

Advanta Dx COVID-19 EASE 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**

Contents

| | | | |
|---|----|--|----|
| Intended Use | 2 | Advanta Dx COVID-19 EASE Assay Controls: Positive, Negative, and Others | 34 |
| Product Description | 2 | Examination and Interpretation of Patient Sample Results | 35 |
| Workflow and Description of Test Steps | 3 | Conditions of Authorizations for Labs | 37 |
| Materials Provided | 4 | Performance Evaluation | 38 |
| Reagents and Consumables | 4 | Limit of Detection (LoD): Analytical Sensitivity | 38 |
| Kit Components and Storage Conditions for Advanta Dx COVID-19 EASE Assay—10 IFCs (102-1477) | 4 | Inclusivity (analytical reactivity) | 39 |
| Kit Components and Storage Conditions for Advanta Dx COVID-19 EASE Assay—2 IFCs (102-1839) | 6 | Cross-reactivity (analytical specificity) | 40 |
| Materials Required but Not Provided | 7 | Endogenous/Exogenous Interference Substances Studies | 41 |
| Warnings, Precautions, and Best Practices | 9 | Sample Stability | 41 |
| Warnings and Precautions | 9 | Clinical Evaluation for Patients Suspected of COVID-19 | 41 |
| Best Practices | 11 | Appendix A: Biomark HD Thermal Cycler Protocol | 42 |
| Limitations of the Procedure | 12 | Appendix B: Related Documents | 42 |
| Sample Collection, Handling, and Storage | 14 | Appendix C: Symbols | 42 |
| Quality Controls | 14 | Revision History | 44 |
| Prepare the Negative and Positive Controls | 16 | | |
| Prepare the Extraction Plate and Extract RNA | 16 | | |
| Prepare the Binding Buffer/RNasequre Mix | 18 | | |
| Prepare the Sample Mixes | 19 | | |
| Prepare the Reverse Transcription- Preamplification Mix | 20 | | |
| Prepare the PCR Mixes | 21 | | |
| Prepare the Advanta Dx 96.16 IFC | 22 | | |
| Load and Run the 96.16 IFC | 24 | | |
| Thermal-Cycle and Collect Real-Time PCR Data | 28 | | |
| Annotate the Real-Time PCR Data | 29 | | |
| Check the Software Options | 29 | | |
| Annotate the Data | 31 | | |
| Assay Results and Interpretation | 34 | | |

Intended Use

The Advanta Dx COVID-19 EASE Assay is a real-time reverse transcription (RT) PCR test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in nasopharyngeal swab, oropharyngeal swab, mid-turbinate nasal swab, and anterior nasal swab specimens from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to laboratories that are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, and meet requirements to perform high-complexity tests.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in upper respiratory specimens 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 patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

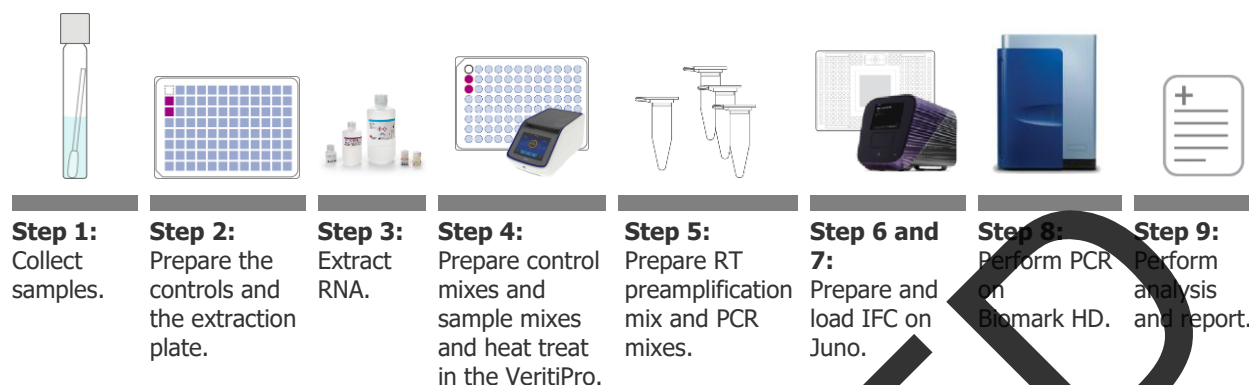
The Advanta Dx COVID-19 EASE Assay is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures.

The Advanta Dx COVID-19 EASE Assay is only for use under the Food and Drug Administration's Emergency Use Authorization.

Product Description

The Advanta Dx COVID-19 EASE Assay is a reverse transcription (RT) and real-time polymerase chain reaction (RT-PCR) test that leverages Standard BioTools™ microfluidics technology. The SARS-CoV-2 primer and probe set(s) is designed to detect RNA from the SARS-CoV-2 in upper respiratory swab samples from patients suspected of COVID-19 by their health care provider. Advanta Dx COVID-19 EASE Assay uses the N1, N2 and RNase P primers and probes, for both the 1-Step RT-preamplification step and the real-time PCR detection step.

Workflow and Description of Test Steps



| Workflow Step | | Hands-On Time | Run Time |
|--------------------------------|---|--------------------|--------------------|
| 1 | Collect samples using CDC-recommended swabs for upper respiratory samples and place into a Saline 0.85% Solution Tube (Thermo Fisher Scientific, R064438). | — | — |
| 2 | Prepare the negative and positive controls (NC, PC) and the extraction plate. | 10 min | — |
| 3 | Extract RNA using the RNeasy Viral Reagent Kit (Qiagen, 74104) | 120 min | — |
| 4 | Prepare the control mixes (NTC, NC, PC) and the sample mixes. Heat-treat controls and samples in the VeritiPro cycler. | 30 min | — |
| 5 | Prepare the RT preamplification mix and PCR mixes. | 15 min | — |
| 6 | Prepare the Advanta Dx 96.16 IFC (integrated fluidic circuit) by injecting control line fluid into the accumulators. Pipet reagents, PCR mixes, and sample mixes into the designated IFC inlets and reservoirs. | 35 min | — |
| 7 | Load the IFC on Juno™. | — | 225 min |
| 8 | Thermal-cycle and collect data on Biomark™ HD. | — | 35 min |
| 9 | Analyze data. Annotate data using the Real-Time PCR Analysis software, then export results and interpret using the Advanta Dx COVID-19 EASE Assay Interpretive Software. | — | — |
| Total time: 7 hr 50 min | | 3 hr 30 min | 4 hr 20 min |

Materials Provided

Reagents and Consumables

10 IFCs

| Bundle | Component | Part Number | Quantity |
|--|--|-------------|--|
| Advanta™ Dx COVID-19 EASE Assay—10 IFCs (102-1477) | Advanta Dx COVID-19 EASE, Module 1—10 IFCs | 102-1473 | 1 kit |
| | Advanta Dx COVID-19 EASE, Module 2—10 IFCs | 102-1474 | 1 kit |
| | Advanta Dx 96.16 IFC, 10-Pack | 102-1476 | 10 IFCs |
| | Advanta Dx Control Line Fluid, 10-Pack | 102-0390 | 20 syringes (2 packs, 10 syringes per pack) |

2 IFCs

| Bundle | Component | Part Number | Quantity |
|---|---|-------------|--|
| Advanta™ Dx COVID-19 EASE Assay—2 IFCs (102-1839) | Advanta Dx COVID-19 EASE, Module 1—2 IFCs | 102-1840 | 1 kit |
| | Advanta Dx COVID-19 EASE, Module 2—2 IFCs | 102-1841 | 1 kit |
| | Advanta Dx 96.16 IFC, 2-pack | 102-1472 | 2 IFCs |
| | Advanta Dx Control Line Fluid, 2-pack | 102-0398 | 4 syringes (2 packs, 2 syringes per pack) |

Kit Components and Storage Conditions for Advanta Dx COVID-19 EASE Assay—10 IFCs (102-1477)

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

102-1473, Advanta Dx COVID 19 EASE Assay, Module 1—10 IFCs

| Part Number | Component | Volume | Quantity | Storage |
|-------------|---------------------------|--------|-----------|------------------|
| 102-1457 | Advanta™ Dx FLR | 70 µL | 2 tubes | -25 °C to -15 °C |
| 102-1458 | Advanta Dx PCR MM | 290 µL | 2 tubes | -25 °C to -15 °C |
| 102-0369 | Advanta Dx PCR Water | 1.8 mL | 4 tubes | -25 °C to -15 °C |
| 102-1460 | Advanta Dx RT PA MM | 240 µL | 2 tubes | -25 °C to -15 °C |
| 102-1462 | Advanta Dx PF 2 | 6.5 mL | 1 bottle | -25 °C to -15 °C |
| 102-1463 | Advanta Dx Binding Buffer | 9.9 mL | 1 bottle | -25 °C to -15 °C |
| 102-1464 | Advanta Dx Wash Buffer | 2.8 mL | 2 bottles | -25 °C to -15 °C |

| | | | | |
|----------|-----------------------------------|--------|-----------|------------------|
| 102-1465 | Advanta Dx EB1 | 2.8 mL | 2 bottles | -25 °C to -15 °C |
| 102-1466 | 2019 nCoV_N1 Primer-Probe Mix | 25 µL | 1 tube | -25 °C to -15 °C |
| 102-1467 | 2019 nCoV_N2 Primer-Probe Mix | 25 µL | 1 tube | -25 °C to -15 °C |
| 102-1468 | RNase P Primer-Probe Mix | 50 µL | 1 tube | -25 °C to -15 °C |
| 102-1469 | Advanta™ Dx Preamp Primer Pool v2 | 520 µL | 2 tubes | -25 °C to -15 °C |

102-1474, Advanta Dx COVID 19 EASE Assay, Module 2—10 IFCs

| Part Number | Component | Volume | Quantity | Storage |
|-------------|---------------------------|--------|----------|----------------|
| 102-1470 | Advanta™ Dx Capture Beads | 500 µL | 2 tubes | +2 °C to +8 °C |
| 102-1830 | SARS-CoV-2 Neg Control | 500 µL | 1 tube | +2 °C to +8 °C |
| 102-1831 | SARS-CoV-2 Pos Control | 500 µL | 1 tube | +2 °C to +8 °C |

102-1476, Advanta Dx 96.16 IFC, 10-IFC pack

| Part Number | Component | Quantity | Storage |
|-------------|-----------------------|----------|------------------|
| 102-1472 | Advanta™ Dx 96.16 IFC | 10 IFCs | +15 °C to +30 °C |

Advanta Dx Control Line Fluid

| Part Number | Component | Quantity | Storage |
|-------------|--------------------------------|-----------------------------------|------------------|
| 102-0390 | Advanta™ Dx Control Line Fluid | 2 packs (10 syringes per pack) | +15 °C to +30 °C |

Kit Components and Storage Conditions for Advanta Dx COVID-19 EASE Assay—2 IFCs (102-1839)

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

102-1840, Advanta Dx COVID 19 EASE Assay, Module 1—2 IFCs

| Part Number | Component | Volume | Quantity | Storage |
|-------------|----------------------------------|--------|----------|------------------|
| 102-1457 | Advanta™ Dx FLR | 70 µL | 1 tube | -25 °C to -15 °C |
| 102-1458 | Advanta Dx PCR MM | 290 µL | 1 tube | -25 °C to -15 °C |
| 102-0369 | Advanta Dx PCR Water | 1.8 mL | 1 tube | -25 °C to -15 °C |
| 102-1460 | Advanta Dx RT PA MM | 240 µL | 1 tube | -25 °C to -15 °C |
| 102-1462 | Advanta Dx PF 2 | 6.5 mL | 1 bottle | -25 °C to -15 °C |
| 102-1463 | Advanta Dx Binding Buffer | 9.9 mL | 1 bottle | -25 °C to -15 °C |
| 102-1464 | Advanta Dx Wash Buffer | 2.8 mL | 1 bottle | -25 °C to -15 °C |
| 102-1465 | Advanta Dx EB1 | 2.8 mL | 1 bottle | -25 °C to -15 °C |
| 102-1466 | 2019 nCoV_N1 Primer-Probe Mix | 25 µL | 1 tube | -25 °C to -15 °C |
| 102-1467 | 2019 nCoV_N2 Primer-Probe Mix | 25 µL | 1 tube | -25 °C to -15 °C |
| 102-1468 | RNase P Primer-Probe Mix | 50 µL | 1 tube | -25 °C to -15 °C |
| 102-1469 | Advanta Dx Preamp Primer Pool v2 | 520 µL | 1 tube | -25 °C to -15 °C |

102-1841, Advanta Dx COVID 19 EASE Assay, Module 2—2 IFCs

| Part Number | Component | Volume | Quantity | Storage |
|-------------|---------------------------|--------|----------|----------------|
| 102-1470 | Advanta™ Dx Capture Beads | 500 µL | 1 tube | +2 °C to +8 °C |
| 102-1830 | SARS-CoV-2 Neg Control | 500 µL | 1 tube | +2 °C to +8 °C |
| 102-1831 | SARS-CoV-2 Pos Control | 500 µL | 1 tube | +2 °C to +8 °C |

102-1472, Advanta Dx 96.16 IFC, 2-IFC pack

| Part Number | Component | Quantity | Storage |
|-------------|-----------------------|----------|------------------|
| 102-1472 | Advanta™ Dx 96.16 IFC | 2 IFCs | +15 °C to +30 °C |

Advanta Dx Control Line Fluid

| Part Number | Component | Quantity | Storage |
|-------------|-------------------------------|----------------------------------|------------------|
| 102-0398 | Advanta Dx Control Line Fluid | 2 packs (2 syringes per pack) | +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. Do not use materials after the indicated expiry date.

| Product Name | Source | Part Number |
|---------------------------------------|---------------------------|-------------|
| RNAAdvance Viral Reagent Kit | Beckman Coulter | C63510 |
| RNAsecure™ RNase Inactivation Reagent | Thermo Fisher Scientific™ | AM7005 |

