ice. Scale up appropriately for multiple runs.

Table 4. 1-step pre-mix

Component	Vol per Reaction (µL)*	Vol for 192 Reactions (µL)†
Pooled primer mix (see Table 1, Table 2, or Table 3)	7	1,484
Advanta RT-Preamp Master Mix (102-0419)	3	636
Total	**10**	**2,120**

* When preparing master mixes for less than 192 reactions, include an additional 10% to the volumes for overage

† Includes overage

3 Cap the tube, vortex, and centrifuge the 1-step pre-mix.

4 Aliquot 128 µL of 1-step pre-mix into each well of two 0.2 mL 8-well strips (see Figure 1).

5 Use an 8-channel pipette to combine the 1-step pre-mix and the samples in 2 new 96-well plates as shown in Figure 1.

a Transfer 10.0 µL of 1-step pre-mix into each well of 2 new 96-well plates.

b Add 5.0 µL of sample to each well of the 96-well plates.

NOTE Only one preamplification reaction is prepared for each sample.

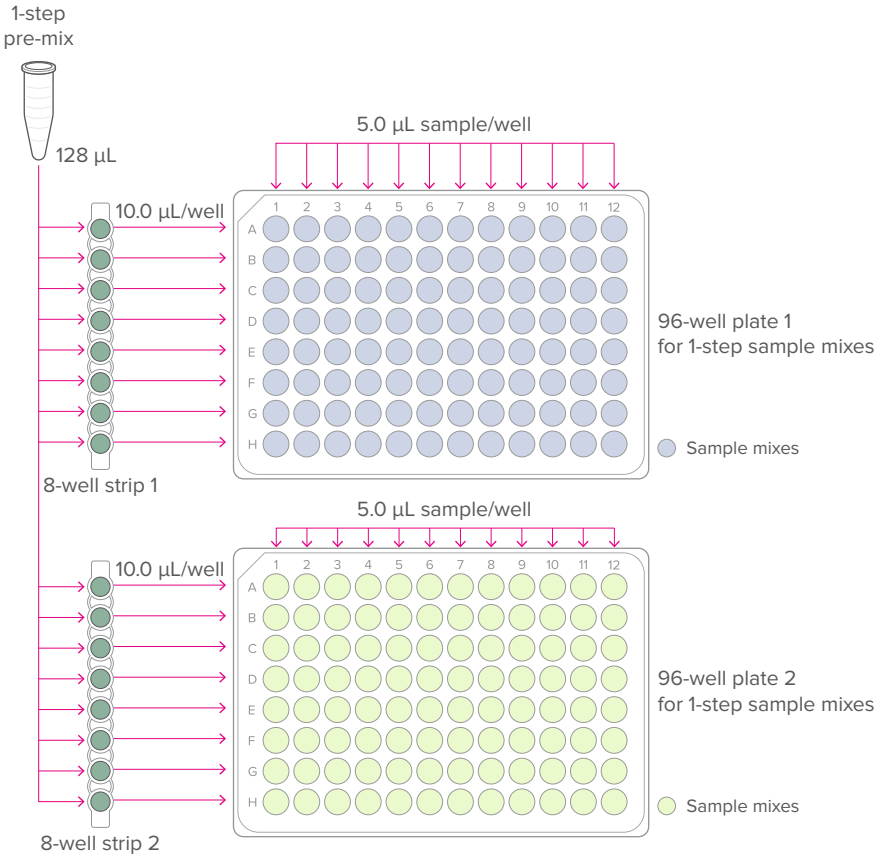


Figure 1. 1-step reaction plates (per-well transfer volumes)

6 Tightly seal the plates with clear adhesive film, then gently vortex and centrifuge them at 3,000 × *g* for 60 sec to mix the reactions.

Perform the 1-Step Reverse Transcription and Preamplification Reactions

1 Place each plate in a 96-well plate thermal cycler and cycle using the program in Table 5:

Table 5. 1-step reverse transcription and preamplification

Temperature	Time	Condition
+50 °C	15 min	RT
+95 °C	2 min	Hot start
+95 °C	15 sec	20 cycles
+60 °C	2 min	
+4 °C	∞	Hold

NOTE The appropriate number of cycles may need to be determined empirically.

Dilute the Preamplified cDNA

IMPORTANT Prepare in the post-PCR area of the facility.

After cycling, dilute the preamplified reactions in the 96-well plates in Dilution Reagent as shown in Table 6 and described as follows:

1 Transfer 13 mL of Dilution Reagent into a new 25 mL reagent reservoir.

NOTE This is sufficient for the dilution of two 96-well plates of preamplified samples.

2 Use an 8-channel pipette to transfer 60 µL of Dilution Reagent into each well containing the preamplified cDNA.

NOTE Any unused Dilution Reagent dispensed in Step 1 should be discarded.

3 Tightly seal plates with clear adhesive film, then gently vortex to mix the dilutions and centrifuge them at 3,000 × *g* for 60 sec. Set aside until ready to prepare the final sample mixes.

STOPPING POINT The diluted, preamplified cDNA can either be assayed immediately or stored at −15 °C to −25 °C for later use.

