



SARS-CoV-2 RNA STAR Complete

Instructions for Use

For Emergency Use Authorization (EUA) Only

Catalog #L018180130096
For *In Vitro* Diagnostic Use
Rx Use Only

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1 Intended Use

LumiraDx SARS-CoV-2 RNA STAR Complete is a rapid, non-isothermal nucleic acid amplification qSTAR (Selective Temperature Amplification Reaction) method intended for the qualitative detection of nucleic acid from SARS-CoV-2 in upper respiratory specimens (such as anterior nasal, mid-turbinate, nasopharyngeal and oropharyngeal swabs) collected from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 USC. §263a that 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 patient history and other diagnostic information is necessary to determine patient 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.

LumiraDx SARS-CoV-2 RNA STAR Complete 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. LumiraDx SARS-CoV-2 RNA STAR Complete is only for use under the Food and Drug Administration's Emergency Use Authorization.

2 Summary and Explanation

The World Health Organization (WHO) have named the disease caused by SARS-CoV-2 virus as coronavirus 2019 disease or COVID-19¹. The common symptoms of COVID-19 are fever, tiredness, and dry cough. Some patients may have aches and pains, nasal congestion, runny nose, sore throat or diarrhea. These symptoms are usually mild and begin gradually. Some people become infected but do not develop any symptoms and do not feel unwell (asymptomatic infection). However, the disease can develop rapidly and have high morbidity in certain populations, especially those with underlying health conditions. The disease can spread from person to person through small droplets from the nose or mouth which are spread when a person with COVID-19 coughs or exhales. Most estimates of the incubation period for COVID-19 range from 2-14 days².

LumiraDx SARS-CoV-2 RNA STAR Complete has been designed to detect a region in ORF1a from nucleic acid sequences within the genome of the SARS-CoV-2 RNA.

¹ World Health Organization: www.who.int.

² Center for Disease Control and Prevention: www.cdc.org.

3 Principles of the Procedure

LumiraDx SARS-CoV-2 RNA STAR Complete is a rapid, non-isothermal nucleic acid amplification technique utilizing qSTAR technology, which detects SARS-CoV-2 viral nucleic acid in under twenty minutes, without needing to perform any specimen purification or extraction. The LumiraDx SARS-CoV-2 RNA STAR Complete Internal Control, Primer and Probe (IC/P) Mix is designed for the qualitative detection of nucleic acid from SARS-CoV-2 in upper respiratory specimens (such as anterior nasal, mid-turbinate, nasopharyngeal and oropharyngeal swabs) collected from individuals suspected of COVID-19 by their healthcare provider.

In a single reaction, SARS-CoV-2 virus can be lysed and amplified from upper respiratory specimens (such as anterior nasal, mid-turbinate, nasopharyngeal and oropharyngeal swabs). The SARS-CoV-2 virions are lysed by the presence of detergents found in the LumiraDx SARS-CoV-2 RNA STAR Complete 10X Extraction Buffer. The nucleic acids present in the lysed swab specimen are reverse transcribed to form cDNA which is then subsequently amplified by qSTAR using primers that target a specific region in the SARS-CoV-2 genome. Amplification of the cDNA by qSTAR is subject to shuttling, a plurality of times, between an upper temperature at which the activity of one of the enzymes, the polymerase, is relatively favored, and a lower temperature at which the activity of the nicking-enzyme is relatively favored.

Controlling enzyme activity by “temperature gating” and optimizing reaction kinetics, the qSTAR amplification method coupled with an extraction buffer has shown consistency and control of amplification, whilst maintaining the sensitivity of detection, to allow for reliable and accurate detection of infectious diseases without performing extraction within minutes. Generated products are specifically detected with molecular beacons designed to anneal to the target amplicon by any of the following instruments: Roche LightCycler 480 II (software version SW 1.5.1), Applied Biosystems 7500 Fast Dx (software version 1.4.1), Applied Biosystems QuantStudio 5 (software version 1.5.1), QuantStudio 7 Flex (software version 1.3), Agilent AriaMx (software version 1.71), or the Agilent Stratagene Mx3005P (software version 4.10) RT-PCR Instruments.

4 Materials Required (Provided)

Component	Description	Volume	Storage
LumiraDx SARS-CoV-2 RNA STAR Complete Positive Control Media (Pos. Ctrl. Med.)	COVID-19 Positive Control (ZeptoMetrix 50,000cp/mL)	500 µL	≤ 8°C
LumiraDx SARS-CoV-2 RNA STAR Complete Negative Control Media (Neg. Ctrl. Med.)	0.85% Saline Solution	1.5 mL	-25°C to -15°C
LumiraDx SARS-CoV-2 RNA STAR Complete 20X Salt Mix	COVID-19 Salt Mix	250 µL	-25°C to -15°C
LumiraDx SARS-CoV-2 RNA STAR Complete 10X Extraction Buffer	Nucleic Acid Extraction Buffer	500 µL	-25°C to -15°C
LumiraDx SARS-CoV-2 RNA STAR Complete 50X Internal Control & Primer Mix (50X IC/P Mix)	COVID-19 Internal Control & Primer Mix	100 µL	-25°C to -15°C
LumiraDx SARS-CoV-2 RNA STAR Complete 2.5X Master Mix	COVID-19 Master Mix	2x 1 mL	-25°C to -15°C

5 Materials Required (But Not Provided)

Consumables	Source	Catalog #
Appropriate Personal Protective Equipment	General lab supplier	n/a
Aerosol Barrier Pipette Tips with Filters	General lab supplier	n/a
Microcentrifuge Tubes (DNase/RNase free), 0.6 to 5mL	General lab supplier	n/a
Powder-Free Nitrile Glove	General lab supplier	n/a
Deep Well 96-Well Plates (U-bottom)	General lab supplier	n/a
Reagent Reservoirs (for minimal dead volume)	General lab supplier	n/a
Sealable Waster Bag or Container	General lab supplier	n/a
KimWipes	General lab supplier	n/a

Reagents	Source	Catalog #
Sodium Hypochlorite Solution (Bleach)	ThermoFisher Scientific	SS290-1
70% Isopropanol (or 70% Ethanol)	VWR	89499-420
DNAZap™ (or equivalent)	ThermoFisher Scientific	AM9890
RNaseZap™ (or equivalent)	ThermoFisher Scientific	AM9782
Molecular Biology Grade Water	Corning	46-000-CM
Compatible Transport Medias ³	General lab supplier	n/a
345TK-2 Viral Transport Medium with Flocked Swab	Ruhof Corporation	345TK-2
Copan Universal Transport Media	Copan	3C047N
Puritan UniTranz-RT Transport System	Puritan Medical Product	UT-316
BD Universal Viral Transport	BD	220220
Transport Medium	Corning	25-500-CM
Viral Transport Medium	Hardy Diagnostics	R99
Saline, 0.85%	Hardy Diagnostics	U157
PBS (pH 7.4), 1X	ThermoFisher Scientific	10010023

Option for PCR Instruments & Recommended Consumables	Source	Catalog #
Roche LightCycler 480 II (software version SW 1.5.1)	Roche Life Science	5015278001
VWR Adhesive Film for Microplates	VWR	60941-070
LightCycler 480 Multiwell Plate 96, Clear	Roche Life Science	5102413001
Applied Biosystems 7500 Fast Dx (software version 1.4.1)	ThermoFisher Scientific	4406984
Applied Biosystems QuantStudio 5 (software version 1.5.1)	ThermoFisher Scientific	A28574
Applied Biosystems QuantStudio 7 Flex (software version 1.3)	ThermoFisher Scientific	4485698
Applied Biosystems Optical Adhesive Covers	ThermoFisher Scientific	4360954
Applied Biosystems MicroAmp Optical 96-Well Plate	ThermoFisher Scientific	4306737
Agilent Aria Mx (software version 1.71)	Agilent Technologies	G8830A
AriaMx 96 Adhesive Seals	Agilent Technologies	401492
AriaMx 96 Well Plates, Skirted, LP	Agilent Technologies	401490
Agilent Stratagene Mx3005P (software version 4.10)	Agilent	401511
Applied Biosystems MicroAmp Clear Adhesive Film	ThermoFisher Scientific	4306311
Eppendorf twin.tec Real-Time PCR Plate 96-Well Semi-Skirted	Eppendorf	951022043
Universal Sealing Films*	General lab supplier	n/a
ThermalSeal A Sealing Film	Research Product International	202545
Heat-Resistant Polypropylene Film for Raised-Rim Plates	VWR	89087-690

*These universal sealing films have been found to be compatible with all instruments listed above.

Equipment	Source	Catalog #
-80°C Laboratory Freezer	General lab supplier	n/a
-25°C to -15°C Laboratory Freezer	General lab supplier	n/a
2°C to 8°C Laboratory Refrigerator	General lab supplier	n/a
Adjustable Multi-Channel Pipettes (2-20µL, 20-200µL)	General lab supplier	n/a
Adjustable Micropipettes (0.5-10µL, 2-20µL, 20-200µL, 100-1000µL)	General lab supplier	n/a
Centrifuges (for 0.6 to 2mL tubes and 96-well plates)	General lab supplier	n/a
PCR Hood	General lab supplier	n/a
Vortex	General lab supplier	n/a
Cold Blocks	General lab supplier	n/a
Racks for Microcentrifuge Tubes	General lab supplier	n/a
USB Flash Drive	General lab supplier	n/a

³ A matrix equivalency study was performed to access the LumiraDx SARS-CoV-2 RNA STAR Complete test performance for use with compatible specimen transport media. SARS Related Coronavirus 2, Isolate was diluted in NP and NS swab matrix near LoD for each of the following were tested: Puritan UniTranz-RT Transport System, BD Universal Viral Transport, Corning Transport Medium, Hardy Diagnostics Viral Transport Medium, Saline, 0.85%, and PBS (pH 7.4), 1X with corresponding NTC in a NP nasal matrix (25% NP, 75% Nasal). Study results demonstrate that the evaluated specimen transport medias are comparable when performed with the LumiraDx SARS-CoV-2 RNA STAR Complete test.

6 Warning and Precautions

- For *in vitro* Diagnostic Use (IVD).
- For prescription (Rx) use only.
- This LumiraDx SARS-CoV-2 RNA STAR Complete Test has not been FDA cleared or approved; but has been authorized for emergency use by the FDA under an EUA for use by authorized laboratories.
- This LumiraDx SARS-CoV-2 RNA STAR Complete Test has been authorized only for the detection of nucleic acid from SARS-CoV-2 virus and not for any other viruses or pathogens.
- This emergency use of LumiraDx SARS-CoV-2 RNA STAR Complete 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.
- Do not eat, drink, smoke, apply cosmetics or handle contact lenses in areas where reagents and human specimens are handled.
- Handle all specimens as if infectious using safe laboratory procedures. Refer to Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with 2019-nCoV (<https://www.cdc.gov/coronavirus/2019-nCoV/lab-biosafety-guidelines.html>).
- Performance characteristics have been determined with human upper respiratory specimens from individuals with signs and symptoms of infection who are suspected of COVID-19.
- Use personal protective equipment such as, but not limited to, gloves and lab coats when handling kit reagents while performing this Test and handling materials including samples, reagents, pipettes, and other equipment and reagents.
- Dispose of unused kit reagents and human specimens according to local, state, and federal regulations.
- 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.
- Laboratories are required to report all results to the appropriate public health authorities.
- Extraction buffers used with this Test described in section 9 include guanidine-containing materials. Highly reactive and/or toxic compounds may form if combined with sodium hypochlorite (bleach).
- Only use listed components provided for LumiraDx SARS-CoV-2 RNA STAR Complete; other LumiraDx products may not contain the same formulations as needed for this Test.

7 Reagent Storage, Handling, and Stability

- Upon receipt, store the LumiraDx SARS-CoV-2 RNA STAR Complete kit between - 15 °C to - 25 °C.
- After initial use, store LumiraDx SARS-CoV-2 RNA STAR Complete Negative Control Media (Neg. Ctrl. Med.), LumiraDx SARS-CoV-2 RNA STAR Complete 20X Salt Mix, LumiraDx SARS-CoV-2 RNA STAR Complete 10X Extraction Buffer, LumiraDx SARS-CoV-2 RNA STAR Complete 50X Internal Control & Primer Mix (50X IC/P Mix), and LumiraDx SARS-CoV-2 RNA STAR Complete 2.5X Master Mix between - 15 °C to - 25 °C.
- Store LumiraDx SARS-CoV-2 RNA STAR Complete Positive Control Media (Pos. Ctrl. Med.) at ≤ 8 °C.
- Always check the expiration date prior to use. Do not use expired reagents.
- Protect fluorogenic probes from light – probes are a component in the LumiraDx SARS-CoV-2 RNA STAR Complete 50X Internal Control & Primer Mix (50X IC/P Mix).
- The Salt, Extraction Buffer, Internal Control/Primer Mix, and Master Mix must be thawed and kept on a cold block at all times during preparation and use.
- The external controls, Pos. Ctrl. Med. and Neg. Ctrl. Med., must also be thawed and kept cold at all times during preparation and use.

8 Specimen Collection, Handling, and Storage

Proper collection of specimens is the most important step in the laboratory diagnosis of infectious diseases. A specimen that is not collected correctly may lead to false negative test results. All testing for SARS-CoV-2 virus should be conducted in consultation with a healthcare provider. Specimens should be collected as soon as possible once a decision has been made to pursue testing, regardless of the time of symptom onset. Training in specimen collection is highly recommended due to the importance of specimen quality. CLSI MM13-A may be referenced as an appropriate resource.

8.1 Collecting Specimens

- Refer to *Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Persons for Coronavirus Disease 2019 (COVID-19)* – <https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html> – or follow specimen collection device manufacturer instructions for proper collection methods.
- Swab specimens should be collected using only swabs with a synthetic tip, such as nylon or Dacron®, and an aluminum or plastic shaft. Calcium alginate swabs are unacceptable and cotton swabs with wooden shafts are not recommended.
- For wet swab collection - Respiratory specimens should be collected and placed into appropriate transport media, such as viral transport media (VTM), 0.85% saline solution, or phosphate buffer saline (PBS – calcium and magnesium free), as described below, based on CDC and WHO guidelines. Swabs provided in up to 3 mL of compatible transport media are acceptable, but in an effort to save on reagents and improve performance, one (1) mL of buffer is suggested. See <https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html> for additional information.
- For dry swab collection - Respiratory specimens should be collected and placed in a sterile, dry transport tube, such as a standard 15 mL Falcon tube. For elution of a dry swab specimen, add 1 mL of compatible transport media (VTM, 0.85% Saline, or PBS), soak the swab for 30 seconds then swirl the solution thoroughly by rotating the swab against the side of the tube 5 times (beware of cross-contamination from splashing). Discard the swab in biohazard waste.

8.2 Transporting Specimens

- Specimens must be packaged, shipped, and transported according to the current edition of the International Air Transport Association (IATA) Dangerous Goods Regulation. Follow shipping regulations for UN 3373 Biological Substance, Category B when sending potential SARS-CoV-2 specimens.
- If wet swab is expressed in a compatible buffer (i.e. viral transport media (VTM), 0.85% saline solution, or phosphate buffer saline (PBS – calcium and magnesium free)) store specimens at 2 to 8 °C for up to 72 hours after collection. If a delay in testing or shipping is expected, store specimens at -70°C or below and ship on dry ice.
- Dry swabs may be shipped in a dry tube without cold chain, with stability established up to 3 days for dry polyester or foam swabs. Additionally, if a delay in testing or shipping is expected, dry swabs can be expressed in saline (1 mL) and frozen for longer storage. Store frozen specimens at -70°C or below and ship on dry ice.

8.3 Storing Specimens

- Dry swab specimens are stable either at room temperature for up to 48-hours or refrigerated (2–8 °C) for up to 72 hours before processing. Wet swab specimens should be stored refrigerated (2–8 °C) for up to 72 hours before processing. If a delay in testing is expected, store specimens at -70°C or below.
- If specimens cannot be tested within 72 hours of collection, both dry-swab (expressed in saline) and wet-swab specimens should be frozen at ≤ -70 °C until tested.

9 LumiraDx SARS-CoV-2 RNA STAR Complete Preparation

9.1 Specimen Preparation

LumiraDx SARS-CoV-2 RNA STAR Complete entirely removes specimen purification and extraction by combining lysis and amplification in a single step. This Test is compatible with swabs stored in an empty (dry) tube or with swabs stored in compatible transport media (VTM, 0.85% Saline, or PBS). It is important for the LumiraDx SARS-CoV-2 RNA STAR Complete Positive Control Media (Pos. Ctrl. Med.) and the LumiraDx SARS-CoV-2 RNA STAR Complete Negative Control Media (Neg. Ctrl. Med.) to be treated as patient specimens and both must be included as external controls in every amplification reaction. See Section 16.1 for more information about the LumiraDx SARS-CoV-2 RNA STAR Controls.

NOTE: Please handle the Pos. Ctrl. Med. with care as it can cause false positives if accidentally spilled or handle carelessly. To avoid cross-contamination, use separate pipette tips for all materials.

1. Thaw LumiraDx SARS-CoV-2 RNA STAR Complete Positive Control Media (Pos. Ctrl. Med.) and LumiraDx SARS-CoV-2 RNA STAR Complete Negative Control Media (Neg. Ctrl. Med.) on a cold block, vortex the Pos. Ctrl. Med. and Neg. Ctrl. Med. for 5 seconds then pulse centrifuge for 5 seconds to collect reagents at the bottom of the tube.
2. To assemble the 1x PCM (Positive Control Media), freshly dilute 40 µL Pos. Ctrl. Med. with 40 µL Neg. Ctrl. Med. in a pre-chilled microcentrifuge tube. To assemble the 1x NCM (Negative Control Media), always freshly pipette 80 µL Neg. Ctrl. Med into a pre-chilled microcentrifuge tube.
3. Upper respiratory specimens (20µL) will be added directly to the sample plate prepared in Section 9.3. If swab is provided dry, transfer swab into a vial, such as a 5mL tube or deep well plate, containing 1 mL compatible transport media and soak the swab for 30 seconds then swirl thoroughly by rotating the swab against the side of the side of the tube 5 times (beware of cross-contamination from splashing). Discard the swab in biohazard waste.

9.2 qSTAR Guidelines

Caution Note: Amplification technologies such as qSTAR, like PCR, are sensitive to accidental introduction of product from previous amplification reactions. Incorrect results could occur if either the clinical specimen or the qSTAR reagents used in the amplification step become contaminated by accidental introduction of amplification product (amplicon). Workflow in the laboratory should always proceed in a unidirectional manner to minimize such contamination events.

