# Advanta SARS-CoV-2 Mutation Assay Panel

## Contents

# About This Protocol

This protocol describes how to detect COVID-19 mutations using the Advanta™ SARS-CoV-2 Mutation Assay Panel and RT-PCR reagents (such as the Advanta Preamp & IFC Reagent Kit—192.24, as shown in this document) with the 192.24 Dynamic Array™ IFC (integrated fluidic circuit) on Biomark™ HD. This protocol only applies to this panel being run with outlined but not provided reagents and does not apply to any other workflows.

**IMPORTANT** Before using this panel, read and understand the detailed instructions and safety guidelines in this document. For complete safety information, see Appendix F. 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).

## 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 SARS-CoV-2 Mutation Assay Panel can be used with saliva specimens or purified nucleic acid samples (RNA or both DNA and RNA) and allows detection of 6 COVID-19 mutations (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.

## Workflow Overview

| | Workflow Step | Run Time* |
|---|---|---|
| 1 | Collect the saliva specimens.<br>(Skip this step if using extracted RNA). | — |
| 2 | Prepare the positive controls. | — |
| 3 | Prepare the saliva specimens, then heat-inactivate the prepared specimens and negative controls.<br>(Skip this step if using extracted RNA.) | — |
| 4 | Prepare the 1-step reverse transcription (RT) and preamplification reactions. | — |
| 5 | Perform the 1-step RT and preamplification reactions. | 70 min |
| 6 | Prepare the final assay and sample mixes for real-time PCR. | — |
| 7 | Prepare the 192.24 IFC (integrated fluidic circuit) by injecting control line fluid | — |
| 8 | Pipet each final assay, sample, and control mix and Actuation Fluid and Pressure Fluid into the IFC, then load the IFC on Juno or IFC Controller RX. | 35 min |
| 9 | Thermal-cycle and collect data on Biomark HD. | 35 min |
| 10 | Annotate data using the Real-Time PCR Analysis software, then export results and interpret using the Biomark Pathogen Detection Software. | |

* Does not include hands-on time

# Materials

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

## Provided Reagents from Fluidigm

**NOTE** The Advanta SARS-CoV-2 Mutation Assay Panel can be used with the reagents in the Advanta Preamp & IFC Reagent Kit–192.24. For a list of the panel components, see Appendix B.

| Product Name | Part Number | Storage |
|---|---|---|
| Advanta™ SARS-CoV-2 Mutation Assay Panel | 102-1340 | −15 °C to −25 °C |

## Additional Kits and Reagents

| Product Name or Description | Source | Part Number |
|---|---|---|
| Advanta™ Preamp & IFC Reagent Kit–192.24* | Fluidigm | 102-1341 |
| PBS, pH 7.4 | Thermo Fisher Scientific™ | 10010023 |
| RNAsecure™ RNase Inactivation Reagent | | AM7005 |
| Twist Synthetic RNA Control 2 (MN908947.3) (wild type) (1,000,000 copies/µL) | Twist Bioscience® | 102024 |
| Normal saliva from pooled human donors confirmed COVID-19 negative | — | — |

* Sufficient for 10 IFCs. For a list of the kit components, see Appendix B.

## Consumables

| Product Name or Description | Source | Part Number |
|---|---|---|
| Sterile container without preservatives for saliva collection | Major laboratory supplier (MLS) | — |
| Disposable microcentrifuge tubes, polypropylene, 1.5 mL, 2 mL, and 5 mL | | — |
| 25 mL reagent reservoir | | — |
| 96-well PCR plates* | | — |
| 8-well PCR tube strips with caps | | — |
| Clear adhesive film for 96-well plates† | | — |

* The PCR plates selected for the workflow should be compatible with the thermal cycler.

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

## Equipment

| Product Name or Description | Source | Part Number |
|---|---|---|
| Biomark™ HD system | Fluidigm | BMKHD-BMKHD |
| Juno™ system or<br>IFC Controller RX | | 101-6455<br>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<br>(for example, Applied Biosystems® Veriti™ 96-Well Thermal Cycler) | | — |
| DNA/template-free hood | | — |
| Class II biological safety cabinet for handling saliva samples | | — |

* Must be capable of 3,000 × *g*

† Recommended: Rainin® pipettes

## Software Requirements

The following software is required for this protocol. For software updates, go to fluidigm.com/software.

- Biomark Data Collection software v4.7.1 or later
- Real-Time PCR Analysis software v4.7.1 or later
- Biomark Pathogen Detection Software v2.1.1 or later

# Sample Requirements

This protocol supports using saliva specimens or purified nucleic acid samples (RNA or both DNA and RNA). Each reaction requires 12 µL diluted saliva or 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 F.

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 foil envelope.
- 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. Control line fluid or air remaining in the syringe tip is normal.
- 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 separate workspaces for control and saliva preparation, 1-step RT preamplification preparation, and IFC preparation.
  - Use a separate set of pipettes, filtered tips, racks, vortexers, centrifuges, generic lab reagents, and supplies at their respective workspaces.
  - Clean the workspaces and pipettes with DNA- or RNA-destroying surface decontaminants, such as 10% bleach or DNAZap™.
  - Change gloves when moving between preparation workspaces.
- To prevent cross-contamination in 96-well sample preparation:
  - Always change the pipette tip after each sample.
  - Do not reuse plate seals.
  - Centrifuge each 96-well plate containing samples and/or pre-mixes at 3,000 × *g* for 1 min to collect contents before removing a plate seal.
  - Press the plate firmly down on a flat surface when removing a plate seal to prevent flicking of fluid between wells.
  - Ensure a secure uniform seal around all wells when sealing the plate with a plate seal.
  - Pipet samples slowly to limit potential aerosols.