Consumables Not Provided

| Product Name | Source | Part Number |
|---|-------------------------------|-------------|
| Thermo Scientific™ Saline 0.85% Solution Tubes | Thermo Fisher Scientific | R064438 |
| MicroAmp® Clear Adhesive Film for 96-well plates | Thermo Fisher Scientific | 4306311 |
| CDC-recommended swab for upper respiratory sample collection | | — |
| Disposable microcentrifuge tubes, polypropylene, 1.5 mL, 2 mL, and 5 mL | | — |
| 25 mL reagent reservoir | Any major laboratory supplier | — |
| 96-well PCR plates* | | — |
| 8-well PCR tube strips with caps | | — |

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

Equipment Not Provided

IMPORTANT: The Advanta Dx COVID-19 EASE Assay Instrument Qualification Method (IQM) Protocol (SVC-00116) must be performed by a Standard BioTools Field Application Specialist using Standard BioTools-qualified COVID-19 EASE material and meet specifications prior to reporting diagnostic results.

| Product Name | Source | Part Number |
|--|-------------------------------|-------------|
| Biomark™ HD system | Standard BioTools | BMKHD-BMKHD |
| Biomark Data Collection software v4.8.1 | | 102-1890 |
| Juno™ system | | 101-6455 |
| Juno system software v3.15.1 | | 102-1889 |
| TX Interface Plate | | 101-6117 |
| Real-Time PCR Analysis software v4.8.1 | | 102-1852 |
| Advanta Dx COVID-19 EASE Interpretive Software v1.0.0 | | 102-2034 |
| 96.16 Sample Plate Map v1 | | SW-00056 |
| 96-well thermal cycler: Applied Biosystems® VeritiPro™ Thermal Cycler, 96 well | Thermo Fisher Scientific | A48141 |
| 2 centrifuges: 1 for microtubes, 1 for 96-well PCR plates NOTE Must be capable of $3,000 \times g$ | 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 respiratory tract samples | | — |
| PCR workstation (for reagent handling) | | — |
| DNA-free hood | | — |
| Freezers and refrigerators | | — |
| Cold block or ice | | — |

Materials Required but Not Provided

| | | |
|---------------------------------------|--|---|
| Tube and plate racks (multiple sizes) | | — |
|---------------------------------------|--|---|

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Warnings, Precautions, and Best Practices

Warnings and Precautions

- The Advanta COVID-19 EASE 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 product 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 authorized laboratories; 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 product 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 product 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.
- The performance of this test was established based on the evaluation of a limited number of clinical samples. Clinical performance has not been established with 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.
- Testing of respiratory tract samples 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.
- 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 the kit components and storage sections on [page 4](#) and [page 6](#) and in [Best Practices on page 11](#).
- Do not use the kit after the indicated expiry date.
- Do not use extraction kits other than those identified in the Reagents not Provided section of this IFU.

- The Advanta Dx COVID-19 EASE 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 sample 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, Standard BioTools recommends the following general safety guidelines in all laboratory and manufacturing areas:
 - Follow necessary precautions when handling samples. 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 COVID-19 EASE Assay is for use only with Biomark HD in conjunction with Juno. For complete instrument safety information, including a full list of the symbols on the instrument, refer to the Juno System User Guide (100-7070) 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](https://www.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 (SDSs) before handling chemicals. To obtain SDSs for chemicals ordered from Standard BioTools, 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.
- Avoid getting control line fluid on the exterior of the IFC or in the inlets because this makes the IFC unusable. If this occurs, use a new IFC.
- During use, take care to avoid the introduction of particulates, reagents, and fluids to the surface of the IFC.

Sample Handling

- To prevent cross-contamination when preparing controls, prepare the negative control mix first.
- 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 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.
- Ensure that the tips fit snugly on the pipettes to avoid volume transfer errors.
- 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.
- Reagent kit modules (Modules 1 and 2) are lot-matched. Do not swap reagents or kit modules between kit lots. Using mixed lots of reagent modules invalidates the results generated.
- Unless otherwise specified, thaw reagents at room temperature (+15 °C to +30 °C), and then use them at room temperature.

- Before use, briefly vortex reagents at medium speed for at least 5 sec, then centrifuge for at least 2 sec to ensure that all reagents are homogeneous.
- During workflow, mix and centrifuge reagents as directed.
- Place all sample and reagent mixes on ice when not in use.

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 bring down all mixes to the bottoms of the wells before pipetting into the IFC inlets. Failure to do so may result in compromised data.
- 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 COVID-19 EASE Assay performance was only established using upper respiratory tract samples from patients suspected of COVID-19 by their health care providers and collected in 0.85% saline. Performance of this assay in persons without signs and symptoms of respiratory infections has not been established.
- Testing of nasopharyngeal swab, oropharyngeal swab, mid-turbinate nasal swab, and anterior nasal swab samples is limited to patients suspected of COVID-19 infection.
- Performance of this test was not evaluated in an asymptomatic patient population from individuals suspected of COVID-19 by their health care providers.
- The Advanta Dx COVID-19 EASE Assay performance was only established using upper respiratory tract samples collected in 0.85% saline. Performance of this assay in other viral transport media has not been established.
- Samples must be collected, transported, and stored using appropriate procedures and conditions. Improper collection, transport, or storage of samples may affect the ability of the assay to perform as indicated.
- 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 COVID-19 EASE Assay cannot rule out respiratory diseases caused by other bacterial or viral pathogens.
- False positive results may arise from:
 - Cross-contamination during sample handling or preparation
 - Cross-contamination between patient samples
 - Sample mix-up
- False negative results may arise from:
 - Improper sample 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 Biomark HD and analyzed by the Real-Time PCR Analysis software do not include the preamplification 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.

Sample Collection, Handling, and Storage

The sample collection device is not included as part of the kit. Refer to the CDC Interim Guidelines for Collecting, Handling, and Testing Clinical Samples for COVID-19 ([cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html](https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html)) while using CDC-recommended swabs for upper respiratory sample collection and the Thermo Scientific™ Saline 0.85% Solution Tubes (see [Consumables not Provided on page 7](#) for more information).

Samples have been demonstrated to be stable at temperatures up to +30 °C for up to 48 hr after collection and prior to RNA extraction. Samples may be subjected to one freeze-thaw cycle at -70°C or lower. If the interval between collection and testing is anticipated to exceed 48 hr, the sample should be stored at -70 °C or lower as recommended by the CDC (<https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html>).

Quality Controls

There are 3 external controls provided with the test kit:

- A no template control (NTC)
- A positive control (PC)
- A negative control (NC)

Each of the 3 controls must be run on the 96-well sample processing plate along with 93 samples. Each control is treated in the same manner as a sample [i.e. diluted in PCR Water (part number 102-0369)].

Controls that will be provided with the test kit include:

- A “no template” negative control (NTC) is needed to test for contamination of reagents and instrumentation with amplifiable material and consists of nuclease-free water in place of the sample. This control is a full process control included in the extraction step. If nonspecific amplification of any of the assays comes up in this control, it is recommended that DNA decontamination of equipment, especially pipettes, occur according to standard protocols and methods (for example, DNA AWAY, Thermo Fisher Scientific, 7010).
- The external positive template control is a full process control needed to detect any failures in extraction, sample processing, reverse transcription, or amplification. The external positive template control is comprised of chemically inactivated SARS-CoV-2 virus suspended in buffer and is used in each assay run at a concentration of 5x the limit of detection following dilution.
- The external negative control consists of chemically inactivated human A549 cells suspended in buffer, from which human RNase P can be detected. The external negative control is used in each assay run.
- An internal control consisting of RNase P which is detected in the patient sample, using the RNase P primer and probe assay, is needed. This control is used to monitor adequate amounts of RNA in the patient samples and its RNA quality. It also monitors adequate RNA release from host cells present in the respiratory tract samples during the extraction 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 presence in the sample of PCR inhibition which may reduce amplification efficiency. This control is used to monitor the

presence or absence of RNase P in each of the 3 external controls as well as each patient sample on the 96-well plate.

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Prepare the Negative and Positive Controls

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

- 1 Briefly vortex and briefly centrifuge the SARS-CoV-2 Neg Control (102-1830) and SARS-CoV-2 Pos Control (102-1831) before use.
- 2 Prepare the negative control (NC):
 - a Add 198 μL of PCR Water (102-0369) to a new, 0.5 mL tube labeled NC.
 - b Add 22 μL of SARS-CoV-2 Neg Control to the NC tube.
 - c Vortex for 5 sec and briefly centrifuge to bring down contents. Store the NC tube on ice or in a cold block until ready to use.
- 3 Prepare the positive control (PC):
 - a Add 190.0 μL of PCR Water (102-0369) to a new 0.5 mL tube labeled PC.
 - b Add 22 μL of the SARS CoV-2 Neg Control to the PC tube.
 - c Add 2.8 μL of the SARS CoV-2 Pos Control to the PC tube.
 - d Vortex for 5 sec and briefly centrifuge to bring down contents. Store the PC tube on ice or in a cold block until ready to use.

Prepare the Extraction Plate and Extract RNA



WARNING POTENTIAL BIOHAZARD. Use universal precautions when handling biological samples. Prior to extraction, the controls and samples should be handled in a BSL-2 environment.

- 1 Use the RNAdvance Viral Reagent Kit (Beckman Coulter, C63510) to extract RNA from each sample and control according to the manufacturer's recommendations. Aliquot the samples and controls (NC, PC) and pipet PCR Water (NTC, 102-0369) as shown in [Figure 1 on page 17](#).

Follow the 96-well plate protocol, **except elute the samples in 20 μL of nuclease-free water.**

IMPORTANT: Eluting in **20 μL nuclease-free water** is required to ensure the appropriate concentration of input sample for this test. Use 20 μL of nuclease-free water to elute the RNA instead of the 40 μL recommended by the RNAdvance Viral Reagent Kit Instructions for Use.

NOTE

- When pipetting samples from the collection tubes to the processing plate for RNA extraction, avoid debris that have collected at the bottom of the tube.
- Overages are not included in the RNAdvance Viral Reagent Kit Instructions for Use.

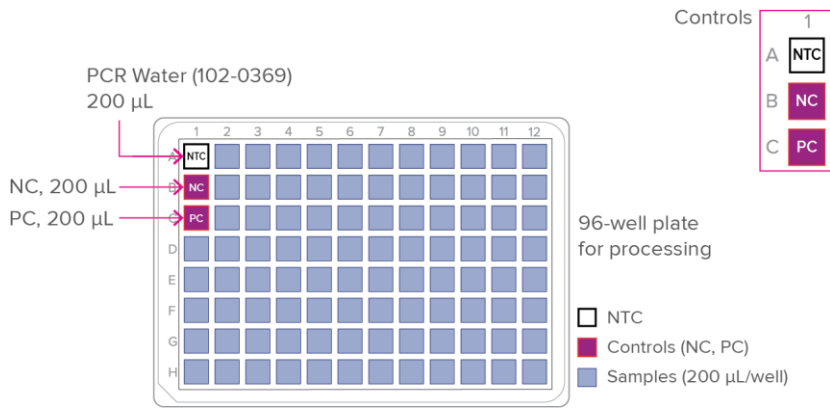


Figure 1. Control mixes and samples in the processing plate

- 2 After transferring the eluted controls and samples to a new 96-well plate, tightly seal the plate using MicroAmp Clear Adhesive Film for 96-well plates (Thermo Fisher Scientific, 4306311) and store extracted RNA at 4 °C or on ice or in a cold block if it is to be used within 4 hr. Long-term storage is not recommended.

REVOKED

Prepare the Binding Buffer/RNasecure Mix

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

- 1 Prepare the binding buffer/RNasecure mix in a new 2 mL tube, as shown in Table 1.

IMPORTANT: Advanta Dx Binding Buffer is viscous. Pipet slowly to ensure that the required volume is transferred.

Table 1. Binding buffer/RNasecure mix

| Component | Vol per IFC (μL) |
|---|-------------------------------|
| Advanta Dx Binding Buffer (102-1463) | 990 |
| RNasecure (Thermo Fisher Scientific, AM7005) | 110 |
| Total | 1,100 |

- 2 Vortex the tube for 5 sec, then centrifuge to bring down contents to the bottom of the tube.
- 3 Distribute 120 μL of binding buffer/RNasecure mix into each well of an 8-strip tube.
- 4 Using an 8-channel pipette, distribute 9 μL of binding buffer/RNasecure mix to each well of a new, labeled 96-well plate and store on ice or in a cold block until ready to prepare the control and sample mixes (Figure 2).

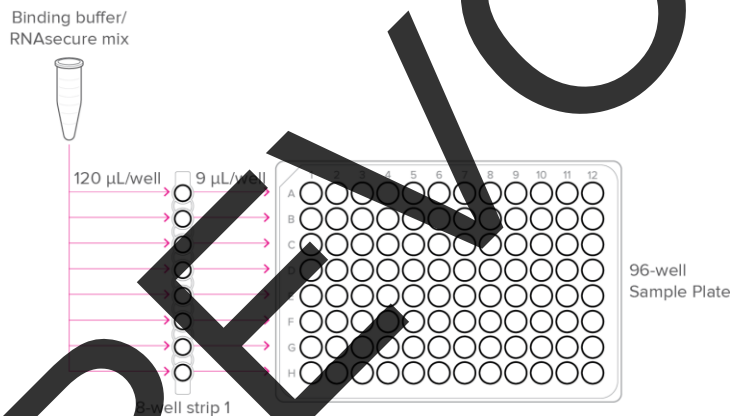


Figure 2. Binding buffer/RNasecure mix pipetting map

Prepare the Sample Mixes

- 1 Thaw the frozen reagents and mixes on ice or in a cold block.
- 2 Centrifuge all reagents and mixes before use.
- 3 Briefly centrifuge the plate containing the eluted controls and samples for 5 sec to bring down contents before removing the seal. Change gloves after removing the plate seal.
- 4 Using an 8-channel pipette, transfer 9 μ L of eluted controls and samples to each well of the sample plate containing the binding buffer/RNasecure mix (Figure 3).