Table 6. Diluted, preamplified cDNA

Component	Vol per Reaction (µL)
Dilution Reagent (100-8730)	60.0
Preamplified cDNA (contained in the 96-well plates)	15.0
Total	**75.0**

Prepare and Perform Real-Time PCR Reactions on the IFC

Prepare the Final Assay Mixes for Loading on the IFC

NOTE Assemble your assays in a 96-well plate and record in a detector map. See the Real-Time PCR Analysis User Guide (68000088) for more information about detector maps.

1 Briefly vortex and centrifuge the reagents before use.

2 In a DNA-free hood, prepare each final assay mix in a new 1.5 mL tube using the components in Table 7, Table 8, or Table 9, as appropriate. The tables show examples for preparing a 50 µL stock, which is sufficient for 10 IFCs. Scale appropriately for multiple runs.

- If using 2019-nCoV RUO assays (6.7 µM primers, forward and reverse; 1.7 µM probe), use components in Table 7 as shown in Figure 2 on page 12.
- If using custom assays (100 µM primers, forward and reverse; 100 µM probe), use components in Table 8 and shown in Figure 3 on page 12.
- If using 20X TaqMan® Gene Expression Assays (18 µM primers, forward and reverse; 5 µM probe), use components in Table 9 and shown in Figure 3 on page 12.

NOTE Unused assay mixes can be stored at −20 °C for at least 6 months.

Table 7. Final 2019-nCoV RUO assay mixes

Component	Vol per Inlet (µL)*	Vol for 50 µL Stock for Each Assay (µL)*
2019-nCoV RUO assays (102-0690 or 10006713)	3.0	37.5
4X Assay Loading Reagent (102-0114)	1.0	12.5
Total	**4.0**	**50.0**

Final concentration: 5 µM primers; 1.28 µM probe

* Includes overage

Table 8. Final custom assay mixes

Component	Vol per Inlet (µL)*	Vol for 50 µL Stock for Each Assay (µL)*
Custom assays:		
• Forward primer	0.36	4.50
• Reverse primer	0.36	4.50
• Probe	0.10	1.25
Dilution Reagent (100-8730)	1.18	14.75
PCR Water (100-5941)	1.0	12.50
4X Assay Loading Reagent (102-0114)	1.0	12.50
Total	**4.0**	**50.00**

Final concentration: 9 µM primers; 2.5 µM probe

* Includes overage

Table 9. Final TaqMan Gene Expression Assay mixes

Component	Vol per Inlet (µL)*	Vol for 50 µL Stock for Each Assay (µL)*
20X TaqMan® Gene Expression Assays	2.0	25.0
PCR Water (100-5941)	1.0	12.5
4X Assay Loading Reagent (102-0114)	1.0	12.5
Total	**4.0**	**50.0**

Final concentration: 9 µM primers; 2.5 µM probe

* Includes overage

3 Pipet 4.0 µL of each assay stock into the respective wells in a new 96-well plate as shown in Figure 2 (for 2019-nCoV RUO assays) or Figure 3 (for custom or TaqMan assays).

NOTE For each unused assay inlet, combine 3.0 µL of PCR Water (100-5941) with 1.0 µL 4X Assay Loading Reagent (102-0114) in the respective wells.

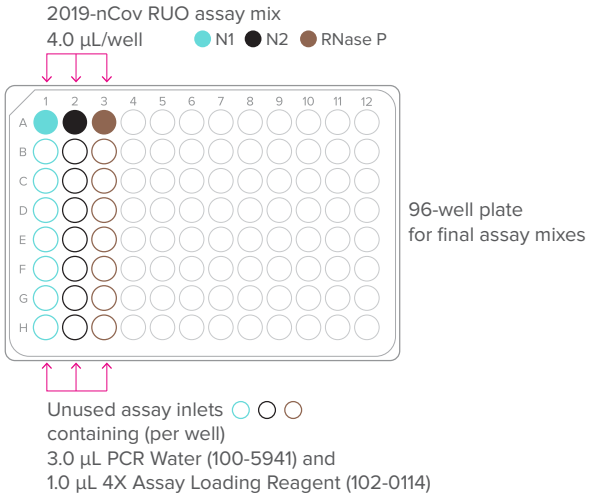


Figure 2. Final 2019-nCoV RUO assay mixes plate (per-well transfer volumes)

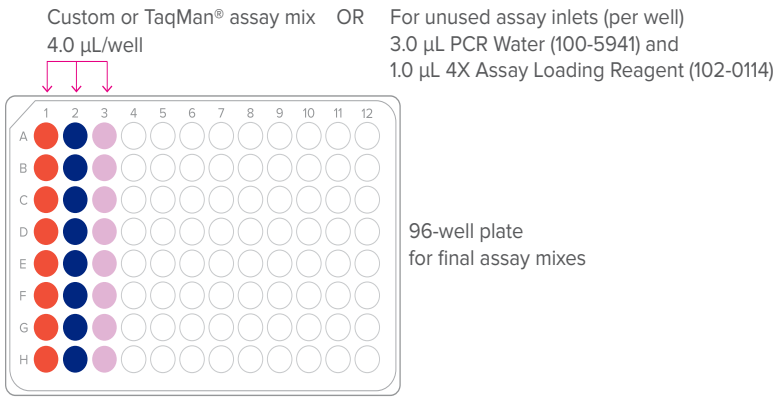


Figure 3. Final custom and TaqMan Gene Expression assay mixes plate (per-well transfer volumes)

Prepare the Final Sample Mixes

1 Thaw the Advanta PCR MM and keep on ice. Briefly vortex and centrifuge the reagents before use.

2 In a DNA-free hood, combine the components shown in Table 10 in a new 1.5 mL tube to make the sample pre-mix and place on ice.