- Maintain separate areas for assay setup and handling of clinical specimens.
- Change aerosol barrier pipette tips between all manual liquid transfers.
- During preparation of samples, compliance with good laboratory techniques is essential to minimize the risk of cross-contamination between samples, and the inadvertent introduction of nucleases into samples during and after the extraction procedure. Proper aseptic technique should always be used when working with nucleic acids.
- Maintain separate, dedicated equipment (e.g., pipettes, microcentrifuges) and supplies (e.g., microcentrifuge tubes, pipette tips) for assay setup and handling of clinical specimens.
- Wear a clean lab coat and powder-free disposable gloves (not previously worn) when setting up assays.
- Change gloves often and whenever contamination is suspected.
- Keep reagent and reaction tubes and plates capped, covered, or sealed as much as possible.
- It is recommended to use a cold block as loose tubes on ice may lead to contamination.
- LumiraDx SARS-CoV-2 RNA STAR Complete 20X Salt Mix, LumiraDx SARS-CoV-2 RNA STAR Complete 10X Extraction Buffer, LumiraDx SARS-CoV-2 RNA STAR Complete 50X IC/P Mix, and LumiraDx SARS-CoV-2 RNA STAR Complete 2.5X Master Mix must be thawed and maintained on a cold block equilibrated to 4 °C at all times during preparation and use. Provided that the reagents are not entirely consumed in the first use, the reagents may be re-frozen no more than three times.
- Work surfaces, pipettes, and centrifuges should be cleaned and decontaminated with cleaning products (e.g. 10% bleach, "DNAZap™", "RNasezap™" or "RNase AWAY®", etc.) to minimize risk of nucleic acid contamination. Residual bleach should be removed using Nuclease Free Water and 70% Ethanol.

9.3 qSTAR Reagent Preparation

It is necessary to make excess Reaction Mix to allow for pipetting error. Additionally, it is recommended to read through the following instructions before attempting to set up the Reaction Mix. All components, including the assembled Reaction Mix, should be thawed and kept on a cold block equilibrated to 4 °C to maintain the integrity of the reagents. Furthermore, it is recommended prior to performing the instructions below to setup the validated thermocycler (i.e. Roche LC 480 II, ABI 7500 Fast Dx, ABI QS 5, ABI QS 7 Flex, Agilent AriaMx, or the Agilent Stratagene Mx3005P) to the step just before starting the instrument to ensure the performance of this Test.

1. Thaw LumiraDx SARS-CoV-2 RNA STAR Complete 20X Salt Mix, LumiraDx SARS-CoV-2 RNA STAR Complete 10X Extraction Buffer, LumiraDx SARS-CoV-2 RNA STAR Complete 50X IC/P Mix, and LumiraDx SARS-CoV-2 RNA STAR Complete 2.5X Master Mix on a cold block. Once thawed, invert each tube to mix (do not vortex samples) then pulse centrifuge for 5 seconds to collect reagents at the bottom of the tube.
2. Determine the number of reactions (N) to be prepared per assay:

Reaction Mix Setup	1 Rxn	100 Rxns	N Rxns
Molecular Biology Grade Water	1.5 µL	150 µL	N x 1.5 µL
LumiraDx SARS-CoV-2 RNA STAR Complete 20X Salt Mix	2.5 µL	250 µL	N x 2.5 µL
LumiraDx SARS-CoV-2 RNA STAR Complete 10X Extraction Buffer	5.0 µL	500 µL	N x 5.0 µL
LumiraDx SARS-CoV-2 RNA STAR Complete 50X IC/P Mix	1.0 µL	100 µL	N x 1.0 µL
LumiraDx SARS-CoV-2 RNA STAR Complete 2.5X Master Mix	20 µL	2000 µL	N x 20 µL
Total Volume	30 µL	3000 µL	N x 30 µL

3. Assuming one reaction is needed, perform the following to make the Reaction Mix:
 - a. Combine 1.5 µL Water and 2.5 µL 20X Salt Mix in a pre-chilled microcentrifuge tube. Vortex for 5 seconds, pulse centrifuge for 5 seconds then place tube back on the cold block.
 - b. Add 5.0 µL 10X Extraction Buffer to the Reaction Mix, mix by slowly pipetting up and down 4 times without introducing bubbles (it is important to not vortex or spin down for an excessive amount of time once the Extraction Buffer has been added), then place tube back on the cold block.
 - c. Add 1.0 µL 50X IC/P Mix to the Reaction Mix, mix by pipetting up and down 4 times without introducing bubbles, centrifuge briefly, then place tube back on the cold block.
 - d. Add 20 µL Master Mix to finalize the Reaction Mix, mix by slowly pipetting up and down 4 times without introducing bubbles, then place tube back on the cold block.
4. Transfer 30 µL of Reaction Mix into an appropriate, pre-chilled, 96-well plate. If there is a delay before adding specimen, this plate should be kept cold (i.e. by using a 96-well PCR plate block, an IsoFreeze PCR rack and/or by storing the plate at 4 °C) and temporarily sealed.
5. Add 20 µL, per well, specimens or external controls into the same well and mix by slowly pipetting up and down 3 times without introducing bubbles (if bubbles occur, centrifuge the 96-well plate at 2000 rpm for 5 seconds as described in Step 8). Seal the 96-well plate using an optically clear adhesive and centrifuge at 2000 rpm for 5 seconds to collect all reagents at the bottom of the plate.
6. Place the 96-well plate in a validated thermocycler and follow instrument specific protocols and analysis procedures detailed below.

9.4 Complete Instructions for Specimen Processing of a Single Swab Format

The following instructions provide an example for the processing of both dry and wet swab specimens, individually, up to the final setup of the sample plate. Swabs provided in up to 3 mL of compatible transport media are acceptable but, in an effort to save on reagents and improve performance, one (1) mL of buffer is suggested.

NOTE: It is recommended to read through the following instructions before attempting to set up the Reaction Mix. All components, including the assembled Reaction Mix, should be thawed and kept on a cold block equilibrated to 4 °C to maintain the integrity of the reagents. Additionally, it is recommended prior to performing the instructions below to setup the validated thermocycler (i.e. Roche LC 480 II, ABI 7500 Fast Dx, ABI QS 5, ABI QS 7 Flex, Agilent AriaMx, or the Agilent Stratagene Mx3005P) to the step just before starting the instrument to ensure the performance of this Test is maintained.

1. Thaw the LumiraDx SARS-CoV-2 RNA STAR Complete kit components in a cold block equilibrated to 4 °C; i.e. LumiraDx SARS CoV-2 RNA STAR Complete Positive Control Media (Pos. Ctrl. Med.), LumiraDx SARS CoV-2 RNA STAR Complete Negative Control Media (Neg. Ctrl. Med.), LumiraDx SARS CoV-2 RNA STAR Complete 20X Salt Mix, LumiraDx SARS CoV-2 RNA STAR Complete 10X Extraction Buffer, LumiraDx SARS CoV-2 RNA STAR Complete 50X Internal Control & Primer Mix (50X IC/P Mix), and LumiraDx SARS CoV-2 RNA STAR Complete 2.5X Master Mix. Invert each tube to mix then pulse centrifuge for 5 seconds to collect reagents at the bottom of the tube (do not vortex samples).
2. If swab is provided dry, transfer one (1) mL of a compatible transport media using a single channel pipette into a suitable tube (e.g. microcentrifuge tube). Swabs (previously) collected in up to 3 mL of compatible transport media (VTM, 0.85% Saline, or PBS) are acceptable, but this higher volume may impact sensitivity.
3. Place and soak the swab for 30 seconds then swirl thoroughly by rotating the swab against the side of the tube up to 5 times (beware of cross-contamination from splashing). Discard the swab in biohazard waste.
4. Assemble fresh 1x PCM (Positive Control Media) by diluting 40 µL Pos. Ctrl. Med. with 40 µL Neg. Ctrl. Med. in a pre-chilled microcentrifuge tube. Assemble the 1x NCM (Negative Control Media) by transferring 80 µL Neg. Ctrl. Med. into a pre-chilled microcentrifuge tube. Excess 1x PCM and 1x NCM should be thrown out.
5. Determine the number of reactions (N) to be prepared per assay and prepare Reaction Mix in a suitable pre-chilled tube (i.e. microcentrifuge tube or 5 mL tube) by following the order in the table below. Between each reagent, slowly mix by pipetting up and down 4 times without introducing bubbles and pulse centrifuge.

Reaction Mix	1 Rxn	100 Rxns	N Rxns
Molecular Biology Grade Water	1.5 µL	150 µL	N x 1.5 µL
20X Salt Mix	2.5 µL	250 µL	N x 2.5 µL
10X Extraction Buffer	5.0 µL	500 µL	N x 5.0 µL
50X IC/P Mix	1.0 µL	100 µL	N x 1.0 µL
2.5X Master Mix	20 µL	2000 µL	N x 20 µL
Total Volume	30 µL	3000 µL	N x 30 µL

6. Transfer 30 µL Reaction Mix per well into an appropriate, pre-chilled, 96-well plate. If there is a delay before adding specimen, this plate should be kept cold (i.e. by using a 96-well PCR plate block, an IsoFreeze PCR rack and/or by storing the plate at 4 °C) and temporarily sealed.
7. Transfer 20 µL, per well, of sample prepared in Step 3 and the external controls prepared in Step 4 to the 96-well plate from Step 6 and mix by slowly pipetting up and down 3 times without introducing bubbles. Seal the 96-well plate using an appropriate optically clear adhesive and centrifuge the plate at 2000 rpm for 5 seconds to collect contents at bottom of plate.
8. Place the 96-well plate in a validated thermocycler and follow instrument specific protocols and analysis procedures detailed below.

9.5 Complete Instructions for Specimen Processing in a Deepwell Format

The following instructions provide an example for the processing of dry swab specimens, using a deepwell plate, up to the final setup of the sample plate. The following language assumes 94 dry swab specimens and two external controls will be processed in which the entirety of the reagents provided in the LumiraDx SARS CoV-2 RNA STAR Complete kit will be consumed.

NOTE: It is recommended to read through the following instructions before attempting to set up the Reaction Mix. All components, including the assembled Reaction Mix, should be thawed and kept on a cold block equilibrated to 4 °C to maintain the integrity of the reagents. Additionally, it is recommended prior to performing the instructions below to setup the validated thermocycler (i.e. Roche LC 480 II, ABI 7500 Fast Dx, ABI QS 5, ABI QS 7 Flex, Agilent AriaMx, or the Agilent Stratagene Mx3005P) to the step just before starting the instrument to ensure the performance of this Test is maintained.

1. Thaw the LumiraDx SARS-CoV-2 RNA STAR Complete kit components in a cold block equilibrated to 4 °C; i.e. LumiraDx SARS CoV-2 RNA STAR Complete Positive Control Media (Pos. Ctrl. Med.), LumiraDx SARS CoV-2 RNA STAR Complete Negative Control Media (Neg. Ctrl. Med.), LumiraDx SARS CoV-2 RNA STAR Complete 20X Salt Mix, LumiraDx SARS CoV-2 RNA STAR Complete 10X Extraction Buffer, LumiraDx SARS CoV-2 RNA STAR Complete 50X Internal Control & Primer Mix (50X IC/P Mix), and LumiraDx SARS CoV-2 RNA STAR Complete 2.5X Master Mix. Once, thawed, invert each tube to mix then pulse centrifuge for 5 seconds to collect reagents at the bottom of the tube (do not vortex samples).
2. Pour 100 mL of a compatible transport media (VTM, 0.85% Saline, or PBS) into a suitable reagent reservoir. Transfer one (1) mL to each deepwell using a multichannel pipette. Leave two designated wells, A1 and A12, empty for the external controls assembled in Step 4.
3. Add the material from a single dry swab specimen to each deepwell in use. Soak the swab for 30 seconds then swirl thoroughly by rotating the swab against the side of the deepwell 5 times (beware of cross-contamination from splashing). Discard the swab in biohazard waste.
4. Assemble the 1x PCM (Positive Control Media) by diluting 500 µL Pos. Ctrl. Med. with 500 µL Neg. Ctrl. Med. in a pre-chilled microcentrifuge tube. Assemble the 1x NCM (Negative Control Media) by transferring 1 mL Neg. Ctrl. Med. into a pre-chilled microcentrifuge tube. Alternatively, the PCM and NCM can be prepared directly in the deepwell in which position A1 should be loaded with the NCM and position A12 should be loaded with the PCM.
5. Assuming 96 reactions are needed, prepare Reaction Mix in a pre-chilled 5mL tube by following the order in the table below. Between each reagent, slowly mix by pipetting up and down 4 times without introducing bubbles.

Reaction Mix	100 Rxns
Molecular Biology Grade Water	150µL
20X Salt Mix	250µL
10X Extraction Buffer	500µL
50X IC/P Mix	100µL
2.5X Master Mix	2x 1000µL
Total	3000µL

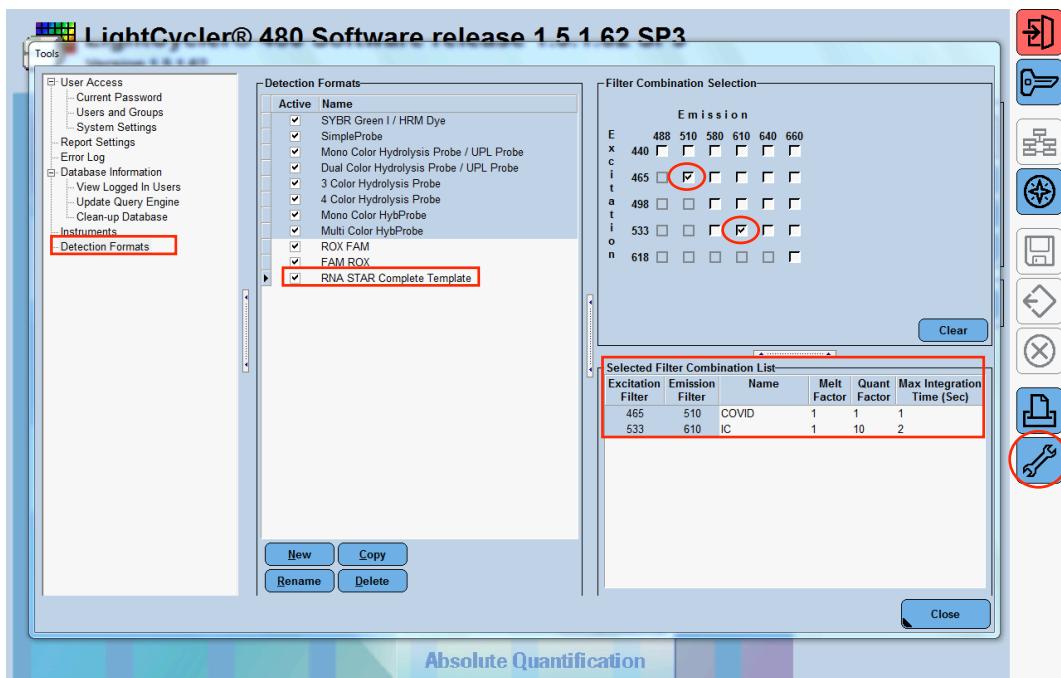
6. Transfer 3 mL Reaction Mix to a pre-chilled reagent reservoir (for minimal dead volume) using a single channel pipette. Then, using a multichannel pipette, transfer 30 µL Reaction Mix per well into an appropriate, pre-chilled, 96-well plate. If there is a delay before adding specimen, this plate should be kept cold (i.e. by using a 96-well PCR plate block, an IsoFreeze PCR rack and/or by storing the plate at 4 °C) and temporarily sealed. Additionally, if the number of reactions prepared is less than 96, consider preparing the sample plate without using a reagent reservoir and multichannel pipette. Excess Reaction Mix was prepared in the previous step to allow for pipetting error and dead volume which accounts for exactly 96 reactions to be prepared.
7. Transfer 20 µL, per well, of samples from the deepwell prepared in Step 3 and, if prepared separately, the external control tubes prepared in Step 4 to the 96-well plate from Step 6 and mix by slowly pipetting up and down 3 times without introducing bubbles. Seal the 96-well plate using an appropriate optically clear adhesive and centrifuge the plate at 2000 rpm for 5 seconds to collect contents at bottom of plate.
8. Place the 96-well plate in a validated thermocycler and follow instrument specific protocols and analysis procedures detailed below.

10 RNA STAR Complete Setup for Roche™ LightCycler 480 II

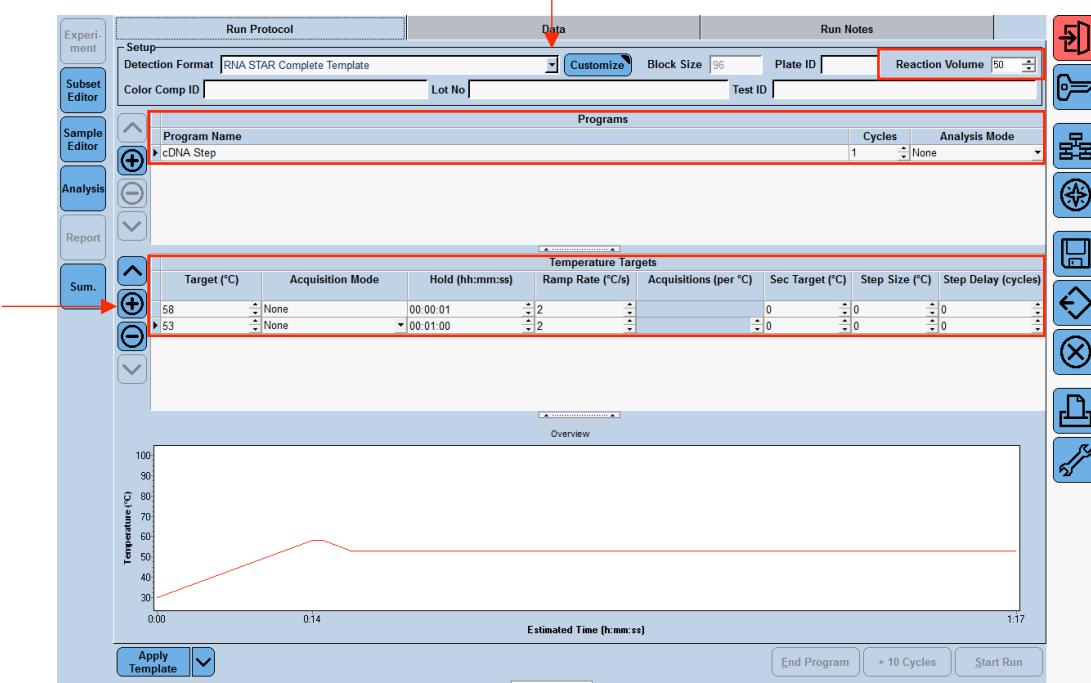
Refer to "User Manual Part Number 05152062001 0208" for additional information.