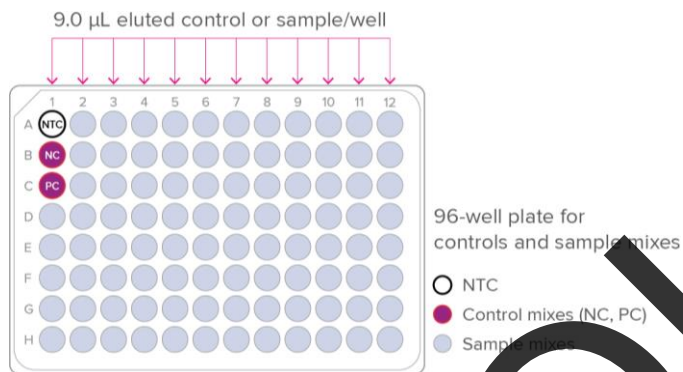


Figure 3. Controls and sample mixes pipetting map

IMPORTANT: For IFC inlets that will not contain a sample, replace the eluted sample with 9 μ L of PCR-grade water.

- 5 Tightly seal the plate using MicroAmp Clear Adhesive Film for 96-well plates (Thermo Fisher Scientific, 4306311), then gently vortex and briefly centrifuge it for 5 sec to bring down contents.
- 6 Heat-treat the samples in the Applied Biosystems VeritiPro Thermal Cycler (Thermo Fisher Scientific, A48141) using the program in Table 2. This removes any secondary structure from the RNA and activates the RNasecure.

IMPORTANT: Pre-heat the thermal cycler block to 75 $^{\circ}$ C before placing the samples in the thermal cycler.

Table 2. Heat treatment of extracted RNA samples

| Temperature | Time |
|------------------|-------|
| +75 $^{\circ}$ C | 5 min |
| +4 $^{\circ}$ C | 5 min |
| +4 $^{\circ}$ C | Hold |

- 7 Briefly centrifuge the sample plate for 20 sec to bring down all liquid to the bottom of the plate.
- 8 Place heat-treated samples on ice or in a cold block until ready to use.

Prepare the Reverse Transcription-Preamplification Mix

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

- 1 Thaw Advanta Dx RT PA MM (102-1460) and keep on ice or in a cold block. Briefly vortex and centrifuge the reagents before use.
- 2 In a DNA-free hood, combine the components shown in Table 3 in a new 0.5 mL tube to make the RT preamplification mix and place on ice or in a cold block.

Table 3. RT preamplification mix

| Component | Vol for 1 IFC (µL)* |
|---|---------------------|
| Advanta Dx RT PA MM (102-1460) | 48.0 |
| Advanta Dx Preamp Primer Pool v2 (102-1469) | 104.0 |
| Advanta Dx FLR (102-1457) | 8.0 |
| Total | 160.0 |

* Includes overage

- 3 Cap the tube, vortex, and centrifuge the RT preamplification mix.

Prepare the PCR Mixes

1 Prepare the PCR pre-mix:

- a Combine the components in Table 4 in a new, labeled 0.5 mL tube.

Table 4. PCR pre-mix

| Component | Vol for 1 IFC (μ L)* |
|---------------------------------|---------------------------|
| Advanta Dx PCR MM (102-1458) | 57.6 |
| Advanta Dx FLR (102-1457) | 5.8 |
| Advanta Dx PCR Water (102-0369) | 51.8 |
| Total | 115.2 |

* Includes overage

- b Cap the tube, vortex, and centrifuge the PCR pre-mix.

2 Prepare the PCR mixes as shown in Table 5:

- a **Prepare the N1 mix:** Combine 21.6 μ L of PCR pre-mix with 2.4 μ L of 2019 nCoV_N1 Primer-Probe Mix (102-1466) in a new, labeled 0.5 mL tube. Vortex thoroughly and store on ice or in a cold block.
- b **Prepare the N2 mix:** Combine 21.6 μ L of PCR pre-mix with 2.4 μ L of 2019 nCoV_N2 Primer-Probe Mix (102-1467) in a new, labeled 0.5 mL tube. Vortex thoroughly and store on ice or in a cold block.
- c **Prepare the RNase P mix:** Combine 43.2 μ L of PCR pre-mix with 4.8 μ L of RNase P Primer-Probe Mix (102-1468) in a new, labeled 0.5 mL tube. Vortex thoroughly and store on ice or in a cold block.

Table 5. PCR mixes

| Component | Vol for 1 IFC (μ L)* | | |
|--|---------------------------|-------------|-------------|
| | N1 Mix | N2 Mix | RNase P Mix |
| PCR premix (see Table 4) | 21.6 | 21.6 | 43.2 |
| 2019 nCoV_N1 Primer-Probe Mix (102-1466) | 2.4 | — | — |
| 2019 nCoV_N2 Primer-Probe Mix (102-1467) | — | 2.4 | — |
| RNase P Primer-Probe Mix (102-1468) | — | — | 4.8 |
| Total | 24.0 | 24.0 | 48.0 |

* Includes overage

Prepare the Advanta Dx 96.16 IFC

IMPORTANT: When injecting control line fluid:

- Follow the best practices for handling IFCs and control line fluid on [page 11](#) of these instructions.
- Only use Advanta Dx Control Line Fluid (102-0390). Each syringe contains 150 μL of control line fluid.

- 1 Remove the Advanta Dx Control Line Fluid syringes (102-0390) from the packaging and remove the Advanta Dx 96.16 IFC (102-1472) from the box and foil envelope.

IMPORTANT:

- Do not remove the waste sponge from around the IFC center (see [Figure 4](#)).
- Do not evacuate air from the syringe prior to injecting control line fluid (Step 4).

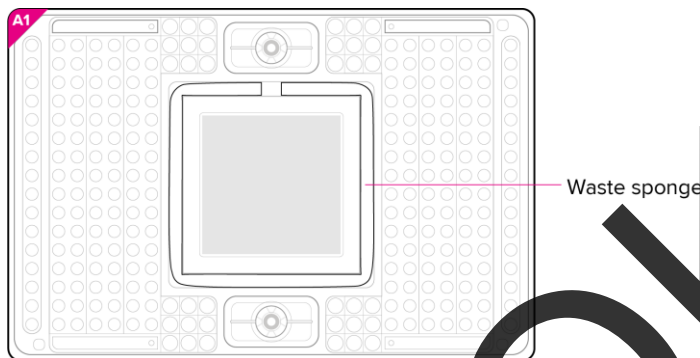


Figure 4. Waste sponge

- 2 Actuate the check valves:

- a Place the IFC on a flat surface.
- b Use a syringe with the shipping cap in place to actuate both check valves with gentle pressure. Ensure that the poppet can move freely up and down ([Figure 5](#)).

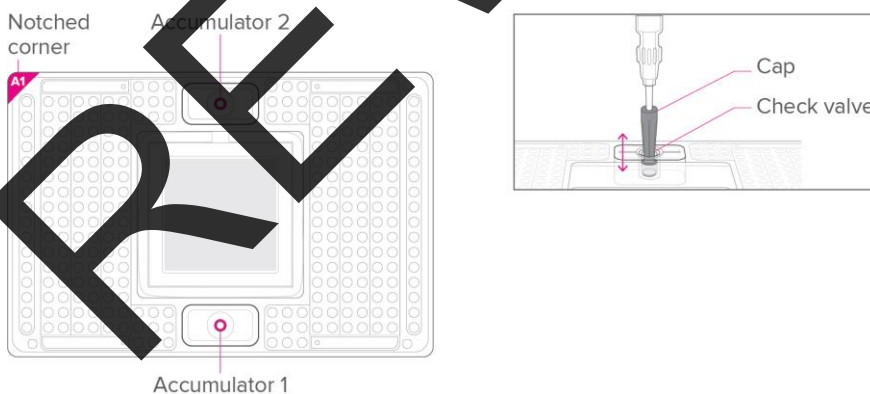


Figure 5. Actuating the check valve in the top accumulator on the 96.16 IFC

- 3 Hold a syringe firmly in 1 hand with tip facing up and away from the IFC, pull back on the plunger slightly to create negative pressure, and remove the shipping cap with the other hand.

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

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 6). Visually confirm that the O-ring has moved.
- 6 Release the control line fluid:
- 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.
 - 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.
 - After fully depressing the plunger, wait approximately 5 sec before withdrawing the syringe. Before removing the syringe from the accumulator, ensure that all of the control line fluid and air are purged from the syringe to avoid dripping fluid on the surface of the IFC. Control line fluid or air remaining in the pipette tip is normal.

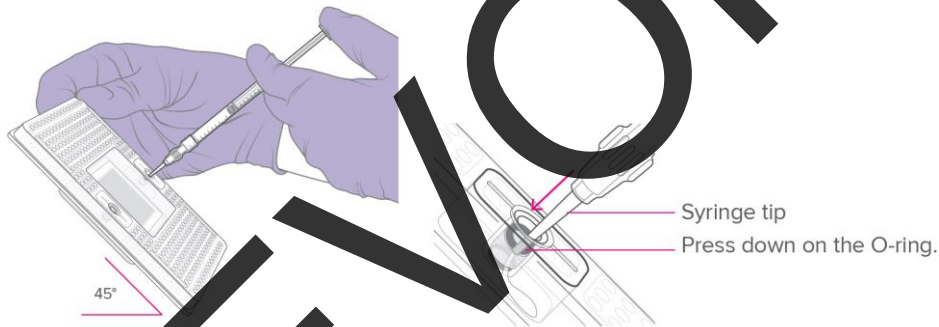


Figure 6. Injecting control line fluid into the accumulators on the 96.16 IFC

- 7 Check to ensure that the O-ring returns to its normal position after the syringe is removed.
- 8 Repeat Steps 3 through 7 for the other accumulator.
- 9 Pull the protective film down and away from the bottom of the IFC. Discard the film.

Load and Run the 96.16 IFC



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

IMPORTANT:

- Vortex all PCR and sample mixes thoroughly, then centrifuge them at $3,000 \times g$ for 1 min at room temperature to bring down contents before removing the plate seals and pipetting the mixes into the IFC inlets. Change gloves after removing the sample plate seal. Failure to do so may result in compromised data.
- While pipetting, do not go past the first stop on the pipette. Doing so may introduce air bubbles into inlets.
- Pipet reagents in the order shown to prevent reagents from wicking into the IFC.

- 1 Pipet 15 μL of Advanta Dx PF 2 (102-1462) in the 36 inlets (O) adjacent to each accumulator, as shown in Figure 7.

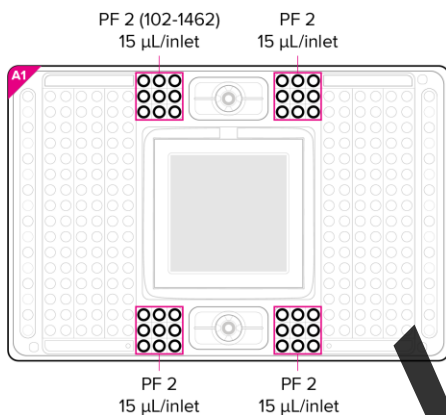


Figure 7. Pipetting map for PF 2

- 2 Pipet 5 μL of each PCR mix (N1, N2) as shown in Figure 8: N1 and N2 each into 4 inlets on the left side of the IFC and RNase P into 8 inlets on the right side of the IFC.

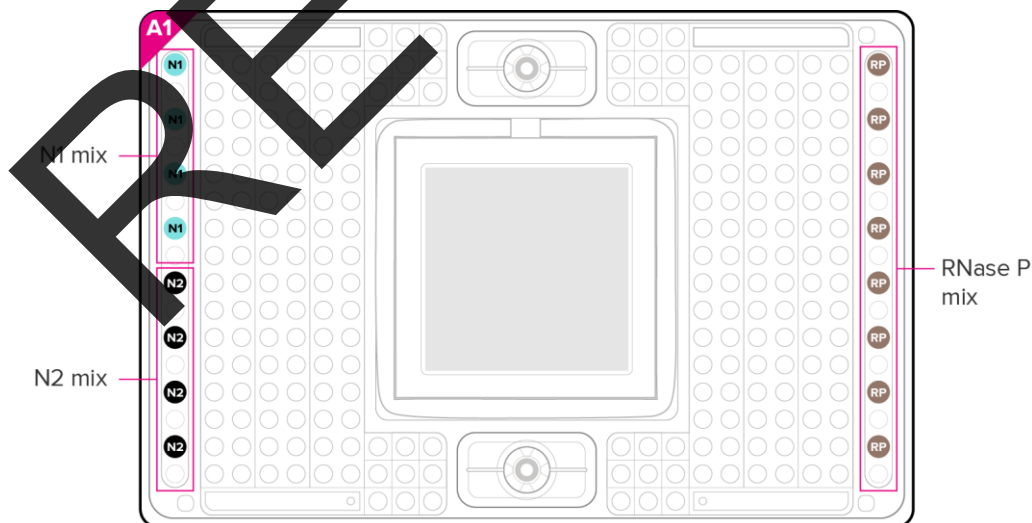


Figure 8. Pipetting map for PCR mixes

- 3 Pipet the buffers and RT preamplification mix into the IFC in the following order, as shown in [Figure 9](#).
 - a Distribute 70 μL of Advanta Dx Wash Buffer (102-1464) into each well of an 8-strip tube, then use an 8-channel pipette to transfer 15 μL into 32 inlets (●).
 - b Distribute 70 μL of Advanta Dx EB1 (102-1465) into each well of an 8-strip tube, then use an 8-channel pipette to transfer 15 μL into 32 inlets (●).
 - c Distribute 18 μL of RT preamplification mix from [Table 3](#) into each well of an 8-strip tube, then use an 8-channel pipette to transfer 4 μL into 32 inlets (●).