NOTE This is enough volume for the entire IFC.

Table 10. Sample pre-mix

Component	Vol per Inlet (µL)*	Sample Pre-Mix for One 192.24 IFC (µL)†
Advanta PCR MM (102-0420)	2.0	460.0
20X GE Sample Loading Reagent (85000735)	0.2	46.0
Total	**2.2**	**506.0**

* Includes overage

† 230 reactions for ease of pipetting

3 Prepare the final sample mixes as shown in Figure 4 on page 14.

a Briefly vortex and centrifuge the sample pre-mix from Table 10.

b Aliquot 60 µL of pre-mix into each well of a new 8-well strip.

c Use an 8-channel pipette to transfer 2.2 µL of sample pre-mix from the 8-well strip into each well of 2 new 96-well plates.

d Remove the plates from the DNA-free hood and prepare the final sample mix by adding 1.8 µL of each diluted, preamplified sample from Table 6 on page 10 to each well.

IMPORTANT Before use, briefly vortex and centrifuge the plates containing the diluted, preamplified cDNA.

NOTE For each unused sample inlet, add 1.8 µL of PCR Water (100-5941) to the 2.2 µL sample pre-mix in the plate.

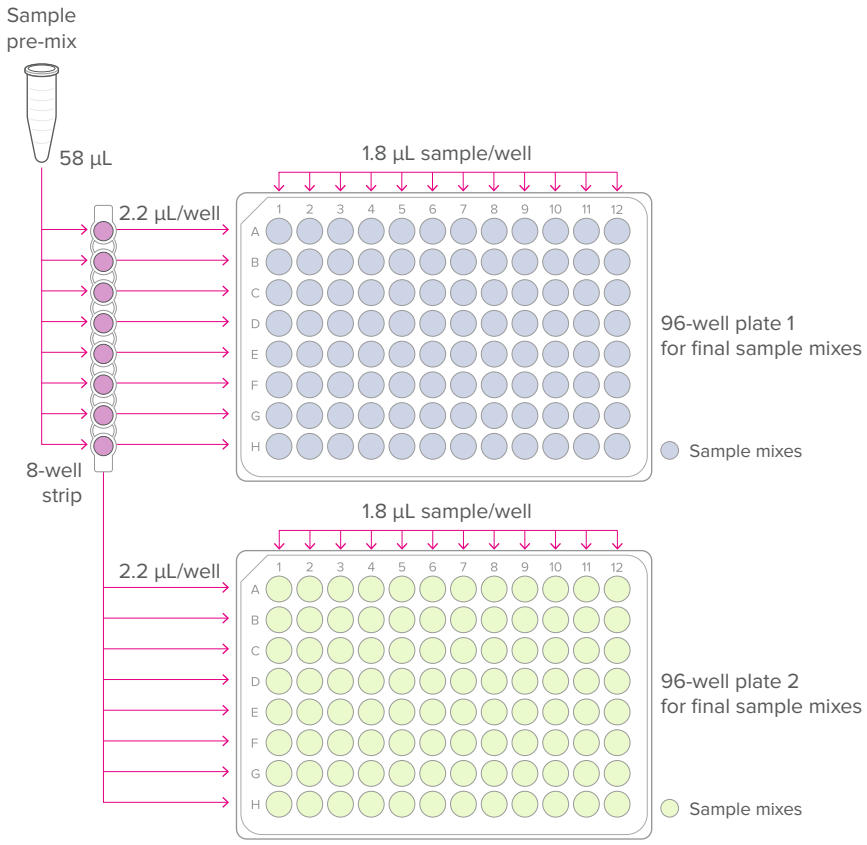


Figure 4. Final sample plates (per-well transfer volumes)

4 Tightly seal the plates with clear adhesive film, then vortex and centrifuge them at 3,000 × *g* for 60 sec.

Prepare the 192.24 IFC

IMPORTANT When injecting control line fluid:
- Follow the best practices for handling IFCs and control line fluid on page 5.
- Only use a 192.24 syringe (100-4058). The syringe is prefilled with 150 µL of control line fluid.

1 Remove the 192.24 Control Line Fluid syringe (100-4058) and the 192.24 Dynamic Array IFC (100-6266) from the packaging.

IMPORTANT Do not evacuate air from the syringe prior to injecting control line fluid (Step 4).

2 Actuate the check valve:

a First, place the IFC on a flat surface.

b Then, use the syringe with the shipping cap in place to actuate the check valve in the top accumulator (closest to the notched A1 corner of the IFC) with gentle pressure. Ensure that the poppet can move freely up and down (Figure 5 on page 15).

IMPORTANT The bottom accumulator is not used.