10.1 Programming the Run Template and Sample Template

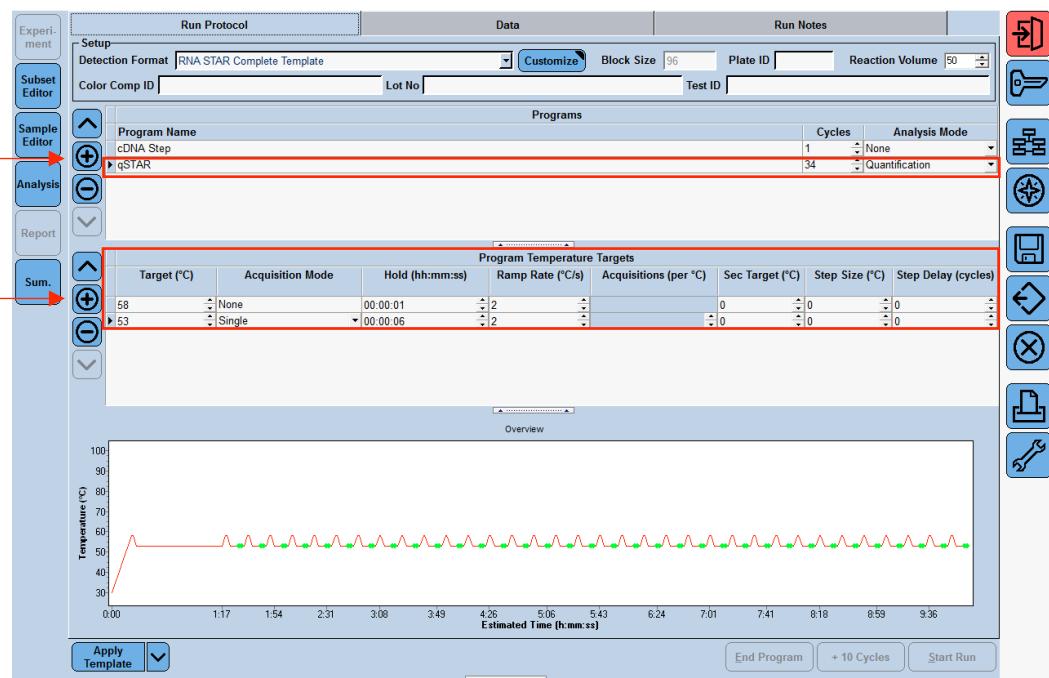
1. Launch the LightCycler (LC) 480 desktop software (version SW 1.5.1). If prompted, 'Log On' to the 'Traceable Database'.



2. Click the 'Tools' button in the startup screen in the lower right of the software. Select 'Detection Formats' and click 'New' in the 'Tools' window that opens. Name the new 'Detection Formats' as "RNA STAR Complete Template". In the 'Filter Combination Selection' section select '465-510' and '533-610'. In the 'Selected Filter' 'Combination List' section under 'Name' type in 'COVID' for '465-510' and type 'IC' for '533-610'. Verify the 'Melt Factor' for '465-510' is set to '1' and for '533-610' is set to '1', the 'Quantification Factor' for '465-510' is set to '1' and for '533-610' is set to '10', and the 'Max Integration Time' for '465-510' is set to '1' and for '533-610' is set to '2'. Click 'Close' to exit the 'Tools' window.
3. To access this newly created 'Detection Format', use the 'Exit the application' button and reload the LightCycler (LC) 480 software (version SW 1.5.1). After closing and reloading the software, in the 'Experiment Creation' section, click either 'White' or 'Clear Plates' and click 'New Experiment'.



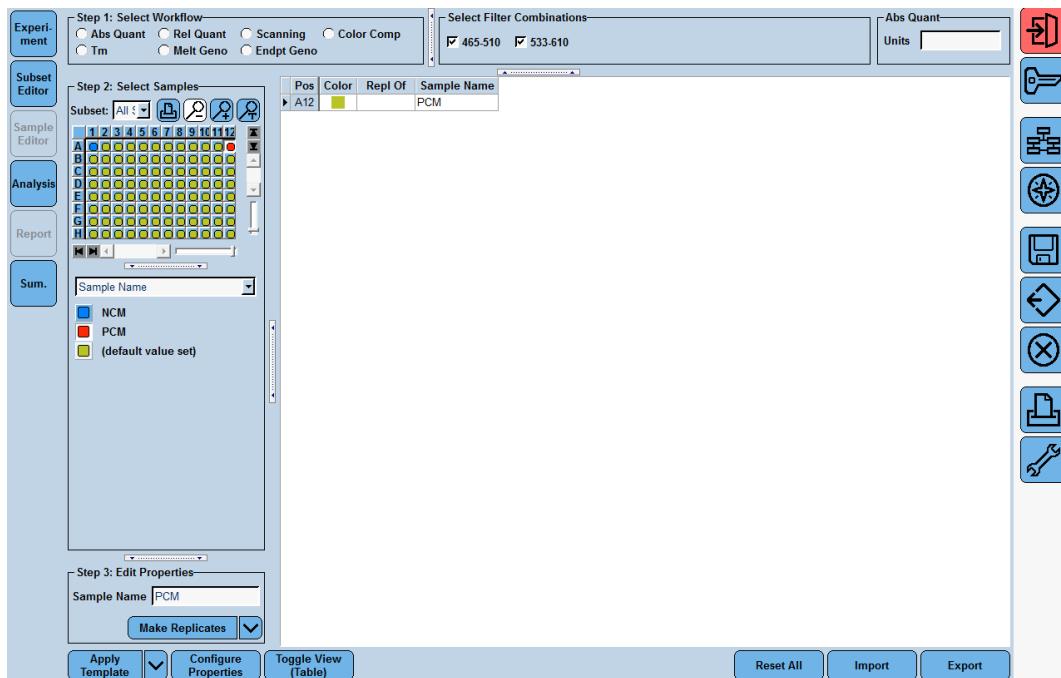
4. Select from the 'Detection Format' drop-down menu "RNA STAR Complete Template". Set 'Reaction Volume' to '50' in the upper right of the software. In the 'Program Name' section type "cDNA Step", set 'Cycles' to '1', and set 'Analysis Mode' to 'None'. Change cDNA Step 'Temperature Targets' as follows: set 'Target (°C)' to '58', set 'Acquisition Mode' to 'None', set 'Hold (hh:mm:ss)' to '0:00:01', set 'Ramp Rate (°C/s)' to '2', and set 'Sec Target (°C)', 'Step Size (°C)', and 'Step Delay (cycles)' to '0'. Select the "+" button on the lower left of the software to add another 'Temperature Target'. Set 'Target (°C)' to '53', set 'Acquisition Mode' to 'None', set 'Hold (hh:mm:ss)' to '0:01:00', set 'Ramp Rate (°C/s)' to '2', and set 'Sec Target (°C)', 'Step Size (°C)', and 'Step Delay (cycles)' to '0'.



5. Click the "+" button on the upper left of the software to add another 'Programs'. In the 'Program Name' section type "qSTAR", set 'Cycles' to '34', and set 'Analysis Mode' to 'Quantification'. Change 'qSTAR Temperature Targets' as follows: set 'Target (°C)' to '58', set 'Acquisition Mode' to 'None', set 'Hold (hh:mm:ss)' to '0:00:01', set 'Ramp Rate (°C/s)' to '2', and set 'Sec Target (°C)', 'Step Size (°C)', and 'Step Delay (cycles)' to '0'. Select the "+" button on the right of the software to add another Temperature Target. Set 'Target (°C)' to '53', set 'Acquisition Mode' to 'Single', set 'Hold (hh:mm:ss)' to '0:00:06', set 'Ramp Rate (°C/s)' to '2', and set 'Sec Target (°C)', 'Step Size (°C)', and 'Step Delay (cycles)' to '0'.

NOTE: The instrument will display a run time that might not be accurate as the instrument does not accurately account for the 1 sec cycle at 58 °C.

- In the lower left corner of the click select the pull-down menu next to the 'Apply Template' button. Select 'Save As Template'. Double-click the 'Templates' folder. Click the 'Run Templates' folder and 'Name' the file "RNA STAR Complete Template" and click the 'Check' button to exit the 'Save Template' window.



- Click the 'Sample Editor Tab' on the left side of the software. Click the 'A1' well location on the 96 well sample plate and in the 'Edit Properties' section define the 'Sample Name' as "NCM" (Negative Control Media). Push the 'Enter' button on the keyboard. Click the 'A12' well and define the 'Sample Name' as "PCM" (Positive Control Media). Push the 'Enter' button on the keyboard.
- In the lower left corner of the click select the pull-down menu next to the 'Apply Template' button. Select 'Save As Template'. Double-click the 'Templates' folder. Click the 'Sample Templates' folder and 'Name' the file "RNA STAR Complete Sample Template" and click the 'Check' button to exit the 'Save Template' window.
- Exit the software. If prompted, click 'No' to not save.

10.2 Programming Initial Run and Creating Analysis Template

It is recommended to setup the instrument up to step 4 before preparing the Reagent Mix as defined in the qSTAR Reagent Preparation section above. The 96-well plate should be recently centrifuged to guarantee all reagents are at the bottom of the plate and kept on a cold block. Use 96-well plates and seals recommended by the instrument manufacturer (see Option for PCR Instruments & Recommended Consumables table above).

Note: The Analysis Template is only established after the initial run has been completed.

- Launch the LightCycler (LC) 480 desktop software (version SW 1.5.1).
- Click 'New Experiment from Template' and in the 'Run Templates' drop-down menu, select 'RNA STAR Complete Template' then click the 'Check' button to exit the 'Create Experiments from Template' window. Proceed to the 'Sample Editor' tab on the left of the software.
- Select 'Apply Template' (do not select the drop-down menu next to the 'Apply Template' button) on the bottom left of the screen. Double-click the Templates folder then double-click the 'Sample Templates' folder and choose the 'RNA STAR Complete Sample Template'. Click the 'Check' button to exit the 'Apply Template' window. The NCM and PCM should be loaded into positions A1 and A12 of the plate. If not already selected, select all wells. Individual 'Sample Names' can be entered in the 'Step 3: Edit Properties' section if desired.
- On the LightCycler 480 II instrument, push the Load Plate "↔" button on the front of the instrument. The instrument drawer will automatically come out. Insert the 96-well plate onto the instrument drawer. Align the notch of the 96-well plate with the notch on the instrument drawer. Push the Load Plate "↔" button to close the plate holder.

5. In the LightCycler (LC) 480 desktop software proceed to the 'Experiment' tab on the upper left of the software. Click 'Start Run' on the bottom right of the software. A 'Plate Sensor if off' warning may appear. Click the 'Check' button to exit this warning. In the 'Save LightCycler 480 Experiment' window that opens, double-click the 'Experiments' folder and save under the 'Name' "RNA STAR Complete [YYMMDD_Plate#]" then click the 'Check' button to exit this window.
6. After the run has finished, proceed to the 'Analysis' tab on the left of the software. Choose 'Abs Quant/Fit Points' in the 'Create new analysis' section. Click the 'Check' button to exit the 'Create New Analysis' pop-up window.
7. Click the 'Background (2-6)' button on the software. Set 'Min Offset' to '0' and 'Max Offset' to '5' and click the 'Check' button to exit the 'Background Settings' window.
8. Confirm that 'Color Compensation' is '(Off)' for all analytes on the bottom of the software and confirm 'First Cycle' is set to '1' and 'Last Cycle' is set to '35'. Proceed to the 'Noise Band' tab at the upper middle of the software.
9. Select from the pull-down menu next to the 'Noiseband (Auto)' button at the bottom of the software select 'Noiseband (STD Mult)'. Click 'Filter Comb (456-510)' and select 'COVID'. Click the 'Check' button to exit the 'Filter Combination' window. Set 'STD Multiplier' to '10.0000'. Click 'Filter Comb (456-510)' and select 'IC'. Click the 'Check' button to exit the 'Filter Combination' window. Set 'STD Multiplier' to '10.0000'.
10. Save the new analysis protocol as a template for future use. In the lower left corner of the screen select the pull-down menu next to the 'Apply Template' button and click 'Save As Template'. Double-click the 'Templates' Folder. Select the 'Analysis Templates' Folder and 'Name' the file "RNA STAR Complete Analysis Template" and click the 'Check' button to exit the 'Save Template' window. Click 'Calculate' at the bottom of the software.
11. To create a report, select the 'Save' button on the right of the software. If prompted, define 'Enter the reason for the changes' as "Report" and click the 'Check' button to exit the window. Proceed to the 'Report' tab on the left of the software.
12. Select the box next to 'Results' in the 'General' tab under the 'Abs Quant/Fit Points' section. Click 'Generate' then click the 'Save' button on the right of the software. If prompted, define 'Enter the reason for the changes' as "Save" and click the 'Check' button to exit the window.
13. Remove the plate from the LightCycler 480 II instrument using the Load Plate " $\leftarrow\rightarrow$ " button on the front of the instrument.
14. Remove the plate and discard the plate in a sealable waste bag or container.

10.3 Using the Run / Sample / Analysis Template

It is recommended to setup the instrument up to step 3 before preparing the Reagent Mix as defined in the qSTAR Reagent Preparation section above. The 96-well plate should be recently centrifuged to guarantee all reagents are at the bottom of the plate and kept on a cold block. Use 96-well plates and seals recommended by the instrument manufacturer (see Option for PCR Instruments & Recommended Consumables table above).

1. Launch the LightCycler (LC) 480 desktop software (version SW 1.5.1).
2. Click 'New Experiment from Template' and in the 'Run Templates' drop-down menu select 'RNA STAR Complete Template' then click the 'Check' button to exit the 'Create Experiments from Template' window. Proceed to the 'Sample Editor' tab on the left of the software.
3. Select 'Apply Template' (do not select the drop-down menu next to the 'Apply Template' button) on the bottom left of the screen. Double-click the Templates folder then double-click the 'Sample Templates' folder and choose the 'RNA STAR Complete Sample Template'. Click the 'Check' button to exit the 'Apply Template' window. The NCM and PCM should be loaded into positions A1 and A12 of the plate. If not already selected, select all wells. Individual 'Sample Names' can be entered in the 'Step 3: Edit Properties' section if desired.
4. On the LightCycler 480 II instrument, push the Load Plate " $\leftarrow\rightarrow$ " button on the front of the instrument. The instrument drawer will automatically come out. Insert the 96-well plate onto the instrument drawer. Align the notch of the 96-well plate with the notch on the instrument drawer. Push the Load Plate " $\leftarrow\rightarrow$ " button to close the plate holder.
5. In the LightCycler (LC) 480 desktop software proceed to the 'Experiment' tab on the upper left of the software. Click 'Start Run' on the bottom right of the software. A 'Plate Sensor if off' warning may appear. Click the 'Check' button to exit this warning. In the 'Save LightCycler 480 Experiment' window that opens, double-click the 'Experiments' folder and save under the 'Name' "RNA STAR Complete [YYMMDD_Plate#]" then click the 'Check' button to exit this window.

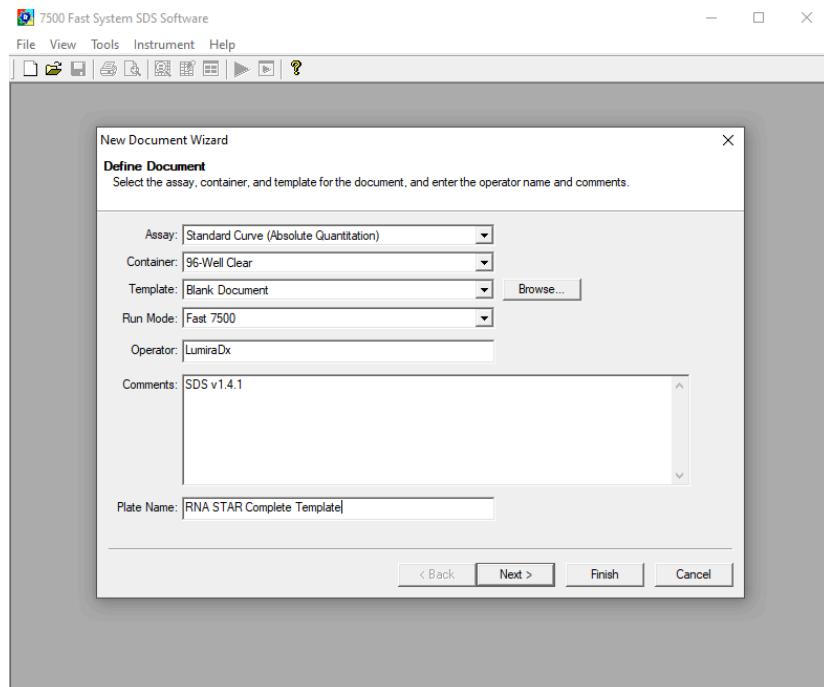
6. After the run has finished, proceed to the 'Analysis' tab on the left of the software. Choose 'Abs Quant/Fit Points' in the 'Create new analysis' section. Click the 'Check' button to exit the 'Create New Analysis' pop-up window.
7. In the lower left corner click 'Apply Template' (do not select the drop-down menu next to the 'Apply Template' button). Double-click the 'Templates' folder. Double click the 'Analysis Templates' folder and select the 'RNA STAR Complete Analysis Template'. Click the 'Check' button to exit the window. An "Apply template to the active analysis" message may appear. Click 'Yes' to proceed.
8. Click 'Calculate' then create a report by selecting the 'Save' button on the right of the software. If prompted, define 'Enter the reason for the changes' as "Report" and click the 'Check' button to exit the window. Proceed to the 'Report' tab on the left of the software.
9. Select the box next to 'Results' in the 'General' tab under the 'Abs Quant/Fit Points' section. Click 'Generate' then click the 'Save' button on the right of the software. If prompted, define 'Enter the reason for the changes' as "Save" and click the 'Check' button to exit the window.
10. Remove the plate from the LightCycler 480 II instrument using the Load Plate " $\leftarrow\rightarrow$ " button on the front of the instrument.
11. Remove the plate and discard the plate in a sealable waste bag or container.