**Reagent Handling**

- Ensure that lab consumables (tubes, tips, plates) used for the RNA handling steps are RNase-free.
- Use separate bottles of Dilution Reagent (100-8730) in their respective workspaces.
- 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.
- 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.
- Ensure that all samples and/or pre-mixes in the 96-well plates are mixed thoroughly at every step.
- Place the sample pre-mixes on ice or in a cold block 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 pre-mixes in 96-well plates 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 at 3,000 × *g* for 1 min 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 plates or tubes 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 and ensure accurate and precise delivery of liquids.
- To avoid creating bubbles in the IFC inlets, pipet vertically and directly into the bottom of the inlet, as shown, 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.

Pipetting into bottom of inlet

- If necessary, remove medium to large bubbles from an IFC inlet by popping them with a new pipette tip, or by removing the contents of the inlet by pipette and then carefully re-pipetting the contents into the inlet.
- Place the IFC on a black surface to reduce glare and make it easier to see the liquid in each inlet when looking for bubbles.

# Collect the Saliva Specimens

If you are using extracted RNA samples, skip this section and go to Prepare the Positive Controls.

**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.

# Prepare the Positive Controls

**IMPORTANT** Prepare the controls in the pre-PCR workspace of the lab.

Prepare controls for the 96-well plates containing samples that will be loaded in the IFC. If you do not have enough samples for the second plate, you do not need to prepare controls for it.

**NOTE** If you are using extracted RNA samples instead of saliva, do not prepare heated negative saliva. For the positive controls, replace the heated negative saliva with water. Do not heat inactivate.

1 Briefly vortex and centrifuge the reagents before use.

2 Prepare the positive controls (PC Wild Type and PC Mutant):

a Combine PBS, saliva that is confirmed COVID-19 negative (not provided), and RNAsecure in a tube at a ratio of 12:12:1. Table 1 shows an example for 1,000 µL of negative saliva mix. Scale as appropriate.

Table 1. Negative saliva mix

| Component | Vol for 1,000 µL Stock (µL) |
|---|---|
| PBS (Thermo Fisher Scientific, 10010023) | 480 |
| Negative saliva specimen | 480 |
| RNAsecure (Thermo Fisher Scientific, AM7005) | 40 |
| **Total** | **1,000** |

b Mix thoroughly and heat the mixture using the program in Table 2 to produce heated negative saliva.

Table 2. Heat treatment of negative saliva

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

3 Prepare each of the positive controls in a new, labeled 1.5 mL tube as follows:

a **Positive Control – wild type (PC Wild Type):** Use the heated negative saliva from Step 2b to dilute Twist Synthetic RNA Control #2 (Twist Bioscience, 102024) to 25 copies/µL (see Table 3), then vortex and centrifuge.

b **Positive Control – mutant (PC Mutant):** Use the heated negative saliva from Step 2b to dilute Mutation Positive Control (102-1335) to 25 copies/µL (see Table 3), then vortex and centrifuge.

Table 3. Dilution of positive controls from 1,000,000 to 25 copies

| Dilution Step | Wild Type or Mutant Control (µL) | Heated Saliva (µL) | Total Vol (µL) | Concentration (copies/µL) |
|---|---|---|---|---|
| First | 5 | 95 | 100 | 50,000 |
| Second | 5 | 95 | 100 | 2,500 |
| Third | 5 | 95 | 100 | 125 |
| Final | 10 | 40 | 50 | 25 |

**IMPORTANT** Dilute Twist synthetic RNA control and Mutation Positive Control in the heated negative saliva at the time of use. Any unused diluted positive control should be discarded.

**IMPORTANT** **DO NOT heat-inactivate PC Wild type or PC Mutant.** They will be added directly to the 1-step RT-preamplification reaction plates.

# Prepare the Saliva Specimens

If you are using extracted RNA samples, skip this section and go to Prepare and Perform the 1-Step Reverse Transcription and Preamplification Reactions on page 11.

**IMPORTANT**

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

1 Prepare the PBS/RNAsecure mix:

a Combine PBS and RNAsecure in a tube, as shown in Table 4. Scale appropriately for multiple runs.

Table 4. PBS/RNAsecure mix

| Reagent | Vol per Reaction (µL) | Vol per 192 Samples (µL)* |
|---|---|---|
| PBS (Thermo Fisher Scientific, 10010023) | 12 | 2730.0 |
| RNAsecure (Thermo Fisher Scientific, AM7005) | 1 | 227.5 |
| **Total** | **13** | **2957.5** |

* Includes overage

b Cap the tube, vortex, and centrifuge the PBS/RNAsecure mix.

c In 2 new 96-well plates, add 13 µL PBS/RNAsecure into all wells except C1 and D1.

**NOTE** The PBS/RNAsecure mix plates can either be used immediately or be tightly sealed with clear adhesive film and stored at −15 °C to −25 °C for up to 3 months.

**NOTE** If you do not have enough samples for 2 plates, leave the unused wells empty.

2 Add the negative controls to the PBS/RNAsecure mix plates:

a **No Template Control (NTC):** Add 12 µL of water into well A1.

b **Negative Extraction Control (NC):** Add 12 µL of negative saliva specimen into well B1.

**NOTE** If you are using extracted RNA samples instead of saliva, use only water for the negative controls (NTC and NC).

3 Add 12 µL of each saliva specimen to the remaining wells containing PBS/RNAsecure mix, as shown in Figure 1.

**IMPORTANT** Leave wells C1 and D1 empty. **Do not** heat-inactivate PC-Wild Type or PC-Mutant. They will be added directly to the 1-step RT-preamplification reaction plates.

