Advanta Dx SARS-CoV-2 RT-PCR Assay

For *In Vitro* Diagnostic Use | For Use Under Emergency Use Authorization Only | Rx Only
For Use Only with Biomark HD in Conjunction with Juno or IFC Controller RX

Contents

IVD

Intended Use

The Advanta Dx SARS-CoV-2 RT-PCR Assay is a real-time Reverse Transcription (RT) PCR test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in saliva specimens collected without preservatives in a sterile container from individuals suspected of COVID-19 by their healthcare provider.

Testing is limited to laboratories which are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, and meet requirements to perform high complexity tests.

This test is also for use with saliva specimens that are self-collected at home with or without the supervision of a healthcare provider (HCP) with the AZOVA COVID-19 Test Collection Kit from individuals suspected of COVID-19 by their HCP.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in saliva during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA. Clinical correlation with individual history and other diagnostic information is necessary to determine individual infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Laboratories within the United States and its territories are required to report all results to the appropriate public health authorities.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for individual management decisions. Negative results must be combined with clinical observations, individual history, and epidemiological information. Negative results for SARS-CoV-2 RNA from saliva should be confirmed by testing of an alternative specimen type if clinically indicated.

The Advanta Dx SARS-CoV-2 RT-PCR Assay is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures. The Advanta Dx SARS-CoV-2 RT-PCR Assay is only for use under the Food and Drug Administration's Emergency Use Authorization.

Product Description

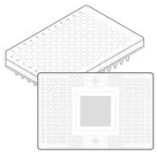
The Advanta Dx SARS-CoV-2 RT-PCR Assay is a reverse transcription (RT) and real-time polymerase chain reaction (RT-PCR) test that leverages Fluidigm microfluidics technology. The SARS-CoV-2 primer and probe set is designed to detect RNA from the SARS-CoV-2 in saliva from patients suspected of COVID-19 by their healthcare providers. The Advanta Dx SARS-CoV-2 RT-PCR Assay uses 2019-nCoV Real-Time RT-PCR Diagnostic Panel (CDC) N1, N2, and RNase P primers and probes developed by the Centers for Disease Control and Prevention (CDC) for both the 1-step RT-preamplification and real-time PCR detection steps.

Table 1. Primers and probes

Target Name	Name	Description	Oligonucleotide Sequence (5′→3′)	Label
N1	2019-nCoV_N1-F	2019-nCoV_N1 forward primer	5′-GAC CCC AAA ATC AGC GAA AT-3′	None
	2019-nCoV_N1-R	2019-nCoV_N1 reverse primer	5′-TCT GGT TAC TGC CAG TTG AAT CTG-3′	None
	2019-nCoV_N1-P	2019-nCoV_N1 probe	5′-FAM-ACC CCG CAT TAC GTT TGG TGG ACC BHQ1-3′	FAM- BHQ-1
N2	2019-nCoV_N2-F	2019-nCoV_N2 forward primer	5′-TTA CAA ACA TTG GCC GCA AA-3′	None
	2019-nCoV_N2-R	2019-nCoV_N2 reverse primer	5′-GCG CGA CAT TCC GAA GAA-3′	None
	2019-nCoV_N2-P	2019-nCoV_N2 probe	5′-FAM-ACA ATT TGC CCC CAG CGC TTC AG-BHQ1-3′	FAM- BHQ-1
RNase P	RP-F	RNase P forward primer	5′-AGA TTT GGA CCT GCG AGC G-3′	None
	RP-R	RNase P reverse primer	5′-GAG CGG CTG TCT CCA CAA GT-3′	None
	RP-P	RNase P probe	5′-FAM-TTC TGA CCT GAA GGC TCT GCG CG BHQ-1-3′	FAM- BHQ-1

Workflow and Description of Test Steps




Step 1: Specimen collection

Step 2: Heat-treat samples.

Step 3: 1-step RT and preamplification

Step 4 and 5: Prepare assay and sample mixes. Prepare IFC.

Step 6: IFC loading and sample/assay mixing

Step 7: qPCR on Biomark HD

Step 8: Analysis and report

Workflow Step		Run Time*
1	Collect and prepare specimens.	—
2	Heat-treat samples.	12 min
3	Prepare and perform the 1-step reverse transcription (RT) and preamplification reactions in Applied Biosystems® Veriti™ 96-Well Thermal Cycler, then dilute sample mixes and controls.	70 min
4	Prepare the final assay mixes, final pre-amplified sample, and final control mixes (generated from output of Step 2 plus PCR reagents) for real-time PCR.	—
5	Prepare the Advanta Dx 192.24 IFC (integrated fluidic circuit) by injecting control line fluid. Pipet each assay and sample mix into the IFC inlets.	—
6	Load the IFC on Juno™ or IFC Controller RX.	35 min
7	Thermal-cycle and collect data on Biomark™ HD.	35 min
8	Analyze data. Annotate data using the Real-Time PCR Analysis software, then export results and interpret using the Advanta Dx SARS-CoV-2 RT-PCR Assay interpretive software.	—
Total run time		**2 hr 32 min**

* Does not include hands-on time

Materials Provided

Reagents and Consumables

Bundle	Component	Part Number	Quantity
Advanta™ Dx SARS-CoV-2 RT-PCR Assay Reagent and IFC Bundle (102-0355)	Advanta Dx SARS-CoV-2 RT-PCR Assay Reagent Kit Module 1	102-0354	1 kit
	Advanta Dx SARS-CoV-2 RT-PCR Assay Reagent Kit Module 2	102-0370	1 kit
	Advanta Dx 192.24 IFC (integrated fluidic circuit)	102-0389	10 IFCs
	Advanta Dx Control Line Fluid (150 µL each)	102-0390	10 syringes

Kit Components and Storage Conditions

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

102-0354, Advanta Dx SARS-CoV-2 RT-PCR Assay Reagent Kit, Module 1

Part Number	Component	Cap Color	Volume	Quantity	Storage
102-0349	Advanta Dx SLR	Blue	250 µL	2 tubes	−15 °C to −25 °C
102-0350	Advanta Dx ALR	Yellow	120 µL	3 tubes	−15 °C to −25 °C
102-0352	Advanta Dx PF 1	Purple	1.5 mL	1 tube	−15 °C to −25 °C
102-0346	Advanta Dx PCR MM	Red	1.2 mL	4 tubes	−15 °C to −25 °C
102-0369	Advanta Dx PCR Water	Natural	1.8 mL	3 tubes	−15 °C to −25 °C
102-0351	Advanta Dx PF 2	Clear	2.04 mL	2 bottles	−15 °C to −25 °C
102-0345	Advanta Dx RT PA MM	Green	3.3 mL	2 bottles	−15 °C to −25 °C
102-0353	Advanta Dx Dilution Reagent	Clear	25 mL	2 bottles	−15 °C to −25 °C

102-0370, Advanta Dx SARS-CoV-2 RT-PCR Assay Reagent Kit, Module 2

Part Number	Component	Cap Color	Volume	Quantity	Storage
102-0353	Advanta Dx Dilution Reagent	Clear	25 mL	5 bottles	−15 °C to −25 °C

102-0389, Advanta Dx 192.24 IFC*, 10 IFC pack (microfluidic chips)

Part Number	Component	Quantity	Storage
102-0392	Advanta Dx 192.24 IFC	10 IFCs	+15 °C to +30 °C

* Integrated fluidic circuit

Advanta Dx Control Line Fluid

Part Number	Component	Quantity	Storage
102-0390	Advanta Dx Control Line Fluid	10 syringes	+15 °C to +30 °C

Materials Required but Not Provided

Reagents Not Provided

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

Product Name	Source	Part Number
2019-nCoV Probe & Primer Kit*, or 2019-nCoV CDC EUA Kit, 500 rxn†,‡	Fluidigm	102-0686 or 10006606
PBS, pH 7.4	Thermo Fisher Scientific™	10010023
RNAsecure™ RNase Inactivation Reagent		AM7005
Heat Inactivated 2019 Novel Coronavirus (used to prepare Positive Control)	ATCC®	VR-1986HK™
Normal saliva from pooled human donors confirmed COVID-19 negative	Lee BioSolutions	991-05-P
	Innovative™ Research	IR100044P

* PN 102-0686 is sufficient for 10 IFCs (each assay is run in 4 replicates)

† PN 10006606 is sufficient for 18 IFCs (each assay is run in 4 replicates)

‡ PN 10006606 is manufactured by IDT and distributed by Fluidigm

Consumables Not Provided

Product Name	Source
Sterile container without preservatives for saliva collection	Any major laboratory supplier
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	
MicroAmp® Clear Adhesive Film for 96-well plates (PN 4306311)	Thermo Fisher Scientific

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

Equipment Not Provided

IMPORTANT: The Advanta Dx SARS-CoV-2 RT-PCR Assay Instrument Qualification Method (IQM) Protocol (SVC-00066) must be performed by a Fluidigm Field Application Specialist using Fluidigm-qualified SARS-CoV-2 material and meet specifications prior to reporting diagnostic results.