11 RNA STAR Complete Setup for Applied Biosystems™ 7500 Fast Dx

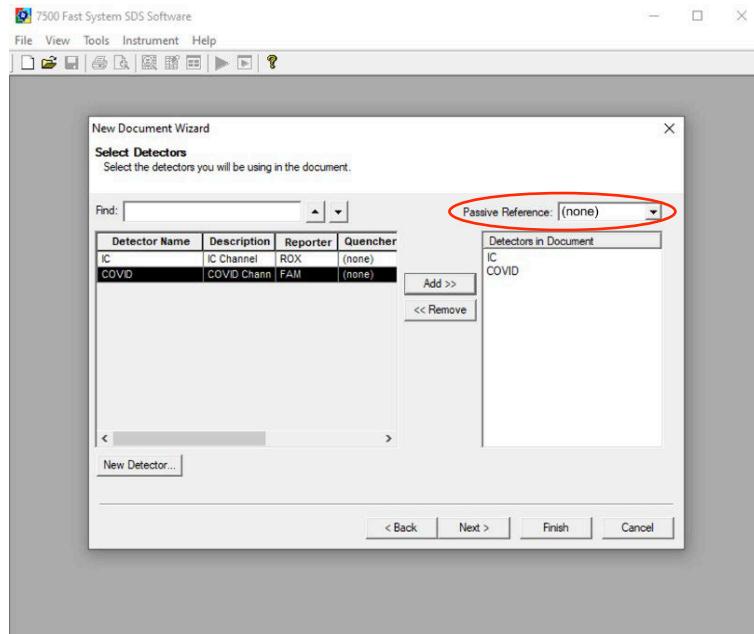
Refer to "User Manual Part Number 4406991" for additional information.

11.1 Programming the Run Template

1. Launch the 7500 Fast System desktop software (version 1.4.1).

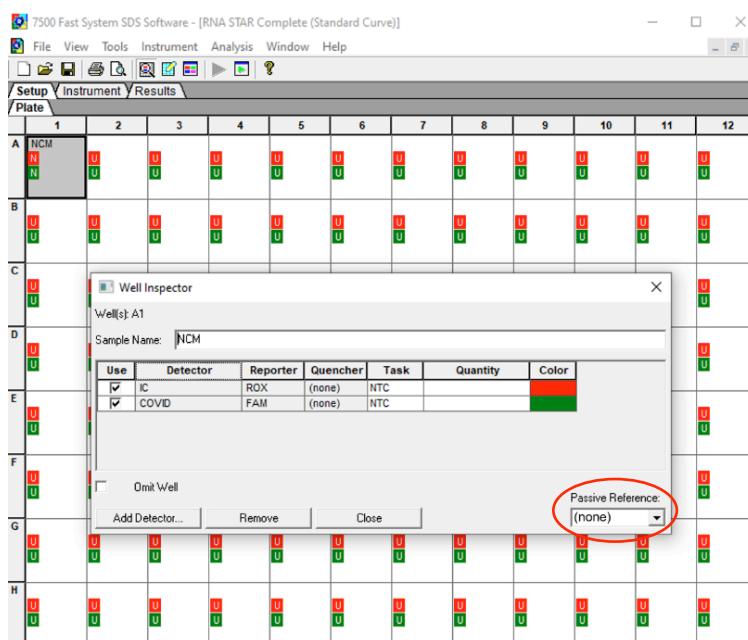


2. The 'Quick Startup document' window will open. Click 'Create New Document' to start the 'New Document Wizard'. In the 'Define Document' window verify the default settings: 'Assay' is set to 'Standard Curve (Absolute Quantification)', 'Container' is set to '96-Well Clear', 'Template' is set to 'Blank Document', 'Run Mode' is set to 'Fast 7500', 'Operator' is defined as "[your operator name]", 'Comments' is set to 'SDS v1.4.1' and 'Plate Name' is defined as "RNA STAR Complete Template". Click 'Next'.



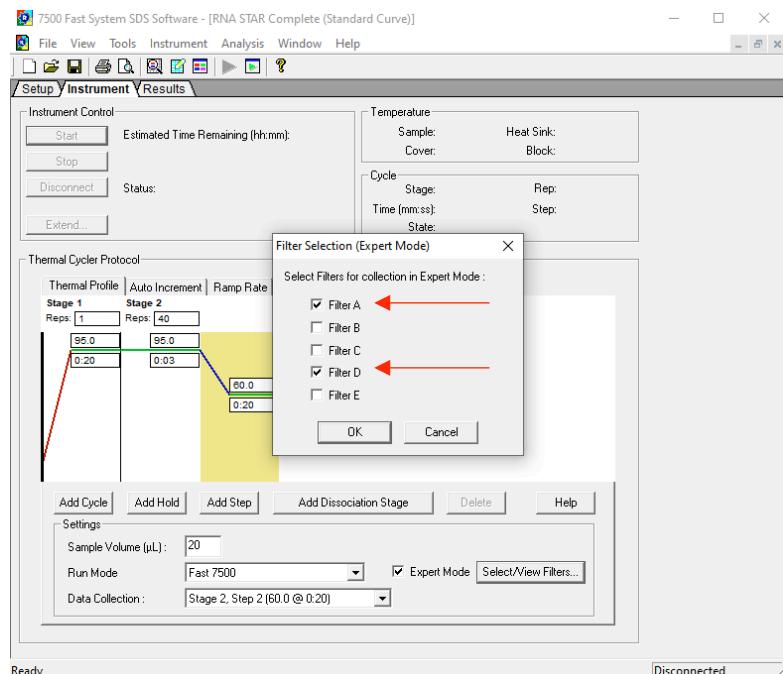
- In the 'Select Detectors' window, click 'New Detector' to open the 'New Detector' pop-up window and define these settings: define 'Name' as "COVID", define 'Description' to "COVID Channel", set 'Reporter' dye to 'FAM', set 'Quencher' to '(none)', and set 'Color' to 'Green'. Select 'OK'. Click 'New Detector' again to open the 'New Detector' pop-up window and define these settings: set 'Name' to "IC", define 'Description' to "IC Channel", set 'Reporter' dye to 'ROX', set 'Quencher' to '(none)', and set 'Color' to 'Red'. Click 'OK'. Highlight each detector (COVID & IC) and click the 'Add >' button to add the detectors to the 'Detectors in Document' column. From the 'Passive Reference' drop-down menu, select '(none)'. Click 'Next'.

NOTE: It is important to make sure the 'Passive Reference' drop-down menu has '(none)' selected. If 'ROX' is not removed as a 'Passive Reference' dye the IC channel will be impacted and the run must be repeated.

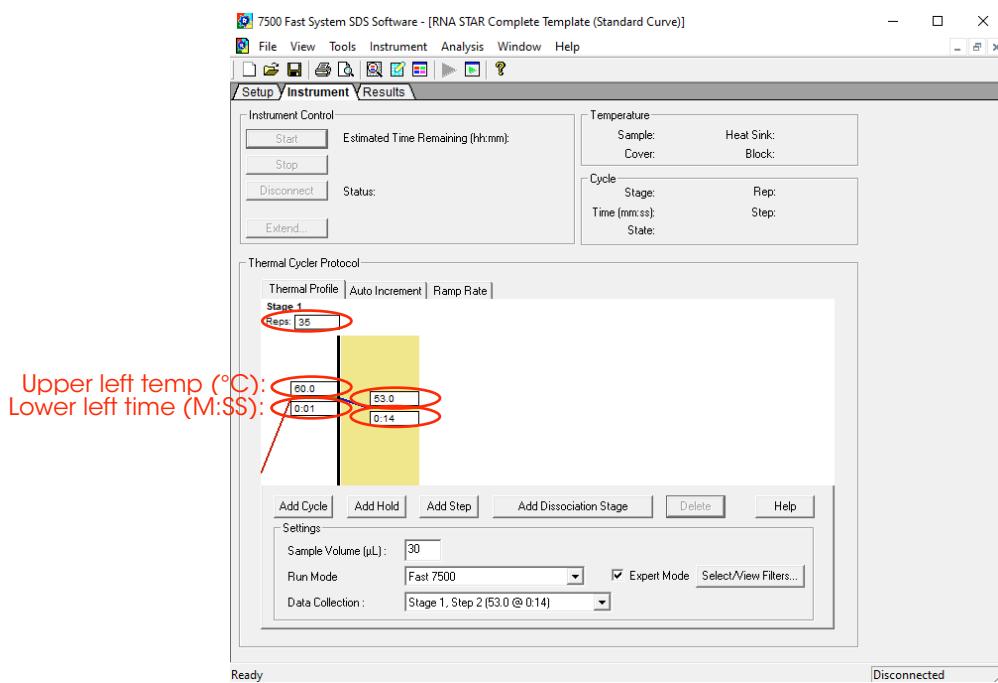


- In the 'Set Up Sample Plate' window click the gray box between "A" and "1" to select all wells then select boxes under the 'Use' section to add the COVID and IC Detector to all wells. Click 'Finish'. The wizard will close, and a screen will display the 'Setup' tab and 'Plate' tab. This will show the sample plate that was set up during the quick start. For the initial set up, select location 'A1', right click and select 'Well Inspector'. In the 'Well Inspector' window, define the 'Sample Name' as "NCM" (Negative Control Media). Select the 'COVID' Detector line, under 'Task' select from the drop-down menu 'NTC'. Select the 'IC' Detector line, under 'Task' select from the drop-down menu 'NTC'. Verify the 'Passive Reference' is set to '(none)'. Click 'Close'.

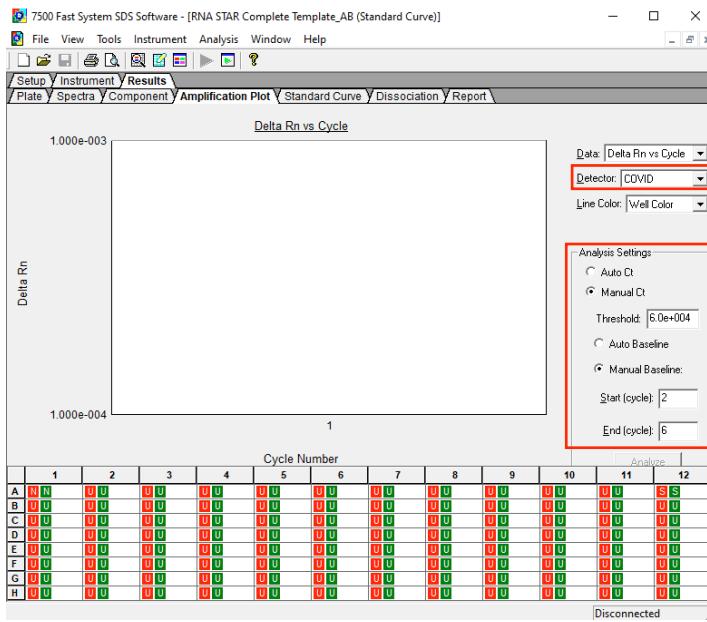
5. Select location 'A12', right click and select 'Well Inspector'. In the 'Well Inspector' window, define the 'Sample Name' as "PCM" (Positive Control Media). Select the 'COVID' Detector line, under 'Task' select from the drop-down menu 'Standard'. Select the 'IC' Detector line, under 'Task' select from the drop-down menu 'Standard'. Verify the 'Passive Reference' is set to '(none)'. Click 'Close'.



6. Select the 'Instrument' tab (do not select 'Instrument' from the menu), check the box next to 'Expert Mode' then click 'Select/View Filters...' and check only the boxes for 'Filter A' and 'Filter D'. Click 'OK'.



7. Modify the default thermal profile as follows: Delete 'Stage 1' by clicking on the left of the stage and dragging the cursor to the right then click 'Delete'. Modify the default thermal profile for 'Stage 1' as follows: set 'Reps' to '35', set 'Temp' (upper left) to '60.0', set 'Time' (lower left) to '0:01', set 'Temp' (upper right) to '53.0', and set 'Time' (lower right) to '0:14'. Under the 'Settings' section perform the following: define 'Sample Volume (µL)' as "30", confirm 'Run Mode' is set to '7500 Fast' (default), and confirm 'Data Collection' is set to 'Stage 1, Step 2(53.0 @ 0:14)'. Proceed to the 'Results' tab to set thresholds for each analyte.

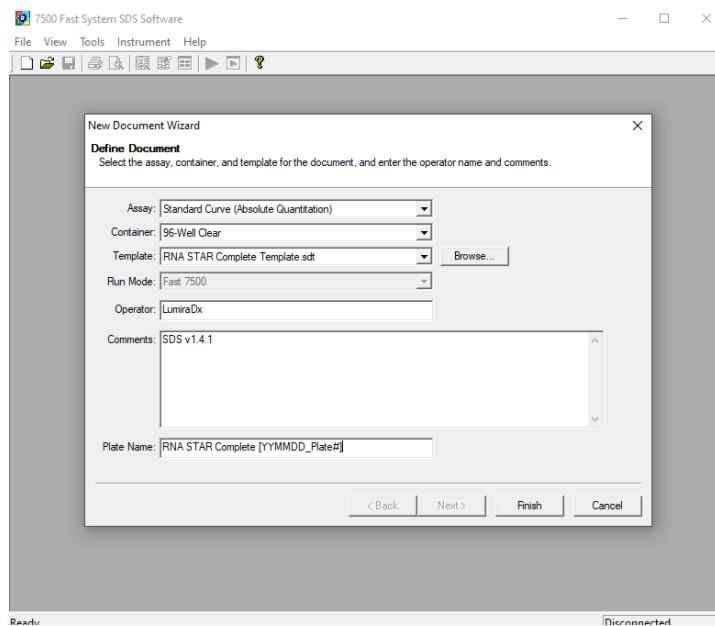


8. Select the 'Amplification Plot' tab. From the 'Detector' drop-down menu in the top right corner, select 'COVID'. In the 'Analysis Settings' window, select the 'Manual Ct' circle and define 'Threshold' as "6.0e+004". In the 'Manual Baseline' window set the 'Start (cycle)' to '2' and set the 'End (cycle)' to '6'.
9. From the 'Detector' drop-down menu again, select 'IC' from the 'Detector'. In the 'Analysis Settings' section, select the 'Manual Ct' circle and define 'Threshold' as "1.2e+004". In the 'Manual Baseline' window set 'Start (cycle)' to '2' and set 'End (cycle)' to '6'.
10. Select 'File' from the menu then click 'Save As'. Save the template in file path "D:\Applied Biosystems\7500 Fast System\Template\" and hit the 'Enter' key to confirm file path. 'Name' the file as "RNA STAR Complete Template". Change 'File Type' to 'SDS Templates (*.sdt)'. Click 'Save'.
11. Exit the software. A prompt may appear to 'Save Changes to Plate 1'. Click 'No'.

11.2 Programming Runs Using the Run Template

If the instrument has been idle, performing a run using a blank plate may be needed to ensure the plate lid starts the run heated. It is recommended to setup the instrument up to step 4 before preparing the Reagent Mix as defined in the qSTAR Reagent Preparation section above. The 96-well plate should be recently centrifuged to guarantee all reagents are at the bottom of the plate and kept on a cold block. Use 96-well plates and seals recommended by the instrument manufacturer (see Option for PCR Instruments & Recommended Consumables table above).

1. Launch the 7500 Fast System desktop software (version 21 CFR Part II Software).



2. The 'Quick Startup document' dialog window will open. Click 'Create New Document'. In the 'Define Document' window verify the default settings: 'Assay' is set to 'Standard Curve (Absolute Quantification)', 'Container' is set to '96-Well Clear', 'Template' is set to 'RNA STAR Complete Template', 'Run Mode' is set to 'Fast 7500', 'Operator' is defined as "[your operator name]", 'Comments' is set to 'SDS v1.4.1' and 'Plate Name' is defined as "RNA STAR Complete [YYMMDD_Plate#]". Click 'Finish'. The wizard will close, and a screen will display the 'Setup' tab and 'Plate' tab.
3. Select all wells that will contain sample, right-click and select 'Well Inspector' from the drop-down menu. Verify the detectors for COVID and IC are selected. Use the 'Well Inspector' window to enter the sample names if desired (optional). Click 'Close'.
4. Select 'File' from the menu and click 'Save'. If prompted, confirm the file 'Name' is "RNA STAR Complete [YYMMDD_Plate#]". Confirm 'File Type' is set to 'SDS Document (*.sds)' and click 'OK'. A window will open asking for the "Reason for change of entry". Type "Run Setup" and select 'OK'. Proceed to the 'Instrument' tab.
5. Push circular button on the front of the Applied Biosystems 7500 Fast Dx instrument. Pull out the instrument drawer and load the plate. Make sure to align A1 on the 96-well plate with the defined A1 position in the instrument drawer. Close the instrument drawer by firmly pressing the circular button till drawer is closed.
6. In the 7500 Fast System desktop software (version 21 CFR Part II Software) click 'Start' to initiate the run in the 'Instrument Control' section.
7. Upon completion of the run, click 'OK' on the 'SDS Software' pop-up window. Go to the 'Results' tab then 'Amplification Plot' to then click 'Analyze'. Alternatively, type "Ctrl G" button on the keyboard.
8. 'Save' the file or type "Ctrl S" on the keyboard. A window will open asking for the "Reason for the change". Type "Data Analysis" and click 'OK'.
9. Click the 'Report' tab then select the whole plate and create report as desired. Remove the plate and discard the plate in a sealable waste bag or container.

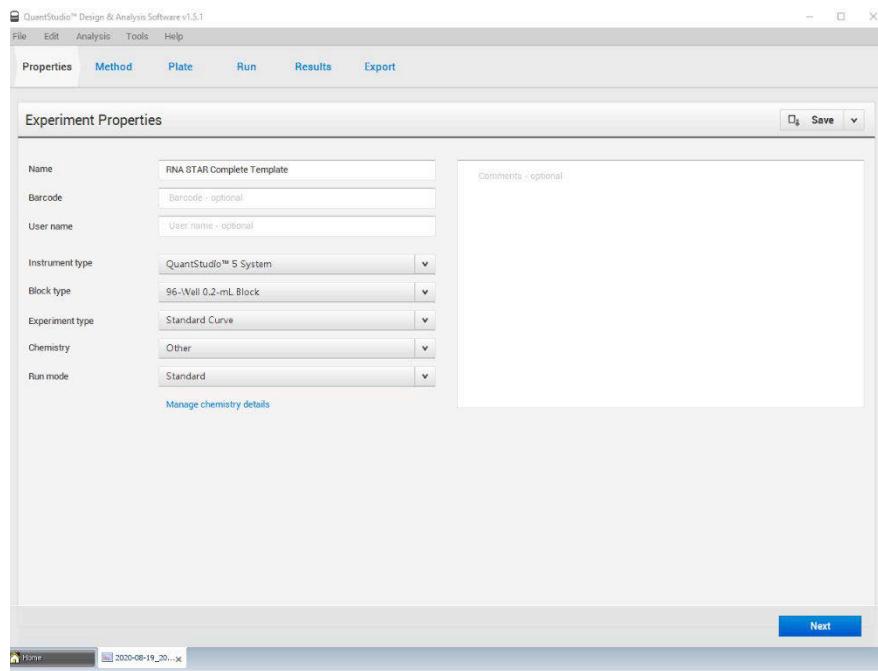
12 RNA STAR Complete Setup for Applied Biosystems™ QuantStudio 5

Refer to "User Manual Part Number MAN0010407" for additional information.