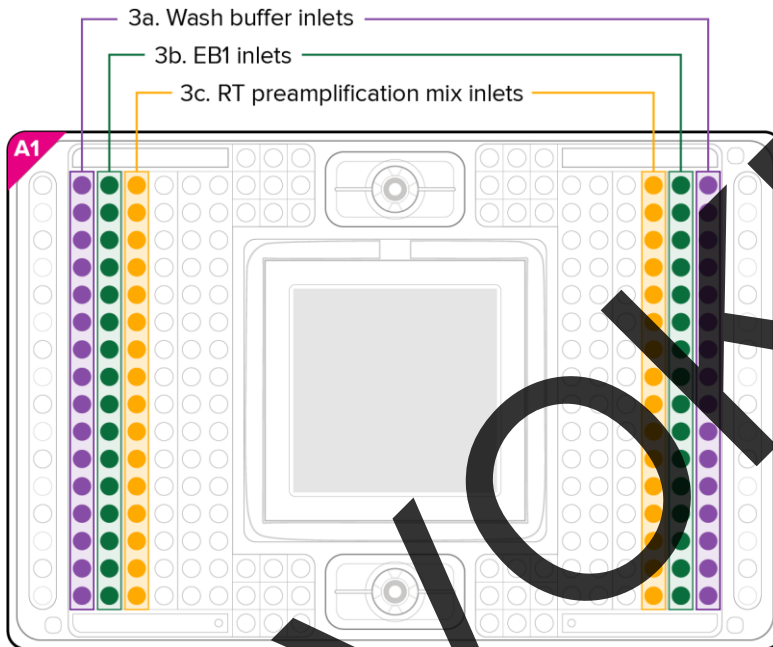


Figure 9. Pipetting buffers and RT preamplification mix for the 96.16 IFC

- 4 Retrieve the sample plate from the thermal cycler or ice or cold block and briefly centrifuge for 5 sec to bring down contents.
- 5 Remove the seal from the sample plate. Change gloves after removing the plate seal.
- 6 Use an 8-channel pipette to transfer the entire volume (16–18 μL) of each sample mix into the sample inlets, as shown in [Figure 10](#) and [Figure 11](#) on page 26.

NOTE There should not be more than 2 μL loss per well from the sample plate. Because of this sample loss, air bubbles in the inlets are expected and do not need to be removed.

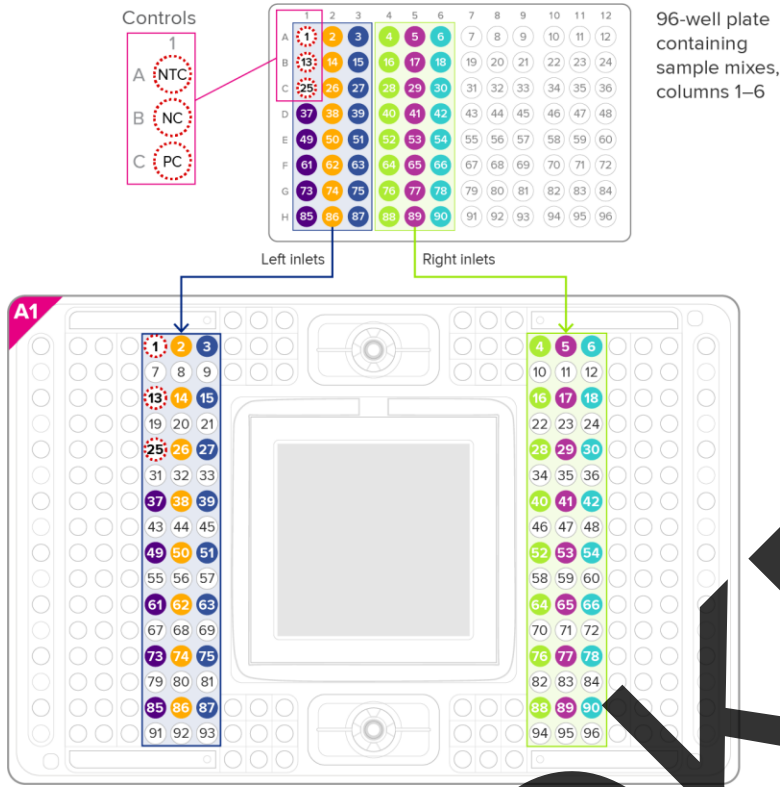


Figure 10. Sample mix pipetting map for sample plate columns 1-6

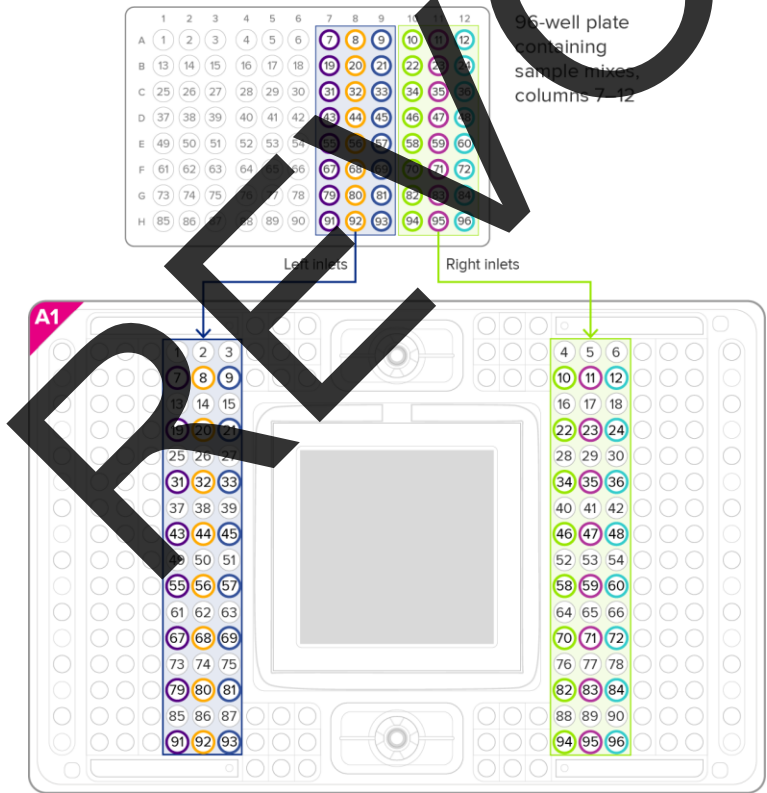



Figure 11. Sample mix pipetting map for sample plate columns 7-12

- 7 Vortex the Advanta Dx Capture Beads (102-1470) vigorously for 5 sec to mix immediately before use to ensure that the beads are fully suspended, then pipet 50 μ L into each of the top 2 reservoirs (R1 and R2, ) , as shown in Figure 12.

IMPORTANT: Start the Advanta Dx Covid-19 EASE script on Juno within 20 min of pipetting the beads.

NOTE Add the beads with the pipette tip held over, but not touching, the inlet hole.

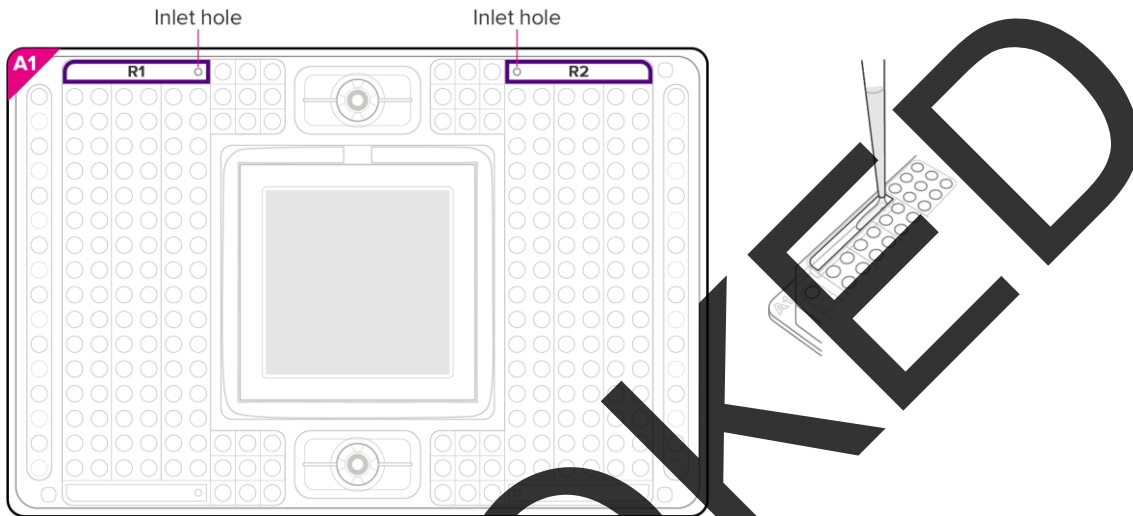
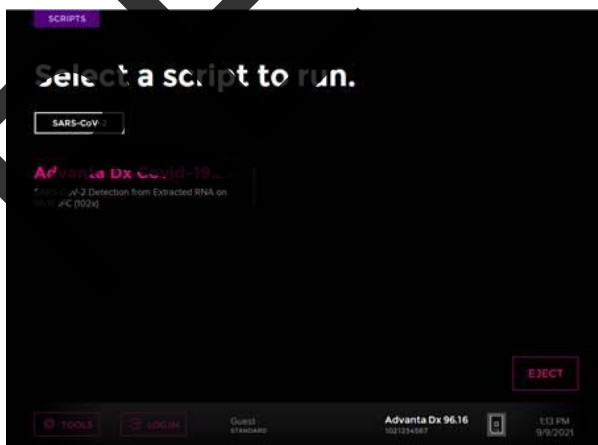


Figure 12. Pipetting beads into the 96.16 IFC reservoirs

- 8 Ensure that the TX Interface Plate is installed in the Juno instrument.
- 9 Place the IFC into 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**.
 - Tap **Advanta Dx Covid-19 EASE**, then tap **Run**. The run takes approximately 225 min to complete.



IMPORTANT: Start the IFC run on Biomark HD within 1 hr of completing the Advanta Dx Covid-19 EASE script.

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.
- 2 Use clear tape to remove any dust particles or debris from the surface of the IFC center, if necessary. Ensure that the waste sponge stays in place (Figure 13).

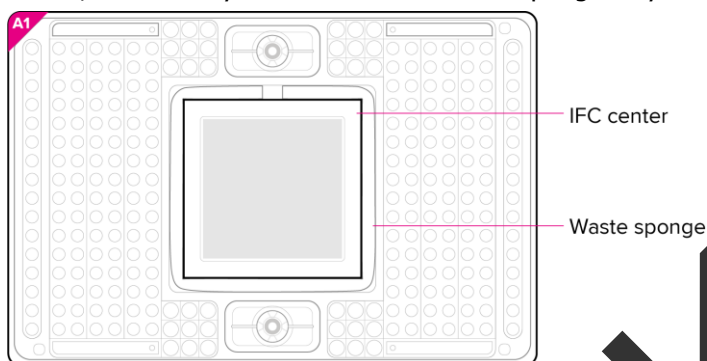
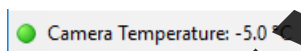


Figure 13. Waste sponge

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

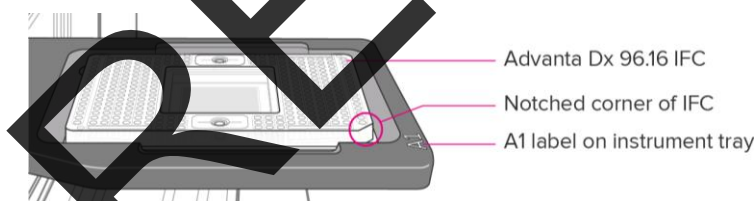


Figure 14. Loaded IFC on instrument tray

- 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: **Advanta Dx Covid-19 EASE.pcl**.

IMPORTANT: Make sure to select Advanta Dx Covid-19 EASE protocol. For a description of the thermal protocol, see [Appendix A](#).

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

13 Confirm that IFC run information is correct and click **Start Run**. The run will take approximately 35 min to complete.

14 After the run is complete, annotate 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 COVID-19 EASE Interpretive Software Quick Reference Guide (FLDM-00683). You can either set up the Real-Time PCR Analysis software to export data directly through the Advanta Dx SARS-CoV-2 Interpretive Software, or you can run the Advanta Dx COVID-19 EASE Interpretive Software from a command line.

Check the Software Options

Before you export the data using the Real-Time PCR Analysis software, check the software options to ensure that the Advanta Dx COVID-19 EASE Interpretive 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 ([Figure 15 on page 30](#)).
- 3 Confirm that the Post Export **Command** is:
C:\Program Files (x86)\Fluidigm\Advanta Dx COVID-19 EASE Interpretive Software\AdvantaDxSC2.exe
- 4 Confirm that the **Additional Arguments** field contains **-re**.

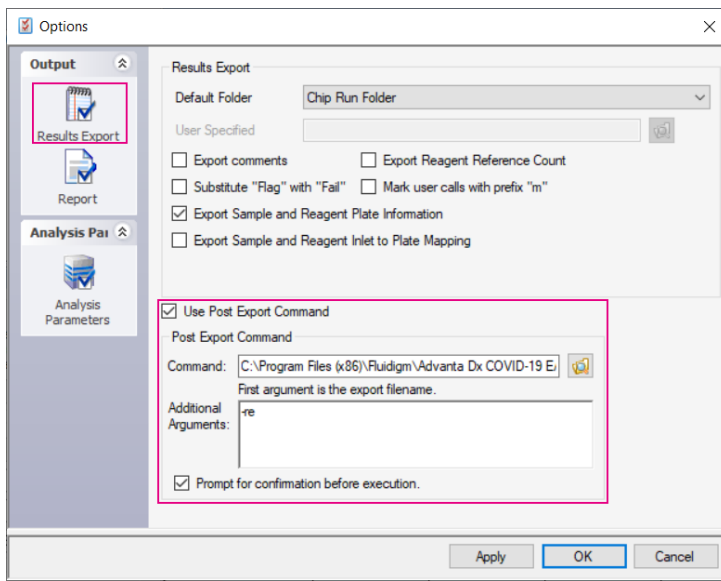


Figure 15. Results Export options

- 5 (Optional) Click **Analysis Parameters** and change the default settings (Figure 16):
 - a Select the **Customize Default Baseline Correction Method** checkbox and select **Linear**.
 - b Select the **Customize Default Ct Threshold Method** checkbox and select **Auto By Control (Global)**.