Product Name	Source	Part Number
Biomark™ HD system	Fluidigm	BMKHD-BMKHD
Biomark Data Collection software v4.7.1		102-0646
Juno™ system with system software v3.14.1, or IFC Controller RX (RX Controller)		101-6455 or IFC-RX
IFC Controller RX system software v2.9, if using IFC Controller RX		100-4739
RX Interface Plate, if using Juno		101-6114
Real-Time PCR Analysis software v4.7.1		102-0555
Advanta™ Dx SARS-CoV-2 RT-PCR Assay interpretive software v1.0.1		102-0323
Applied Biosystems® Veriti™ 96-Well Thermal Cycler	Thermo Fisher Scientific	4375786
2 centrifuges: 1 for microtubes, 1 for 96-well PCR plates	Any major laboratory supplier	—
Pipettes (P2–P1000) and appropriate filtered, low-retention tips		—
8-channel pipettes and appropriate filtered, low-retention tips		—
Vortexer		—

Class II biological safety cabinet for handling saliva samples		—

Warnings, Precautions, and Best Practices

Warnings and Precautions

- The Advanta Dx SARS-CoV-2 RT-PCR Assay is for *in vitro* diagnostic use under Emergency Use Authorization only.
- Federal Law restricts this device to sale by or on the order of a licensed practitioner.
- For *in vitro* diagnostic use only (IVD)
- This test has not been FDA cleared or approved but has been authorized for emergency use by FDA under an Emergency Use Authorization (EUA) for use by laboratories certified under the Clinical Laboratory Improvement Amendments (CLIA) of 1988, 42 U.S.C. §263a, that meet the requirements to perform high complexity tests.
- This test has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.
- The emergency use of this test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of *in vitro* diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug, and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated or authorization is revoked sooner.
- **IMPORTANT: Due to the nested PCR design of this test, strict quality control and physical separation of areas with potential amplicon or positive control material contamination is critical.**
- Testing of saliva specimens is limited to patients suspected of COVID-19.
- Negative results do not preclude infection with SARS-CoV-2 virus and should not be used as the sole basis for treatment or other patient management decision.
- Positive results are indicative of the presence of SARS-CoV-2 RNA and laboratories within the United States and its territories are required to report all results to the appropriate public health authorities.
- Before collection of the saliva specimen, ensure that the person has not used oral hygiene products within at least 30 minutes prior to collection.
- Samples and controls should always be treated as if infectious and/or biohazardous in accordance with safe laboratory procedures.
- Always use pipette tips with aerosol barriers. Tips that are used must be free from DNases and RNases.
- Reagents must be stored and handled as specified in Kit Components and Storage Conditions on page 5.
- Do not use the kit after the indicated expiry date.
- The Advanta Dx SARS-CoV-2 RT-PCR Assay workflow should be performed by qualified and trained staff to avoid the risk of erroneous results. Use separate areas for the preparation of samples and controls to prevent false positive results. Reagents may be

handled under a laminar airflow hood. Use universal precautions when handling biological samples in a Class II biological safety cabinet.

- Modifications to assay reagents, assay protocol, or instrumentation are not permitted, and are in violation of the product Emergency Use Authorization.
- Reliable results depend on proper specimen collection, storage, and handling procedures.
- Avoid contamination from positive controls and samples by following good laboratory practices.
- In addition to your site-specific safety requirements, Fluidigm recommends the following general safety guidelines in all laboratory and manufacturing areas:
  - Follow necessary precautions when handling specimens. Use personal protective equipment (PPE) consistent with current guidelines for the handling of potentially infectious samples.
  - 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 cosmetic products in lab areas.
  - Maintain clean work areas.
  - Wash hands before leaving the lab.
- The Advanta Dx SARS-CoV-2 RT-PCR Assay is for use only with Biomark HD in conjunction with Juno or an IFC Controller RX. 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).
- 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.
- 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.
- Read and understand the safety data sheets (SDS) 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.
- Dispose of waste in compliance with local, state, and federal regulations.

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 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 the cross-contamination of samples and controls with pre-amplified amplicons:
  - Designate space for the preparation of controls and 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.
  - 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.
- 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.

Limitations of the Procedure

- The Advanta Dx SARS-CoV-2 RT-PCR Assay performance was only established using saliva specimens from patients suspected of COVID-19 by their healthcare provider. Performance of this assay in persons without signs and symptoms of respiratory infections has not been established.
- Testing of saliva is limited to patients suspected of COVID-19 infection.
- Oral products such as toothpaste and mouth rinse should not be used for at least 30 min prior to collecting the saliva sample.
- Performance of this test was not evaluated in an asymptomatic patient population from individuals suspected of COVID-19 by their healthcare providers.

- Specimens must be collected, transported, and stored using appropriate procedures and conditions. Improper collection, transport, or storage of specimens may affect the ability of the assay to perform as indicated.
- Oral products such as toothpaste and mouth rinse should not be used for at least 30 min prior to collecting the saliva sample.
- Nasal gel products may interfere with the detection of low positive samples.
- The impacts of vaccines, antiviral therapeutics, antibiotics, chemotherapeutic or immunosuppressant drugs, or homeopathic medications have not been evaluated.
- The Advanta Dx SARS-CoV-2 RT-PCR Assay cannot rule out respiratory diseases caused by other bacterial or viral pathogens.
- False positive results may arise from:
  - Cross-contamination during specimen handling or preparation
  - Cross-contamination between patient samples
  - Specimen mix-up
  - RNA contamination during product handling: please ensure that the pre-amplification products are handled and diluted in a separate area from the amplification on the microfluidic chip to avoid contamination with pre-amplification product.
- False negative results may arise from:
  - Improper specimen collection
  - The presence of RT-PCR inhibitors
  - The presence of sequence variants in the pathogen targets of the assay
  - Degradation of the SARS-CoV-2 RNA during shipping/storage
  - Use of unauthorized assay reagents
  - Mutation in the SARS-CoV-2 virus
  - Failure to follow Instructions for Use
  - Analyte concentrations below the limit of detection
- Negative results do not preclude infection with SARS-CoV-2 virus and should not be the sole basis of a patient management decision.
- All results from this and other tests must be considered in conjunction with the clinical history, epidemiological data, and other data available to the clinician evaluating the patient.
- The results of this test should not be used as the sole basis for diagnosis, treatment, or other patient management decisions.
- This test is a qualitative test and the Ct values do not provide a quantitative assessment of SARS-CoV-2. The Ct values of the Real-Time PCR performed on the Biomark HD and analyzed by the Real-Time PCR analysis software do not include the pre-amplification cycles and therefore Ct results do not compare to other conventional Real-Time PCR tests.
- This device may not be able to differentiate newly emerging SARS-CoV-2 subtypes.

- As with any molecular test, if the virus mutates in the target region, SARS-CoV-2 RNA may not be detected or may be detected less predictably.
- The clinical performance has not been established in all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2 and their prevalence, which change over time.

Specimen Collection, Handling, and Storage

The specimen collection device is not included as part of the kit. Refer to the CDC Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens for COVID-19 (cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html).

Saliva specimens have been demonstrated to be stable at ambient temperature for up to 120 hrs after collection and prior to heat inactivation (see Table 2 on page 16 for heat inactivation). If the interval between collection and testing is anticipated to exceed 120 hrs, the specimen should be stored at −20 °C or lower.

Quality Control Procedures

Control materials to be used with Advanta Dx SARS-CoV-2 RT-PCR Assay:

- A “no template” negative control (NTC) is needed to test for amplicon contamination of reagents and instrumentation and consists of nuclease-free water in place of the sample. It is a full process control. If nonspecific amplification of any of the assays comes up in this sample, it is recommended that DNA decontamination of equipment, especially pipets, occur according to standard protocols and methods (for example, DNA AWAY™, Thermo Fisher Scientific, PN 7010).
- An internal control consisting of the RNase P primer and probe assay is needed. This control is used to monitor adequate amounts of RNA in the patient specimens and its RNA quality. It also monitors adequate RNA release from host cells present in the saliva specimens during the heating step and monitors reagent failure and the efficiency of the 1-step RT-preamplification and real-time PCR detection steps. This process control also monitors for inhibitors in the specimen that may reduce amplification efficiency.

Additional controls that are required but not provided with the test kit:

- The negative extraction control (NC) consists of normal saliva pooled from human donors confirmed COVID-19 negative combined with PBS and RNAsecure. The NC is heated at +90 °C for 10 min. Human RNA is detected using the RNase P primer and probe set.
- The positive template control (PC) is needed to control for adequate release of RNA and any failure in reverse transcription and amplification reagents and sample processing steps. The positive template control consists of heat-inactivated SARS-CoV-2 virus (ATCC® PN VR-1986HK) spiked into normal saliva from confirmed SARS-CoV-2 negative pooled human donors at 50 genome equivalents (GE)/µL. The positive control also serves as an extraction control because it is processed in the same manner as the saliva samples. Human RNA is detected using the RNase P primer and probe set.

All 3 quality controls [“no template” control (NTC), negative extraction control (NC), and positive control (PC)] must be run on each 96-well sample processing plate along with 93 samples. Each control is treated in the same manner as the sample (diluted in PBS and RNAsecure and then heated). If the negative or positive control fails, then it invalidates the 96-well plate run. A root cause investigation should be performed and once a root cause is identified the run must be repeated with the controls and specimens first. If the controls and specimens fail upon the second run, recollection of the specimens, re-processing of

the specimens, and fresh aliquots of the controls are necessary before performing the next run.

Prepare the Controls and Saliva Specimens

Collect the Saliva Specimens

IMPORTANT: Use universal precautions when handling biological samples.

