

Advanta SARS-CoV-2 Mutation Assay Panel

This quick reference describes how to detect COVID-19 mutations using the Advanta™ SARS-CoV-2 Mutation Assay Panel, RT-PCR reagents (such as the Advanta Preamp & IFC Reagent Kit—192.24, as shown in this document) and the 192.24 Dynamic Array™ IFC (integrated fluidic circuit) on Biomark™ HD. This document only applies to this panel being run with outlined but not provided reagents as specified in the Advanta SARS-CoV-2 Mutation Assay Panel Protocol (FLDM-00479) and does not apply to any other workflows.

IMPORTANT Before using this document, read and understand the safety guidelines in the Advanta SARS-CoV-2 Mutation Assay Panel Protocol (FLDM-00479). For detailed instructions on instrument and software operation, and complete instrument safety information, 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).

Workflow

Workflow Step	Run Time*
1 Collect the saliva specimens. (Skip this step if using extracted RNA).	—
2 Prepare the negative and positive controls.	—
3 Prepare the saliva specimens, then heat inactivate them. (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 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 sample, control, and assay mix, 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

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 controls in pre-PCR workspace of 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.

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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 Pre-amplification Reactions](#).

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:

- 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

- Cap tube, vortex, and centrifuge PBS/RNAsecure mix.
- 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:

- No Template Control (NTC):** Add 12 μL of water into well A1.
- 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-pre-amplification reaction plates.

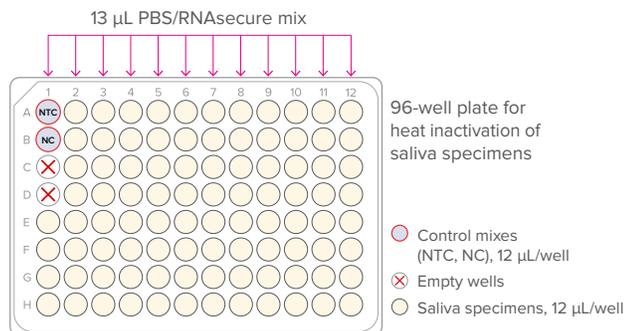


Figure 1. Heat-inactivation plate

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

- 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 Pre-amplification Reactions

Prepare the 1-Step Reverse Transcription and Pre-amplification Reactions

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

- Thaw Advanta RT-Preamp Master Mix and keep on ice or in a cold block. Briefly vortex and centrifuge reagents before use.
- In a DNA/template-free hood, combine 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

- Cap the tube, vortex, and centrifuge the 1-step pre-mix.
- Prepare the 1-step reaction plates as shown in [Figure 2](#) on [page 3](#).

- 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.

- 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.
- Add 5.0 μL of each control into wells A1 (NTC), B1 (NC), C1 (PC Wild Type), and D1 (PC Mutant) of each plate.
- 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 pre-amplification reaction is prepared for each sample.

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

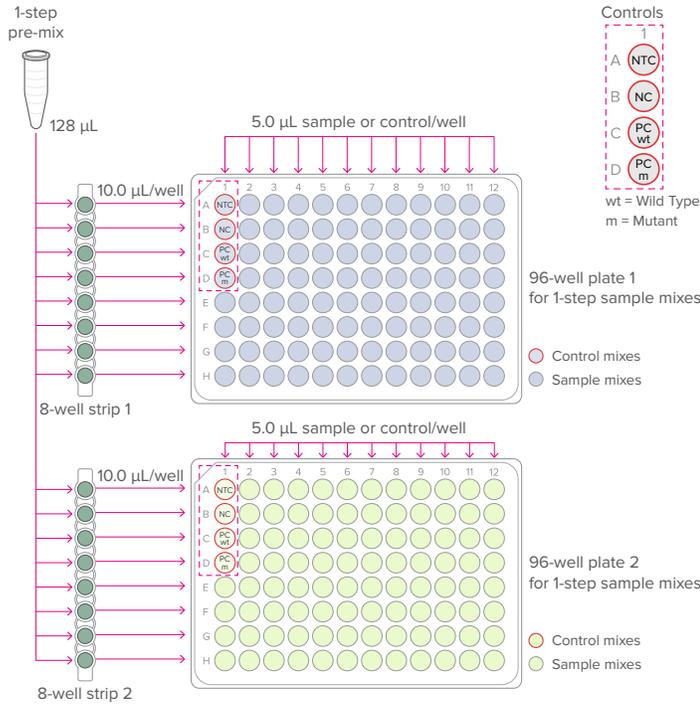


Figure 2. 1-step reaction plates (per-well transfer volumes)

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

Dilute the Preamplified cDNA

IMPORTANT Perform 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 $3,000 \times g$ 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 dilutions and centrifuge them at $3,000 \times g$ 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 4 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.