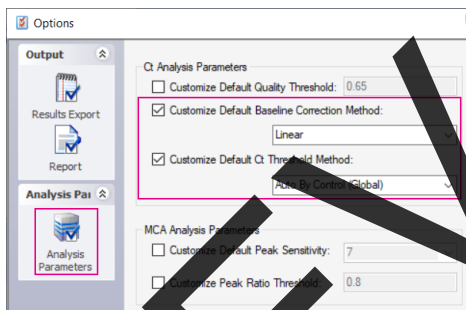




Figure 16. Analysis Parameters options

- 6 Click **OK** to save the changes.

Annotate the Data

To annotate the real-time PCR data:

- 1 Double-click the **Real-Time PCR Analysis** icon () on the desktop to launch the Real-Time PCR Analysis software.
- 2 Click  (**Open**), then browse to and select the **chiprun.bml** file to open it in the Real-Time PCR Analysis software.
- 3 Annotate the samples for the first analysis of a new IFC run (Figure 17):
 - a In the **Chip Explorer** pane, click **Sample Setup**.
 - b In the **Task** pane, click **New**.
 - c For **Container Type** select **Sample Inlets**, then click **OK**. The Plate Setting Source displays AD 96.16 (102x) : Sample Inlets.
 - d Annotate the samples in one of the following ways:
 - If the sample organization was recorded in the 96.16 Sample Plate Map (SW-00056), copy the entire 6 × 16 grid containing the sample names and paste it into the Sample Setup. Select **Sample Name** as the **Data** item.
 - Click **Import** to import the sample information from a plate file (.plt) or a comma separated values (CSV) file for the sample plate.
 - In the **Sample Setup** pane, click **Editor** to annotate the samples in each plate well-by-well.

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**.
- Controls must be named specifically **NTC**, **NC**, and **PC**, entered exactly as shown.
- Sample names cannot include commas.

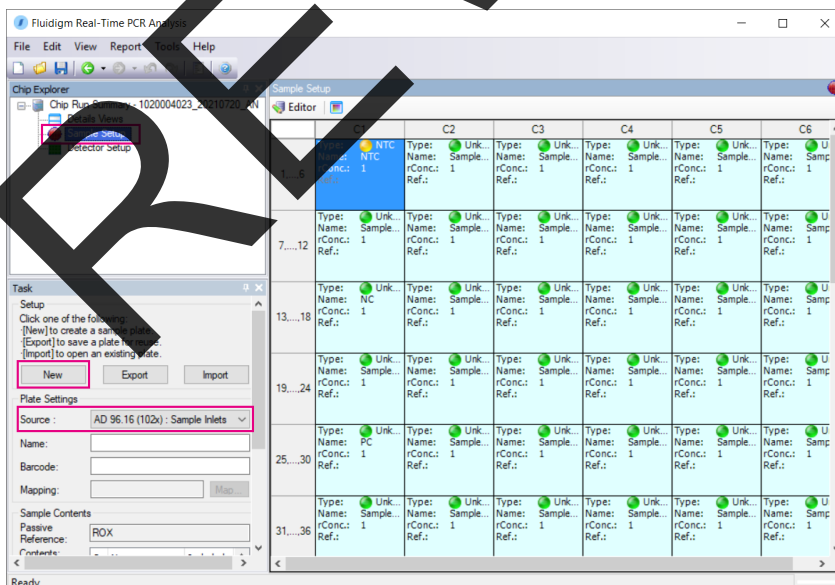


Figure 17. Sample Setup pane

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

IMPORTANT: The assay names **N1**, **N2**, and **RNase P** 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).

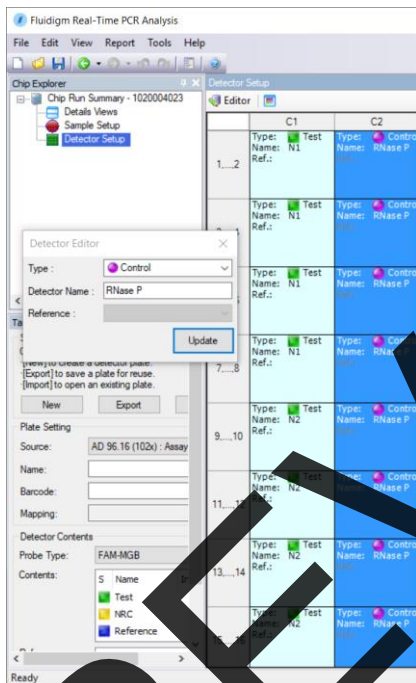


Figure 18. Detector Setup pane

- 5 Click **Details Views**.

- 6 Set the following Analysis Settings, then click **Analyze** to analyze the IFC run (Figure 19).

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

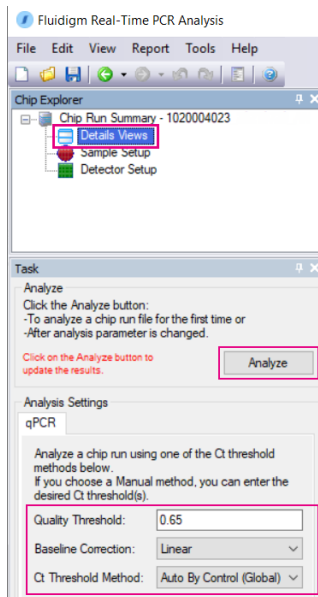


Figure 19. Details Views

- 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.

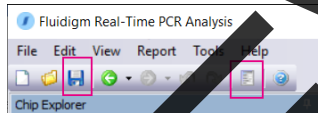
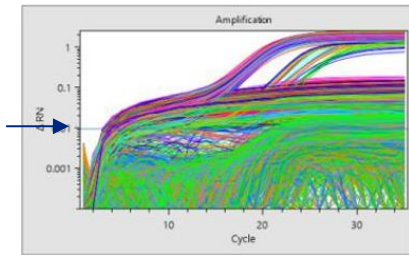


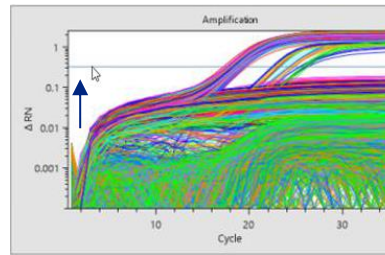
Figure 20. Save and Export buttons

- 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:
- In the Real-Time PCR Analysis software, open the IFC run.
 - In the **Details Views** pane, show the **Heat Map View**, then select all rows with assays (RNase P, N1, N2).
 - Click **Expand/Collapse** in the secondary toolbar to expand the Normalized Intensity and Amplification plots.
 - Click **Threshold** to show the threshold line in the Amplification plot, then click **Log Graph** to show more detail.
 - 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 (Figure 21).



Ct threshold in the baseline in the Amplification plot



Ct threshold adjusted manually

Figure 21. Adjusting the Ct threshold

- h** In the **Task** pane, click **Analyze** to analyze the data with the updated Ct Threshold Method.
- i** Export the interpretative report and review the expected interpretations for the controls, and confirm that most samples are not called inconclusive.

Assay Results and Interpretation

All test controls are evaluated by the Advanta Dx COVID-19 EASE Interpretive Software. If the controls meet their expected control results, all patient results indicate a Plate QC Result of "Pass" in the interpretive software report. If the controls fail to meet their expected control results, all patient results indicate a Plate QC Result of "Fail" in the interpretive software report.

Advanta Dx COVID-19 EASE Assay Controls: Positive, Negative, and Others

The 3 external quality controls referenced in [Quality Controls on page 14](#) will be included on each IFC generated (3 controls and 93 samples). See [Table 6](#) for expected control results.

Table 6. Expected Control Results

| Control Type | N1 Result | N2 Result | RNase P Result |
|---------------------------|-----------|-----------|----------------|
| Positive Control (PC) | + | + | + |
| Negative Control (NC) | - | - | + |
| No Template Control (NTC) | - | - | - |

Examination and Interpretation of Patient Sample Results

Interpretation of Advanta Dx COVID-19 EASE Assay patient results should be performed after the positive control, negative control, and no template control (NTC) results have been determined to be acceptable. If any control fails to meet expected results on a given IFC, then it invalidates all sample results (as indicated by a Plate QC Result of "Fail"). Such 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 NTC fails to meet expected control results, it is recommended that standard laboratory DNA decontamination procedures and/or additional training of test operators are performed. If the controls fail upon retest, a new kit is necessary before performing additional retests.

The assay targets N1, N2 and RNase P. There are 4 assay replicates per assay per sample for N1 and N2, and 8 assay replicates per sample for RNase P. The patient result interpretation consists of the following steps:

- 1** Interpretation of all 4 assay replicates for each sample based on a Ct of 32 as the cutoff and the "Ct Call". The "Ct Call" is a sample QC produced by the Biomark HD analysis software that indicates the quality of the amplification curve in comparison to a perfect sigmoid curve.
- 2** Interpretation for each assay target for each sample based on the 4 replicate results.
- 3** Interpretation of the sample based on the assay interpretation for the N1, N2 and RNase P targets (assays; see [Table 7 on page 36](#)).

Sample Interpretation

Table 7. Sample Interpretation from assay N1, N2 and RNase P results.

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

| N1 Result | N2 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 | Re-extract and Retest. If the result of the retest is No Template or Inconclusive, obtain a new sample. |
| – | ? | – | Inconclusive | |
| – | + | – | Inconclusive | |
| ? | – | – | Inconclusive | |
| ? | ? | – | Inconclusive | |
| ? | + | – | Inconclusive | |
| + | – | – | Inconclusive | |
| + | ? | – | Inconclusive | |
| – | – | ? | Inconclusive | |
| – | ? | ? | Inconclusive | |
| – | + | ? | Inconclusive | |
| ? | – | ? | Inconclusive | |
| ? | ? | ? | Inconclusive | |
| ? | + | ? | Inconclusive | |
| + | – | ? | Inconclusive | |
| + | ? | ? | Inconclusive | |
| – | ? | + | Inconclusive | |
| ? | – | + | Inconclusive | |
| ? | ? | + | Inconclusive | |

Conditions of Authorizations for Labs

Conditions of Authorization for the Advanta Dx COVID-19 EASE Assay¹ 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: <https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/in-vitro-diagnostics-euas>

However, to assist clinical laboratories using the Advanta Dx COVID-19 EASE Assay¹ the relevant Conditions of Authorization are listed below:

- A** Authorized laboratories using this product¹ 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 Standard BioTools Inc. (via email: techsupport@fluidigm.com) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of this product of which they become aware.
- F** All laboratory personnel using this product must be appropriately trained in RT-PCR techniques, the specific processes and instruments used in Advanta Dx COVID-19 EASE Assay¹ and use appropriate laboratory and personal protective equipment when handling this kit, and use this product in accordance with the authorized labeling.
- G** Standard BioTools Inc. 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 Advanta Dx COVID-19 EASE 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."

Performance Evaluation

Limit of Detection (LoD): Analytical Sensitivity

To determine the limit of detection (LoD) of the Advanta Dx COVID-19 EASE Assay, contrived samples at known concentrations of viral genome were prepared using inactivated SARS-CoV-2 virus [Zeptomatrix, Part Number NATSARS(COV2)-ERC] diluted in pooled negative nasopharyngeal clinical matrix. Contrived samples were subsequently spiked into negative nasopharyngeal clinical matrix in decreasing 2-fold serial dilutions to create samples at 2 copies/ μL , 1 copy/ μL , 0.5 copies/ μL , 0.25 copies/ μL , 0.125 copies/ μL , 0.063 copies/ μL , 0.031 copies/ μL , and 0.016 copies/ μL to determine the preliminary LoD. Each replicate was extracted using the Beckman Coulter RNAdvance Viral Reagent Kit and tested using the Advanta Dx COVID-19 EASE Assay. The results of the preliminary LoD range-finding study are provided in Table 8.

Table 8. Summary of preliminary detection in solution-based study results

| Copies/ μL | Number of Replicates Tested | Number of Positive Replicates | Mean Ct | | | Percentage Positive |
|-----------------------|-----------------------------|-------------------------------|-------------|-------------|-------------|---------------------|
| | | | N1 | N2 | RNase P | |
| 2 | 3 | 3 of 3 | 15.5 | 15.1 | 9.8 | 100% |
| 1 | 3 | 3 of 3 | 16.3 | 16.9 | 9.3 | 100% |
| 0.5 | 3 | 3 of 3 | 18.0 | 17.2 | 9.4 | 100% |
| 0.25 | 6 | 6 of 6 | 18.9 | 18.9 | 11.2 | 100% |
| 0.125 | 6 | 6 of 6 | 20.1 | 20.9 | 10.6 | 100% |
| 0.063 | 3 | 1 of 3 | ND* | 20.6 | 10.7 | 33% |
| 0.031 | 3 | 2 of 3 | ND | 23.7 | 10.5 | 67% |
| 0.016 | 3 | 1 of 3 | ND | 20.9 | 10.7 | 33% |

* ND = Not detected

The lowest concentration at which 6 of 6 replicates were positive was 0.125 copies/ μL and was considered the preliminary LoD.

Following the determination of preliminary LoD, verification of the preliminary LoD was performed. To verify the preliminary LoD, 20 replicates each were contrived in the manner described above at 3 concentrations (0.5 copies/ μL , 0.25 copies/ μL , and 0.125 copies/ μL). Each replicate was extracted using the Beckman Coulter RNAdvance Viral Reagent Kit and tested using the Advanta Dx COVID-19 EASE Assay. The results of the final LoD determination study are provided in Table 9.

Table 9. Summary of final detection in solution-based study results

| Copies/ μL | Number of Replicates Tested | Number of Positive Replicates | Mean Ct | | | Percentage Positive |
|-----------------------|-----------------------------|-------------------------------|-------------|-------------|-------------|---------------------|
| | | | N1 | N1 | N1 | |
| 0.5 | 20 | 20 of 20 | 18.6 | 18.6 | 18.6 | 100% |
| 0.25 | 20 | 20 of 20 | 18.9 | 18.9 | 18.9 | 100% |
| 0.125 | 20 | 19 of 20 | 20.1 | 20.1 | 20.1 | 95% |

The final LoD of the Advanta Dx COVID-19 EASE Assay was determined to be the lowest concentration resulting in positive detection of at least 19 out of 20 replicates, and is 0.125 copies/ μ L.