Collect saliva specimen in a sterile container. Transport and test specimens as soon as possible after collection. Specimens are stable for up to 120 hrs at ambient temperature.

IMPORTANT: Due to the nested PCR design of this test, strict quality control and physical separation of areas with potential amplicon or positive control material contamination is critical. Sample receiving activities and sample and control preparation activities need to be physically separated from the area/s in which the preamplification and amplification reactions are set-up to mitigate the risk of contamination.

Prepare the Negative and Positive Controls

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

1. Briefly vortex and centrifuge the reagents before use.
2. Prepare each control with PBS (Thermo Fisher Scientific, 10010023) in a new, labeled 1.5 mL tube as follows:
   - **No Template Control (NTC):** Mix 25 µL of water with 25 µL of PBS, then vortex and centrifuge.
   - **Negative Extraction Control (NC):** Mix 25 µL of negative saliva specimen with 25 µL of PBS, then vortex and centrifuge.
   - **Positive Control (PC):** Dilute heat-inactivated virus (ATCC, VR-1986HK) in 25 µL of negative saliva specimen at 50 GE/µL. Add 25 µL of PBS, then vortex and centrifuge.

     IMPORTANT: Dilute the heat-inactivated virus in saliva at the time of use. Any unused diluted positive control should be discarded.
3. Aliquot 24 µL of each control to a new, labeled 1.5 mL tube.
4. Add 1 µL of RNAsecure (Thermo Fisher Scientific, AM7005) to each tube, then vortex and centrifuge to mix.
5. Set aside until ready to heat inactivate together with the saliva specimens.

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).

2 Aliquot 24 µL of the saliva/PBS mix 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 and the 3 controls (NTC, NC, PC) in a thermal cycler using the program in Table 2.

Table 2. 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 samples on ice until ready to use.

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

This section describes the preparation of the pooled assay mixes and sample mixes for the preamplification of a single run of 192 tests in two 96-well plates. Each sample requires the preparation of a single preamplification reaction. No fluorescence data is collected from the 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 and Probe 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 (6.7 µM primer, forward and reverse) in a new 2 mL tube, as shown in Table 3. The assay mix should be prepared and used immediately.

Table 3. Pooled and diluted primer and probe mix

Component	Vol for 3 Assays/ 1 Reaction (µL) *	Vol for 3 Assays/ 192 Reactions (µL) †
2019-nCoV Probe & Primer Kit (102-0686) or 2019-nCoV CDC EUA Kit (10006606; IDT)		
• 2019 nCoV_N1	0.112	25.0
• 2019 nCoV_N2	0.112	25.0
• RNase P (RP)	0.022	5.0
Advanta Dx Dilution Reagent (102-0353)	6.754	1,509

Total	**7.0**	**1,564**

* When preparing master mixes for less than 192 reactions, include an additional 10% to the volumes for overage. Remaining volumes may not be stored and are discarded.

† Includes overage

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

Prepare the 1-Step Reverse Transcription and Preamplification Reactions

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

1 Thaw Advanta Dx RT PA 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 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 and probe mix (see Table 3)	7	1,484
Advanta Dx RT PA MM (102-0345)	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.

5 Using an 8-channel pipette, combine the 1-step pre-mix and the samples or controls in 2 new 96-well plates as shown in Figure 1 on page 18.

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

b Next, add 5.0 µL of each control into wells A1 (NTC), B1 (NC), and C1 (PC) of each plate.

c Last, add 5.0 µL of sample to each remaining well of the 96-well plates.

NOTE: Only 1 preamplification reaction is prepared for each sample

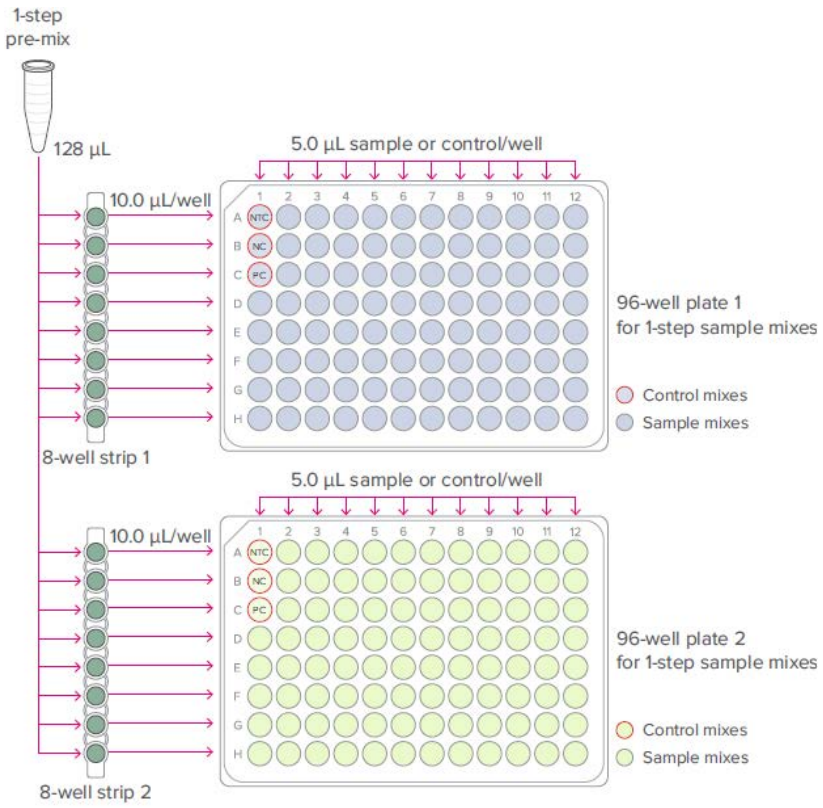


Figure 1. 1-step reaction plates (per-well transfer volumes)

6 Tightly seal the plates using MicroAmp Clear Adhesive Film for 96-well plates (Thermo Fisher Scientific, 4306311) 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

Place each plate in the Applied Biosystems Veriti 96-Well Thermal Cycler (Thermo Fisher Scientific, 4375786) and cycle using the program in Table 5:

Table 5. 1-step reverse transcription and preamplification

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

Dilute the Pre-amplified cDNA

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

After cycling, dilute the pre-amplified reactions in the 96-well plates in Advanta Dx Dilution Reagent as shown in Table 6 and described as follows.

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

NOTE: This is sufficient for the dilution of two 96-well plates of pre-amplified samples.

2 Use an 8-channel pipette to transfer 60 µL of Advanta Dx Dilution Reagent into each well containing the pre-amplified cDNA or control.

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

3 Tightly seal the plates using MicroAmp Clear Adhesive Film for 96-well plates (Thermo Fisher Scientific, 4306311), 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, pre-amplified cDNA and controls can either be assayed immediately or stored at −15 °C to −25 °C for later use.

Table 6. Diluted, pre-amplified cDNA and controls

Component	Vol per Reaction (µL)
Advanta Dx Dilution Reagent (102-0353)	60.0
Pre-amplified cDNA or control (contained in the 96-well plates)	15.0
Total	**75.0**

Prepare and Perform the Real-Time PCR Reactions on the IFC

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

This section describes the preparation of the final assay mixes, sample mixes, and integrated fluidics circuit (IFC) for the collection of real-time PCR amplification results.

Prepare the Final Assay Mixes for Loading on the IFC

NOTE:
- For each patient sample, each assay (N1, N2, RNase P) is automatically run in 4 replicates in each IFC.
- Assemble your assays in a 96-well plate and record in a detector map.

1. Briefly vortex and centrifuge the reagents before use.
2. In a DNA-free hood, prepare the assay mixes using the 2019-nCoV Probe & Primer Kit (102-0686) or the 2019-nCoV CDC EUA Kit (10006606; IDT) as shown in Figure 2.

   NOTE: For unused assay inlets, replace the assays with 3.0 µL of Advanta Dx PCR Water (102-0369).

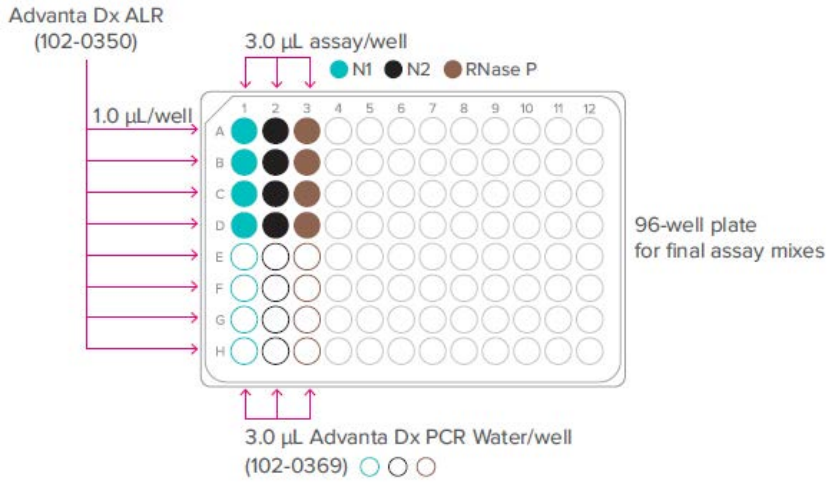


Figure 2. Final assay mixes plate (per-well transfer volumes)

Prepare the Final Sample Mixes

1. Thaw Advanta PCR MM and keep on ice. Briefly vortex and centrifuge the reagents before use.
2. In a DNA-free hood, combine the components (Table 7 on page 21) in a sterile 1.5 mL tube to make the sample pre-mix and place on ice. Scale up appropriately for multiple runs.