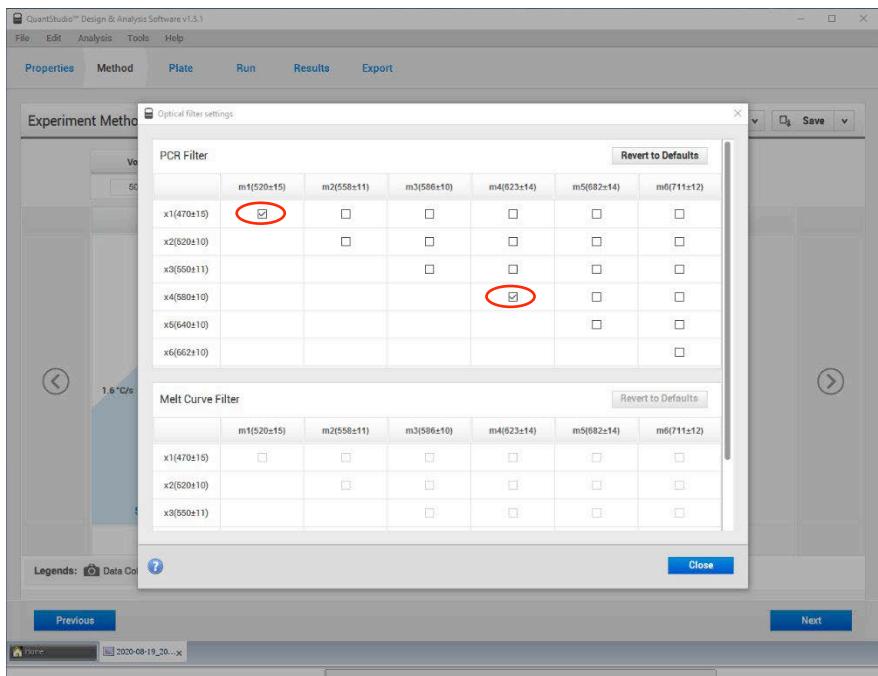
12.1 Programming Instructions for PC/Laptop-connected Instrument

It is recommended to setup the instrument up to step 9 before preparing the Reagent Mix as defined in the qSTAR Reagent Preparation section above. The 96-well plate should be recently centrifuged to guarantee all reagents are at the bottom of the plate and kept on a cold block. Use 96-well plates and seals recommended by the instrument manufacturer (see Option for PCR Instruments & Recommended Consumables table above).

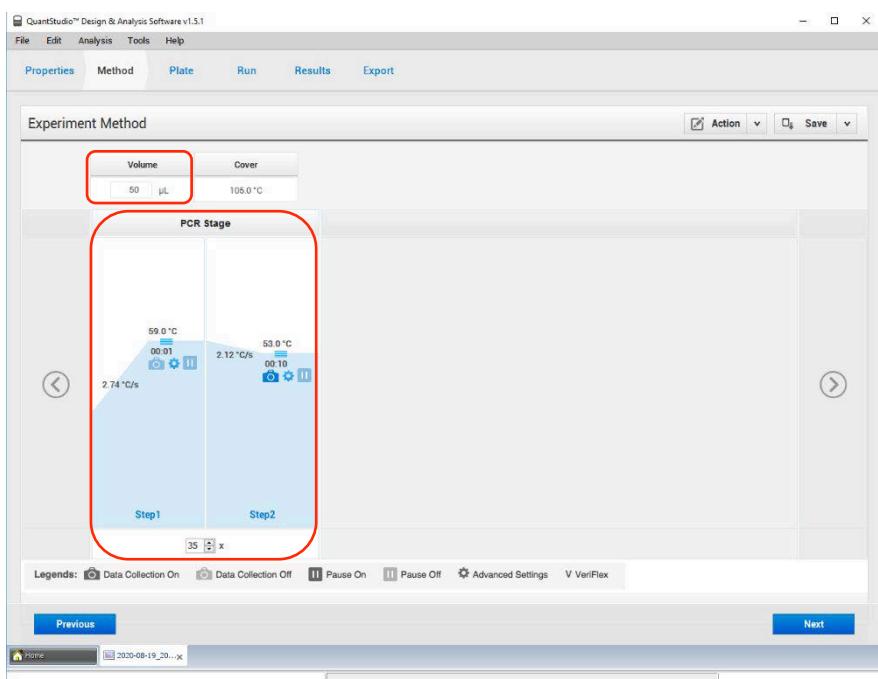
1. If the instrument touchscreen is in sleep mode (dark screen), touch the screen anywhere to activate the instrument. Sleep mode lowers the temperature of the heated cover. It is important that the heated cover idle at 105°C before loading a 96-well plate.



2. Launch the 'QuantStudio Design & Analysis' Desktop Software (version v1.5.1) and select 'Create New Experiment'. A window will open with the 'Properties' tab selected.
3. Enter the experiment properties as follows: name the experiment "RNA STAR Complete Template", set 'Instrument type' to 'QuantStudio 5 System', set 'Block type' to '96-Well 0.2mL Block', set 'Experiment type' to 'Standard Curve', set 'Chemistry' to 'Other', and set 'Run mode' to 'Standard'. Proceed to the 'Method' tab.

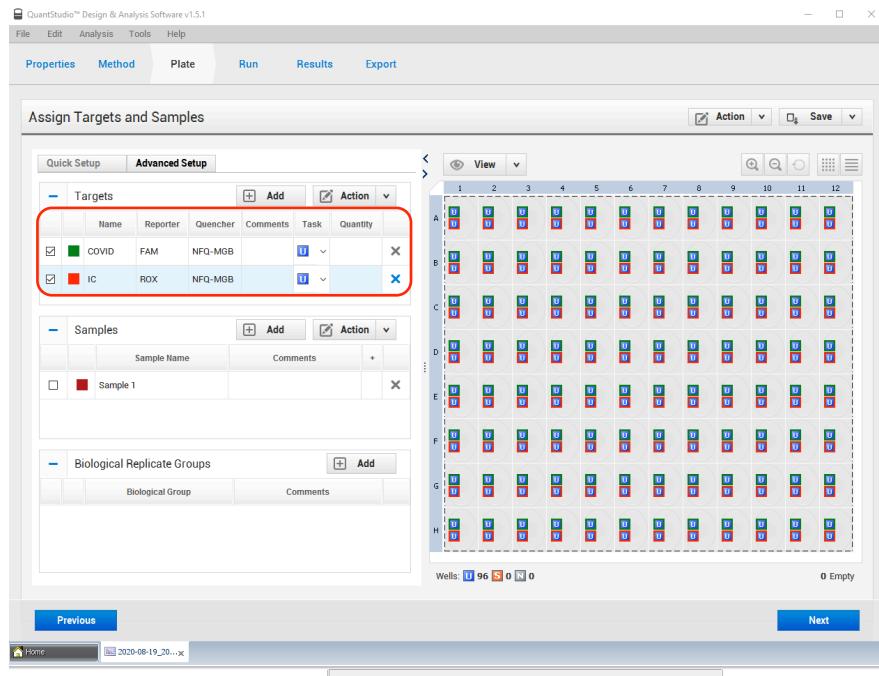


4. Click 'Action' and select 'Optical Filter Settings' from the drop-down menu. In the 'PCR Filter' table, uncheck all filter combinations except the following:
x1(470±15), m1(520±15), x4(580±10), m4(623±14)



5. Click 'Close' to return to the 'Method' tab. Set the 'Volume' to '50 μL'. Select the 'Hold Stage' and delete both steps by clicking the “-” button so no 'Hold Stage' segments remain. In the 'PCR Stage', change 'Step 1' settings as follows: set ramp rate to 2.74°C/s (default ramp rate), set temp to 59°C, and set time to 00:01. Change 'Step 2' settings as follows: set ramp rate to 2.12°C/s (default ramp rate), set temp to 53°C, and set time to 00:10 (data collection step). At the bottom of window, set 'Cycles' to '35'. Then proceed to the 'Plate' tab.

6. Change 'Passive Reference' to 'None' in the 'Plate Attributes' window. Select all the wells from the plate layout then proceed to the 'Advanced Setup' tab in the 'Assign Targets and Samples' section.



7. Under the 'Targets' section, rename 'Target 1' to 'COVID', change the default color to green, select 'FAM' as the 'Reporter' (the 'Quencher' is automatically entered as 'NFQ-MGB'), and checkmark the empty box to the left of the colored box to apply it to the wells. 'Add' a second target then rename to 'IC', change the default color to red, select 'ROX' as the 'Reporter' (the 'Quencher' is automatically entered as 'NFQ-MGB'), and checkmark the empty box to the left of the colored box to apply it to the wells.
8. (Optional) Under the 'Samples' section, the 'Sample Name' can be added individually or pasted into the 96-well plate layout from an Excel file. Then proceed to the 'Run' tab.
9. Save the experiment as a template for subsequent runs by clicking the 'Save' down arrow and selecting the 'Save As' option. Name the template "RNA STAR Complete Template" and click 'Save'.
10. Load the plate by pressing the 'Arrow' icon on the QuantStudio 5 instrument touchscreen at the top right of the window. Place the sample plate on the amplification block that automatically comes out. Make sure to align A1 on the 96-well plate with the defined A1 position on the instrument drawer. Press the 'Arrow' icon on the instrument touchscreen to close the drawer.
11. Return to the desktop software 'Run' tab and click the 'START RUN' button. When instrument connection is made, the instrument number will appear in a drop-down menu below the 'START RUN' button. Select the instrument number to initiate the run. Change the 'File Name' to "LumiraDx SARS-CoV-2 RNA STAR [YYMMDD_Plate#]" and click 'Save'.
12. Following the run, the instrument touchscreen indicates when the run is 'Complete'. Close the run screen by pressing 'Done'. Remove the plate and discard the plate in a sealable waste bag or container.

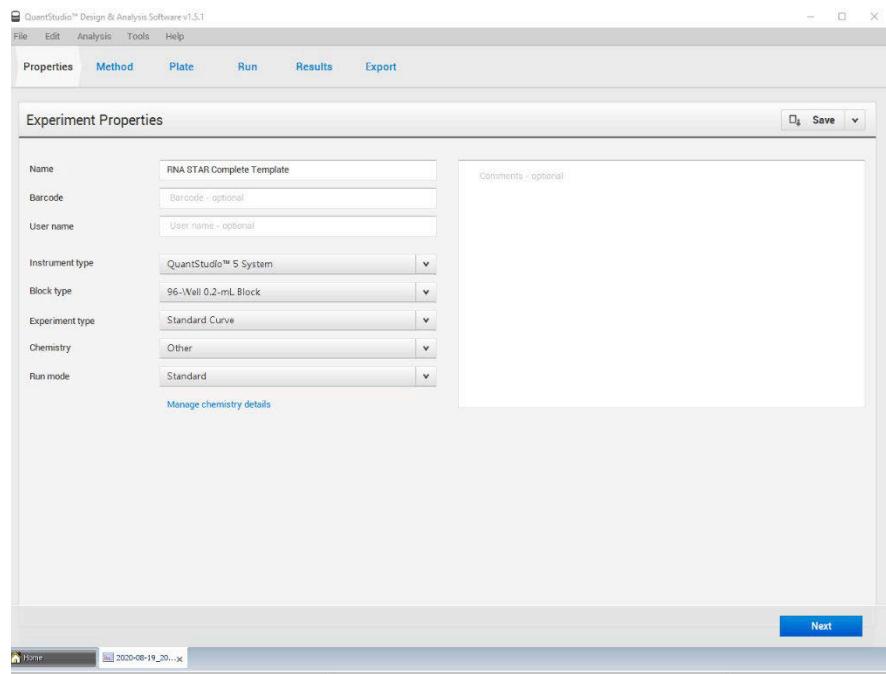
12.2 Analysis Instructions

1. Modify the analysis settings using the 'Results' tab in the 'QuantStudio Design & Analysis' Desktop Software (version v1.5.1). Select the desired wells for analysis in the Plate Layout then click on the Eye symbol above the 'Amplification Plot' to configure the plot as follows: set 'Plot Type' to ' ΔRn vs Cycle', set 'Graph Type' to 'Log', and set 'Plot Color' to 'Target'. All other content remains unchanged. Click out of the window to accept changes.
2. Click the 'Gear' icon to the right of the 'Analyze' button in the top left of the software to open the 'Analysis Settings' window. In the 'Ct settings' tab, uncheck the 'Default settings' box under the 'Ct settings for COVID' section, uncheck the 'Automatic Threshold' box, and uncheck the 'Automatic Baseline' box. Enter "25,000" for the 'Threshold', set 'Baseline Start Cycle' to '4' and set 'End Cycle' to '8'. Select the 'IC' line then uncheck the 'Default settings' box, the 'Automatic Threshold' box, and the 'Automatic Baseline' box. Enter "20,000" for the 'Threshold', set 'Baseline Start Cycle' to '4' and set 'End Cycle' to '8'. Click 'Apply' to proceed the 'Export' tab.
3. Define the 'File Name' for the exported file ("RNA STAR Complete [YYMMDD_Plate#]"). Define the 'File name', choose the 'File Type' (default is QuantStudio as an .xls file), choose the 'Location' to export the file, and choose the 'Content' to be exported (the 'Results' box needs to be checked for Ct values). Click 'Customize' to proceed to what is to be exported within each Content item.
4. Select the 'All Fields' box to uncheck all content then check the boxes for the following content: 'Well', 'Well Position', 'Sample Name', 'Target Name', 'Task', 'Reporter', 'Quencher', and 'CT'. Click 'Close' to return to the 'Export' tab screen.
5. Click 'Save' to save the modified settings.
6. Click the 'Export' button to generate the export data file. The exported file will include a 'Results' section which contains the sample Ct values.
7. Close the software.

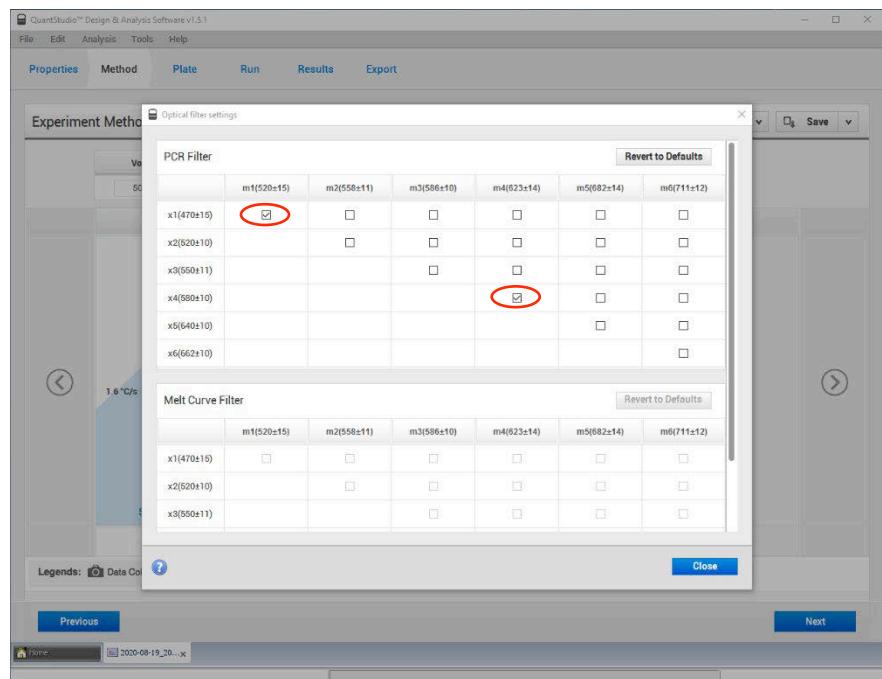
12.3 Programming Instructions for Stand-Alone Instrument

It is recommended to setup the instrument up to step 12 before preparing the Reagent Mix as defined in the qSTAR Reagent Preparation section above. The 96-well plate should be recently centrifuged to guarantee all reagents are at the bottom of the plate and kept on a cold block. Use 96-well plates and seals recommended by the instrument manufacturer (see Option for PCR Instruments & Recommended Consumables table above).

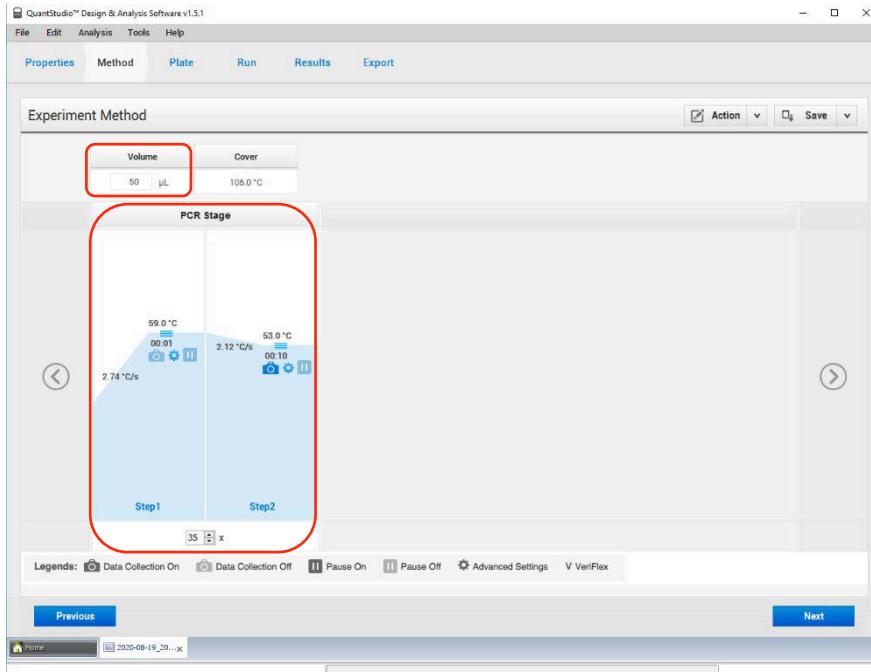
1. If the instrument touchscreen is in sleep mode (dark screen), touch the screen anywhere to activate the instrument. Sleep mode lowers the temperature of the heated cover. It is important that the heated cover idle at 105°C before loading a 96-well plate.