Inclusivity (analytical reactivity)

An *in silico* analysis was performed using the RADx ROSALIND DXM online tool (<https://radx.onramp.bio>) to compare the assay primers and probes to all published SARS-CoV-2 sequences in the GSAID database as of January 16th, 2022. The sequences used in the comparison are updated weekly from the GISAID Initiative by the ROSALIND tool. Table 10 and Table 11 list the results of the *in silico* analysis.

When sequences were analyzed for mismatches, there were less $\leq 1\%$ of sequences with mutations occurring within 5 bp of the 3' end for both, the N1 and the N2 primers. All variant sequences with mismatches occurred with frequencies $\leq 1\%$ with the exception of one variant mismatch affecting the N1 probe [ACTCCGCATTACGTTTGGTGGACC] which is currently present in the majority of SARS-CoV-2 sequences in the GSAID database. The mismatch is at the 3rd basis of the 5' end of the probe and is not expected to result in a failure to detect the N1 target. However, due to the dual target design of the Advanta Dx COVID-19 EASE assay and based on the in-silico analysis and current monitoring the performance of the Advanta Dx COVID-19 EASE assay is predicted to be unaffected by the currently circulating variants and continues to have reliable detection of current VOCs and VOIs defined by the CDC and WHO.

Standard BioTools continuously monitors emerging strains/sequence variants of SARS-CoV-2 and assessing sequence variations for impact on the test performance.

Table 10: Number of US sequences with mismatches to the test's N1 primers and probes from December 20, 2021 to January 19, 2022

| | N1 Forward (Fwd) Primer | N1 Reverse (Rev) Primer | N1 Probe | 30-Day Frequency |
|--------------------------|-------------------------|-------------------------|----------|--|
| Total primer length (nt) | 20 | 24 | 24 | — |
| Total strains | 12846 | 12846 | 12846 | — |
| 100% match | 12548 | 12774 | 11906 | — |
| 1 mismatch | 298 | 72 | 935 | N1-Fwd: 2.3% N1-Rev: 0.6% N1-Probe: 7.3% |
| 2 mismatches | 0 | 0 | 5 | N1-Fwd: 0% N1-Rev: 0% N1-Probe: 0.04% |
| 3 mismatches | 0 | 0 | 0 | — |
| >3 mismatches | 0 | 0 | 0 | — |

Table 11: Number of US sequences with mismatches to the test's N2 primers and probes from December 20, 2021 to January 19, 2022

| | N2 Forward (Fwd) Primer | N2 Reverse (Rev) Primer | N2 Probe | 30-Day Frequency |
|--------------------------|-------------------------|-------------------------|----------|--|
| Total primer length (nt) | 20 | 18 | 23 | — |
| Total strains | 12846 | 12846 | 12846 | — |
| 100% match | 12736 | 12846 | 12794 | — |
| 1 mismatch | 110 | 0 | 50 | N2-Fwd: 0.9% N2-Rev: 0% N2-Probe: 0.4% |
| 2 mismatches | 0 | 0 | 2 | N2-Fwd: 0% N2-Rev: 0% N2-Probe: 0.02% |
| 3 mismatches | 0 | 0 | 0 | — |
| >3 mismatches | 0 | 0 | 0 | — |

Cross-reactivity (analytical specificity)

An *in silico* analysis of the organisms listed in Table 12 was performed.

Table 12. Organisms used for *in silico* cross-reactivity results analysis

| High priority organisms from the same genetic family | High priority organisms likely present in a respiratory sample | |
|--|--|-----------------------------------|
| Virus | Virus | Bacteria |
| Human coronavirus 229E | Adenovirus (e.g. Cl Ad. 71) | <i>Chlamydia pneumoniae</i> |
| Human coronavirus OC43 | Human Metapneumovirus (hMPV) | <i>Haemophilus influenzae</i> |
| Human coronavirus HKU1 | Parainfluenza virus 1-4 | <i>Legionella pneumophila</i> |
| Human coronavirus NL63 | Influenza A & B | <i>Mycobacterium tuberculosis</i> |
| SARS-CoV-1 | Enterovirus (e.g. EV68) | <i>Streptococcus pneumoniae</i> |
| MERS-coronavirus | Respiratory syncytial virus | <i>Streptococcus pyogenes</i> |
| — | Rhinovirus | <i>Bordetella pertussis</i> |
| — | — | <i>Mycoplasma pneumonia</i> |
| — | — | <i>Candida albicans</i> |
| — | — | <i>Pseudomonas aeruginosa</i> |
| — | — | <i>Staphylococcus epidermis</i> |
| — | — | <i>Streptococcus salivarius</i> |

Microbial interference studies were not performed for this submission. BLAST analysis demonstrated that for each bacterial, viral, or fungal organism sequence, with the exception of SARS-CoV-1 isolates, no Advanta Dx COVID-19 EASE primers/probe set aligned with $\geq 80\%$ primer/probe set (N1, N2, or RNase P) homology for more than one oligonucleotide component for any of the organisms evaluated. Amplification of these organism is not anticipated because hybridization of both primers is required to produce an amplicon, and the three assay components are necessary to generate a signal.

Endogenous/Exogenous Interference Substances Studies

The Advanta Dx COVID-19 EASE Assay uses conventional, well-established nucleic acid extraction methods and interference from common endogenous substances is not expected.

Sample Stability

Samples have been demonstrated to be stable at +30 °C for up to 48 hr after collection and prior to RNA extraction. For storage beyond 48 hr after collection and prior to RNA extraction, store at –70°C or lower. Samples can be subjected to one freeze-thaw cycle as shown in [Table 13](#).

Table 13. Results of sample stability study at 48 hours

| Test samples | Test Condition | Time Point | Acceptance Criteria | Number of Positives Detected | Result |
|--------------|----------------|------------|---------------------|------------------------------|--------|
| 5X LoD | +30 °C | 24 hours | 100% Positive | 10 of 10 (100%) | Pass |
| 2X LoD | +30 °C | 24 hours | ≥ 95% Positive | 30 of 30 (100%) | Pass |
| Negative | +30 °C | 24 hours | 100% Negative | 10 of 10 (100%) | Pass |
| 5X LoD | –80 °C | 24 hours | 100% Positive | 10 of 10 (100%) | Pass |
| 2X LoD | –80 °C | 24 hours | ≥ 95% Positive | 29 of 30 (96.6%) | Pass |
| Negative | –80 °C | 24 hours | 100% Negative | 10 of 10 (100%) | Pass |
| 5X LoD | +30 °C | 48 hours | 100% Positive | 10 of 10 (100%) | Pass |
| 2X LoD | +30 °C | 48 hours | ≥ 95% Positive | 29 of 30 (96.6%) | Pass |
| Negative | +30 °C | 48 hours | 100% Negative | 10 of 10 (100%) | Pass |

Clinical Evaluation for Patients Suspected of COVID-19

A total of 78 nasopharyngeal clinical samples (36 positive and 42 negative) were tested with the Advanta Dx COVID-19 EASE Assay. These 78 samples were processed per this Instructions for Use document, including interpretation of results based on a Ct of 32 as the cutoff as described in the result interpretation section on [page 34](#). The 78 samples were also tested using a high-sensitivity EUA comparator assay. The results of the clinical evaluation are summarized in [Table 14](#).

Table 14. Summary of results for clinical samples

| | | EUA Comparator | | | |
|---|------------------|--------------------------------|------------------|--------------|-----------|
| | | Positive (#) | Inconclusive (#) | Negative (#) | Total (#) |
| Advanta Dx COVID-19 EASE Assay | Positive (#) | 35 | 0 | 2 | 37 |
| | Inconclusive (#) | 0 | 0 | 0 | 0 |
| | Negative (#) | 1 | 0 | 40 | 41 |
| | Total (#) | 36 | 0 | 42 | 78 |
| Positive Percent Agreement (PPA) | | 97.2% (95% CI: 85.8% to 99.5%) | | | |

| EUA Comparator | | | |
|--------------------------------|------------------|--------------|-----------|
| Positive (#) | Inconclusive (#) | Negative (#) | Total (#) |
| 95.2% (95% CI: 84.2% to 98.7%) | | | |

Negative Percent Agreement (NPA)

Appendix A: Biomark HD Thermal Cycler Protocol

Advanta Dx Covid-19 EASE thermal cycling parameters







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





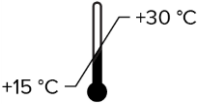
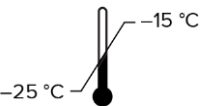
Appendix B: Related Documents

Go to fluidigm.com to download these related documents.

| Title | Document Number |
|--|-----------------|
| Juno System User Guide | 100-7070 |
| Biomark HD Data Collection User Guide | 100-2451 |
| Real-Time PCR Analysis Software User Guide | 68000088 |
| Advanta Dx COVID-19 EASE Interpretive Software Quick Reference Guide | FLDM-00683 |

Appendix C: Symbols

| Symbol | Indicates |
|---|---|
|  | Medical device manufacturer |
|  | Use by date |
|  | Batch code |
|  | Catalog number |
|  | Manufacturer's serial number |
|  | <i>In vitro</i> diagnostic medical device |

| Symbol | Indicates |
|--|-------------------------------|
|  | Consult Instructions for Use |
|  | Caution |
|  | Negative control |
|  | Positive control |
|  | Indicates ambient temperature |
|  | Indicates frozen temperature |

| | | | |
|---|------------------|--|------------------------------------|
|  | Biological risks |  | Indicates refrigerated temperature |
|---|------------------|--|------------------------------------|

REVOKED

Revision History

| Revision | Date | Description of change |
|----------|---------|------------------------------------|
| 02 | 05/2022 | Updated company name and branding. |
| 01 | 02/2022 | Initial release. |

REVOCKED



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Advanta Dx COVID-19 EASE Interpretive Software

For *In Vitro* Diagnostic Use | For Use with the Advanta Dx COVID-19 EASE Assay Under Emergency Use Authorization Only | Rx Only

The Advanta™ Dx COVID-19 EASE Interpretive Software is used on datasets that are derived from the Biomark™ HD and Juno™ systems, which are non-cleared instruments operated with RUO software that have been validated as part of the EUA-authorized Advanta Dx COVID-19 EASE Assay. Please note:

- This product has not been FDA cleared or approved, but has been authorized by FDA under an Emergency Use Authorization (EUA) for use by authorized laboratories; 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 product 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 product 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.

Contents

| | | | |
|--|---|--|----|
| About the Software | 2 | Appendix A: Run the Advanta Dx COVID-19 EASE Interpretive Software from a Command Line | 9 |
| Computer Requirements | 2 | Appendix B: Reporting Ct Values | 10 |
| Software and Input File Requirements | 2 | Revision History | 11 |
| Install the Advanta Dx COVID-19 EASE Interpretive Software | 2 | | |
| Use the Advanta Dx COVID-19 EASE Interpretive Software | 3 | | |
| Interpretation | 5 | | |
| Specifications | 5 | | |
| Interpretation of Control Results | 5 | | |
| Interpretation of Patient Specimen Results | 6 | | |
| Output File Format | 7 | | |



About the Software

The Advanta Dx COVID-19 EASE Interpretive Software is a command-line software tool used to interpret real-time PCR data generated by Biomark HD and the Real-Time PCR Analysis software. The Advanta Dx COVID-19 EASE Interpretive Software is for use only with Table Results exported from the Real-Time PCR Analysis software v4.8.1. The Advanta Dx COVID-19 EASE Interpretive Software can either be initiated within the Real-Time PCR Analysis software as a post export command or run separately from a command line.

The Detector Setup must be annotated in the Real-Time PCR Analysis software using the names **N1, N2, RNase P**. The Advanta Dx COVID-19 EASE Interpretive Software uses those 3 assays to interpret results.

Computer Requirements

The minimum computer requirements for running the Advanta Dx COVID-19 EASE Interpretive Software are:

- Operating system: Microsoft® Windows® 10 (32- or 64-bit)
- CPU: Intel® Core™ i5 or later
- Memory: 4 GB of RAM

Software and Input File Requirements

The Advanta Dx COVID-19 EASE Interpretive Software can only interpret files exported as **Table Results (*.csv)** from the Real-Time PCR Analysis software version v4.8.1.

Install the Advanta Dx COVID-19 EASE Interpretive Software

- 1 Download the software installer file from fluidigm.com/covid-19-EASE.
- 2 Double-click the **Advanta Dx COVID-19 EASE Interpretive Software Setup** icon ().
- 3 Follow the wizard prompts to accept the license agreement and install the software.

IMPORTANT: The Advanta Dx COVID-19 EASE Interpretive Software Setup application is for use only in installing or uninstalling the software. Double-clicking the icon does not run the software. You can delete the setup application after the software is installed.

You can either set up the Real-Time PCR Analysis software to export data directly through the Advanta Dx COVID-19 EASE Interpretive Software or you can run the Advanta Dx COVID-19 EASE Interpretive Software from a command line to interpret the Ct results and save the interpretation as a CSV file.

Use the Advanta Dx COVID-19 EASE Interpretive Software



Before using the Advanta Dx COVID-19 EASE Interpretive Software, annotate the real-time PCR data as described in the Advanta Dx COVID-19 Assay Instructions for Use (FLDM-00675).

Run the Advanta Dx COVID-19 EASE Interpretive Software from the Real-Time PCR Analysis Software. The Advanta Dx COVID-19 EASE interpretive software can also be run from a command line (see [Appendix A](#) for instructions).