   NOTE: This is enough volume for the entire IFC.

Table 7. Sample pre-mix

Component	Vol per Inlet (µL)*	Sample Pre-Mix for One 192.24 IFC (µL)†
Advanta Dx PCR MM (102-0346)	2.0	460.0
Advanta Dx SLR (102-0349)	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 3.

a First, briefly vortex and centrifuge the sample pre-mix from Table 7.

b Next, aliquot 60 µL of pre-mix into each well of a new 8-well strip.

c Next, 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 Last, remove the plates from the DNA-free hood and prepare the final sample mix by adding 1.8 µL of each diluted, pre-amplified sample and control from Table 6 on page 19 to each well.

NOTE: For unused sample inlets, replace the diluted, pre-amplified cDNA with 1.8 µL of Advanta Dx PCR Water (102-0369).

IMPORTANT: Before use, briefly vortex and centrifuge the plates containing the diluted, pre-amplified cDNA and controls.

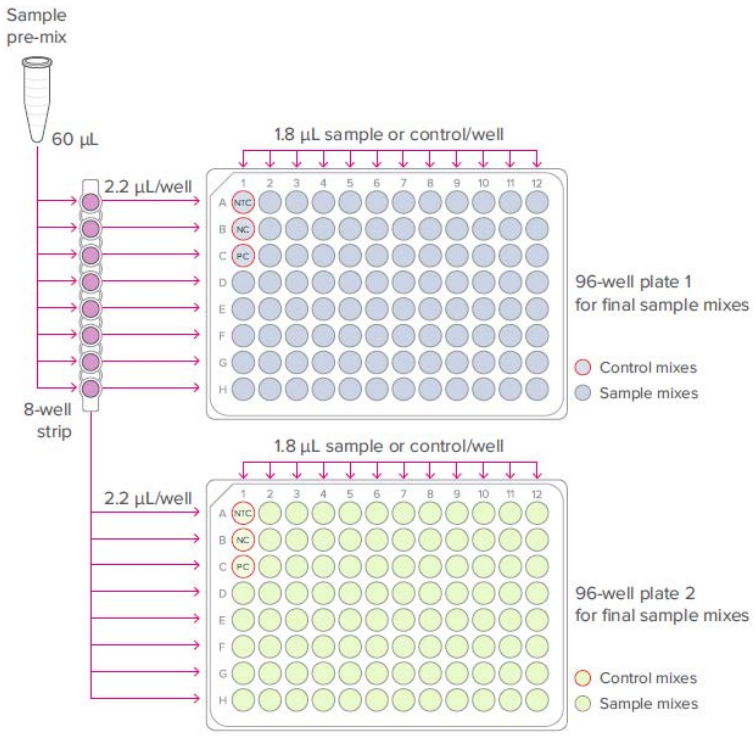


Figure 3. Final sample plates (per-well transfer volumes)

4 Tightly seal the plates using MicroAmp Clear Adhesive Film for 96-well plates (Thermo Fisher Scientific, 4306311), then vortex and centrifuge them at 3,000 × *g* for 60 sec.

Prepare the Advanta Dx 192.24 IFC

IMPORTANT: When injecting control line fluid:
- Follow the best practices for handling IFCs and control line fluid on page 10 of these instructions.
- Only use an Advanta Dx Control Line Fluid syringe (102-0390). The syringe contains 150 µL of control line fluid.

1 Remove the Advanta Dx Control Line Fluid syringe (102-0390) and the Advanta Dx 192.24 IFC (102-0389) 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 4).

IMPORTANT: The bottom accumulator is not used.

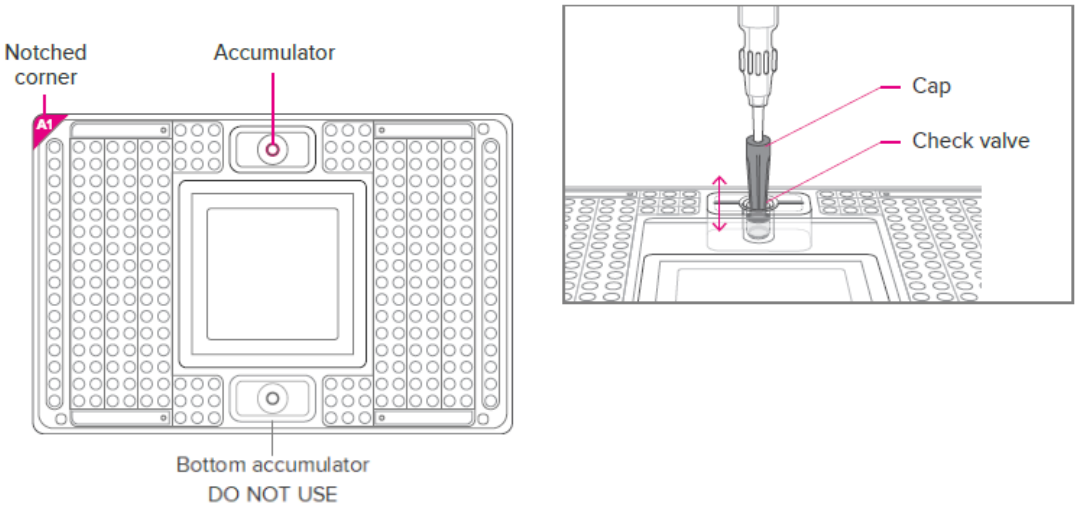


Figure 4. Actuating the check valve in the top accumulator on the 192.24 IFC

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 5 on page 23).

IMPORTANT:
- Avoid bending the syringe tip. Be careful when removing the 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 5). 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.

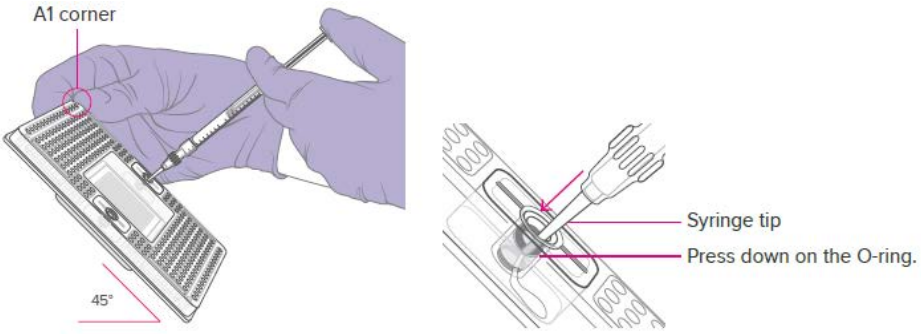


Figure 5. Injecting control line fluid into the accumulators on the 192.24 IFC

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 on page 24 when pipetting final sample, control, and assay mixes, PF 1, and PF 2 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 or 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. This enables each sample to be amplified by 4 replicates of each assay on the IFC.

4 Pipet 150 µL of Advanta Dx PF 1 (102-0352) into the P1 reservoir ( ) on the IFC.

5 Pipet 150 µL of Advanta Dx PF 2 (102-0351) into each of the P2 and P3 reservoirs (▭) on the IFC.

6 Pipet 20 µL of Advanta Dx PF 2 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 within 1 hr of completing the Load Mix script.