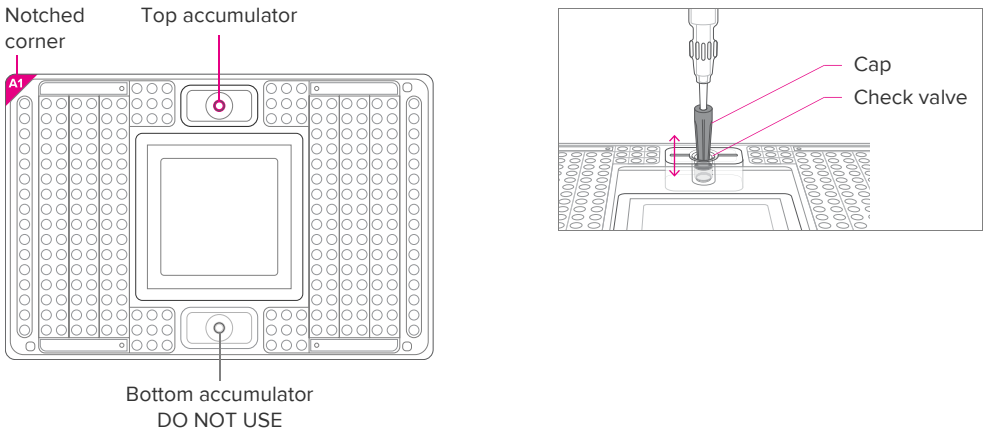


Figure 5. Actuating the check valve in the top accumulator on the 192.24 IFC

3 Hold the syringe firmly in one hand with tip facing up and away from the IFC and remove the shipping cap with the other hand.

4 Holding the IFC at a 45° angle, insert the syringe tip into the top accumulator (Figure 6).

IMPORTANT

- Avoid bending the syringe tip. Be careful when removing syringe cap to prevent drips.
- Avoid getting control line fluid on the exterior of the IFC or in the inlets because this makes the IFC unusable. If this occurs, use a new IFC.

5 Use the syringe tip to press down gently on the black O-ring to move it (Figure 6). Visually confirm that the O-ring has moved.

6 Release the control line fluid:

a First, press the syringe plunger to release the control line fluid into the accumulator while maintaining the 45° angle to allow the fluid to flow away from the O-ring.

b Next, slowly inject the control line fluid by pushing down on the syringe plunger. The control line fluid flows into the accumulator through the open check valve. Use the entire contents of the syringe.

c Last, after fully depressing the plunger, wait approximately 5 sec before withdrawing the syringe.

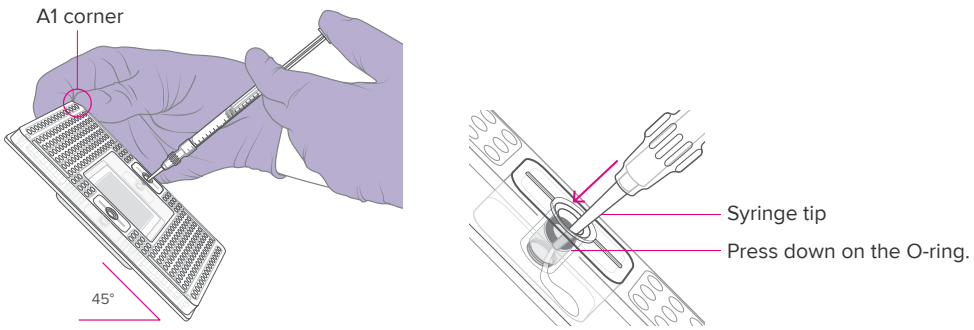


Figure 6. Injecting control line fluid into the accumulators on the 192.24 IFC

7 Check to ensure that the O-ring returns to its normal position after the syringe is removed.

8 Pull the protective film down and away from the bottom of the IFC. Discard the film.

Load the IFC

For detailed instructions about using Juno, see the Juno System User Guide (100-7070). For detailed instructions about using the IFC Controller RX, see the IFC Controller RX User Guide (100-3385).

IMPORTANT

- Vortex thoroughly and centrifuge all assay and sample solutions before pipetting into the IFC inlets. Failure to do so may result in a decrease in data quality.
- While pipetting, do not go past the first stop on the pipette. Doing so may introduce air bubbles into inlets.

Refer to Figure 6 (for 2019-nCoV RUO assays) or Figure 7 (for custom or TaqMan assays) on page 17 when pipetting final sample and assay mixes, Actuation Fluid, and Pressure Fluid into the IFC.

1. If using Juno, ensure that the RX Interface Plate is installed in the Juno instrument.
2. Pipet 3 µL of each final sample mix into the respective sample inlets on the IFC.
3. Pipet 3 µL of each final assay mix into the respective assay inlets on the IFC
4. Pipet 150 µL of Actuation Fluid (100-6250) into the P1 reservoir ( ) on the IFC.
5. Pipet 150 µL of Pressure Fluid (100-6249) into each of the P2 and P3 reservoirs ( ) on the IFC.
6. Pipet 20 µL of Pressure Fluid into each of the P4 and P5 inlets ( ) on the IFC.
7. Blot the IFC surface with a dry, lint-free cloth.
8. Place the IFC into the controller:
   - Juno: Tap **OPEN** to open the instrument tray and align the notched corner of the IFC to the white notch on the tray. Tap **LOAD**.
   - RX: Press **EJECT** to open the instrument tray and align the notched corner of the IFC to the A1 mark. Press **Load Chip**.
9. Run the Load Mix script:
   - Juno: Tap **Load Mix 192.24 GE**, then tap **Run**.
   - RX: Select **Load Mix (169x)** and press **Run Script**.