- 1 Set up the post export command in the Real-Time PCR Analysis software:

NOTE Setting up the post export command is only required before the first analysis unless settings for this option have been changed between analyses.

a In the Real-Time PCR Analysis software, select **Tools > Options**.

b Click **Results Export in the Output** section ([Figure 1](#) on [page 4](#)).

c (Optional) Specify a default folder for the exported results files.

d Select the **Use Post Export Command** checkbox, then browse to:
C:\Program Files (x86)\Fluidigm\Advanta Dx COVID-19 EASE Interpretive Software

NOTE If you are running the 32-bit version of the Windows operating system, browse to:

C:\Program Files\Fluidigm\Advanta Dx COVID-19 EASE Interpretive Software

e Select the **AdvantaDxSC2** application.

f In the Additional Arguments field, enter **-re**

NOTE Entering “-re” in the Additional Arguments field of the Real-Time PCR Analysis software enables the software to display a message when the Advanta Dx COVID-19 EASE Interpretive Software encounters an error.

g Confirm that the **Prompt for confirmation before execution** checkbox is checked, then click **OK**.

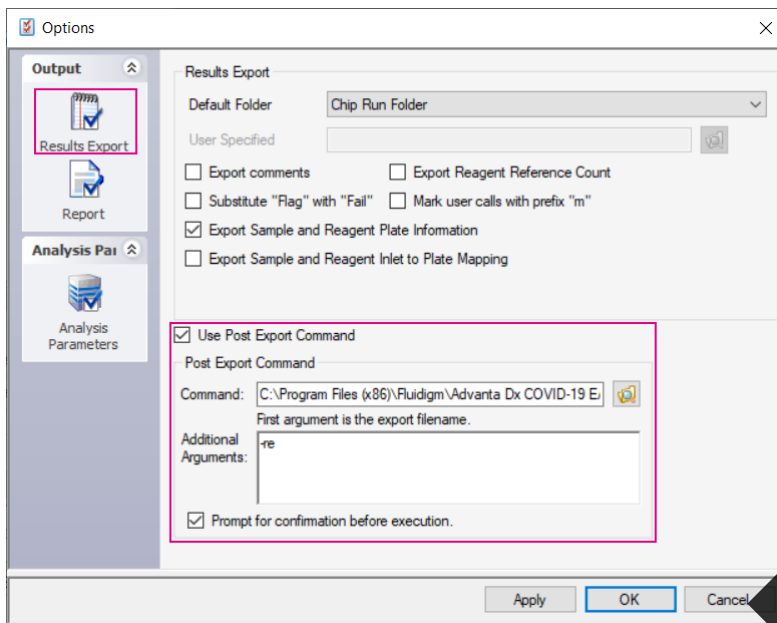


Figure 1. Results Export options

- 2 After annotating the real-time PCR data, export the analysis results as type **Table Results (*.csv)** to interpret the Ct results and save them to the designated CSV file.
- 3 Click **Yes** when prompted to execute the post export command.

Interpretation



For more information about the interpretation of results, see the Advanta Dx COVID-19 EASE Assay Instructions for Use (FLDM-00675).

Specifications

- Maximum positive Ct: A Ct of 32 is the maximum value allowed to call a reaction positive.
- Number of positives: Requires 2 or more positive replicate reactions to call an assay positive.
- Interpretive assays: Must be named **N1, N2, RNase P**.
N1 and N2 must be of type **Test**.
RNase P assay must be of type **Control**.
- Test samples: Must be of type **Unknown**.
- Control samples: Must be named **PC, NC, NTC**.
No template control must be of type **NTC**.
Negative and positive control must be of type **Unknown**.
- Empty samples: Must be of type **Blank**.
- Quality Threshold: Must be **0.65**.
- Baseline Correction: Must be set to **Linear***.
- Ct Threshold Method: Must be set to **Auto By Control (Global)***.

*You can set these as the default selections in the Real-Time Analysis PCR software.

Interpretation of Control Results

NOTE + = Positive, - = Negative; Results must match table below to be considered passing

| Control Description | N1 Result | N2 Result | RNase P Result | Control Result |
|---------------------------|-----------|-----------|----------------|----------------|
| Positive control (PC) | + | + | + | Pass |
| Negative control (NC) | - | - | + | Pass |
| No template control (NTC) | - | - | - | Pass |

Interpretation of Patient Specimen Results

NOTE The IFC QC results that are reported in the interpretive software do not modify the interpretations. If any control on the IFC fails, patient results are not reportable.

NOTE + = **Positive**, - = **Negative**, ? = **Inconclusive**

| N1 Result | N2 Result | RNase P Result | Sample Interpretation | Action |
|-----------|-----------|----------------|-----------------------|---|
| + | + | + | Positive | Report as positive for SARS-CoV-2. |
| + | + | - | Positive | |
| + | + | ? | Positive | Report result to public health authorities. |
| + | - | + | Positive | |
| + | ? | + | Positive | |
| - | + | + | Positive | Report as negative for SARS-CoV-2. Report result to public health authorities. |
| ? | + | + | Positive | |
| - | - | + | Negative | |
| - | - | - | No Template | Reextract and Retest. |
| - | ? | - | Inconclusive | |
| - | + | - | Inconclusive | 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 | |

Output File Format

The interpretive output file format is a comma-separated values (.csv) file with the following information:

- Line 1: File format name
- Line 2: IFC barcode
- Line 3: Biomark scan date and time
- Line 4: Biomark system ID
- Line 5: Real-Time PCR Analysis software version
- Lines 6–8: Analysis parameters used by the Real-Time PCR Analysis software
- Line 9: Advanta Dx COVID-19 EASE Interpretive Software version
- Line 10: Assay names
- Lines 11–13: Positive parameters used by the Advanta Dx COVID-19 EASE Interpretive Software
- Line 15: Controls for successful interpretation or errors, if encountering errors
- Line 16: (Headers)
- Control Name: Name of the control entered in the Sample Setup (in the Real-Time PCR Analysis software)
 - IFC Inlet Location: Sample inlet ID (IFC inlet map)
 - Plate Name: Name of the sample plate
 - Plate Barcode: Barcode of the sample plate
 - Plate Position: Position of the sample in the plate
 - N1: N1 assay result
 - N2: N2 assay result
 - RNase P: RNase P assay result
 - Result: Interpretation according to the assay results.
- Lines 17–22: Results for each control
- NOTE** The number of lines needed for the control results depends on the number of controls that are defined. For example, if only 3 controls are defined (Lines 17–19), the following line numbers will shift accordingly (Lines 21, 22, 23–).
- Line 24: Samples for successful interpretation or errors, if encountering errors
- Line 25: (Headers)
- Sample Name: Name entered in the Sample Setup (in the Real-Time PCR Analysis software)
 - IFC Inlet Location: Sample inlet ID (IFC inlet map)
 - Plate Name: Name of the sample plate
 - Plate Barcode: Barcode of the sample plate
 - Plate Position: Position of the sample in the plate
 - N1: N1 assay result
 - N2: N2 assay result
 - RNase P: RNase P assay result
 - Interpretation: Interpretation according to the assay results.
 - Plate QC Result: Quality control results for the plate
- Lines 26– : Results for each sample

Example of a Successful Output File

| | A | B | C | D | E | F | G | H | I | J | K |
|----|--|--------------------------|------------|---------------|----------------|----|----|---------|----------------|-----------------|---|
| 1 | Advanta Dx COVID-19 EASE Interpretive Software Output | | | | | | | | | | |
| 2 | IFC Barcode | 1020004023 | | | | | | | | | |
| 3 | Biomark Scan Date and Time | 7/20/2021 16:42 | | | | | | | | | |
| 4 | Biomark System ID | BIOMARKHD542 | | | | | | | | | |
| 5 | Real-Time PCR Analysis Software Version 4.8.1 | | | | | | | | | | |
| 6 | Quality Threshold | 0.65 | | | | | | | | | |
| 7 | Baseline Correction Method | Linear | | | | | | | | | |
| 8 | Ct Threshold Method | Auto By Control (Global) | | | | | | | | | |
| 9 | Advanta Dx COVID-19 EASE Interpretive Software Version 1.0.0 | | | | | | | | | | |
| 10 | Assays | N1 | N2 | RNase P | | | | | | | |
| 11 | Maximum Detected Ct | 32 | 32 | 32 | | | | | | | |
| 12 | Number of Detected Assay Replicates | 2 | 2 | 2 | | | | | | | |
| 13 | Assay Detection Rules | Defined Detected | | | | | | | | | |
| 14 | Controls | | | | | | | | | | |
| 16 | Control Name | IFC Inlet Location | Plate Name | Plate Barcode | Plate Position | N1 | N2 | RNase P | Result | | |
| 17 | NTC | S01 | NA | NA | NA | - | - | - | Pass | | |
| 18 | NC | S13 | NA | NA | NA | - | - | + | Pass | | |
| 19 | PC | S25 | NA | NA | NA | + | + | + | Pass | | |
| 20 | Samples | | | | | | | | | | |
| 22 | Sample Name | IFC Inlet Location | Plate Name | Plate Barcode | Plate Position | N1 | N2 | RNase P | Interpretation | Plate QC Result | |
| 23 | Sample_14 | S03 | NA | NA | NA | - | + | + | Positive | Pass | |
| 24 | Sample_06 | S02 | NA | NA | NA | + | + | + | Positive | Pass | |
| 25 | Sample_62 | S09 | NA | NA | NA | - | - | + | Negative | Pass | |
| 26 | Sample_54 | S08 | NA | NA | NA | - | + | + | Positive | Pass | |
| 27 | Sample_46 | S07 | NA | NA | NA | - | + | + | Positive | Pass | |
| 28 | Sample_15 | S15 | NA | NA | NA | + | + | + | Positive | Pass | |
| 29 | Sample_07 | S14 | NA | NA | NA | + | + | + | Positive | Pass | |
| 30 | Sample_63 | S21 | NA | NA | NA | - | - | + | Negative | Pass | |

Example of an Output with a Failed Control

NOTE If any control fails, all rows in the Plate QC Result column are marked Fail.

| | A | B | C | D | E | F | G | H | I | J | K |
|----|--|--------------------------|------------|---------------|----------------|----|----|---------|----------------|-----------------|---|
| 1 | Advanta Dx COVID-19 EASE Interpretive Software Output | | | | | | | | | | |
| 2 | IFC Barcode | 1020004023 | | | | | | | | | |
| 3 | Biomark Scan Date and Time | 7/20/2021 16:42 | | | | | | | | | |
| 4 | Biomark System ID | BIOMARKHD542 | | | | | | | | | |
| 5 | Real-Time PCR Analysis Software Version 4.8.1 | | | | | | | | | | |
| 6 | Quality Threshold | 0.65 | | | | | | | | | |
| 7 | Baseline Correction Method | Linear | | | | | | | | | |
| 8 | Ct Threshold Method | Auto By Control (Global) | | | | | | | | | |
| 9 | Advanta Dx COVID-19 EASE Interpretive Software Version 1.0.0 | | | | | | | | | | |
| 10 | Assays | N1 | N2 | RNase P | | | | | | | |
| 11 | Maximum Detected Ct | 32 | 32 | 32 | | | | | | | |
| 12 | Number of Detected Assay Replicates | 2 | 2 | 2 | | | | | | | |
| 13 | Assay Detection Rules | Defined Detected | | | | | | | | | |
| 14 | Controls | | | | | | | | | | |
| 16 | Control Name | IFC Inlet Location | Plate Name | Plate Barcode | Plate Position | N1 | N2 | RNase P | Result | | |
| 17 | NTC | S01 | NA | NA | NA | - | - | - | Pass | | |
| 18 | NC | S13 | NA | NA | NA | - | - | + | Pass | | |
| 19 | PC | S25 | NA | NA | NA | + | + | - | Fail | | |
| 20 | Samples | | | | | | | | | | |
| 22 | Sample Name | IFC Inlet Location | Plate Name | Plate Barcode | Plate Position | N1 | N2 | RNase P | Interpretation | Plate QC Result | |
| 23 | Sample_14 | S03 | NA | NA | NA | - | + | + | Positive | Fail | |
| 24 | Sample_06 | S02 | NA | NA | NA | + | + | + | Positive | Fail | |
| 25 | Sample_62 | S09 | NA | NA | NA | - | - | + | Negative | Fail | |
| 26 | Sample_54 | S08 | NA | NA | NA | - | + | + | Positive | Fail | |
| 27 | Sample_46 | S07 | NA | NA | NA | - | + | + | Positive | Fail | |
| 28 | Sample_15 | S15 | NA | NA | NA | + | + | + | Positive | Fail | |
| 29 | Sample_07 | S14 | NA | NA | NA | + | + | + | Positive | Fail | |
| 30 | Sample_63 | S21 | NA | NA | NA | - | - | + | Negative | Fail | |

Example of an Error

| | A | B | C | D |
|----|--|--------------------------|----|---------|
| 1 | Advanta Dx COVID-19 EASE Interpretive Software Output | | | |
| 2 | IFC Barcode | 1020004023 | | |
| 3 | Biomark Scan Date and Time | 7/20/2021 16:42 | | |
| 4 | Biomark System ID | BIOMARKHD542 | | |
| 5 | Real-Time PCR Analysis Software Version | 4.8.1 | | |
| 6 | Quality Threshold | 0.65 | | |
| 7 | Baseline Correction Method | Linear | | |
| 8 | Ct Threshold Method | Auto By Control (Global) | | |
| 9 | Advanta Dx COVID-19 EASE Interpretive Software Version | 1.0.0 | | |
| 10 | Assays | N1 | N2 | RNase P |
| 11 | Maximum Detected Ct | | 32 | 32 |
| 12 | Number of Detected Assay Replicates | | 2 | 2 |
| 13 | Assay Detection Rules | Defined Detected | | |
| 14 | | | | |
| 15 | Errors | | | |
| 16 | The input data file contains incompatible assay names | Rnasep | | |

Appendix A: Run the Advanta Dx COVID-19 EASE Interpretive Software from a Command Line

- 1 Export the analysis results as type Table Results (*.csv) to interpret the Ct results and save it to the designated CSV file.
- 2 In the Windows taskbar, select **Start** button > **Windows System** > **Command Prompt**.
- 3 In the **Command Prompt** window, change the directory to the C drive, if necessary.
C:

- 4 Navigate to the folder that contains the Advanta Dx COVID-19 EASE Interpretive Software:
`cd "\Program Files (x86)\Fluidigm\Advanta Dx COVID-19 EASE Interpretive Software"`

NOTE If you are running the 32-bit version of the Windows operating system, enter:
`cd "\Program Files\Fluidigm\Advanta Dx COVID-19 EASE Interpretive Software"`

- 5 Run the command: `AdvantaDxSC2 [input filename] [output filename]`

NOTE Do not include the brackets ([]) when entering the filenames; they are not part of the command.