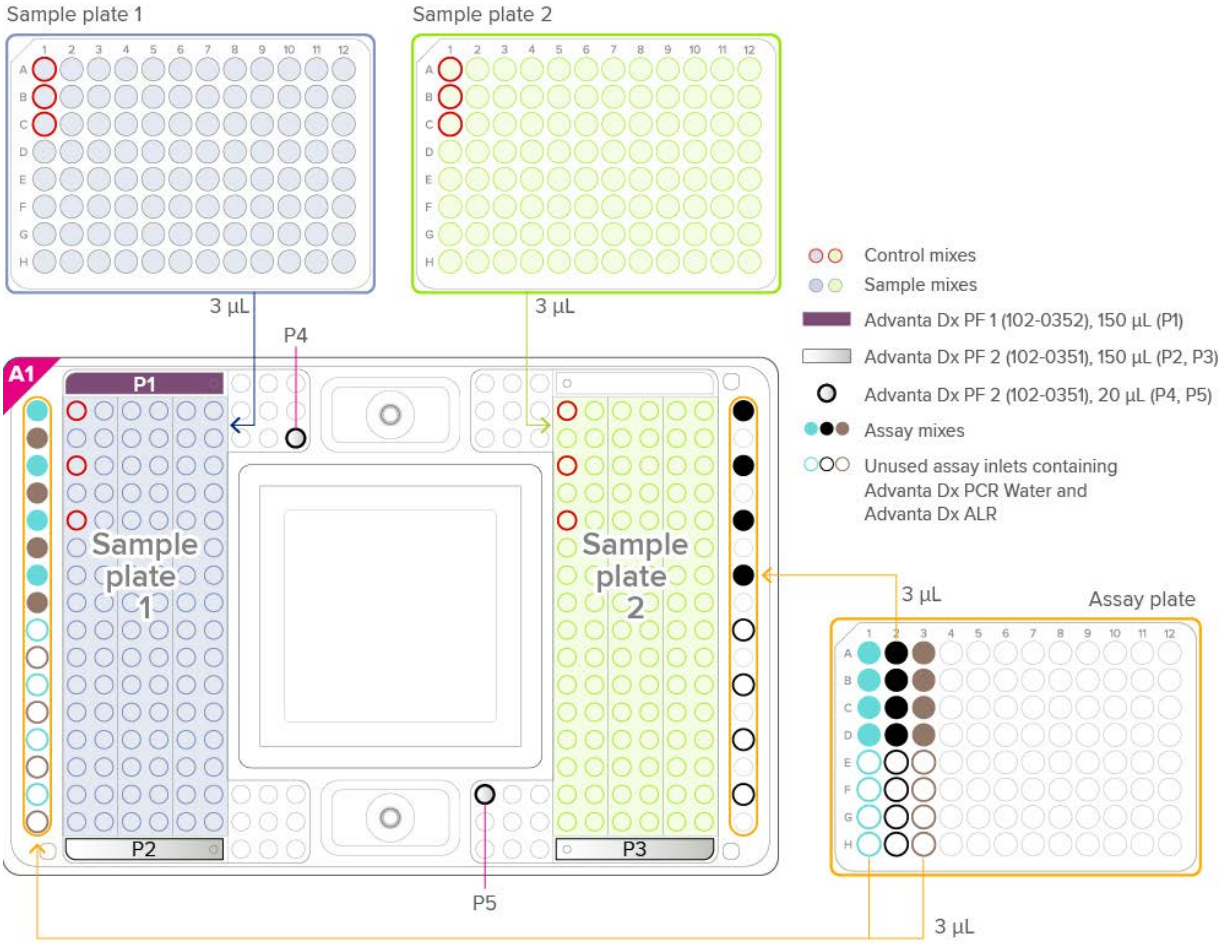


Figure 6. Pipetting map for the 192.24 IFC

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).

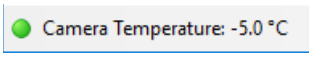
1 Remove the loaded IFC from Juno or IFC Controller RX.

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

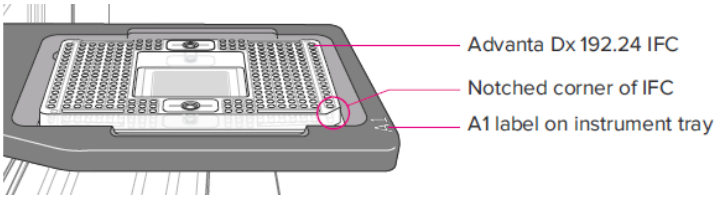


Figure 7. Loaded IFC on instrument tray

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

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 protocols, see Appendix A.

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 Advanta Dx SARS-CoV-2 RT-PCR Assay Interpretive Software Quick Reference Guide (FLDM-00162). You can either set up the Real-Time PCR Analysis software to export data directly through the Advanta Dx SARS-CoV-2 RT-PCR Assay interpretive software or you can run the Advanta Dx SARS-CoV-2 RT-PCR Assay interpretive software from a command line.

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.

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**, or **Empty**.
- Type: RNase P assays are type **Control**. Empty assay inlets are type **Blank**. N1 and N2 assays are type **Test**.

IMPORTANT: The names **N1**, **N2**, **RNase P**, and **Empty** are case-sensitive and must be entered exactly as shown.

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).

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

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)**

NOTE: To set Linear and Auto By Control (Global) as the default settings, select **Tools** > **Options** > **Analysis Parameters**. Check the checkbox for **Customize Default Baseline Correction Method** and select **Linear**. Check the checkbox for **Customize Default Ct Threshold Method** and select **Auto By Control (Global)**. Click **OK** to save the changes.

7 After analyzing the data, click (**Save**), then click (**Export**) and use the Advanta Dx SARS-CoV-2 RT-PCR Assay interpretive software to interpret the Ct results and save the interpretation as a CSV file.

8 Review the interpreted data. If any of the controls fail, 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 (RNase P, N1, N2).

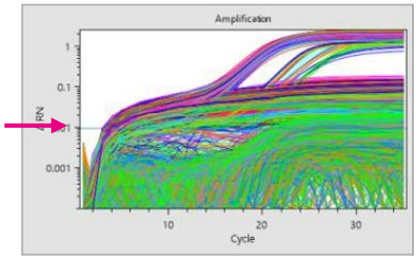
c Click (**Expand / Collapse**) in the secondary toolbar to expand the Normalized Intensity and Amplification plots.

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

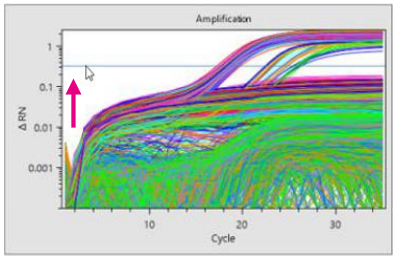
e Confirm that the Ct threshold needs to be adjusted by visually confirming that the horizontal Ct Threshold line is in the baseline in the Amplification plot.

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.

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

Assay Results and Interpretation

All test controls must be examined prior to interpretation of patient results. If the positive or negative controls are not valid, the patient results cannot be interpreted, and all patient specimens should be retested after a root cause has been identified and eliminated.

Examination and Interpretation of Control Results

The 3 quality controls referenced in Quality Control Procedures on page 14 are included on each 96-well sample plate generated (3 controls and 93 samples). Two 96-well sample plates are used to load each integrated fluidic circuit (IFC). Therefore, there are 6 controls in one IFC run, 3 per 96-well sample plate. The definitions of positive (+) and negative (–) results are in Table 8. Control results are interpreted manually on a per plate basis. Therefore, if the controls in one 96-well sample plate fail to meet the expectation, it will only affect the samples in the same plate.

Table 8. Expected control results from Fluidigm Biomark HD

Control Description	N_1 Result	N_2 Result	RNase P Result	Interpretation	Control Result (Manual)
Positive control (PC)	+	+	+	Positive	Pass
Negative extraction control (NC)	–	–	+	Negative	Pass
No template control (NTC)	–	–	–	No Template	Pass

If the NTC, NC, or PC fail to meet expected control results for a given 96-well plate, then it invalidates the plate and the results are not reportable. A root cause investigation shall be completed prior to repeating the test. Upon elimination of the root cause, a retest may be performed. If the controls and specimens fail upon the second run, recollection of the samples, re-processing of the samples and fresh aliquots of the controls and reagents are necessary before performing the next run.

If the NTC fails to meet expected control results, it is recommended that standard laboratory DNA decontamination procedures are implemented and/or additional training of test operators.

Interpretation of Patient Specimen Results

Interpretation of specimen results is performed using the Advanta Dx SARS-CoV-2 RT-PCR Assay interpretive software based on a Ct 32 cutoff and according to Table 9 on page 29.

NOTE: Ct values of the Real-Time PCR step performed on the Biomark HD do not include the pre-amplification cycles, and therefore results do not compare to other conventional Real-Time PCR tests.

For detailed instructions about using the interpretive software, see the Advanta Dx SARS-CoV-2 RT-PCR Assay Interpretive Software Quick Reference Guide (FLDM-00162).

Table 9. Patient sample interpretation from assay N1, N2 and RNase P results.

NOTE: **+ = Positive, – = Negative, ? = Inconclusive**

N_1 Result	N_2 Result	RNase P Result	Sample Interpretation	Action
+	+	+	Positive	Report as positive for SARS-CoV-2. Report result to public health authorities.
+	+	–	Positive	
+	+	?	Positive	
+	–	+	Positive	
+	?	+	Positive	
–	+	+	Positive	
?	+	+	Positive	
–	–	+	Negative	Report as negative for SARS-CoV-2. Report result to public health authorities.
–	–	–	No Template	Retest. If the result of the retest repeats as No Template or as Inconclusive, obtain a new specimen.
–	?	–	Inconclusive	
–	+	–	Inconclusive	
?	–	–	Inconclusive	
?	?	–	Inconclusive	
?	+	–	Inconclusive	
+	–	–	Inconclusive	
+	?	–	Inconclusive	
–	–	?	Inconclusive	
–	?	?	Inconclusive	
–	+	?	Inconclusive	
?	–	?	Inconclusive	
?	?	?	Inconclusive	
?	+	?	Inconclusive	
+	–	?	Inconclusive	
+	?	?	Inconclusive	

–	?	+	Inconclusive
?	–	+	Inconclusive
?	?	+	Inconclusive

Conditions of Authorizations for Labs

The Advanta Dx SARS-CoV-2 RT-PCR Assay Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients, and authorized labeling, are available on the FDA website: fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/vitro-diagnostics-euas.

To assist clinical laboratories using the Advanta Dx SARS-CoV-2 RT-PCR Assay ("your product" in the conditions below), the relevant Conditions of Authorization are listed below:

A Authorized laboratories using this product[1] must include with test result reports, all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.

B Authorized laboratories using this product must use this product as outlined in the authorized labeling. Deviations from the authorized procedures, including the authorized instruments, authorized extraction methods, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents, and authorized materials required to use this product, are not permitted.

C Authorized laboratories that receive this product must notify the relevant public health authorities of their intent to run this product prior to initiating testing.

D Authorized laboratories using this product must have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.

E Authorized laboratories must collect information on the performance of this product and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and Fluidigm (via email: techsupport@fluidigm.com) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of your product of which they become aware.