   IMPORTANT Start the IFC run on the Biomark HD or Biomark instrument within 1 hr of completing the Load Mix script.
10. If necessary, turn on the Biomark HD or Biomark system (computer and instrument). For Biomark, also launch the Data Collection software, and turn on the lamp. The lamp takes 20 min to warm up.

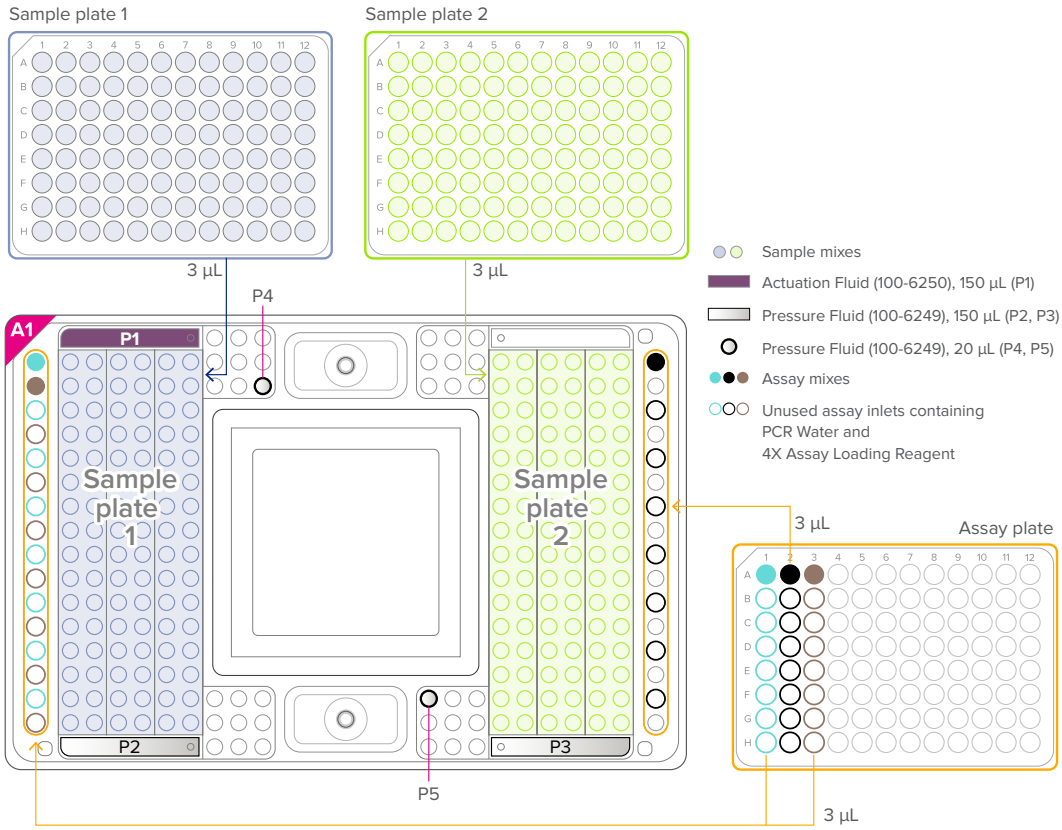


Figure 6. Pipetting map for the 192.24 IFC for 2019-nCoV RUO assays

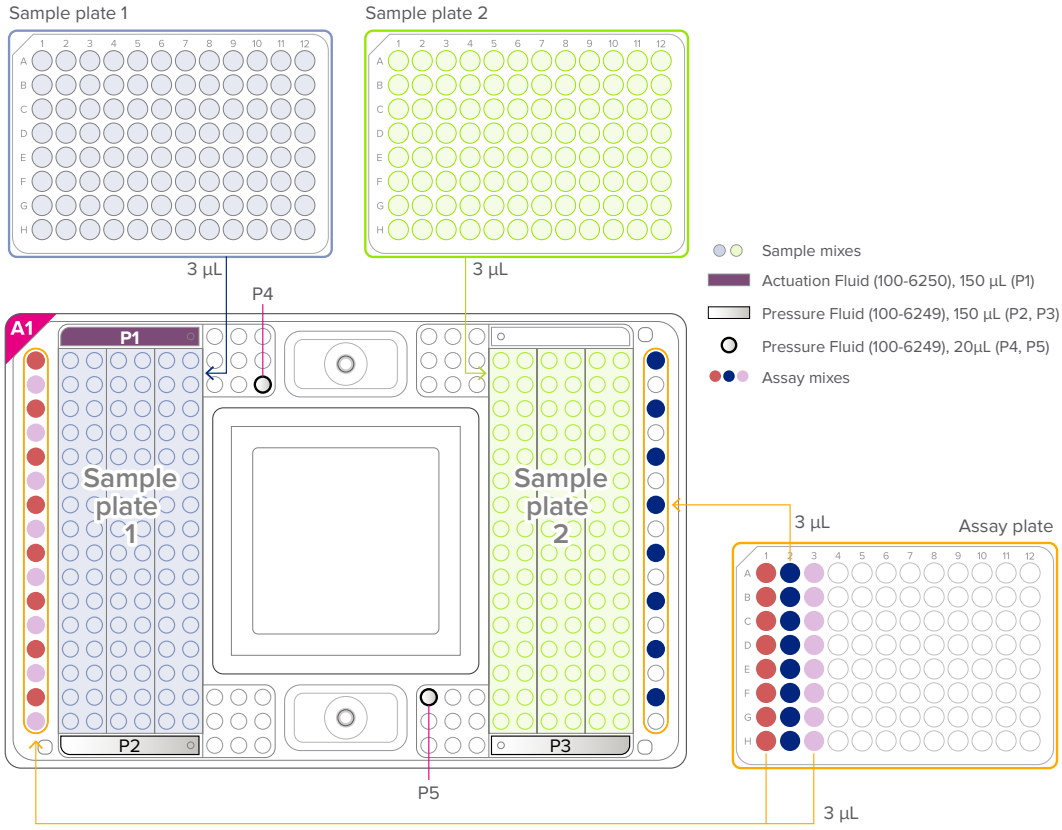


Figure 7. Pipetting map for the 192.24 IFC for custom and TaqMan assays

Thermal-Cycle and Collect Real-Time PCR Data

For detailed instructions about using the Data Collection software, see the Biomark HD Data Collection User Guide (100-2451) or Biomark/EP1 Data Collection User Guide (68000127).

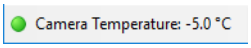
1 Remove the loaded IFC from Juno or IFC Controller RX.

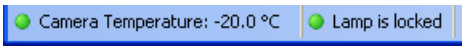
2 Use clear tape to remove any dust particles or debris from the IFC surface, if necessary.

3 If necessary, double-click the **Data Collection** icon (or) on the desktop of the Biomark HD or Biomark computer to launch the software.

4 Click **Start a New Run**.

5 Confirm that the camera status indicator and the lamp status indicator (Biomark only) at the bottom of the window are green.

Biomark HD: 

Biomark: 

6 Place the loaded IFC on the instrument tray and align the notched A1 corner on the IFC with the A1 label on the tray (Figure 7). In the Data Collection software, click **Load**.

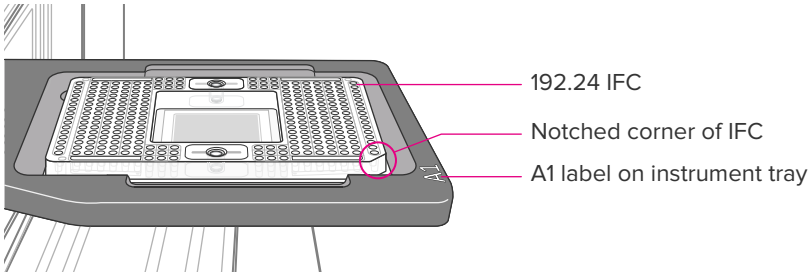


Figure 7. Loaded IFC on instrument tray

7 In the Data Collection software, confirm the IFC barcode and IFC type and then click **Next**.

8 Complete the Chip Run section by selecting either a new or a pre-defined run.

NOTE To pre-define a run, see the Biomark HD Data Collection User Guide or Biomark/EP1 Data Collection User Guide.

9 Complete the Chip Run Name and Location section:

a Enter a run name or select the checkbox to use the IFC barcode as the run name.

b Select a file storage location for a new IFC run or browse to select a pre-defined run file and click **Next**.