This command interprets the input .csv file (exported from Real-Time PCR Analysis software) and saves it to the output .csv filename. Include the directory information when entering the filename.

IMPORTANT: If the output filename is not specified, the input file will be overwritten with the interpretive results.

NOTE To display the name, version, and build number for the Advanta Dx COVID-19 EASE Interpretive Software, enter: `AdvantaDxSC2 -nvb`

Appendix B: Reporting Ct Values

You can configure the Advanta Dx COVID-19 EASE Interpretive Software to include the Ct values in the output file for detected assay replicates by entering **-ct** in the Additional Arguments field or at the end of the command line.

NOTE If you are entering **-ct** after **-re**, separate the 2 arguments with a space (**-re -ct**).

In the output file, the control Ct values appear after the Results column and the sample Ct values appear after the Plate QC Results column, with the following headings:

- N1 # of Detected: Number of detected N1 assay replicates
- N2 # of Detected: Number of detected N2 assay replicates
- RNase P # of Detected: Number of detected RNase P assay replicates
- N1 Ct: Average Ct value for the detected N1 assay replicates
- N2 Ct: Average Ct value for the detected N2 assay replicates
- RNase P Ct: Average Ct value for the detected RNase P assay replicates

NOTE The Ct values are only reported for positive results. Negative results are not reported (NA).

Example of a Successful Output File with exported Ct values

| | A | B | C | D | E | F | G | H | I | J | K | L | M | N | O | P |
|----|--|--------------------------|------------|---------------|----------------|----|----|---------|----------------|---------------------------------|---------------------------------|--------------------------------------|--------------------------------------|----------------------|---------------------------|---------------------------|
| 1 | Advanta Dx COVID-19 EASE Interpretive Software Output | | | | | | | | | | | | | | | |
| 2 | IFC Barcode | 1020004023 | | | | | | | | | | | | | | |
| 3 | Biomark Scan Date and Time | 7/20/2021 16:42 | | | | | | | | | | | | | | |
| 4 | Biomark System ID | BIOMARKHDS42 | | | | | | | | | | | | | | |
| 5 | Real-Time PCR Analysis Software Version | 4.8.1 | | | | | | | | | | | | | | |
| 6 | Quality Threshold | 0.65 | | | | | | | | | | | | | | |
| 7 | Advanta Dx COVID-19 EASE Interpretive Software Output | | | | | | | | | | | | | | | |
| 8 | IFC Barcode | 1020004023 | | | | | | | | | | | | | | |
| 9 | Biomark Scan Date and Time | 7/20/2021 16:42 | | | | | | | | | | | | | | |
| 10 | Biomark System ID | BIOMARKHDS42 | | | | | | | | | | | | | | |
| 11 | Real-Time PCR Analysis Software Version | 4.8.1 | | | | | | | | | | | | | | |
| 12 | Quality Threshold | 0.65 | | | | | | | | | | | | | | |
| 13 | Baseline Correction Method | Linear | | | | | | | | | | | | | | |
| 14 | Ct Threshold Method | Auto By Control (Global) | | | | | | | | | | | | | | |
| 15 | Advanta Dx COVID-19 EASE Interpretive Software Version | | | | | | | | | | | | | | | |
| 16 | Assays | N1 | N2 | RNase P | | | | | | | | | | | | |
| 17 | Maximum Detected Ct | 32 | 32 | 32 | | | | | | | | | | | | |
| 18 | Number of Detected Assay Replicates | 2 | 2 | 2 | | | | | | | | | | | | |
| 19 | Assay Detection Rules | Defined | | | | | | | | | | | | | | |
| 20 | | | | | | | | | | | | | | | | |
| 21 | Controls | | | | | | | | | | | | | | | |
| 22 | Control Name | IFC Inlet Location | Plate Name | Plate Barcode | Plate Position | N1 | N2 | RNase P | Result | N1 # of Passed Assay Replicates | N2 # of Passed Assay Replicates | RNase P # of Passed Assay Replicates | N1 Average Passed Ct | N2 Average Passed Ct | RNase P Average Passed Ct | |
| 23 | NTC | S01 | NA | NA | NA | - | - | - | Pass | 0 | 0 | 0 | 999 | 999 | 999 | |
| 24 | NC | S15 | NA | NA | NA | - | - | - | Pass | 0 | 0 | 0 | 999 | 999 | 8.15 | |
| 25 | PC | S25 | NA | NA | NA | + | + | + | Pass | 4 | 4 | 8 | 18.93 | 19.42 | 8.19 | |
| 26 | | | | | | | | | | | | | | | | |
| 27 | Samples | | | | | | | | | | | | | | | |
| 28 | Sample Name | IFC Inlet Location | Plate Name | Plate Barcode | Plate Position | N1 | N2 | RNase P | Interpretation | Plate QC Result | N1 # of Passed Assay Replicates | N2 # of Passed Assay Replicates | RNase P # of Passed Assay Replicates | N1 Average Passed Ct | N2 Average Passed Ct | RNase P Average Passed Ct |
| 29 | Sample_34 | S03 | NA | NA | NA | - | + | + | Positive | Pass | 0 | 4 | 4 | 8 | 999 | 20.55 18.08 |
| 30 | Sample_05 | S02 | NA | NA | NA | + | + | + | Positive | Pass | 4 | 4 | 8 | 19.1 | 19.29 | 18.47 |
| 31 | Sample_62 | S09 | NA | NA | NA | - | - | + | Negative | Pass | 0 | 0 | 0 | 999 | 999 | 17.55 |
| 32 | Sample_54 | S08 | NA | NA | NA | - | + | + | Positive | Pass | 0 | 4 | 8 | 999 | 20.85 | 17.57 |
| 33 | Sample_46 | S07 | NA | NA | NA | - | + | + | Positive | Pass | 0 | 4 | 8 | 999 | 20.8 | 16 |
| 34 | Sample_15 | S15 | NA | NA | NA | + | + | + | Positive | Pass | 4 | 4 | 8 | 17.85 | 18.88 | 19.8 |
| 35 | Sample_07 | S14 | NA | NA | NA | + | + | + | Positive | Pass | 4 | 4 | 8 | 19.4 | 18.76 | 18.22 |

Revision History

| Revision | Date | Description of change |
|----------|---------|------------------------------------|
| 02 | 05/2022 | Updated company name and branding. |
| 01 | 02/2022 | Initial release. |

REVOKED



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

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Advanta Dx COVID-19 EASE Assay Instrument Qualification Method (IQM) Protocol

For in vitro diagnostic use

Rx Only

For use under Emergency Use Authorization (EUA) only

| Instrument | Serial Number |
|--|----------------------|
| Biomark™ HD System With Biomark Data Collection software v4.8.1 and Real Time PCR Analysis software v4.8.1 | Serial Number: _____ |
| Juno™ System With Juno system software v3.15.1 | Serial Number: _____ |

Company Name: _____

Date of IQM Execution: _____ Work Order Number: _____

Test performed by FAS (Print): _____ FAS Initials: _____

FAS Signature: _____



Contents

| | | |
|---|----------------------------------|---|
| 1 | Warnings | 3 |
| 2 | Using this Protocol | 3 |
| 3 | Roles | 3 |
| 4 | Customer Protocol Approval | 4 |
| 5 | Required Materials and Equipment | 4 |
| 6 | Procedure | 4 |
| 7 | Results of Protocol Execution | 5 |
| 8 | Instrument Labeling | 6 |
| 9 | Final Approval | 6 |

REVOKED

1 Warnings

- A. This product has not been FDA cleared or approved, but has been authorized for emergency use by FDA under an EUA for use by authorized laboratories; use by laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C § 263a, and meet requirements to perform high-complexity tests.
- B. This product has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.
- C. The emergency use of this product 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 and the Advanta™ Dx COVID-19 EASE 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. Standard BioTools 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

Standard BioTools 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 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 COVID-19 EASE Assay Instructions for Use (FLDM-00675) for required materials and equipment.

| Component | Part Number | Quantity Required |
|--|-------------|-------------------|
| Advanta™ Dx COVID-19 EASE Assay—2 IFCs | 102-1839 | 1 Kit |

6 Procedure

This procedure is intended to follow the IFU while using the Positive Control and Negative Control to assess assay performance as interpreted using the Advanta Dx COVID-19 EASE Interpretive Software v1.0.0 (102-2034).

NOTE Prior to beginning this procedure, ensure all consumables used adhere to the materials list provided in the Advanta™ Dx COVID-19 EASE Assay Instructions for Use (FLDM-00675).

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

- 1 Clean the area and pipettes thoroughly with DNA destroying surface decontaminants then change gloves.
- 2 Pulse vortex and briefly centrifuge the SARS CoV-2 Neg Control (102-1830) and SARS CoV-2 Pos Control (102-1831) before use.
- 3 Prepare 11 negative control (NC) tubes:
 - a. Add 198 μ L of PCR Water (102-0369) to each new, 0.5 mL tube labeled NC 1 through NC 11.
 - b. Add 22 μ L of SARS CoV-2 Neg Control to each NC tube.
 - c. Vortex each tube for 5 sec and pulse centrifuge to bring down contents. Store the NC tubes on ice or in a cold block until ready to use.
- 4 Prepare 21 positive control (PC) tubes:
 - a. Add 190 μ L of PCR water (102-0369) to each new, 0.5 mL tube labeled PC 1 through PC 21.
 - b. Add 22 μ L of the SARS CoV-2 Neg Control to each PC tube.

- c. Add 8.0 μ L of the SARS CoV-2 Pos Control to each PC tube.
- d. Vortex each tube for 5 sec and pulse centrifuge to bring down contents. Store the PC tubes on ice or in a cold block until ready to use.

NOTE NC 1 and PC 1 will be used as controls in the IQM run. All others will be used as test samples.

- 5 Process 10 replicates of NC and 20 replicates of PC following the Advanta™ Dx COVID-19 EASE Assay Instructions for Use, beginning with the “**Prepare the Extraction Plate and Extract RNA**” section.

7 Results of Protocol Execution

| Parameter | Specification | Results |
|--|--|---|
| Run validity | Results for NTC, NC 1 and PC 1 (controls) meet expected control results per the Instructions for Use | <input type="checkbox"/> Controls Meet Expectations <input type="checkbox"/> Controls Do Not Meet Expectations <input type="checkbox"/> Pass <input type="checkbox"/> Fail |
| Negative Test Samples (NC 2 through NC 11) | All 10 replicates are negative based upon Interpretive Software result | <input type="checkbox"/> 10 / 10 replicates are negative <input type="checkbox"/> < 10 replicates are negative <input type="checkbox"/> Pass <input type="checkbox"/> Fail |
| Positive Test Samples (PC 2 through PC 21) | At least 19 of 20 replicates are positive based upon Interpretive Software result | <input type="checkbox"/> \geq 19 replicates are positive <input type="checkbox"/> < 19 replicates are positive <input type="checkbox"/> Pass <input type="checkbox"/> 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 7, apply the label below to each Standard BioTools instrument (Biomark HD and Juno) included in the execution of this Instrument Qualification Method.

| |
|---|
| Emergency Use Authorization Only This instrument is authorized for use with the Advanta™ Dx COVID-19 EASE 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 Standard BioTools Research Use Only instruments for use with the Advanta Dx COVID-19 EASE 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 COVID-19 EASE Assay and the laboratory may use Standard BioTools' Research Use Only instruments tested in this qualification to report patient results while using the Advanta Dx COVID-19 EASE Assay.

Authorized Customer Name and Signature

Date:

Revision History

| Rev | Author | CO Number | Change Description |
|-----|----------------|------------|---|
| 02 | Jason Erickson | CHG-007001 | Updated company name throughout document. |
| 01 | Jason Erickson | CHG-006249 | Initial release |

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For *In Vitro* Diagnostic Use. For Emergency Use Authorization Only. RX only. This product has not been FDA cleared or approved but has been authorized by FDA under an EUA for use by authorized laboratories. This product 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 product 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 Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated, or authorization is revoked sooner.

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Instruments and Software Used with the Advanta Dx COVID-19 EASE Assay

For *In Vitro* Diagnostic Use | For Use with the Advanta Dx COVID-19 EASE Assay Under Emergency Use Authorization Only | Rx Only

The following Standard BioTools™ instruments and software were validated for use with the Advanta™ Dx COVID-19 EASE Assay and are therefore acceptable to be used for diagnostic testing for COVID-19.

| Product Name | Part Number |
|---|-------------|
| Biomark™ HD system | BMKHD-BMKHD |
| Biomark Data Collection software v4.8.1 | 102-1890 |
| Juno™ with system software v3.15.1 | 102-1889 |
| Real-Time PCR Analysis software v4.8.1 | 102-1852 |

Warnings:

- This product has not been FDA cleared or approved, but has been authorized for emergency use by FDA under an EUA for use by authorized laboratories; use by laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C § 263a, and meet requirements to perform high-complexity tests.
- This product 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 product 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.

Revision History

| Revision | Date | Description of Change |
|----------|---------|------------------------------------|
| 02 | 05/2022 | Updated company name and branding. |
| 01 | 02/2022 | Initial release. |

IVD

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The Advanta™ Dx COVID-19 EASE Assay is:
IVD, Rx Only, For use under the Emergency Use Authorization (EUA) only

- 1 Please contact techsupport@fluidigm.com if you require a printed copy of the complete instructions free of charge.
- 2 This is not the complete instructions. All product documentation may be found at fluidigm.com/covid-19-EASE.
- 3 Note the following statements from the Letter of Authorization:
 - a This product 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 product 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 product is only for 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.



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FLDM-00572 Rev 02