F All laboratory personnel using your product must be appropriately trained in RT-PCR techniques and the specific processes and instruments used in the Advanta Dx SARS-CoV-2 RT-PCR Assay, use appropriate laboratory and personal protective equipment when handling this kit, and use your product in accordance with the authorized labeling.

G Fluidigm Corporation, authorized distributors, and authorized laboratories using this product must ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records will be made available to FDA for inspection upon request.

[1] "This product" refers to the Fluidigm Advanta Dx SARS-CoV-2 RT-PCR Assay. The letter of authorization refers to, "Laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform high complexity tests" as "authorized laboratories."

H Authorized laboratories testing specimens self-collected using the AZOVA COVID-19 Test Collection Kit must have in place a suitable specimen receipt and accessioning SOP.

Performance Evaluation

All performance data were collected and analyzed using the Fluidigm Real-Time PCR Analysis software and interpreted using the Advanta Dx SARS-CoV-2 RT-PCR Assay interpretive software.

Limit of Detection (LoD): Analytical Sensitivity

To determine the limit of detection (LoD), 3 independent SARS-CoV-2 negative saliva sample pools from 2 different commercial suppliers were used for this study to test the impact of natural saliva variation on the test's sensitivity.

Heat-inactivated SARS-CoV-2 virus (ATCC PN VR-1986HK, 3.75 x 10^5 genome equivalents (GE)/µL Lot 70035039) was spiked into the negative saliva pools with a starting concentration of 50 GE/µL and then serially diluted in the negative saliva pools in decreasing 2-fold dilutions down to 0.391 GE/µL. After the dilutions were performed, samples were processed as described in this Instructions for Use, including all pre-processing and inactivation steps. Two Fluidigm Advanta Dx 192.24 IFCs loaded from the same pre-amplified material and one each was processed on the IFC Controller RX and the Juno instrument.

The results are shown in the Table 10 and Table 11. 95% or higher positive detection rate of the SARS-CoV-2 amplicons led to an LoD of 3.125 GE/µL for Saliva Pools 2 and 3 and 6.25 GE/µL for Saliva Pool 1. Thus, the final LoD for the Advanta Dx SARS-CoV-2 RT-PCR Assay was determined to be 6.25 GE/µL. The LoD results were independent of the controller used to load the IFC with samples and assays.

Table 10. Summary table of IFC loaded with IFC Controller RX (Saliva Pools 1, 2, and 3)

Target Level GE/µL	Valid Tested Replicates	Detected Replicates per Result Interpretation	SARS-CoV-2 N1 Positive			SARS-CoV-2 N2 Positive			Internal Control Positive		
			n	Mean Ct	Detection Rate	n	Mean Ct	Detection Rate	n	Mean Ct	Detection Rate
50	60	60 (100%)	60	20.2	100%	60	22.2	100%	60	18.4	100%
25	60	60 (100%)	60	20.8	100%	60	23.1	100%	60	18.4	100%
12.5	60	60 (100%)	60	22.1	100%	60	24.2	100%	60	18.4	100%
6.25	**60**	**59 (98%)**	**57**	**23.1**	**95%**	**54**	**25.6**	**90%**	**60**	**18.4**	**100%**
3.125	60	56 (93%)	52	23.4	87%	43	25.6	72%	60	17.8	100%
1.563	60	44 (73%)	32	24.0	53%	29	26.0	48%	60	17.9	100%
0.781	60	24 (40%)	20	24.2	33%	9	25.8	15%	60	17.7	100%
0.391	60	16 (27%)	10	24.2	17%	11	25.8	18%	60	17.8	100%
0	84	0 (0%)	0	UD*	0%	0	UD	0%	84	18.0	100%

* UD = Undetected

Table 11. Summary table of IFC loaded with Juno (Saliva Pools 1, 2, and 3)

Target Level GE/µL	Valid Tested Replicates	Detected Replicates per Result Interpretation	SARS-CoV-2 N1 Positive			SARS-CoV-2 N2 Positive			Internal Control Positive		
			n	Mean Ct	Detection Rate	n	Mean Ct	Detection Rate	n	Mean Ct	Detection Rate
50	60	60 (100%)	60	20.1	100%	60	22.0	100%	60	18.3	100%
25	60	60 (100%)	60	20.8	100%	60	23.0	100%	60	18.3	100%
12.5	60	60 (100%)	60	22.1	100%	60	24.2	100%	60	18.4	100%
6.25	**60**	**59 (98%)**	**57**	**23.2**	**95%**	**54**	**25.6**	**90%**	**60**	**18.4**	**100%**
3.125	60	56 (93%)	52	23.6	87%	43	25.7	72%	60	18.0	100%
1.563	60	44 (73%)	32	24.1	53%	29	26.0	48%	60	18.0	100%
0.781	60	24 (40%)	20	24.3	33%	9	26.1	15%	60	17.9	100%
0.391	60	16 (27%)	10	24.5	17%	11	26.1	18%	60	18.0	100%
0	84	0 (0%)	0	UD*	0%	0	UD	0%	84	18.0	100%

* UD = Undetected

Inclusivity (Analytical Sensitivity)

The Advanta Dx SARS-CoV-2 RT-PCR Assay uses the same primers and probes as the CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel. The CDC has granted right-of-reference to assay developers to utilize the data generated in its authorized CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel EUA.

Cross-Reactivity

An *in silico* analysis was performed for the authorized CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel that determined that the primer and probe sequences used in the Advanta Dx SARS-CoV-2 RT-PCR Assays do not have significant homology to other respiratory pathogens. CDC has granted right-of-reference for the data in the authorized CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel EUA.

Given the use of a direct saliva lysate in the Advanta Dx SARS-CoV-2 RT-PCR wet testing was conducted for the organisms, listed in Table 12 on page 36. High priority microorganisms provided by Microbiologics Respiratory (21 Targets) Control Panel PN 8217 were spiked into 3 independent negative saliva sample pools from 2 different commercial suppliers and were each tested in triplicates. As a positive control for SARS-CoV-2, heat-inactivated SARS-CoV-2 virus (ATCC PN VR-1986HK) diluted in the negative saliva pools in the absence of the other high priority microorganisms was tested. Negative controls (unspiked saliva sample pools) were run in parallel. Cross-reactive organisms were spiked into the negative background saliva pool in large excess of SARS-CoV-2.

All samples were processed according to the Instructions for Use, including the heat treatment using both controllers, the IFC Controller RX and the Juno instrument.

As seen in Table 12, none of the high priority microorganisms tested generated a detectable response. All positive control replicates were positive for both the N1 and N2 SARS-CoV-2 targets (3 out of 3 replicates), and all negative controls were positive for RNase P and negative for the SARS-CoV-2 targets (8 out of 8 replicates). No detectable amplification for the SARS-CoV-2 N1

and N2 targets as well as RNase P occurred for the NTCs (32 out of 32 replicates were negative). No difference between the controllers or between the different saliva pools was observed.

Table 12. Cross-reactivity results

Virus/Bacteria/Parasite	Strain	Source/Sample Type	Minimum Copies/Reaction	Saliva Pool 1 Results	Saliva Pool 2 Results	Saliva Pool 3 Results
Virus						
Adenovirus	Type 6	Inactivated virus	10000	0/3 reps detected	0/3 reps detected	0/3 reps detected
Human coronavirus	229E	Inactivated virus	1000	0/3 reps detected	0/3 reps detected	0/3 reps detected
	HKU1, recombinant	Inactivated recombinant bacteria	1000	0/3 reps detected	0/3 reps detected	0/3 reps detected
	NL63, recombinant	Inactivated recombinant bacteria	1000	0/3 reps detected	0/3 reps detected	0/3 reps detected
	OC43 Strain 1	Inactivated recombinant bacteria	1000	0/3 reps detected	0/3 reps detected	0/3 reps detected
	OC43 Strain 2	Inactivated recombinant bacteria	1000	0/3 reps detected	0/3 reps detected	0/3 reps detected
Human metapneumovirus		Inactivated recombinant bacteria	10000	0/3 reps detected	0/3 reps detected	0/3 reps detected
Human parainfluenza virus 1		Inactivated virus	1000	0/3 reps detected	0/3 reps detected	0/3 reps detected
Human parainfluenza virus 2		Inactivated virus	1000	0/3 reps detected	0/3 reps detected	0/3 reps detected
Human parainfluenza virus 3		Inactivated virus	1000	0/3 reps detected	0/3 reps detected	0/3 reps detected
Human parainfluenza virus 4a	4a, recombinant	Inactivated recombinant bacteria	1000	0/3 reps detected	0/3 reps detected	0/3 reps detected
Human respiratory syncytial virus		Inactivated virus	10000	0/3 reps detected	0/3 reps detected	0/3 reps detected
Human rhinovirus		Inactivated virus	10000	0/3 reps detected	0/3 reps detected	0/3 reps detected
Influenza A		Inactivated virus	10000	0/3 reps detected	0/3 reps detected	0/3 reps detected
	Subtype H1	Inactivated virus	10000	0/3 reps detected	0/3 reps detected	0/3 reps detected
	Subtype H1-2009	Inactivated virus	10000	0/3 reps detected	0/3 reps detected	0/3 reps detected
	Subtype H3	Inactivated virus	10000	0/3 reps detected	0/3 reps detected	0/3 reps detected
Influenza B		Inactivated virus	10000	0/3 reps detected	0/3 reps detected	0/3 reps detected
Bacteria						
Bordetella parapertussis		Inactivated bacteria	10000	0/3 reps detected	0/3 reps detected	0/3 reps detected
Bordetella pertussis		Inactivated bacteria	10000	0/3 reps detected	0/3 reps detected	0/3 reps detected
Chlamydia pneumoniae		Inactivated bacteria	10000	0/3 reps detected	0/3 reps detected	0/3 reps detected
Mycoplasma pneumoniae		Inactivated bacteria	10000	0/3 reps detected	0/3 reps detected	0/3 reps detected

Endogenous Interference Substances Studies

An endogenous interference study was performed to determine interference substances that could be found in saliva samples and to evaluate the extent, if any, of potential Advanta Dx SARS-CoV-2 RT-PCR Assay inhibition or interference. Three saliva pools were tested with 3 replicates each using both controllers, generating a total of 18 replicates per interfering substance. Saliva pools without interfering substances were included as experimental sample controls. The results of this study are presented in Table 13.