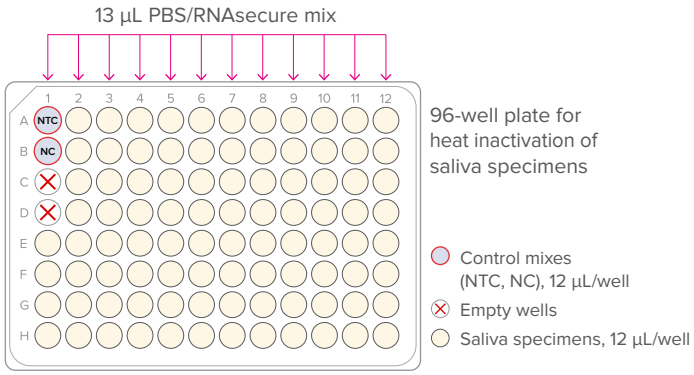



Figure 1. Heat-inactivation plate

4 Tightly seal the plates with clear adhesive film, briefly vortex and then centrifuge at 3,000 × *g* for 1 min to collect contents.

5 Heat-inactivate the prepared saliva specimens and 2 negative controls (NTC and NC) in a thermal cycler using the program in Table 5:

Table 5. Heat inactivation of saliva samples

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

6 After +4 °C for 2 min, place the samples on ice or in a cold block until ready to use.

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

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

**IMPORTANT** Prepare in a pre-PCR workspace of the lab.

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

2 In a DNA/template-free hood, combine the components shown in Table 6 in a new 5 mL tube to make the 1-step pre-mix and place on ice or in a cold block. Scale appropriately for multiple runs or if additional overage is needed based on pipette performance.

Table 6. 1-step pre-mix

| Component | Vol per Reaction (µL)* | Vol for 192 Reactions (µL)† |
|---|---|---|
| Mutation Preamp Primer Pool (102-1324) ○ | 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% in the volumes for overage.

† Includes overage

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

4 Prepare the 1-step reaction plates as shown in Figure 2.

a In a DNA/template-free hood, aliquot 128 µL of 1-step pre-mix into each well of two 0.2 mL 8-well strips.

**NOTE** If reusing pipette tips for this dispense, be sure to pre-wet tips prior to first dispense.

b In a DNA/template-free hood, use an 8-channel pipette to transfer 10.0 µL of 1-step pre-mix into each well of 2 new 96-well plates. Remove the plates from the hood.

c Add 5.0 µL of each control into wells A1 (NTC), B1 (NC), C1 (PC Wild Type), and D1 (PC Mutant) of each plate.

d Add 5.0 µL of each sample to the remaining wells of the 96-well plates that already contain 1-step pre-mix.

**NOTE** Only 1 preamplification reaction is prepared for each sample.

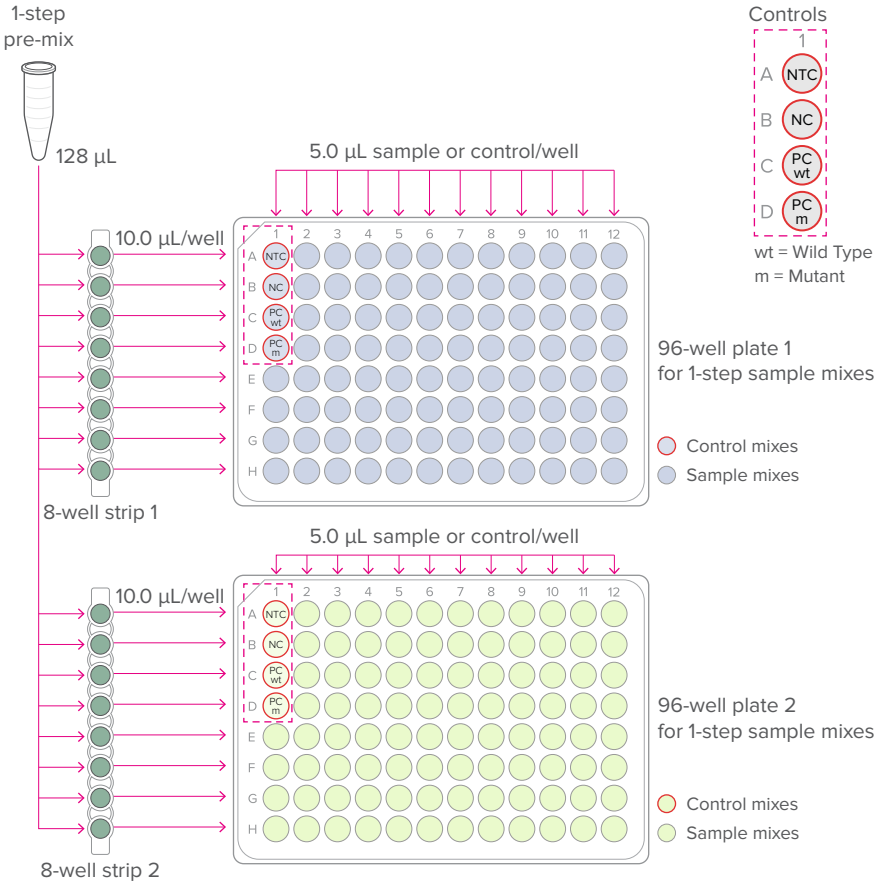



Figure 2. 1-step reaction plates (per-well transfer volumes)

**5** Tightly seal the plates with clear adhesive film, gently vortex and then centrifuge them at 3,000 × *g* for 1 min to mix and collect the reactions.

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

**1** Place each 96-well plate in a compatible thermal cycler and cycle using the program in Table 7:

Table 7. 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** Perform the dilution in a post-PCR workspace of the lab.

After cycling, dilute the preamplified reactions in the 96-well plates in Dilution Reagent as shown in Table 8 and described as follows:

**IMPORTANT** Briefly vortex the plates containing the preamplified cDNA and then centrifuge them at 3,000 × *g* for 1 min to collect contents before removing the plate seals.

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. Scale volumes as appropriate.

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

**IMPORTANT** Dispense slowly to prevent aerosolization.

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

3 Tightly seal the plates with clear adhesive film, then gently vortex to mix the dilutions and centrifuge them at 3,000 × *g* for 1 min to collect contents. 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 8. 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

**IMPORTANT** Open and handle diluted cDNA only in a post-PCR workspace of the lab.

## Prepare the Final Assay Mixes for Loading on the IFC

**NOTE**

- For each sample, the N1, N2, and RNase P assays are run in 4 replicates in each IFC. All other assays are run singly.
- Assemble the assays in a 96-well plate and record locations for later use during data analysis.