2. An experiment template is required for the QuantStudio 5 instrument when using the touch screen. Create the template by launching the 'QuantStudio Design & Analysis' Desktop Software (version v1.5.1). Select 'Create New Experiment'. A window will open with the 'Properties' tab selected.
3. Enter the experiment properties as follows: name the experiment "RNA STAR Complete Template", set 'Instrument type' to 'QuantStudio 5 System', set 'Block type' to '96-Well 0.2mL Block', set 'Experiment type' to 'Standard Curve', set 'Chemistry' to 'Other', and set 'Run mode' to 'Standard'.



4. Proceed to the 'Method' tab. Click 'Action' and select 'Optical Filter Settings' from the drop-down menu. In the 'PCR Filter' table, uncheck all filter combinations except the following: x1(470±15), m1(520±15), x4(580±10), m4(623±14)



5. Click 'Close' to return to the 'Method' tab. Set the 'Volume' to '50 μ L'. Select the 'Hold Stage' and delete both steps by clicking the "-" button so no 'Hold Stage' segments remain. In the 'PCR Stage', change 'Step 1' settings as follows: set ramp rate to 2.74°C/s (default ramp rate), set temp to 59°C, and set time to 00:01. Change 'Step 2' settings as follows: set ramp rate to 2.12°C/s (default ramp rate), set temp to 53°C, and set time to 00:10 (data collection step). At the bottom of window, set 'Cycles' to '35'. Then proceed to the 'Plate' tab.
6. Change 'Passive Reference' to 'None' in the 'Plate Attributes' window. Select all the wells from the plate layout then proceed to the 'Advanced Setup' tab in the 'Assign Targets and Samples' section.
7. Under the 'Targets' section, rename 'Target 1' to "COVID", change the default color to green, select 'FAM' as the 'Reporter' (the 'Quencher' is automatically entered as 'NFQ-MGB'), and checkmark the empty box to the left of the colored box to apply it to the wells. 'Add' a second target then rename to "IC", change the default color to red, select 'ROX' as the 'Reporter' (the 'Quencher' is automatically entered as 'NFQ-MGB'), and checkmark the empty box to the left of the colored box to apply it to the wells.
8. (Optional) Under the 'Samples' section, the 'Sample Name' can be added individually or pasted into the 96-well plate layout from an Excel file. Then proceed to the 'Run' tab.
9. Save the experiment as a template for subsequent runs by clicking the 'Save' down arrow and selecting the 'Save As' option. Name the template "RNA STAR Complete Template" and click 'Save'.
10. Copy the template (.edt) file to a USB drive.
11. Insert the USB drive into the designated slot in the front of the QuantStudio 5 instrument. On the instrument touchscreen, press 'Load Experiment'. Select 'USB' from the options provided, scroll to locate the saved template (.edt) file and select the file to import it.
12. Change the 'Run File Name' to "RNA STAR Complete [YYMMDD_Plate#]" then press 'Start Run'.
13. An alert will prompt the user to insert the sample plate. Press 'Open Drawer' to open instrument drawer, place the sample plate on the amplification block. Make sure to align A1 on the 96-well plate with the defined A1 position on the instrument drawer. Press 'Close Drawer' to close the instrument drawer and press 'Start Run'.
14. Following the run, the instrument touchscreen indicates when the run is 'Complete'. Press 'Transfer file' to export the file to the USB drive and remove the USB drive from the instrument.
15. Open the instrument drawer to remove the plate by pressing the arrow icon at the top right of the instrument touch screen. The instrument drawer will open.
16. Remove the plate and discard the plate in a sealable waste bag or container.

12.4 Analysis Instructions

1. Launch the QuantStudio Design & Analysis Desktop Software (version v1.5.1). Click 'Open' in the 'Open Existing Experiment' section then locate the experiment file (.eds) to be analyzed and click 'Open'.
2. Modify the analysis settings using the 'Results' tab in the QuantStudio Design & Analysis Desktop Software (version v1.5.1). Select the desired wells for analysis in the Plate Layout then click on the Eye symbol above the 'Amplification Plot' to configure the plot as follows: set 'Plot Type' to ' ΔRn vs Cycle', set 'Graph Type' to 'Log', and set 'Plot Color' to 'Target'. All other content remains unchanged. Click out of the window to accept changes.
3. Click the 'Gear' icon to the right of the 'Analyze' button in the top left of the software to open the 'Analysis Settings' window. In the 'Ct settings' tab, uncheck the 'Default settings' box under the 'Ct settings for COVID' section, uncheck the 'Automatic Threshold' box, and uncheck the 'Automatic Baseline' box. Enter "25,000" for the 'Threshold', set 'Baseline Start Cycle' to '4' and set 'End Cycle' to '8'. Select the 'IC' line then uncheck the 'Default settings' box, the 'Automatic Threshold' box, and the 'Automatic Baseline' box. Enter "20,000" for the 'Threshold', set 'Baseline Start Cycle' to '4' and set 'End Cycle' to '8'. Click 'Apply' to proceed the 'Export' tab.
4. Define the 'File Name' for the exported file ("RNA STAR Complete [YYMMDD_Plate#]"). Define the 'File name', choose the 'File Type' (default is QuantStudio as an .xls file), choose the 'Location' to export the file, and choose the 'Content' to be exported (the 'Results' box needs to be checked for Ct values). Click 'Customize' to proceed to what is to be exported within each Content item.
5. Select the 'All Fields' box to uncheck all content then check the boxes for the following content: 'Well', 'Well Position', 'Sample Name', 'Target Name', 'Task', 'Reporter', 'Quencher', and 'CT'. Click 'Close' to return to the 'Export' tab screen.
6. Click 'Save' to save the modified settings. Click the 'Export' button to generate the export data file. The exported file will include a 'Results' section which contains the sample Ct values.
7. Close the software.

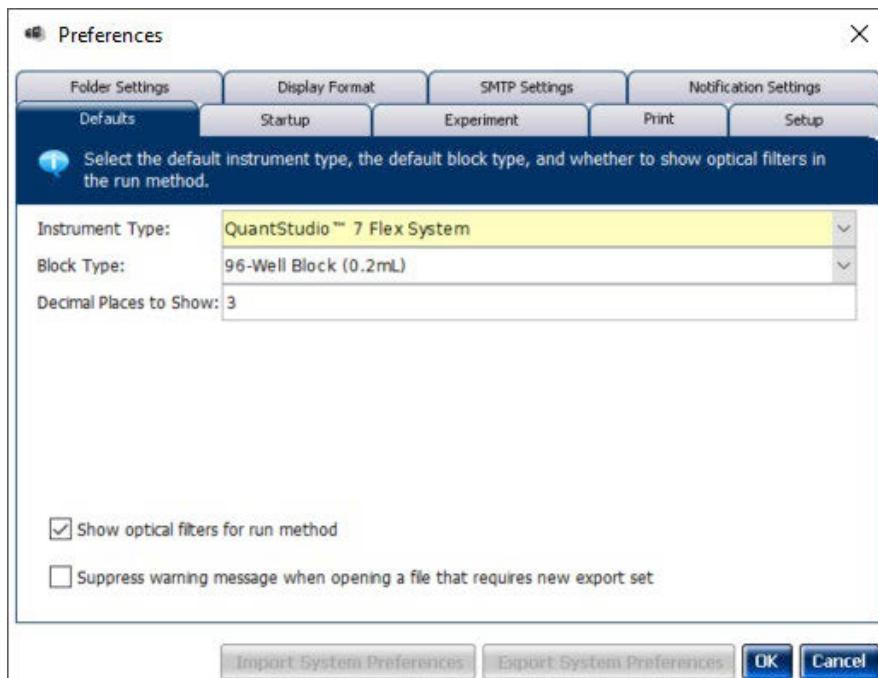
13 RNA STAR Complete Setup for Applied Biosystems™ QuantStudio 7 Flex

Refer to "User Manual Part Number 4489821" for additional information. The instrument programming instructions are intended for setup of an entire 96-well plate. If the entire plate is not used, please deselect wells as appropriate during analysis.

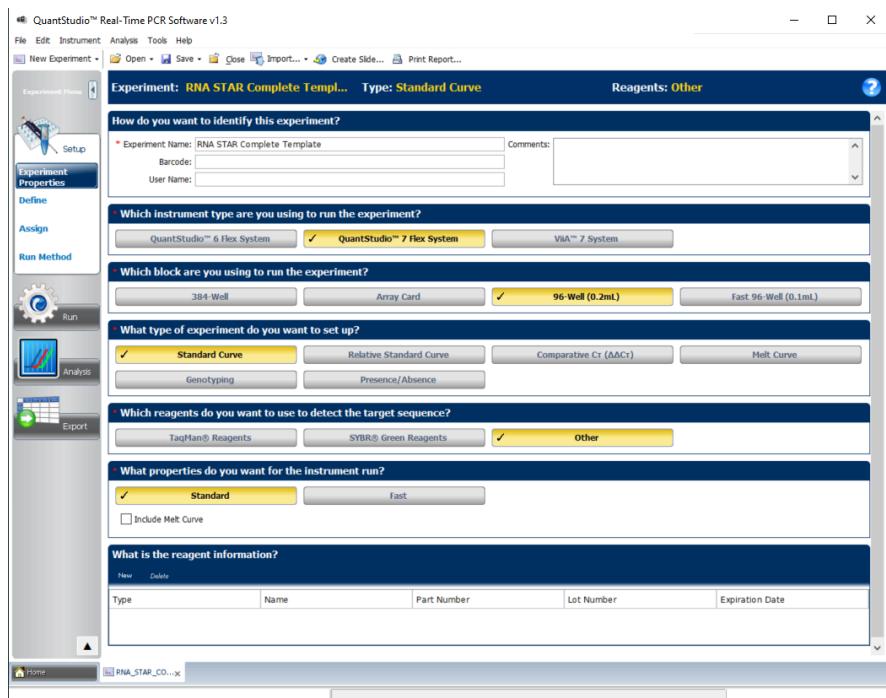
13.1 Programming Instructions for PC/Laptop-connected Instrument

It is recommended to setup the instrument up to step 11 before preparing the Reagent Mix as defined in the qSTAR Reagent Preparation section above. The 96-well plate should be recently centrifuged to guarantee all reagents are at the bottom of the plate and kept on a cold block. Use 96-well plates and seals recommended by the instrument manufacturer (see Option for PCR Instruments & Recommended Consumables table above).

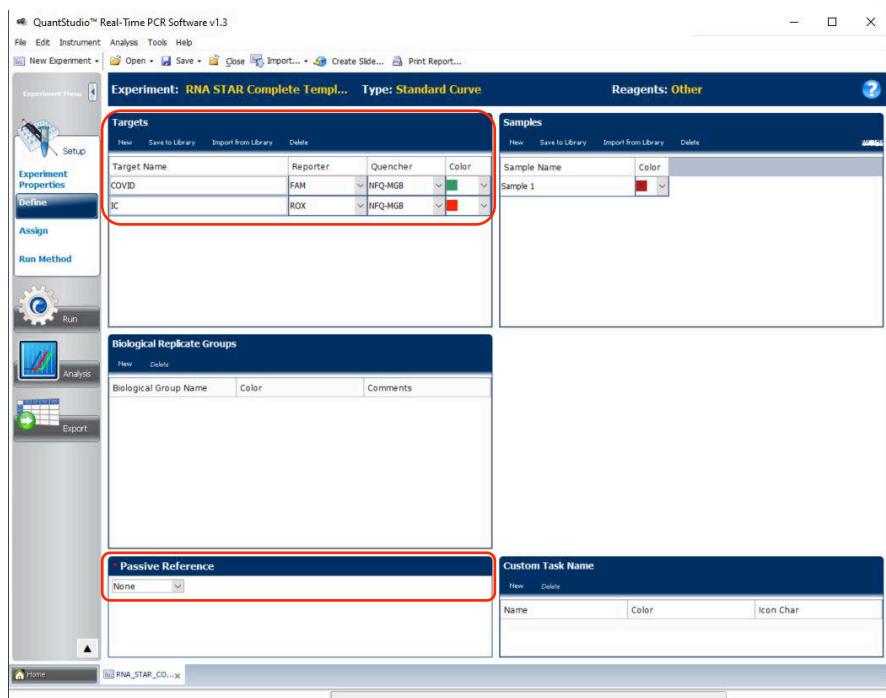
1. If the instrument touchscreen is in sleep mode (dark screen), touch the screen anywhere to activate the instrument. Sleep mode lowers the temperature of the heated cover. It is important that the heated cover idle at 105°C before loading a 96-well plate.
2. Launch the 'QuantStudio Real-Time PCR Software' Desktop Software (version v1.3). On the toolbar select 'Tools' then 'Properties'. This opens the 'Preferences' window which opens to the 'Defaults' tab.



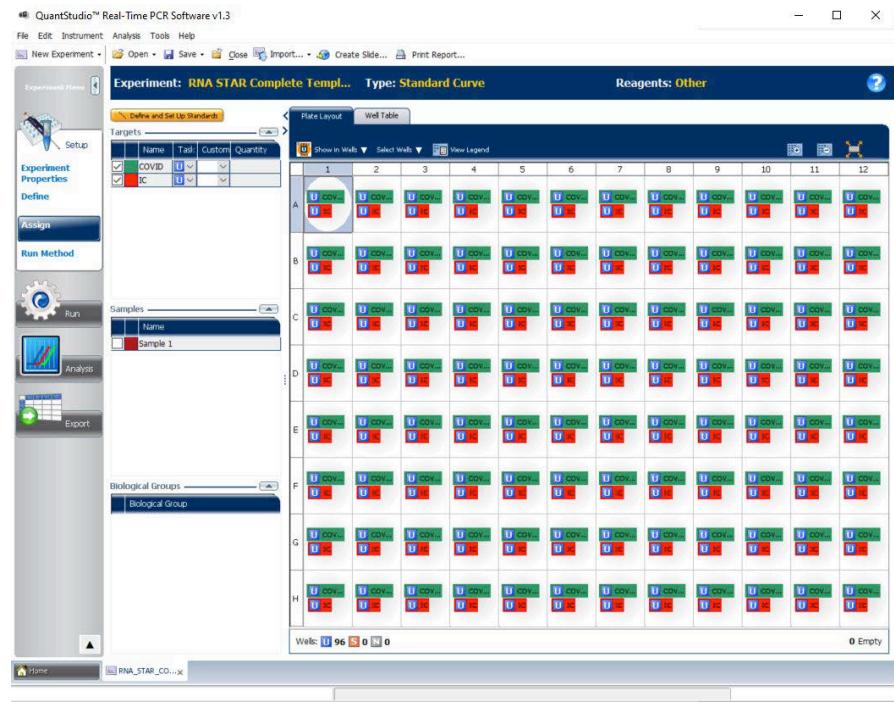
3. Confirm that the 'Instrument Type:' is set to 'QuantStudio™ 7 Flex System', the 'Block Type:' is set to '96-Well Block (0.2mL)', and that the 'Decimal Places to Show:' is set to '3'. Click the box next to 'Show optical filters for run method' to select this feature. Click on 'OK'.
4. Select 'Create New Experiment'. A window will open with the 'Properties' tab selected.



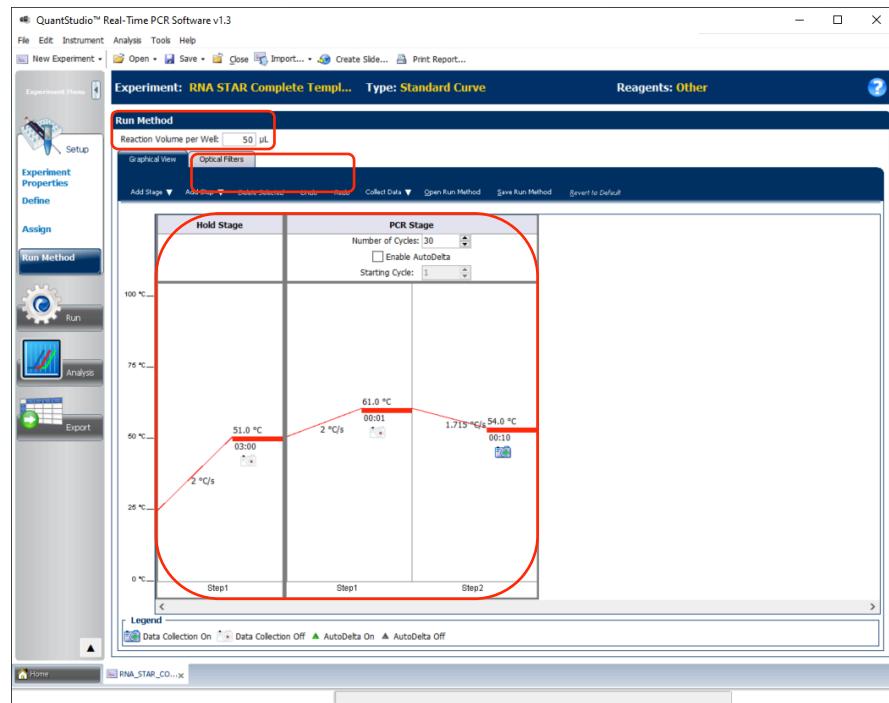
5. On the left of the screen, the 'Experiment Properties' tab will be selected. Enter the 'Experiment Properties' as follows: define the 'Experiment Name' as "RNA STAR Complete Template", select the instrument type as 'QuantStudio™ 7 Flex System', select the block being used for the run as '96-Well (0.2mL)', select the experiment type being set up as 'Standard Curve', select the reagents being used to detect target sequences as 'Other', and select properties for the instrument run as 'Standard'. On the left of the screen, proceed to the 'Define' tab.