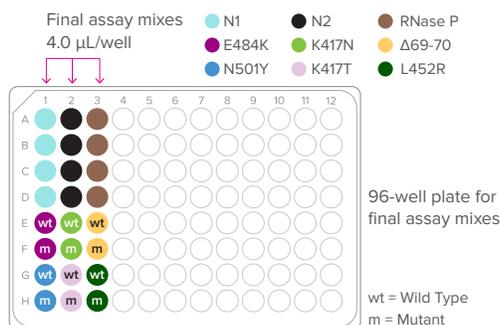


Figure 3. Final assay plate (per-well transfer volume)

- 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.

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.
 - a Briefly vortex and centrifuge 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 final sample mix by adding 1.8 μL of each diluted, preamplified sample from Table 8 on page 3 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).

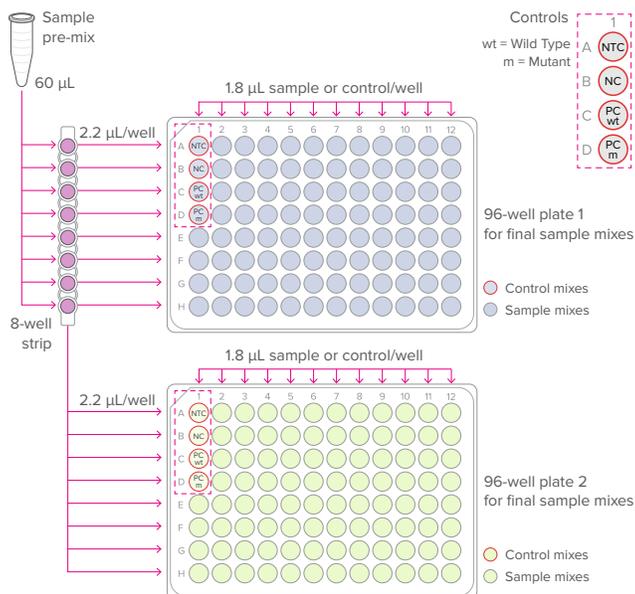


Figure 4. Final sample plates (per-well transfer volumes)

- 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

For detailed instructions about injecting control line fluid, see the Control Line Fluid Loading Procedure (68000132).

IMPORTANT

- Use the IFC within 24 hr of opening the foil envelope.
- Only use a 192.24 syringe (100-4058). The syringe is prefilled with 150 μL of control line fluid.
- Do not evacuate air from the syringe prior to injecting control line fluid (Step 3).
- Avoid getting control line fluid on exterior of IFC or in the inlets because this makes IFC unusable. If this occurs, use a new IFC.

- 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.
- 2 Place the IFC on a flat surface and actuate the check valve for the top accumulator by pressing gently with the syringe cap.

IMPORTANT The bottom accumulator is not used.

- 3 Inject control line fluid into the top accumulator on the IFC (see Figure 5). Use the entire contents of the syringe.
- 4 Check to ensure that the O-ring returns to its normal position after the syringe is removed.
- 5 Remove and discard the protective film from bottom of IFC.

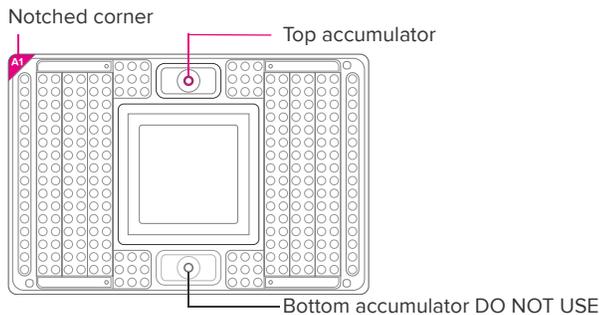


Figure 5. Top accumulator on the 192.24 IFC

Load the IFC

IMPORTANT

- Vortex all assay and sample mixes thoroughly, then centrifuge them at $3,000 \times g$ 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.