1 Briefly vortex and centrifuge the reagents before use.

**2** In a DNA/template-free hood, prepare each final assay mix in a new 1.5 mL tube using the assays in the Advanta SARS-CoV-2 Mutation Assay Panel (102-1340). Table 9 and Table 10 show examples for preparing sufficient volumes of final assay mix for 10 IFCs.

**NOTE** If you are preparing assay mixes for 1 IFC, you can combine 1 µL of 4X Assay Loading Reagent with 3 µL of each assay directly in a new 96-well plate in the order shown in Figure 3 on page 15 and skip to Step 4 of this section.

**a** Combine each N1, N2, and RNase P assay (6.7 µM primers, forward and reverse; 1.7 µM probe) as shown in Table 9. Scale appropriately for multiple runs.

Table 9. Final N1, N2, and RNase P assay mixes

| Component | Vol per Inlet (µL)* | Vol per Assay for 10 IFCs (µL)† |
|---|---|---|
| N1, N2, RNase P assays (102-0678, 102-088, 102-0689) | 3.0 | 150 |
| 4X Assay Loading Reagent (102-0114) | 1.0 | 50 |
| **Total** | **4.0** | **200** |

Final concentration: 5 µM primers; 1.28 µM probe

* Includes overage

† 230 reactions (per replicate) for ease of pipetting. Each assay is run in 4 replicates.

**b** Combine each Wild Type and Mutant assays as shown in Table 10. Scale appropriately for multiple runs.

Table 10. Final wild type and mutant assay mixes

| Component | Vol per Inlet (µL)* | Vol per Assay for 10 IFCs (µL)† |
|---|---|---|
| Wild Type and Mutant Oligo Mixes (102-1325, 102-1326, 102-1327, 102-1328, 102-1329, 102-1330, 102-1331, 102-1332, 102-1333, 102-1334, 102-1336, 102-1337) | 3.0 | 37.5 |
| 4X Assay Loading Reagent (102-0114) | 1.0 | 12.5 |
| **Total** | **4.0** | **50.0** |

* Includes overage

† 230 reactions for ease of pipetting. Each assay is run singly.

**c** Cap the tubes, vortex, and centrifuge the final assay mixes.

**d** Transfer the final assay mixes to a new 96-well plate in the order shown in Figure 3.

**NOTE** Prepared final assay mixes can either be used immediately or stored at −20 °C for at least 6 months.

**3** In a DNA/template-free hood, use an 8-channel pipette to transfer 4.0 µL of each final assay mix into the respective wells in a new 96-well plate as shown in Figure 3.

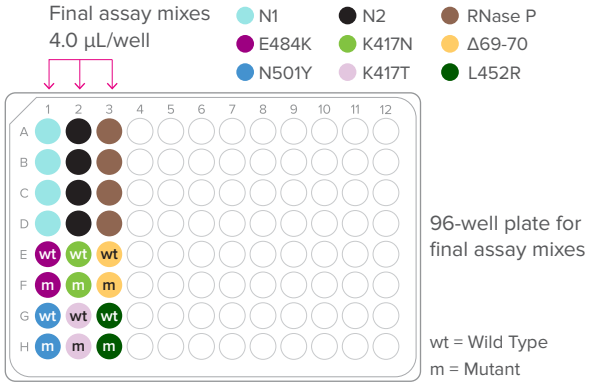



Figure 3. Final assay plate (per-well transfer volume)

4 Tightly seal the plates with clear adhesive film, then centrifuge them at 3,000 × *g* for 1 min to mix and collect the contents.

## Prepare the Final Sample Mixes

1 Thaw the Advanta PCR MM and keep on ice or in a cold block. Briefly vortex and centrifuge the reagents before use.

2 In a DNA/template-free hood, combine the components shown in Table 11 in a new 1.5 mL tube to make the sample pre-mix and place on ice or in a cold block. Scale appropriately for multiple runs or if additional overage is needed based on pipette performance.

**NOTE** This is enough volume for 1 IFC.

Table 11. 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 16.

a Briefly vortex and centrifuge the sample pre-mix from Table 11.

b In a DNA/template-free hood, aliquot 60 µL of pre-mix into each well of a new 8-well strip.

c In a DNA/template-free hood, 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 plates from the DNA/template-free hood and prepare the final sample mix by adding 1.8 µL of each diluted, preamplified sample from Table 8 on page 13 to each well.

**NOTE** Sample pre-mix and diluted cDNA volumes can be scaled as appropriate while keeping the ratio the same.

**IMPORTANT** Before use, briefly vortex the plates containing the diluted, preamplified cDNA and then centrifuge them at 3,000 × *g* for 1 min to collect contents.

**NOTE** For IFC inlets that do not correspond to a diluted cDNA sample, replace the diluted, preamplified cDNA with 1.8 µL of PCR Water (100-5941).