10 Complete the Application, Reference and Probes section and then click **Next**.

For	Select
Application	**Gene Expression**
Passive reference	**ROX™**
Assay	**Single probe**
Probes	**FAM-MGB**

11 Browse to and select the thermal protocol:

For	Select
Biomark HD	**GE 192x24 Fast v1.pcl**
Biomark*	**GE 192x24 Quick v1.pcl**

* See Appendix D for more information about the GE 192x24 Quick thermal protocol.

NOTE For a description of the thermal protocols, see Appendix D.

12 Confirm that **Auto Exposure** is selected. Click **Next**.

13 Confirm that IFC run information is correct and click **Start Run.**

14 After the run is complete, analyze your data using the Real-Time PCR Analysis software.

Appendix A: Saliva Preparation

Materials

IMPORTANT Store reagents as soon as they are received, according to manufacturer's storage recommendations.

Reagents

Product Name	Source	Part Number
PBS, pH 7.4	Thermo Fisher Scientific™	10010023
RNAsecure™ RNase Inactivation Reagent		AM7005

Consumables

Product Name	Source
Sterile container without preservatives for saliva collection	Major laboratory supplier
96-well PCR plates and clear adhesive film for 96-well plates* or 8-well PCR tube strips with caps	

* Recommended: MicroAmp® Clear Adhesive Film (Thermo Fisher Scientific, 4306311)

Equipment

Product Name	Source	Part Number
Pipettes (P2–P1000) and appropriate filtered, low-retention tips*	MLS	—
Centrifuge		
Vortexer		—
Thermal cycler for 96-well plates		—

* Recommended: Rainin® pipettes

Collect the Saliva Specimens

IMPORTANT Use universal precautions when handling biological samples.

Collect saliva specimen in a sterile container. Store specimens at −20 °C to −80 °C and ship on dry ice. Transport and test specimens as soon as possible after collection. Specimens are stable for up to 120 hr at ambient temperature.

Process the Saliva Specimens

IMPORTANT

- Prepare in the pre-PCR area of the facility.
- Use universal precautions when handling biological samples. Prior to heat inactivation, the saliva specimens should be handled in a BSL-2 environment.