Refer to Figure 6 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.
- 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 notched corner of IFC to white notch on 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.

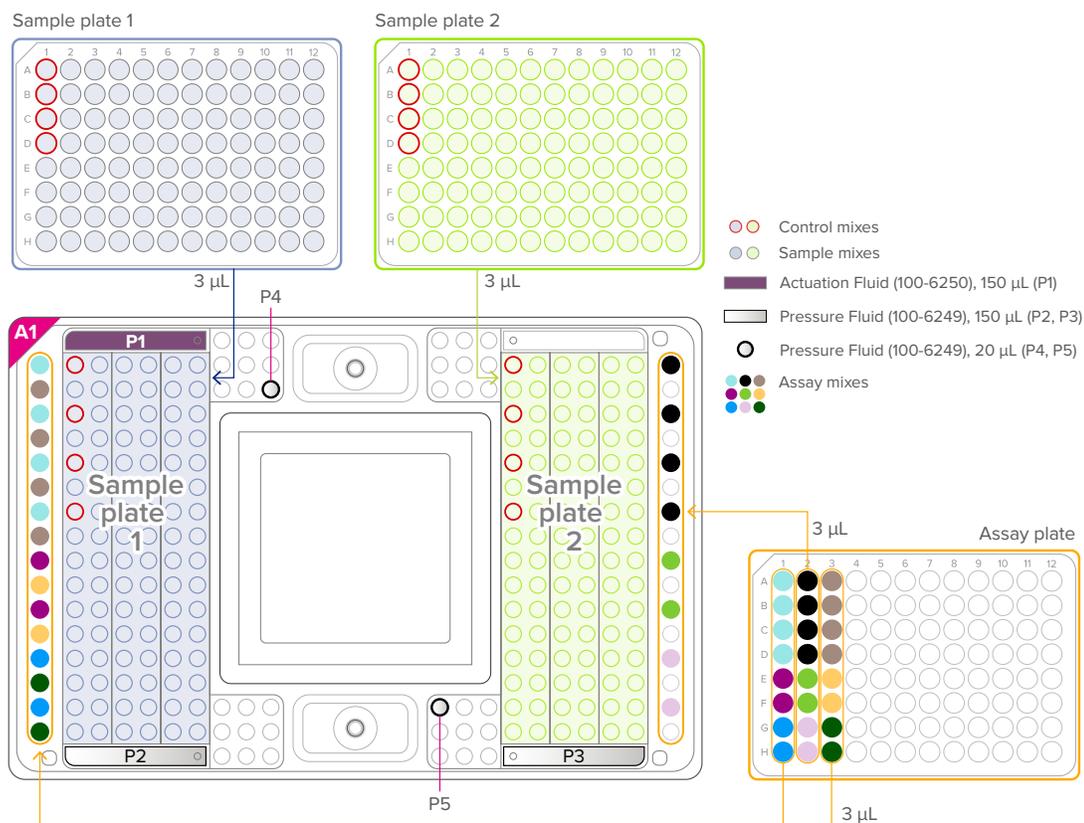


Figure 6. Pipetting map for the 192.24 IFC for Advanta SARS-CoV-2 Mutation Assays

Thermal-Cycle and Collect Real-Time PCR Data

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

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 **chiplun.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:

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**.

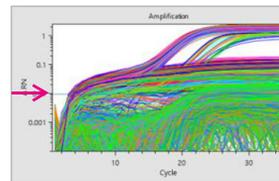
NOTE After you annotate the assays for the first time, you can export the detector setup as a plate file (.plt) for reuse. To reuse the exported plate file, click **Import** instead of **New** in **Step 4b**, then select the detector setup plate file (.plt).

- 5 Click **Details Views**.
- 6 Set the following Analysis Settings, then click **Analyze** to analyze the IFC run.

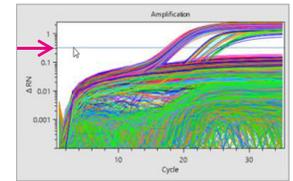
For	Select
Quality Threshold	0.65
Baseline Correction	Linear
Ct Threshold Method	Auto By Control (Global)

- 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 in the Amplification plot

- 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.

For technical support visit techsupport.fluidigm.com.

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