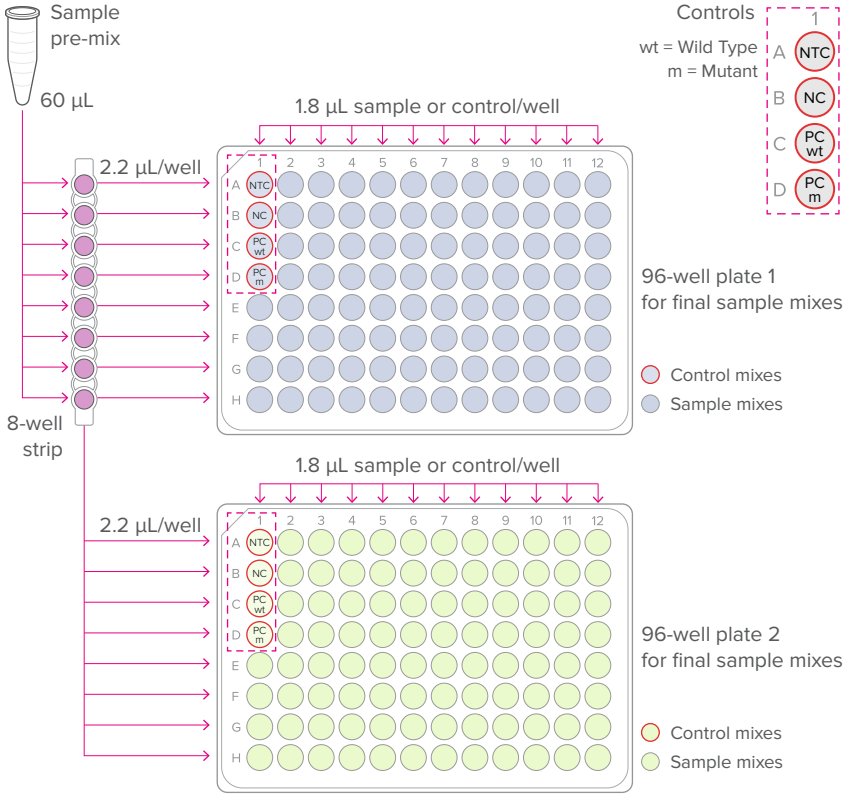



Figure 4. Final sample plates (per-well transfer volumes)

**4** Tightly seal the plates with clear adhesive film, vortex and then centrifuge them at 3,000 × *g* for 1 min to collect contents.

## Prepare the 192.24 IFC

**IMPORTANT** When injecting control line fluid:
- Follow the best practices for handling IFCs and control line fluid on page 6.
- 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) from the packaging and the 192.24 Dynamic Array™ IFC for Gene Expression (100-6266) from the box and foil envelope.

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

2 Actuate the check valve:

a Place the IFC on a flat surface.

b 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).

**IMPORTANT** The bottom accumulator is not used.

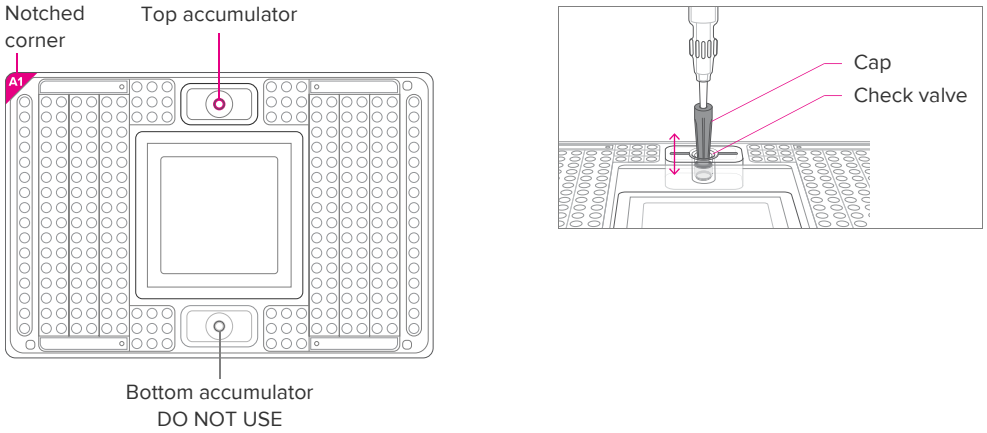



Figure 5. Actuating the check valve in the top accumulator on the 192.24 IFC

3 Hold the syringe firmly in 1 hand with tip facing up and away from the IFC, pull back on the plunger slightly to create negative pressure, 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 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 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 After fully depressing the plunger, wait approximately 5 sec before withdrawing the syringe.

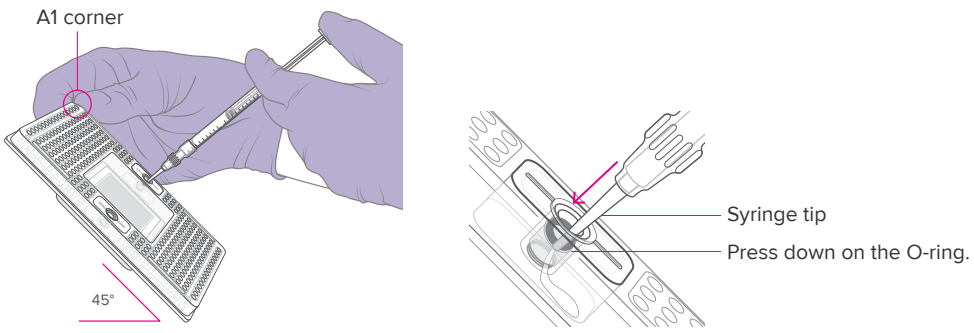



Figure 6. Injecting control line fluid into the accumulators on the 192.24 IFC

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 all assay and sample mixes thoroughly, then centrifuge them at 3,000 × *g* for 1 min to collect contents before removing the plate seals and pipetting the mixes 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.
- Follow the best practices for preventing bubbles on page 7.

Refer to Figure 7 on page 19 when pipetting final sample, control, 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 and control 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 Inspect the sample and assay inlets for bubbles and to ensure liquid delivery. See the best practices on page 7 for more details.

5 Pipet 150 µL of Actuation Fluid (100-6250) into the P1 reservoir ( ) on the IFC.

6 Pipet 150 µL of Pressure Fluid (100-6249) into each of the P2 and P3 reservoirs ( ) on the IFC.

7 Pipet 20 µL of Pressure Fluid into each of the P4 and P5 inlets ( ) on the IFC.

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

**IMPORTANT** Start the Load Mix script on the controller within 10 min of pipetting the assay and sample mixes and fluids.