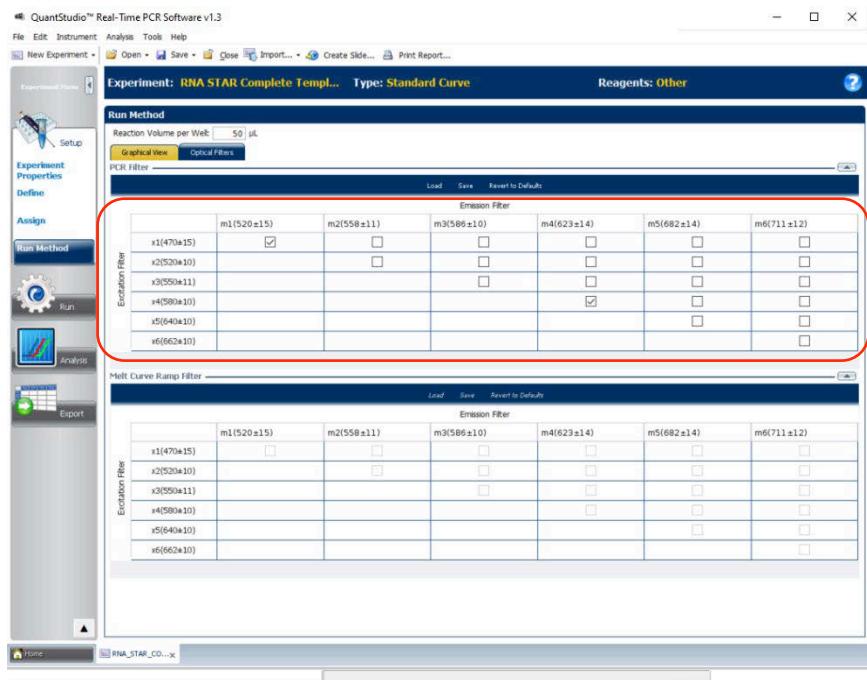
6. Under the 'Targets' section: define the 'Target Name' for 'Target 1' as "COVID", select 'FAM' as the 'Reporter', change the 'Color' to green, and confirm the 'Quencher' is automatically entered as 'NFQ-MGB'. Add a second target by clicking 'New' (just above 'Target Name') then rename to 'IC', change the default color to red, select 'ROX' as the 'Reporter' (the 'Quencher' is automatically entered as 'NFQ-MGB'). Using the drop-down menu in the 'Passive Reference' section, change the setting to 'None'.
 7. (Optional) Under the 'Samples' section, the 'Sample Name' can be added individually. Then proceed to the 'Assign' tab.



8. Select all wells from the plate layout. On the left of the screen in the 'Targets' section, check the boxes next to 'COVID' and 'IC' to apply these targets to the wells. If 'Samples' have been defined for each well, select individual wells and check the box next to the appropriate 'Name' in the 'Samples' section. Proceed to the 'Run Method' tab.



9. Under the 'Run Method' section, define the 'Reaction Volume per Well' to "50" (μL). In the 'Graphical View' section, select the 'Hold Stage' and delete one step by clicking the step and click 'Delete Selected' so one 'Hold Stage' segment remains. Change the 'Hold Stage' 'Step 1' settings as follows: set ramp rate to '2 °C/s', set temp to '51.0 °C', and set time to '03:00'. Change 'Step 1' in 'PCR Stage' as follows: set ramp rate (middle) to '2 °C/s', set temp (upper middle) to '61 °C', and set time (lower middle) to '00:01'. Change 'Step 2' in 'PCR Stage' as follows: set ramp rate (left) to '1.715 °C/s', set temp (upper right) to '54 °C', and set time (lower right) to '00:10'. 'Step 2' in the 'PCR Stage' is the data collection step. At the top of the 'PCR Stage', set 'Number of Cycles' to '30'.



- Near the top of the window select 'Optical Filters'. Just below in the 'PCR Filter' section, uncheck all 'Emission Filter' combinations except the following:
x1(470±15), m1(520±15), and x4(580±10), m4(623±14)
- Save the experiment as a template for subsequent runs by clicking the 'Save' drop-down menu and selecting 'Save As'. Name the template "RNA STAR Complete Template" and click 'Save'.
- Load the plate by pressing the red 'Arrow' icon on the QuantStudio 7 Flex instrument touchscreen at the bottom right of the window. Place the sample plate on the amplification block that automatically comes out. Make sure to align A1 on the 96-well plate with the defined A1 position on the instrument drawer. Press the red 'Arrow' icon on the instrument touchscreen to close the drawer.
- Return to the desktop software 'Run' tab and click the 'START RUN' button. When instrument connection is made, the instrument number will appear in a drop-down menu below the 'START RUN' button. Select the instrument number to initiate the run. Change the 'File Name' to "LumiraDx SARS-CoV-2 RNA STAR [YYMMDD_Plate#]" and click 'Save'.
- Following the run, the instrument touchscreen indicates when the run is 'Complete'. Remove the plate and discard the plate in a sealable waste bag or container.

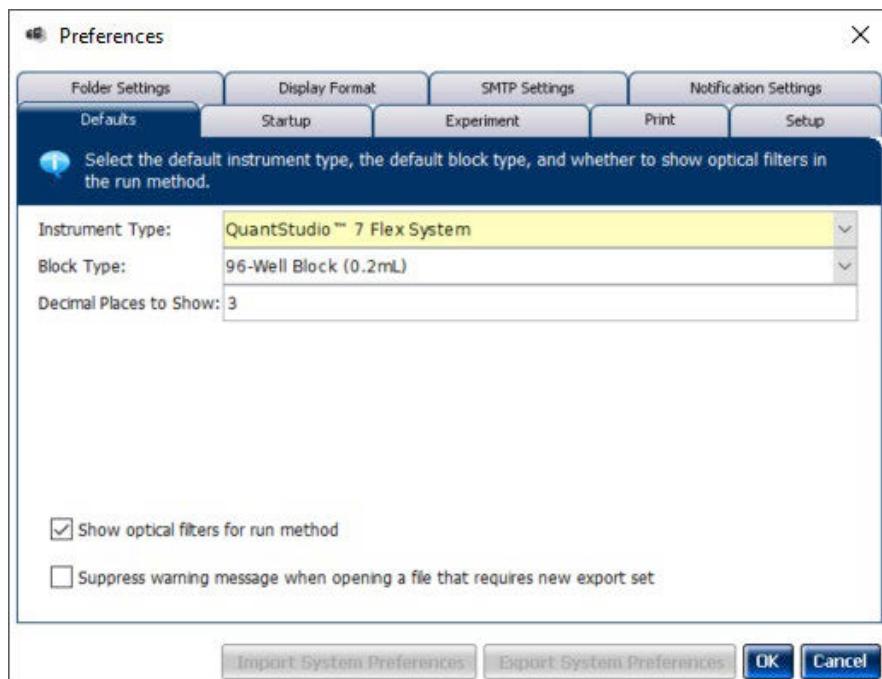
13.2 Analysis Instructions

- Modify the analysis settings using the 'Results' tab in the 'QuantStudio Real-Time PCR Software' Desktop Software (version v1.3). Select the desired wells for analysis in the Plate Layout then configure the 'Amplification Plot' as follows: set 'Plot Type' to 'ΔRn vs Cycle', set 'Graph Type' to 'Log', and set 'Plot Color' to 'Target'.
- Click the 'Analysis Settings' to the right of the 'Analyze' button in the top left of the software to open the 'Analysis Settings' window. In the 'Ct settings' tab, uncheck the 'Default settings' box under the 'Ct settings for COVID' section, uncheck the 'Automatic Threshold' box, and uncheck the 'Automatic Baseline' box. Enter "80,000" for the 'Threshold', set 'Baseline Start Cycle' to '1' and set 'End Cycle' to '3'. Select the 'IC' line then uncheck the 'Default settings' box, the 'Automatic Threshold' box, and the 'Automatic Baseline' box. Enter "10,000" for the 'Threshold', set 'Baseline Start Cycle' to '1' and set 'End Cycle' to '3'. Click 'Apply' to proceed to the 'Export' tab.
- Define the 'File Name' for the exported file ("RNA STAR Complete [YYMMDD_Plate#]"). Define the 'File name', choose the 'File Type' (default is QuantStudio as an .xls file), choose the 'Location' to export the file, and choose the 'Content' to be exported (the 'Results' box needs to be checked for Ct values). Click 'Customize' to proceed to what is to be exported within each Content item.
- Select the 'All Fields' box to uncheck all content then check the boxes for the following content: 'Well', 'Well Position', 'Sample Name', 'Target Name', 'Task', 'Reporter', 'Quencher', and 'CT'. Click 'Close' to return to the 'Export' tab screen.
- Click 'Save' to save the modified settings.
- Click the 'Export' button to generate the export data file. The exported file will include a 'Results' section which contains the sample Ct values.
- Close the software.

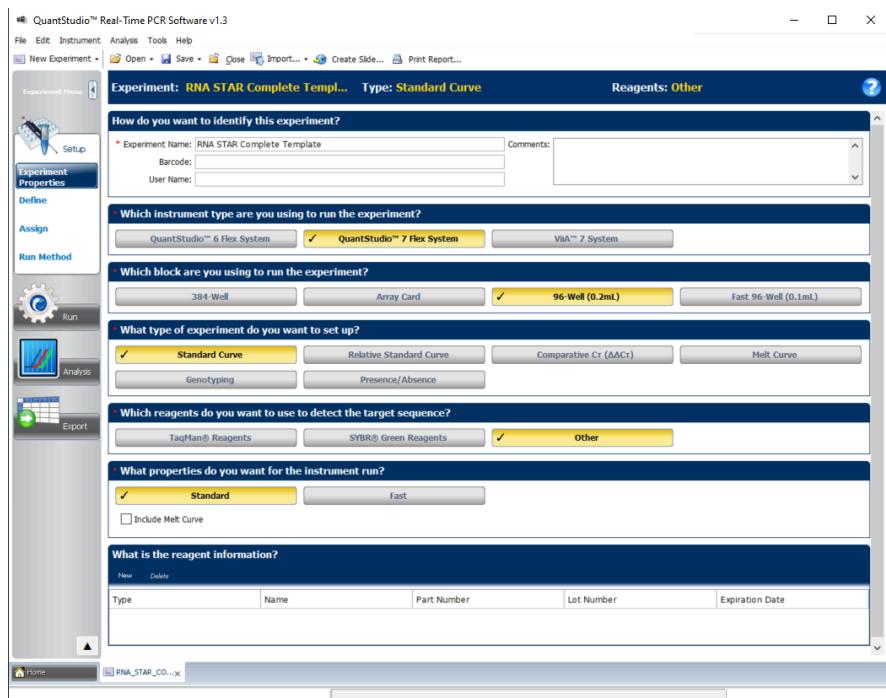
13.3 Programming Instructions for Stand-Alone Instrument

It is recommended to setup the instrument up to step 12 before preparing the Reagent Mix as defined in the qSTAR Reagent Preparation section above. The 96-well plate should be recently centrifuged to guarantee all reagents are at the bottom of the plate and kept on a cold block. Use 96-well plates and seals recommended by the instrument manufacturer (see Option for PCR Instruments & Recommended Consumables table above).

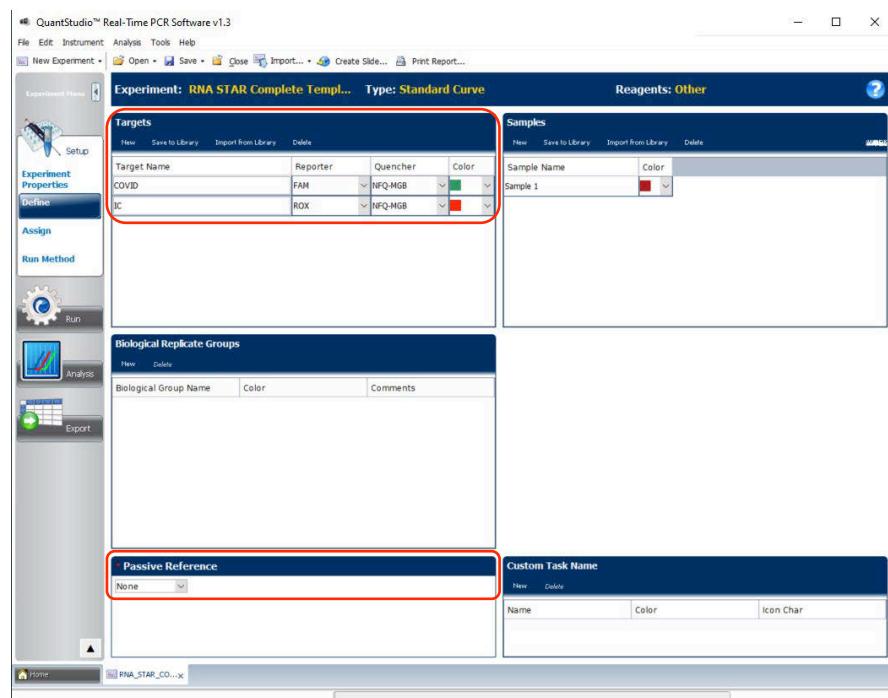
1. If the instrument touchscreen is in sleep mode (dark screen), touch the screen anywhere to activate the instrument. Sleep mode lowers the temperature of the heated cover. It is important that the heated cover idle at 105°C before loading a 96-well plate.
2. Launch the 'QuantStudio Real-Time PCR Software' Desktop Software (version v1.3). On the toolbar select 'Tools' then 'Properties'. This opens the 'Preferences' window which opens to the 'Defaults' tab.



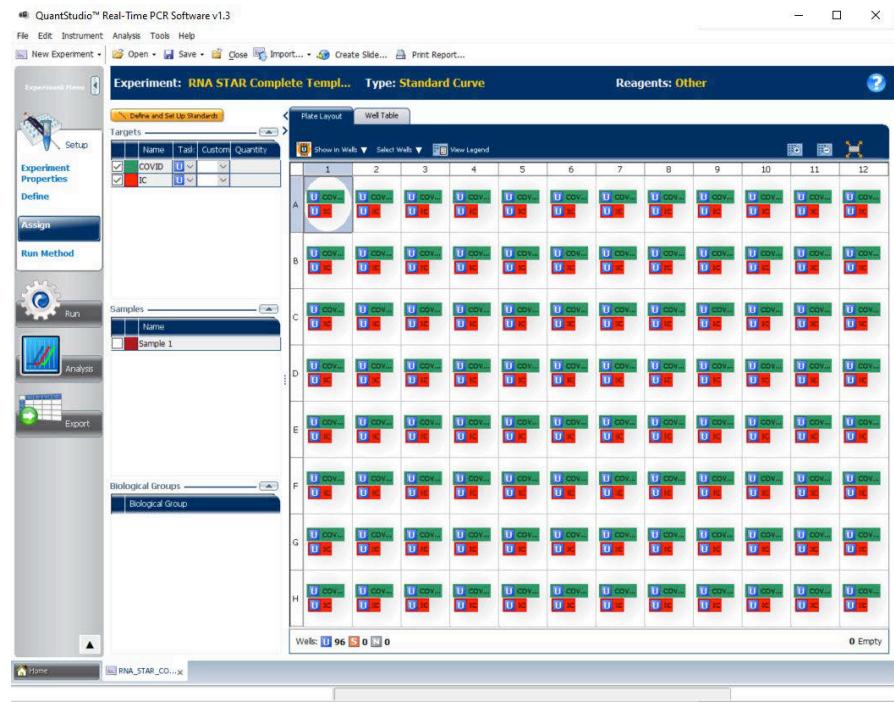
3. Confirm that the 'Instrument Type:' is set to 'QuantStudio™ 7 Flex System', the 'Block Type:' is set to '96-Well Block (0.2mL)', and that the 'Decimal Places to Show:' is set to '3'. Click the box next to 'Show optical filters for run method' to select this feature. Click 'OK'.
4. Select 'Create New Experiment'. A window will open with the 'Properties' tab selected.



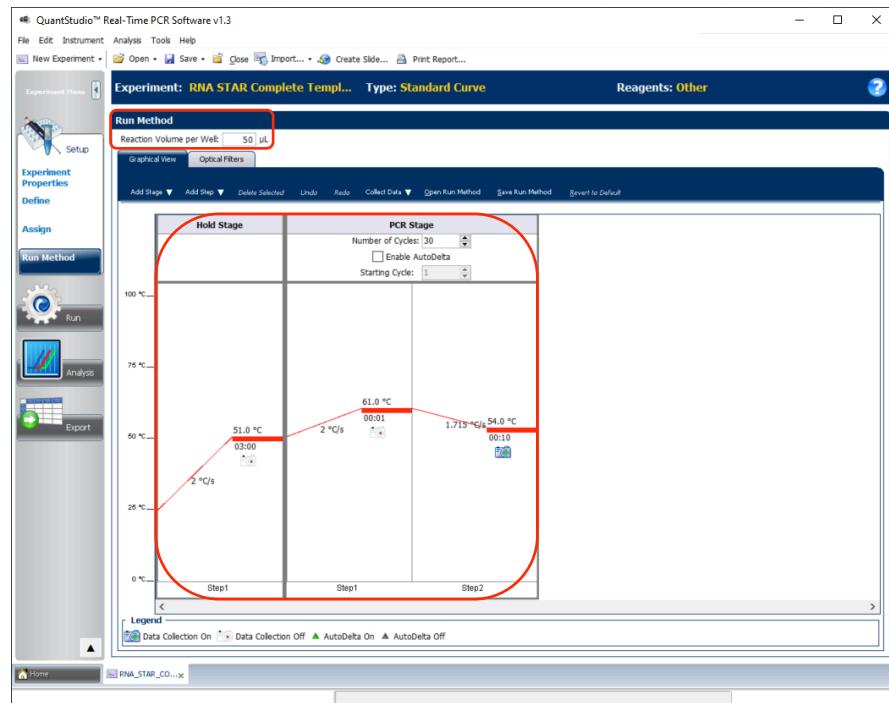
5. On the left of the screen, the 'Experiment Properties' tab will be selected. Enter the 'Experiment Properties' as follows: define the 'Experiment Name' as "RNA STAR Complete Template", select the instrument type as 'QuantStudio™ 7 Flex System', select the block being used for the run as '96-Well (0.2mL)', select the experiment type being set up as 'Standard Curve', select the reagents being used to detect target sequences as 'Other', and select properties for the instrument run as 'Standard'. On the left of the screen, proceed to the 'Define' tab.