1 Mix each saliva specimen with an equal volume of nuclease-free PBS (Thermo Fisher Scientific, 10010023). For example, if 15 µL of saliva specimen was collected, add 15 µL of PBS.

2 Aliquot 24 µL of each saliva/PBS mix into a tube or plate and add 1 µL of RNAsecure (Thermo Fisher Scientific, AM7005) to the mix, then briefly vortex and centrifuge.

3 Heat-inactivate the prepared saliva specimens in a thermal cycler using the program in Table 11:

Table 11. Heat-inactivation of saliva samples

Temperature	Time
+90 °C	10 min
+4 °C	2 min
+4 °C	∞

4 After 2 min at +4 °C, you can place the samples on ice until ready to use.

5 Use 5 µL of each heat-inactivated saliva sample in the 1-step reverse transcription and preamplification reactions on page 8.

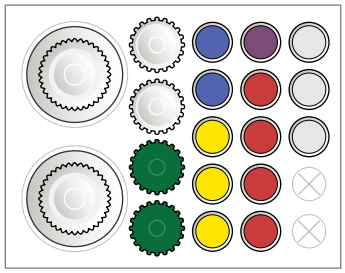
Appendix B: Advanta RT-PCR Kit Bundled Components

Bundle	Component	Part Number	Quantity
Advanta™ RT-PCR Reagent Kit—192.24, 10 IFCs (102-0424)	Advanta RT-PCR Reagent Kit—192.24, 10 IFCs, Module 1	102-0422	1 kit
	Advanta RT-PCR Reagent Kit—192.24, 10 IFCs, Module 2	102-0423	1 kit
	192.24 Dynamic Array IFC	100-6266	10 IFCs
	Control Line Fluid for 192.24 IFCs (150 µL each)	100-4058	10 syringes

Bundle	Component	Part Number	Quantity
Advanta™ RT-PCR Reagent Kit—192.24, 2 IFCs (102-0525)	Advanta RT-PCR Reagent Kit—192.24, 2 IFCs, Module 1	102-0524	1 kit
	192.24 Dynamic Array IFC	100-6266	2 IFCs
	Control Line Fluid for 192.24 IFCs (150 µL each)	101-0383	2 syringes

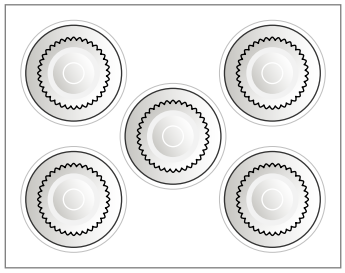
Appendix C: Advanta RT-PCR Kit Components

Advanta RT-PCR Reagent Kit–192.24, 10 IFCs Module 1 (102-0422)



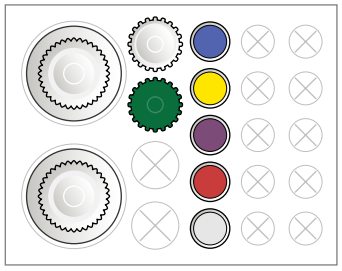
- 2 tubes of 20X GE Sample Loading Reagent, 250 µL (85000735)
- 3 tubes of 4X Assay Loading Reagent, 120 µL (102-0114)
- 1 tube of Actuation Fluid, 1.5 mL (100-6250)
- 4 tubes of Advanta™ PCR MM, 1.2 mL (102-0420)
- 3 tubes of PCR Water, 1.8 mL (100-5941)
- 2 bottles of Pressure Fluid, 2.04 mL (100-6249)
- 2 bottles of Advanta RT Preamp Master Mix, 3.3 mL (102-0419)
- 2 bottles of Dilution Reagent, 25 mL (100-8730)

Advanta RT-PCR Reagent Kit–192.24, 10 IFCs, Module 2 (102-0423)



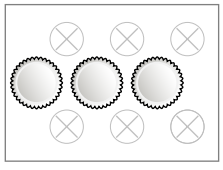
- 5 bottles, Dilution Reagent, 25 mL (100-8730)

Advanta RT-PCR Reagent Kit–192.24, 2 IFCs (102-0524)



- 1 tube of 20X GE Sample Loading Reagent, 250 µL (85000735)
- 1 tube of 4X Assay Loading Reagent, 120 µL (102-0114)
- 1 tube of Actuation Fluid, 1.5 mL (100-6250)
- 1 tube of Advanta™ PCR MM, 1.2 mL (102-0420)
- 1 tube of PCR Water, 1.8 mL (100-5941)
- 1 bottle of Pressure Fluid, 2.04 mL (100-6249)
- 1 bottle of Advanta RT-Preamp Master Mix, 3.3 mL (102-0419)
- 2 bottles of Dilution Reagent, 25 mL (100-8730)

2019-nCoV Probe & Primer RUO Kit (102-0690)



- 1 tube of RP, 170 µL (102-0687)
- 1 tube of 2019-nCoV_N1, 370 µL (102-0688)
- 1 tube of 2019-nCoV_N2, 370 µL (102-0689)

Appendix D: Research Use Only 2019-Novel Coronavirus (2019-nCoV) Real-time RT-PCR Primers and Probes

The Centers for Disease Control and Prevention (CDC) has released primer and probe sequences which target SARS-CoV-2 viral sequences for the purposes of respiratory virus surveillance and research. These primers and probes can be ordered as custom assays from major oligo suppliers. This protocol utilizes 100 µM primers (forward and reverse), and 100 µM probes.