**9** 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**.

**10** 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 instrument within 1 hr of completing the Load Mix script.

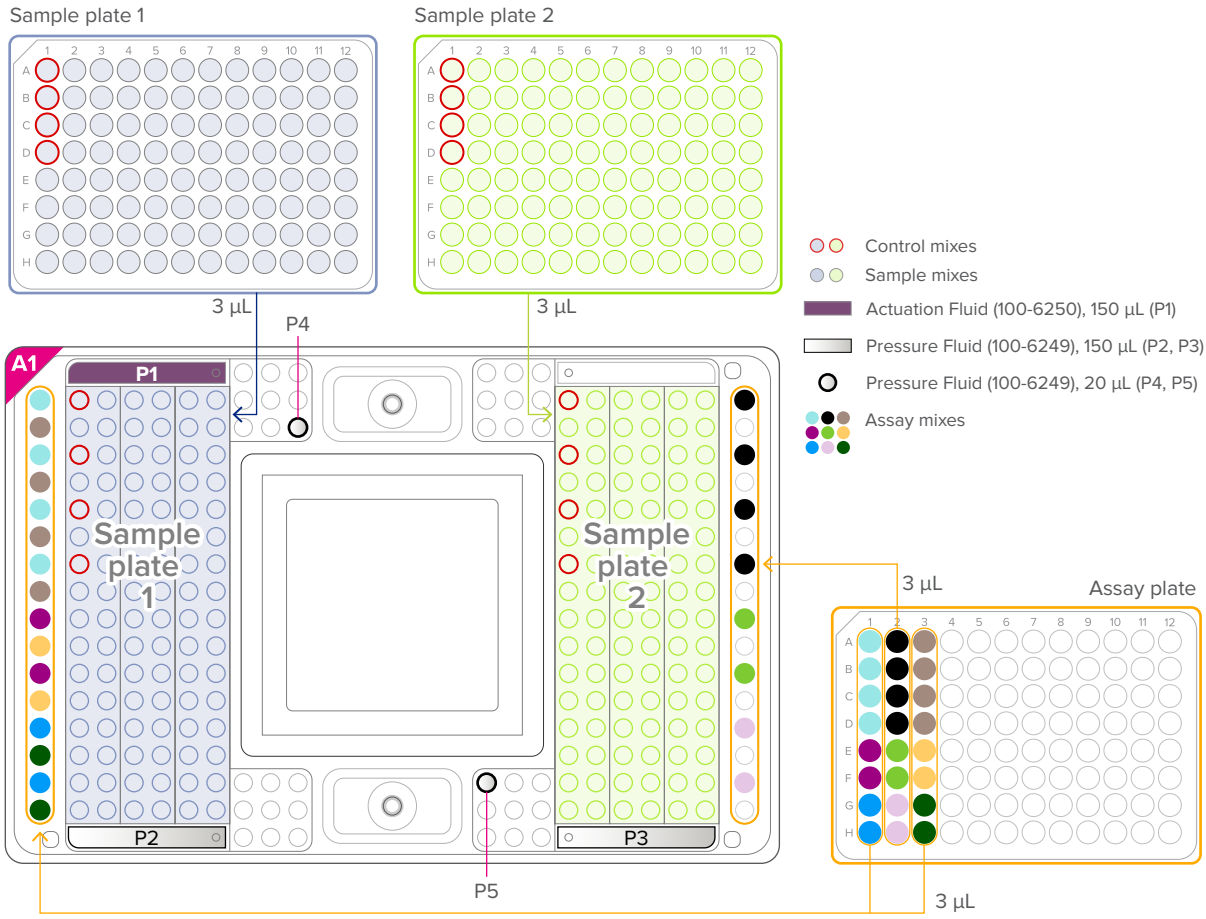



Figure 7. Pipetting map for the 192.24 IFC for Advanta SARS-CoV-2 Mutation Assays

## 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).

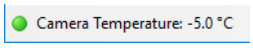
1 Remove the loaded IFC from Juno or IFC Controller RX.

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 ( ) on the desktop of the Biomark HD computer to launch the software.

4 Click **Start a New Run**.

5 Confirm that the camera status indicator at the bottom of the window is green.




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 8). In the Data Collection software, click **Load**.

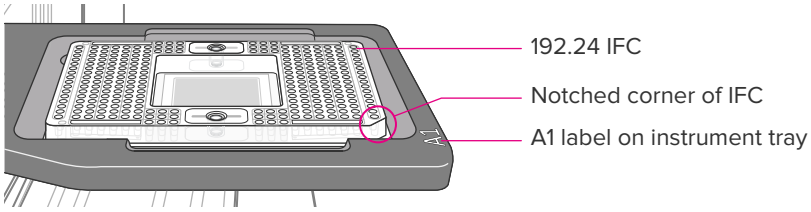



Figure 8. Loaded IFC on instrument tray

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.

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: **GE 192x24 Fast v1.pcl.**

**NOTE** For a description of the thermal protocol, see Appendix C.

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.

# Annotate the Real-Time PCR Data

For detailed instructions about using the Real-Time PCR Analysis software, see the Real-Time PCR Analysis Software User Guide (68000088). For detailed instructions about installing, setting up, and using the interpretive software, see the Biomark Pathogen Detection Software Quick Reference Guide (FLDM-00557). You can either set up the Real-Time PCR Analysis software to export data directly through the Biomark Pathogen Detection Software, or run the Biomark Pathogen Detection Software from a command line.

Before you export the data using the Real-Time PCR Analysis software, check the software options to ensure that the Biomark Pathogen Detection Software is being used as the post-run command and the plate quality control (QC) results are being reported in the output file. You can also change the default analysis settings, if desired.

1 Select **Tools** > **Options**.

2 Click **Results Export** in the **Output** section. Check the **Export Sample and Reagent Inlet to Plate Mapping** checkbox.