Table 13. Testing of potentially interfering substances

Potential Interfering Substances for Saliva	Concentration of Interfering Substance	Positive Samples at No More than 3x LoD (18.75 GE/µL)			Negative Samples		
		Detected			Detected		
		Saliva Pool 1	Saliva Pool 2	Saliva Pool 3	Saliva Pool 1	Saliva Pool 2	Saliva Pool 3
Mucin: bovine submaxillary gland, type I-S	2.5 mg/mL	6/6*	6/6	6/6	0/6	0/6	0/6
White blood cells/ leukocytes	1 to 5 × 10^6 cells/mL	6/6	6/6	6/6	0/6	0/6	0/6
Afrin® Original nasal spray	15% v/v	6/6	6/6	6/6	0/6	0/6	0/6
NeilMed® NasoGel®	1.25%	6/6	6/6	**4/6**	0/6	0/6	0/6
Cepacol® (benzocaine/ menthol lozenges)	3 mg/mL	6/6	6/6	6/6	0/6	0/6	0/6
Chloraseptic® Sore Throat spray/solution	5% v/v	6/6	6/6	6/6	0/6	0/6	0/6
Toothpaste (Colgate®)	0.5% v/v	6/6	6/6	**4/6**	0/6	0/6	0/6
Crest mouthwash	5% v/v	**2/6**	6/6	6/6	0/6	0/6	0/6
Nicotine	0.03 mg/mL	6/6	6/6	6/6	0/6	0/6	0/6
Human genomic DNA	10 ng/µL	6/6	6/6	6/6	0/6	0/6	0/6
		Positive Samples at No More than 3x LoD (18.75 GE/µL)			Negative Samples		
Experimental sample control without inhibitors		64/66			0/8		

* 3 replicates on each of 2 controllers

None of the interfering substances caused false positives in this study. However, 3 substances were found to potentially interfere with detection of low positive samples: NeilMed NasoGel, Crest mouthwash, and Colgate toothpaste.

Clinical Evaluation

A total of 77 retrospective, blinded saliva specimens (43 positive and 34 negative) collected at one clinical site were tested with the Advanta Dx SARS-CoV-2 RT-PCR Assay. These specimens had matching nasopharyngeal (NP) test results with FDA EUA SARS-Cov-2 Real-Time PCR tests authorized for use with NP swabs.

These 77 specimens were processed per this Instructions for Use document including interpretation of results based on a Ct 32 cutoff as described in the result interpretation section (above). Results are summarized in Table 14.

Table 14. Summary of results for blinded clinical samples comparing nasopharyngeal and saliva sample types

		EUA Comparator with Nasopharyngeal Swab			
		Positive (#)	Inconclusive (#)	Negative (#)	Total (#)
Saliva samples tested with Advanta™ Dx SARS-CoV-2 RT-PCR Assay	Positive (#)	43	0	0	43
	Inconclusive (#)	0	0	0	0
	Negative (#)	0	0	34	34
	Total (#)	43	0	34	77
Positive percent agreement (PPA)	100% (95% CI: 91.8% - 100%)				
Negative agreement (%)	100% (95% CI: 89.9% - 100%)				

Mean Ct values for saliva and nasopharyngeal swab specimens showed no trend between Ct values from different samples from the same patient. The results support saliva as a specimen type for use with the Advanta Dx SARS-CoV-2 RT-PCR Assay.

FDA SARS-CoV-2 Reference Panel Testing

The evaluation of sensitivity and MERS-CoV cross-reactivity was performed using reference material (T1), blinded samples and a standard protocol provided by the FDA. The study included a range finding study and a confirmatory study for LoD. Blinded sample testing was used to establish specificity and to confirm the LoD. The instruments used were IFC Controller RX and Biomark HD. The results are summarized in Table 15.

Table 15. Summary of LoD confirmation result using the FDA SARS-CoV-2 reference panel

Reference Materials Provided by FDA	Specimen Type	Product LoD	Cross-Reactivity
SARS-CoV-2	Saliva	5.4×10^4 NDU/mL	N/A
MERS-CoV		N/A	ND

NDU/mL = RNA NAAT detectable units/mL
N/A: Not applicable
ND: Not detected

Appendix A: Biomark HD Thermal Cycler Protocol

GE 192x24 Fast v1 thermal cycling parameters

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

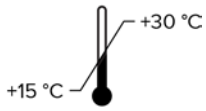
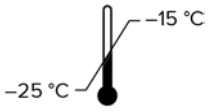
Appendix B: Related Documents

Go to fluidigm.com to download these related documents.

Title	Document Number
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 Software User Guide	68000088
Advanta Dx SARS-CoV-2 RT-PCR Assay Interpretive Software Quick Reference Guide	FLDM-00162

Appendix C: Symbols

Symbol	Indicates
	Medical device manufacturer
	Use-by date
LOT	Manufacturer's batch code
REF	Manufacturer's catalog number
SN	Manufacturer's serial number
IVD	*In vitro* diagnostic medical device

Symbol	Indicates
	Biological risks
	Consult Instructions for Use
	Caution
	Indicates ambient temperature to which the *in vitro* diagnostic medical device can safely be exposed
	Indicates frozen temperature to which the *in vitro* diagnostic medical device can safely be exposed

Revision History

Revision	Date	Description of change
10	02/2021	Updated Intended Use for home collection. Referenced Instrument Qualification Method in the Equipment Not Provided section.
09	12/2020	Updated assay products. Added clarification for PCR plate selection. Clarified controls and control interpretation. Corrected typo in 2019-nCoV_N1-P sequence.
08	11/2020	Software instructions for Biomark Data Collection and Real-Time PCR Analysis software updated to v4.7.1. IFC Controller RX system software updated to v2.9. Updated warnings and precautions to remove need for use of sterile pipette tips. Revised positive control instructions. Corrected typos.
07	09/2020	Updated cap color for Advanta Dx PCR MM (102-0346). Added instructions for sample and assay (detector) annotation in the Real-Time PCR Analysis software for ease of use. Added FDA SARS-CoV-2 Reference Panel Testing section. Corrected typos.
06	08/2020	Updated regulatory information, changed IDT product procurement.
05	08/2020	Revised IDT part number and corrected typos.
04	08/2020	Updated regulatory information, updated IDT part number, added interfering substances table.
03	06/2020	Updated regulatory information, added detailed control line fluid loading procedure, removed reference to 68000132, and updated cross-references.
02	06/2020	Updated regulatory language and corrected figures.
01	06/2020	Initial release.

Fluidigm Corporation
2 Tower Place Suite 2000
South San Francisco, CA 94080

For technical support visit techsupport.fluidigm.com.

North America +1 650 266 6100 | Toll-free (US/CAN): 866 358 4354 | techsupport@fluidigm.com Latin America +1 650 266 6100 | techsupportlatam@fluidigm.com
Europe/Middle East/Africa/Russia +33 1 60 92 42 40 | eu.support@fluidigm.com China (excluding Hong Kong) +86 21 3255 8368 | techsupportchina@fluidigm.com
Japan +81 3 3662 2150 | techsupportjapan@fluidigm.com All other Asian countries/India/Australia +1 650 266 6100 | techsupportasia@fluidigm.com

For *In Vitro* Diagnostic Use. For Emergency Use Authorization Only.

Advanta™ Dx SARS-CoV-2 RT-PCR Assay Instrument Qualification Method (IQM) Protocol

For in vitro diagnostic use

Rx Only

For use under Emergency Use Authorization (EUA) only

Biomark™ HD System ID: _____

Work Order: _____

Company name: _____

Test performed by certified FAS (Print):_____

FAS Initials:_____

FAS Signature: _____

Date of execution: _____

Contents

1 Warnings

- This test has not been FDA cleared or approved, but has been authorized for emergency use by FDA under an EUA for use by authorized laboratories;
- This test has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens; and
- The emergency use of this test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug, and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated or authorization is revoked sooner.

2 Using this Protocol

A. Use this IQM Protocol to verify the performance of the Biomark™ HD system in combination with the Juno™ System or the IFC Controller RX (RX Controller) and the Advanta Dx SARS-CoV-2 RT-PCR Assay to ensure that the criteria for acceptance in this procedure are met prior to use for diagnostic purposes.

B. Documents including test results generated during the execution of this protocol must be attached as objective evidence.