Table 12. RUO2019-Novel Coronavirus (2019-nCoV) Real-time RT-PCR Primers and Probes

Name	Description	Oligonucleotide Sequence (5′→3′)	Label*
2019-nCoV_N1-F	2019-nCoV_N1 Forward Primer	GAC CCC AAA ATC AGC GAA AT	None
2019-nCoV_N1-R	2019-nCoV_N1 Reverse Primer	TCT GGT TAC TGC CAG TTG AAT CTG	None
2019-nCoV_N1-P	2019-nCoV_N1 Probe	FAM-ACC CCG CAT TAC GTT TGG TGG ACC-BHQ1	FAM, BHQ-1
2019-nCoV_N2-F	2019-nCoV_N2 Forward Primer	TTA CAA ACA TTG GCC GCA AA	None
2019-nCoV_N2-R	2019-nCoV_N2 Reverse Primer	GCG CGA CAT TCC GAA GAA	None
2019-nCoV_N2-P	2019-nCoV_N2 Probe	FAM-ACA ATT TGC CCC CAG CGC TTC AG-BHQ1	FAM, BHQ-1
RP-F	RNase P Forward Primer	AGA TTT GGA CCT GCG AGC G	None
RP-R	RNase P Reverse Primer	GAG CGG CTG TCT CCA CAA GT	None
RP-P	RNase P Probe	FAM-TTC TGA CCT GAA GGC TCT GCG CG-BHQ1	FAM, BHQ-1

* Probes are labeled at the 5′-end with the reporter molecule 6-carboxyfluorescein (FAM) and with the quencher, Black Hole Quencher® 1 (BHQ-1) (Biosearch Technologies, Inc., Novato, CA) at the 3′-end.

These oligonucleotide sequences from the CDC are current as of May 29, 2020 and are subject to future changes as the 2019-Novel Coronavirus evolves. For the most up-to-date sequences, visit https://www.cdc.gov/coronavirus/2019-ncov/lab/rt-pcr-panel-primer-probes.html.

Appendix E: Biomark HD and Biomark Thermal Cycler Protocols

GE 192x24 Fast v1 thermal cycling parameters (for Biomark HD only, 5.5 °C/sec ramp rate)

Temperature	Time	Cycles	Description	
95 °C	60 sec	1	Hot start	
96 °C	5 sec	35	PCR	Denaturation
60 °C	20 sec			Annealing

GE 192x24 Quick v1 thermal cycling parameters (for 1-step workflow on Biomark only, 2 °C/sec ramp rate)

Use the Protocol Editor in the Biomark Data Collection software to create the **GE 192x24 Quick v1.pcl** thermal protocol. See the Biomark/EP1 Data Collection User Guide (68000127) for instructions.

Temperature	Time	Cycles	Description	
95 °C	60 sec	1	Hot start	
96 °C	5 sec	35	PCR	Denaturation
60 °C	20 sec			Annealing

Appendix F: Related Documents

Go to fluidigm.com to download these related documents.

Title	Document Number
Pathogen Detection Using the Advanta RT-PCR Kit Quick Reference	FLDM-00192
Control Line Fluid Loading Procedure	68000132
Juno System User Guide	100-7070
IFC Controller RX User Guide	100-3385
Biomark HD Data Collection User Guide	100-2451
Biomark/EP1 Data Collection User Guide	68000127
Real-Time PCR Analysis User Guide	68000088

Appendix G: Safety

General Safety

In addition to your site-specific safety requirements, Fluidigm recommends the following general safety guidelines in all laboratory and manufacturing areas:

- Use the appropriate personal protective equipment (PPE): safety glasses, fully enclosed shoes, lab coats, and gloves, according to your laboratory safety practices.

- Know the locations of all safety equipment (fire extinguishers, spill kits, eyewashes/showers, first-aid kits, safety data sheets, etc.), emergency exit locations, and emergency/injury reporting procedures.
- Do not eat, drink, smoke, or apply cosmetics in lab areas.
- Maintain clean work areas.
- Wash hands before leaving the lab.

Instrument Safety

For complete instrument safety information, including a full list of the symbols on the instrument, refer to the Juno System User Guide (100-7070) or IFC Controller RX User Guide (100-3385) and Biomark HD Data Collection User Guide (100-2451) or Biomark/EP1 Data Collection User Guide (68000127).

WARNING BIOHAZARD. When handling biohazardous materials or when using biohazardous material on the instrument, use appropriate personal protective equipment and adhere to Biosafety in Microbiological and Biomedical Laboratories (BMBL), a publication from the Centers for Disease Control and Prevention, and to your lab's safety protocol to limit biohazard risks. If biohazardous materials are used, properly label the equipment as a biohazard. For more information, see the BMBL guidelines online at cdc.gov/biosafety/publications/index.htm.

Chemical Safety

The responsible individuals must take the necessary precautions to ensure that the surrounding workplace is safe and that instrument operators are not exposed to hazardous levels of toxic substances. When working with any chemicals, refer to the applicable safety data sheets (SDSs) provided by the manufacturer or supplier.

Disposal of Products

Used IFCs and reagents should be handled and disposed of in accordance with federal, state, regional, and local laws for hazardous waste management and disposal.

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