3 Confirm that the **Post Export Command** is:
C:\Program Files (x86)\Fluidigm\Advanta Dx SARS-CoV-2 Interpretive Software\BiomarkPD.exe

4 (Optional) Click **Analysis Parameters** and change the default settings:

   a Check the **Customize Default Baseline Correction Method** checkbox and select **Linear**.

   b Check the **Customize Default Ct Threshold Method** checkbox and select **Auto By Control (Global)**.

5 Click **OK** to save the changes.

To annotate the real-time PCR data:

1 Double-click the **Real-Time PCR Analysis** icon (  ) on the desktop to launch the Real-Time PCR Analysis software.

2 Click  (**Open**), then browse to and select the **chiprun.bml** file to open it in the Real-Time PCR Analysis software.

3 Annotate the samples for the first analysis of a new IFC run:

   a In the **Chip Explorer** pane, click **Sample Setup**.

   b In the **Task** pane, click **New**.

   c For **Container Type** select **SBS Plate**, for **Container Format** select **SBS96**, then click **OK**.

   d Annotate the samples:

   - Click **Import** to import the sample information from a plate file or a comma-separated values (CSV) file for both sample plates 1 and 2, or
   - In the **Sample Setup** pane, click **Editor** to annotate the samples in each plate well by well. To switch plates, select the Source (**96 Wellplate #1** or **96 Wellplate #2**) in the **Task** pane, or

- If the sample organization was recorded in a spreadsheet in a plate map 8 × 12 grid, copy the entire 8 × 12 grid containing the sample names and paste it into the Sample Setup. Select **Sample Name** as the **Data** item.

**IMPORTANT** Annotate no template controls with type **NTC** and negative and positive controls with type **Unknown**. Annotate all samples with type **Unknown**. Any empty sample inlets must be annotated with type **Blank**.

e Click **Map**, select **192-Sample-SBS96-Left&Right.cdsp**, then click **Open**.

4 Annotate the detectors (assays) for the first analysis of a new IFC run:

a In the **Chip Explorer** pane, click **Detector Setup**.

b In the **Task** pane, click **New**.

c For **Container Type** select **SBS Plate**, for **Container Format** select **SBS96**, then click **OK**.

d In the **Detector Setup** pane, click **Editor** and annotate the assays:

- Detector names (see Appendix D for more information about the mutation assays):

| | | |
|---|---|---|
| N1 | N2 | RNase P |
| E484K Wild Type | K417N Wild Type | Del69&70 Wild Type |
| E484K Mutant | K417N Mutant | Del69&70 Mutant |
| N501Y Wild Type | K417T Wild Type | L452R Wild Type |
| N501Y Mutant | K417T Mutant | L452R Mutant |

**IMPORTANT** The detector names are case-sensitive and must be entered exactly as shown.

**NOTE** N1, N2, and RNase P are repeated in the first 4 rows of the Detector Setup (cells 1–12).

- Type: RNase P assays are type **Control**. N1, N2, and wild type and mutant assays are type **Test**.

e Click **Map**, select **24-Assay-SBS96-Left3.dsp**, then click **Open**.

**NOTE** After you annotate the assays for the first time, you can export the detector setup as a plate file (.plt) for reuse. To reuse the exported plate file, click **Import** instead of New in Step 4b, then select the detector setup plate file (.plt).

5 Click **Details Views**.

6 Set the following Analysis Settings, then click **Analyze** to analyze the IFC run.

| For | Select |
|---|---|
| Quality Threshold | **0.65** |
| Baseline Correction | **Linear** |
| Ct Threshold Method | **Auto By Control (Global)** |

7 After analyzing the IFC run, click  (**Save**), then click  (**Export**) and use the Biomark Pathogen Detection Software to interpret the Ct results and save the interpretation as a CSV file.

8 Review the interpreted data. Review the Ct threshold to ensure that it resides within the exponential phase of the amplification curve. If the Ct threshold falls outside of the exponential phase, adjust the Ct threshold manually as follows:

a In the Real-Time PCR Analysis software, open the IFC run.

b In the **Details Views** pane, show the **Heat Map View**, then select all rows with assays (N1, N2, RNase P, E484K Wild Type, E484K Mutant, N501Y Wild Type, N501Y Mutant, K417N Wild Type, K417N Mutant, K417T Wild Type, K417T Mutant, Del69&70 Wild Type, Del69&70 Mutant, L452R Wild Type, and L452R Mutant).

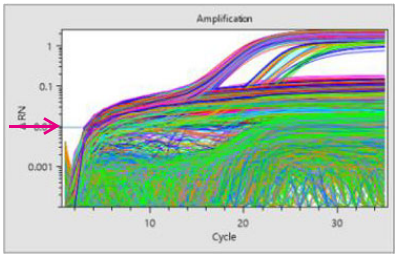
c Click (**Expand/Collapse**) in the secondary toolbar to expand the Normalized Intensity and Amplification plots.

d Click **Threshold** to show the threshold line in the Amplification plot, then click **Log Graph** to show more detail.

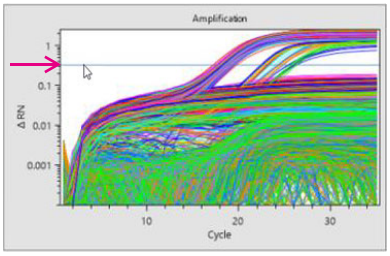
e Confirm that the Ct threshold needs to be adjusted by visually confirming that the horizontal Ct Threshold line is in the baseline in the Amplification plot.

f In the **Analysis Settings** pane, change the Ct Threshold Method to **User (Global)**.

g In the **Details Views** pane, click **Edit**, then click and drag the threshold line so that it falls above the background signal and within the exponential phase of the fluorescence curves.