C. Record exceptions and resolutions in the exceptions section.

D. Only the authorized customer can adequately determine whether this IQM meets their company's regulatory requirements. As such, the customer is responsible for performing such assessment. Fluidigm makes no claims that this IQM Protocol satisfies any requirement of any governing, regulating, or tracking body, including the United States Food and Drug Administration (FDA).

3 Roles

Fluidigm Field Application Specialist (FAS)

A. Maintain records of the protocol execution by making comments in appropriate sections.

B. Record each exception and provide description of each resolution.

C. Sign and date the Instrument Qualification Method (IQM) Final Approval section upon completion of this protocol.

Authorized Customer

A. Approve the use of this protocol prior to its execution.

B. Sign and date the Instrument Qualification Method (IQM) Final Approval section upon completion of this protocol.

4 Customer Protocol Approval

The Biomark HD System Instrument Qualification Method (IQM) Protocol is understood by the customer and is approved to be performed.

_____ _____

Authorized Customer Name and Signature Date:

5 Required Materials and Equipment

NOTE See the Advanta Dx SARS-CoV-2 RT-PCR Assay Instructions for Use (FLDM-00161) for required materials and equipment.

6 Procedure

This procedure is intended to follow the IFU while utilizing samples contrived by diluting heat-inactivated virus (ATCC, Part Number VR-1986HK) in negative pooled saliva (Innovative Research, Part Number IR100044P or Lee BioSolutions, Part Number 991-05-P) at two concentrations and a negative pooled saliva sample to assess assay performance.

NOTE Prior to beginning this procedure, ensure all consumables used adhere to the materials list provided in the Advanta™ Dx SARS-CoV-2 RT-PCR Assay Instructions for Use (FLDM-00161).

1. Locate the concentration of ATCC viral particles on the Lot Specific CoA provided by ATCC. Attach a copy of the CoA to this record.

   Concentration: _____ genome copies (GC)/µL*
   *GC/µL and GE/µL are equivalent

2. Use the concentration of ATCC viral particles to determine the dilution factor required to prepare Dilution 1 (100,000 GE/µL).

   For example:

   a. **Assuming** that the concentration of the ATCC viral particles is 375,000 GE/µL, the calculation of dilution factor to achieve 100,000 GE/µL is:

      (*375,000* GE/µL) / (100,000 GE/µL) = 3.75

      The calculation for dilution 1 is dependent upon the concentration of the ATCC viral particles provided in the Lot Specific CoA (see step 1 of this procedure)

NOTE Perform the following steps in a Class II Biological Safety Cabinet.

3. Clean the area and pipettes thoroughly with DNA destroying surface decontaminants then change gloves.
4. Label eight microcentrifuge tubes as Dilution 1, Dilution 2, Dilution 3, Dilution 4, Dilution 5, Dilution 6, negative saliva, and positive stock.

5 Remove negative pooled saliva from storage and thaw. Mix thoroughly by vortexing and aliquot 1.5 mL into the tube labeled negative pooled saliva for use in preparation of Dilutions. Return the bulk negative saliva to frozen storage.

6 Remove ATCC viral particles from storage and thaw on ice. It is recommended to use a fresh vial of ATCC viral particles that has never been previously thawed. Mix thoroughly by vortexing and aliquot 5µL into the tube labeled positive stock for use in preparation of Dilution 1 (step 8, below). Aliquot the remainder into single use volumes prior to returning to frozen storage.

7 Clean the prep area and pipettes thoroughly with DNA destroying surface decontaminants then change gloves.

8 Prepare the initial dilution of the ATCC viral particles (Dilution 1) according to the table below.

Dilution	Final Volume Required	ATCC Vial Particles Concentration	Dilution Factor	Final Concentration	ATCC Viral Particle Volume Required (µL)	Negative Saliva Volume Required (µL)
1	10 µL	See Step 1 _____	See step 2a _____	100,000 GE/µL	= 10 µL ÷ Dilution Factor _____ µL	= 10 µL – ATCC Viral Particle Volume Required _____ µL

9 Prepare a serial dilution series (Dilutions 2, 3, 4, 5, and 6) using Dilution 1 prepared in step 8 as the starting material, according to the table below. Store on ice following each preparation.

NOTE: Following the preparation of each dilution, mix by vortexing to ensure a homogenous mixture followed by a brief centrifugation before proceeding to preparing the next dilution.

Dilution	Final Volume Required	Previous Dilution Concentration	Dilution Factor	Final Concentration	Volume of Previous Dilution Required (µL)	Negative Saliva Volume Required (µL)
2	20 µL	100,000 GE/µL	10	10,000 GE/µL	2.0	18.0
3	50 µL	10,000 GE/µL	10	1,000 GE/µL	5.0	45.0
4	400 µL	1,000 GE/µL	10	100 GE/µL	40.0	360.0
5	600 µL	100 GE/µL	2	50 GE/µL	300.0	300.0
6	600 µL	50 GE/µL	2.67	18.75 GE/µL	225.0	375.0

10 Dilution 5 and Dilution 6 will be used as positive saliva specimens in the Advanta Dx SARS-CoV-2 RT-PCR Assay at 8x LoD (50 GE/uL) and 3x LoD (18.75 GE/uL), respectively. Pooled, negative saliva will be used as negative saliva specimens.

11 Process 10 replicates of Dilution 5, 20 replicates of Dilution 6 and 10 replicates of pooled, negative saliva following the Advanta™ Dx SARS-CoV-2 RT-PCR Assay Instructions for Use,

beginning with the "Process the Saliva Specimens" section. Any remaining volume of the Dilutions should be discarded as biohazardous waste according to the laboratory's procedures.

7 Results of Protocol Execution

Parameter	Specification	Results	
Run validity	All controls meet expected control results per the Instructions for Use	☐ Controls Meet Expectations ☐ Controls Do Not Meet Expectations	☐ Pass ☐ Fail
Dilution 5: 8x LoD Sample – (50 GE/µL)	All 10 replicates are positive based upon Interpretive Software result	☐ 10 / 10 replicates are positive ☐ < 10 replicates are positive	☐ Pass ☐ Fail
Dilution 6: 3x LoD Sample – (18.75 GE/µL)	At least 19 of 20 replicates are positive based upon Interpretive Software result	☐ ≥ 19 replicates are positive ☐ < 19 replicates are positive	☐ Pass ☐ Fail
SARS-CoV-2 Negative Saliva	All 10 replicates are negative based upon Interpretive Software result	☐ 10 / 10 replicates are negative ☐ < 10 replicates are negative	☐ Pass ☐ Fail

NOTE The qualification passes if the results for each parameter in the table above pass.

Exceptions: ☐ No, line through the exceptions box below and initial/date ☐ Yes, see below

Exceptions:

8 Instrument Labeling

Following successful completion of this protocol and meeting the specifications in section 6, apply the label below to each Fluidigm instrument included in the execution of this Instrument Qualification Method.

Emergency Use Only

This instrument is authorized for use with the Advanta Dx SARS-CoV-2 RT-PCR Assay

9 Final Approval

I certify the results obtained from the execution of this Instrument Qualification Method are valid and demonstrate acceptable performance of the Fluidigm Research Use Only instruments for use with the Advanta Dx SARS-CoV-2 RT-PCR Assay.

_____ _____

FAS Name and Signature Date:

Customer Acceptance

The results from the execution of this protocol have been accepted as objective evidence that the system tested meets the intended performance requirements for the Advanta Dx SARS-CoV-2 RT-PCR Assay and the laboratory may use Fluidigm's Research Use Only instruments tested in this qualification to report patient results while using the Advanta Dx SARS-CoV-2 RT-PCR Assay.

Authorized Customer Name and Signature

Date:

Revision History

Rev	Author	CO Number	Change Description
01	Jason Erickson	CHG-003617	Initial release

Fluidigm Corporation
2 Tower Place Suite 2000
South San Francisco, CA 94080

For technical support visit techsupport.fluidigm.com.

North America +1 650 266 6100 | Toll-free (US/CAN): 866 358 4354 | techsupport@fluidigm.com Latin America +1 650 266 6100 | techsupportlatam@fluidigm.com
Europe/Middle East/Africa/Russia +33 1 60 92 42 40 | eu.support@fluidigm.com China (excluding Hong Kong) +86 21 3255 8368 | techsupportchina@fluidigm.com
Japan +81 3 3662 2150 | techsupportjapan@fluidigm.com All other Asian countries/India/Australia +1 650 266 6100 | techsupportasia@fluidigm.com

For *In Vitro* Diagnostic Use. For Emergency Use Authorization Only.

The Advanta™ Dx SARS-CoV-2 RT-PCR Assay is:

1. IVD, Rx Only, For use under the Emergency Use Authorization (EUA) only
2. Please contact techsupport@fluidigm.com if you require a printed copy free of charge
3. Instructions/webpage links to the full labeling documents
4. Printed matter statements from Letter of Authorization:
   a. This test has not been FDA cleared or approved, but has been authorized for emergency use by FDA under an EUA for use by authorized laboratories;
   b. This test has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens; and
   c. The emergency use of this test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1)of the Federal Food, Drug and Cosmetic Act, 21 U.S.C. § 360bbb-3 (b)(1), unless the declaration is terminated or authorization is revoked sooner.

BRANDING Manufacturer Symbol Address FLDM-XXXXX Rev 01