Ct threshold in the baseline in the Amplification plot



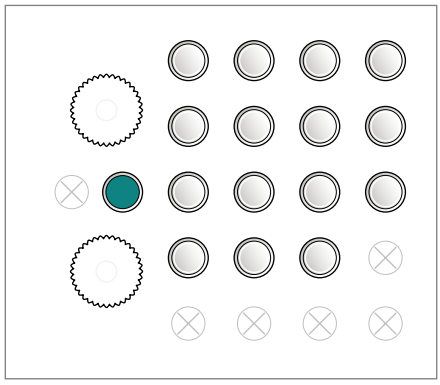
Ct threshold adjusted manually

h In the Task pane, click **Analyze** to analyze the data with the updated Ct Threshold Method.

9 Export the interpretative report and review control expected interpretations, confirm most samples are not called inconclusive.

# Appendix A: Advanta SARS-CoV-2 Mutation Assay Panel Components

## Advanta SARS-CoV-2 Mutation Assay Panel (102-1340)



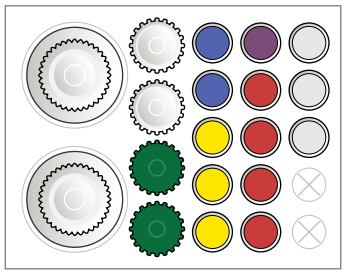
- 1 tube of RP Oligo Mix (RUO), 170 µL/tube (102-0687)
- 1 tube of 2019-nCoV_N1 Oligo Mix (RUO), 370 µL/tube (102-0688)
- 1 tube of 2019-nCoV_N2 Oligo Mix (RUO), 370 µL/tube (102-0689)
- 2 bottles of Mutation Preamp Primer Pool, 7.5 mL/bottle (102-1324)
- 1 tube of Δ69-70 Mutant Oligo Mix, 30 µL/tube (102-1325)
- 1 tube of Δ69-70 Wild Type Oligo Mix, 30 µL/tube (102-1326)
- 1 tube of E484K Mutant Oligo Mix, 30 µL/tube (102-1327)
- 1 tube of E484K Wild Type Oligo Mix, 30 µL/tube (102-1328)
- 1 tube of K417N Mutant Oligo Mix, 30 µL/tube (102-1329)
- 1 tube of K417N Wild Type, 30 µL/tube (102-1330)
- 1 tube of K417T Mutant Oligo Mix, 30 µL/tube (102-1331)
- 1 tube of K417T Wild Type Oligo Mix, 30 µL/tube (102-1332)
- 1 tube of L452R Mutant Oligo Mix, 30 µL/tube (102-1333)
- 1 tube of L452R Wild Type Oligo Mix, 30 µL/tube (102-1334)
- 1 tube of Mutation Positive Control, 20 µL/tube (102-1335)
- 1 tube of N501Y Mutant Oligo Mix, 30 µL/tube (102-1336)
- 1 tube of N501Y Wild Type Oligo Mix, 30 µL/tube (102-1337)

# Appendix B: Advanta Preamp & IFC Reagent Kit Components

## Advanta™ Preamp & IFC Reagent Kit —192.24 (102-1341)

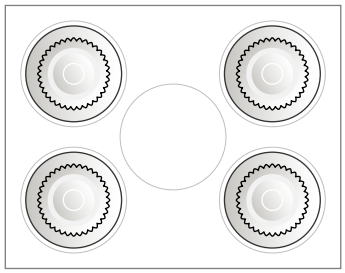
| Bundle | Component | Part Number | Quantity |
|---|---|---|---|
| Advanta™ Preamp & IFC Reagent Kit —192.24 (102-1341) | Advanta Preamp & IFC Reagent Kit, Module 1 | 102-1338 | 1 kit |
| | Advanta Preamp & IFC Reagent Kit, Module 2 | 102-1339 | 1 kit |
| | 192.24 Dynamic Array IFC for Gene Expression | 100-6266 | 10 IFCs |
| | Control Line Fluid for 192.24 IFCs (150 µL each) | 100-4058 | 10 syringes |

### Advanta Preamp & IFC Reagent Kit, Module 1 (102-1338)



- 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 Preamp & IFC Reagent Kit, Module 2 (102-1339)



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

# Appendix C: Biomark HD Thermal Cycler Protocols

**GE 192x24 Fast v1 thermal cycling parameters (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 |

# Appendix D: Mutation Assays

| Product Name | Nucleotide Position | WT Nucleotide | Mutation | AA Change | Found in [Strain] |
|---|---|---|---|---|---|
| K417T Wild Type, K417T Mutant | 22812 | A | C | K417T | Brazil P1 |
| K417N Wild Type, K417N Mutant | 22813 | G | T | K417N | SA B.1.351 |
| L452R Wild Type, L452R Mutant | 22917 | T | G | L452R | CA B.1.427, B.1.429 |
| E484K Wild Type, E484K Mutant | 23012 | G | A | E484K | SA B.1.351; Brazil P1; NY B.1.526 |
| N501Y Wild Type, N501Y Mutant | 23063 | A | T | N501Y | UK B.1.1.7; SA B.1.351; BrazilP1 |
| Del69&70 Wild Type, Del69&70 Mutant | Δ69/70 | — | — | — | UK B.1.1.7 |

# Appendix E: Related Documents

Go to fluidigm.com to download these related documents.

| Title | Document Number |
|---|---|
| Advanta SARS-CoV-2 Mutation Assay Panel Quick Reference | FLDM-00480 |
| Control Line Fluid Loading Procedure Quick Reference | 68000132 |
| Juno System User Guide | 100-7070 |
| IFC Controller RX User Guide | 100-3385 |
| Biomark HD Data Collection User Guide | 100-2451 |
| Real-Time PCR Analysis User Guide | 68000088 |
| Biomark Pathogen Detection Software Quick Reference Guide | FLDM-00557 |

# Appendix F: 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).

**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.

**For technical support visit techsupport.fluidigm.com.**

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**Japan** +81 3 3662 2150 | techsupportjapan@fluidigm.com **All other Asian countries/India/Australia** +1 650 266 6100 | techsupportasia@fluidigm.com