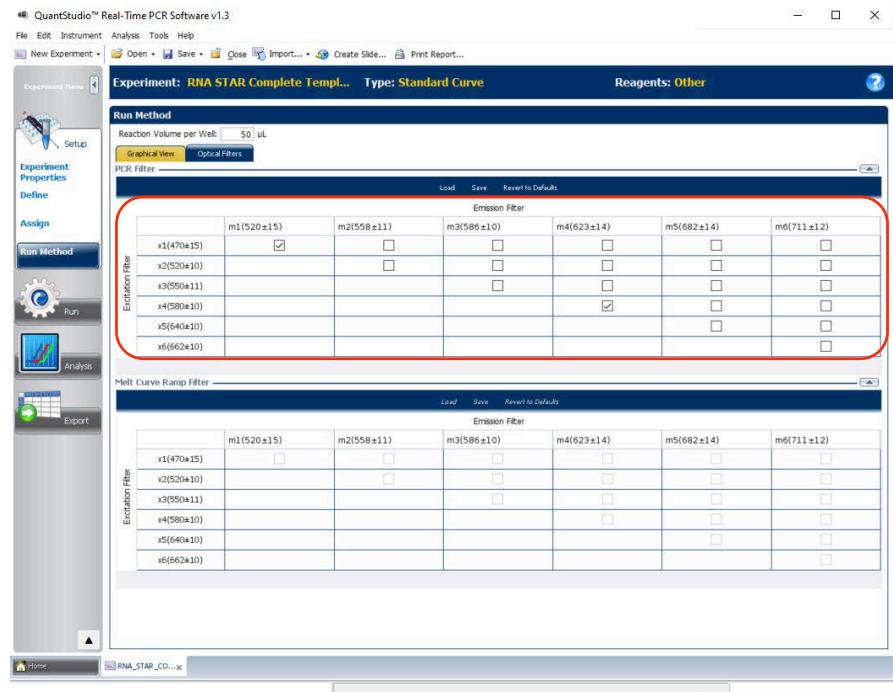
6. Under the 'Targets' section: define the 'Target Name' for 'Target 1' as "COVID", select 'FAM' as the 'Reporter', change the 'Color' to green, and confirm the 'Quencher' is automatically entered as 'NFQ-MGB'. Add a second target by clicking 'New' (just above 'Target Name') then rename to 'IC', change the default color to red, select 'ROX' as the 'Reporter' (the 'Quencher' is automatically entered as 'NFQ-MGB'). Using the drop-down menu in the 'Passive Reference' section, change the setting to 'None'.
7. (Optional) Under the 'Samples' section, the 'Sample Name' can be added individually. Then proceed to the 'Assign' tab.



8. Select all wells from the plate layout. On the left of the screen in the 'Targets' section, check the boxes next to 'COVID' and 'IC' to apply these targets to the wells. If 'Samples' have been defined for each well, select individual wells and check the box next to the appropriate 'Name' in the 'Samples' section. Proceed to the 'Run Method' tab.



9. Under the 'Run Method' section, define the 'Reaction Volume per Well' to "50" (μL). In the 'Graphical View' section, select the 'Hold Stage' and delete one step by clicking the step and click 'Delete Selected' so one 'Hold Stage' segment remains. Change the 'Hold Stage' 'Step 1' settings as follows: set ramp rate to '2 °C/s', set temp to '51.0 °C', and set time to '03:00'. Change 'Step 1' in 'PCR Stage' as follows: set ramp rate (middle) to '2 °C/s', set temp (upper middle) to '61 °C', and set time (lower middle) to '00:01'. Change 'Step 2' in 'PCR Stage' as follows: set ramp rate (left) to '1.715 °C/s', set temp (upper right) to '54 °C', and set time (lower right) to '00:10'. 'Step 2' in the 'PCR Stage' is the data collection step. At the top of the 'PCR Stage', set 'Number of Cycles' to '30'.



10. Near the top of the window select 'Optical Filters'. Just below in the 'PCR Filter' section, uncheck all 'Emission Filter' combinations except the following:
11. x1(470±15), m1(520±15), and x4(580±10), m4(623±14)
12. Save the experiment as a template for subsequent runs by clicking the 'Save' drop-down menu and selecting 'Save As'. Name the template "RNA STAR Complete Template" and click 'Save'.
13. Insert the USB drive into the designated slot in the front of the QuantStudio 7 Flex instrument. On the instrument touchscreen, press 'View Templates'. Select the 'folder' icon and then select 'USB' from the options provided, scroll to locate the saved template (.edt) file and select the file and press 'Start Run' to import it.
14. Change the 'Run File Name' to "RNA STAR Complete [YYMMDD_Plate#]" then press 'Start Run Now'.
15. Following the run, the instrument returns to the 'Home' screen. Insert a USB drive into the designated slot in the front of the QuantStudio 7 Flex instrument. Press 'Collect Results'. Select the experiment then touch 'Save to USB'. Remove the USB drive from the instrument.
16. Open the instrument drawer to remove the plate by pressing the arrow icon at the top right of the instrument touch screen. The instrument drawer will open.
17. Remove the plate and discard the plate in a sealable waste bag or container.

13.4 Analysis Instructions

1. Launch the 'QuantStudio Real-Time PCR Software' Desktop Software (version v1.3). Click 'Analyze Experiment' in the 'Analyze' section then locate the experiment file (.eds) to be analyzed and click 'Open'.
2. Modify the analysis settings using the 'Results' tab in the 'QuantStudio Real-Time PCR Software' Desktop Software (version v1.3). Select the desired wells for analysis in the Plate Layout then configure the 'Amplification Plot' as follows: set 'Plot Type' to 'ΔRn vs Cycle', set 'Graph Type' to 'Log', and set 'Plot Color' to 'Target'.
3. Click the 'Analysis Settings' to the right of the 'Analyze' button in the top left of the software to open the 'Analysis Settings' window. In the 'Ct settings' tab, uncheck the 'Default settings' box under the 'Ct settings for COVID' section, uncheck the 'Automatic Threshold' box, and uncheck the 'Automatic Baseline' box. Enter "80,000" for the 'Threshold', set 'Baseline Start Cycle' to '1' and set 'End Cycle' to '3'. Select the 'IC' line then uncheck the 'Default settings' box, the 'Automatic Threshold' box, and the 'Automatic Baseline' box. Enter "10,000" for the 'Threshold', set 'Baseline Start Cycle' to '1' and set 'End Cycle' to '3'. Click 'Apply' to proceed to the 'Export' tab.
4. Define the 'File Name' for the exported file ("RNA STAR Complete [YYMMDD_Plate#]"). Define the 'File name', choose the 'File Type' (default is QuantStudio as an .xls file), choose the 'Location' to export the file, and choose the 'Content' to be exported (the 'Results' box needs to be checked for Ct values). Click 'Customize' to proceed to what is to be exported within each Content item.

5. Select the 'All Fields' box to uncheck all content then check the boxes for the following content: 'Well', 'Well Position', 'Sample Name', 'Target Name', 'Task', 'Reporter', 'Quencher', and 'CT'. Click 'Close' to return to the 'Export' tab screen.
6. Click 'Save' to save the modified settings.
7. Click the 'Export' button to generate the export data file. The exported file will include a 'Results' section which contains the sample Ct values.
8. Close the software.

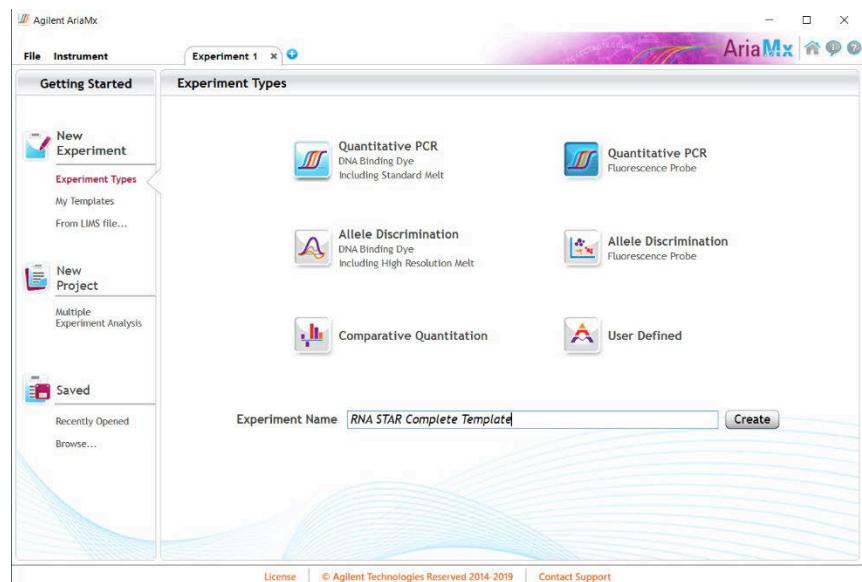
14 RNA STAR Complete Setup for Agilent™ AriaMx

Refer to "User Manual Part Number G8830A" for additional information.

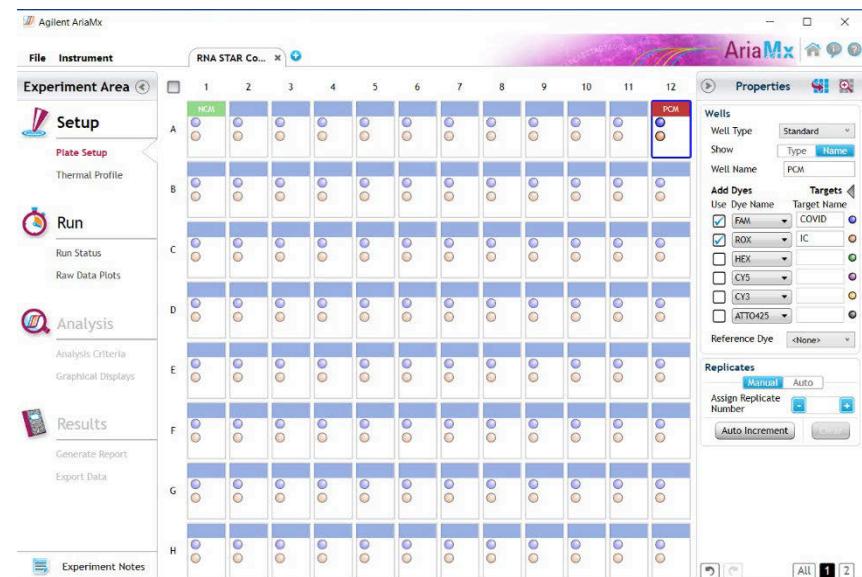
14.1 Programming Instructions for PC/Laptop-connected Instrument

It is recommended to setup the instrument up to step 6 before preparing the Reagent Mix as defined in the qSTAR Reagent Preparation section above. The 96-well plate should be recently centrifuged to guarantee all reagents are at the bottom of the plate and kept on a cold block. Use 96-well plates and seals recommended by the instrument manufacturer (see Option for PCR Instruments & Recommended Consumables table above).

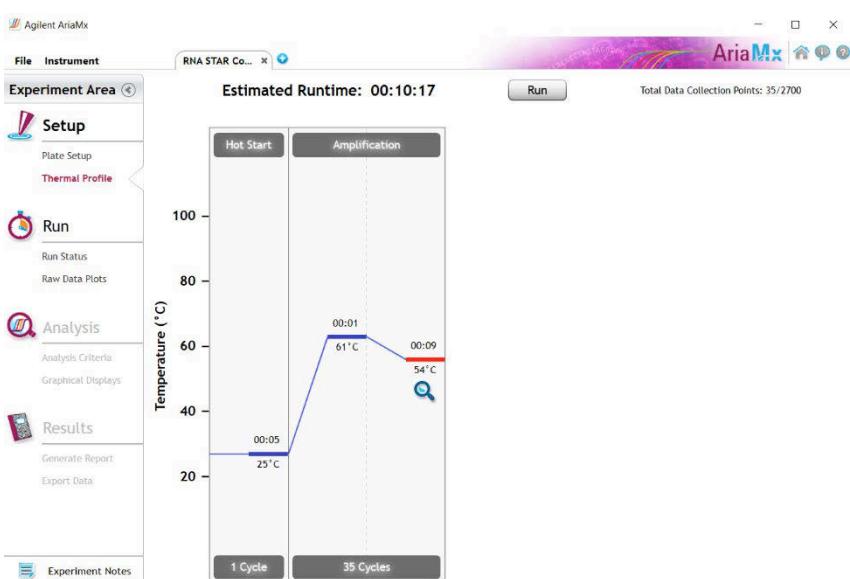
1. Launch the Agilent AriaMx desktop software (version 1.71). Select 'Quantitative PCR Fluorescence Probe' in the 'Experiment Types' section (do not double click). Define the 'Experiment Name' as "RNA STAR Complete Template" and click 'Create'.



2. Select all the wells and in the 'Properties' section on the right of the screen set 'Well Type' to 'Unknown', select the 'FAM' and 'ROX' boxes, and toggle the 'Target' arrow (across from 'Add Dyes'). Set the 'Target Name' for 'FAM' to "COVID" and 'ROX' to "IC".
3. Select just well 'A1' and re-set 'Well Type' to 'NTC' (No Template Control) from the drop-down menu. Click the 'Name' button next to and define the 'Well Name' as "NCM" (Negative Control Media). Select just well 'A12' and re-set 'Well Type' to 'Standard' from the drop-down menu. Click the 'Name' button next to and define the 'Well Name' as "PCM" (Positive Control Media). The other wells will remain 'Unknown' unless defined as Samples Names (optional).



4. Proceed to the 'Thermal Profile' tab on the left of the software and modify the default thermal profile for 'Hot Start' as follows: set 'Temp' to '25°C', set 'Time' to '00:05' and confirm 'Cycle' is automatically set to '1'. Modify the default thermal profile for 'Amplification' as follows: set 'Temp' (left) to '61°C', set 'Time' (left) to '00:01', set 'Temp' (right) to '54°C', set 'Time' (right) to '00:09' (Data collection step), and set 'Cycle' to '35'.
 5. Proceed to save the file by clicking 'File' from the menu and choose 'Save As Template' from the drop-down menu. Name the file "RNA STAR Complete Template" and click 'Save'.
 6. Click 'Run' and, once instrument connection is made, click 'Send Config'. The software will prompt you to save the experiment again. Click 'Save' and define 'File Name' as "RNA STAR Complete [YYMMDD_Plate#]" and click 'Save'.
 7. On the Agilent AriaMx instrument touchscreen, locate and press the 'Monitor' icon () at the bottom of the touch screen. Press 'Open Primed Experiment' from pop-up menu. Press the 'Thermal Profile' tab on the left side of the touch screen then press 'Run Experiment' to initiate the run. The instrument lid will begin its Warm-Up procedure (~2 minutes). Press the 'Pause' button when it is activated (green arrow). The 'Pause' button is active once the lid reaches 100 °C. Lift up the instrument door, pull the block lid out to unlock then lift the block lid and load the plate.
- Note:** Be cautious when loading the plate since the lid is heated. Position A1 is not defined in the instrument but load the plate so that position A1 on the 96-well plate is the top, left-most well.
- Close and lock the block lid then close the instrument door. Press the 'Resume' button to now start the run.
8. Upon completion of the run, close the 'Run Data Plots' screen by selecting the "X". Remove the plate and discard the plate in a sealable waste bag or container.



14.2 Analysis Instructions

1. In the Agilent AriaMx desktop software (version 1.71), select 'Analysis Criteria' from the 'Experiment Area' panel on the left side of the software then select the appropriate wells for analysis using the plate map.
2. Select 'Graphical Displays' from the 'Experiment Area' panel on the left side of the screen, then confirm in the 'Amplification Plots' panel on the right side of the software that 'Fluorescence Term' is set to 'ΔR' and 'Smoothing' is 'On'. Expand the section under 'Amplification Plots' by toggling the 'Arrow' icon (if needed) then click 'Adjust'. In the 'Baseline Correction' window, click 'Select All' to highlight all the wells to be analyzed. Set 'Start Cycle' to '5' and set 'End Cycle' to '8' then click 'Apply'. To exit the window, click 'OK'.
3. In the 'Amplification Plots' panel, change 'Graph Type' to 'Log'. Change automatically generated 'Threshold Fluorescence' values by defining 'COVID' as '120' then click the 'Unlock' icon immediately next to the newly defined threshold to lock the setting, additionally, define 'IC' as '100' then click the 'Unlock' icon immediately next to the newly defined threshold to lock the setting. Set 'Background Based Threshold' for 'Cycle Range' as '1' thru '1' and set 'Sigma Multiplier' to '10'.
4. 'Threshold Fluorescence' values are automatically generated for 'COVID' and 'IC'. Set 'Background Based Threshold' for 'Cycle Range' as '1' thru '1' and set 'Sigma Multiplier' to '10'.

5. Select 'Generate Report' from the 'Experiment Area' panel on the left side of the screen, then confirm in the 'Report Configuration' panel that 'Definition' is set to 'Default'. The 'Report' type can be set to 'PDF' or 'PowerPoint'. In the 'Items' section, select the Pencil button next to the 'Tabular Results' line and set 'Include Target Information' to 'Yes'. Check mark the following results: 'Well', 'Well Type', 'Well Name', 'Dye', 'Target', 'Cq (ΔR)', and 'Final Call (ΔR)'. All other headings should be unchecked. Click 'OK' to exit the 'Tabular Results Properties' window. All other report features *can* remain unchanged. Click 'Generate Report', define 'File Name' and click 'Save'.
6. Select 'Export Data' from the 'Experiment Area' panel on the left side of the screen, then confirm in the 'Export Configuration' panel that 'Definition' is set to 'Default'. The file type can be set to 'Excel', 'Text', 'LIMS', 'RDML'. In the 'Items' section, select the Pencil button next to the 'Tabular Results' line and set 'Include Target Information' to 'Yes'. Check mark the following results: 'Well', 'Well Type', 'Well Name', 'Dye', 'Target', 'Cq (ΔR)', and 'Final Call (ΔR)'. All other headings should be unchecked. Click 'OK' to exit the 'Column Options' window then click 'Export Data' to generate the report.
7. To save the run, click 'File' from the menu and choose 'Save' from the drop-down menu.
8. Close the software.

14.3 Programming Instructions Using Instrument Touch Screen

It is recommended to setup the instrument up to step 4 before preparing the Reagent Mix as defined in the qSTAR Reagent Preparation section above. The 96-well plate should be recently centrifuged to guarantee all reagents are at the bottom of the plate and kept on a cold block. Use 96-well plates and seals recommended by the instrument manufacturer (see Option for PCR Instruments & Recommended Consumables table above).

1. On the Agilent AriaMx instrument touchscreen, press the 'Home' button then press 'New Experiment' and press 'Quantitative PCR Fluorescence Probe'.
2. On the 'Plate Setup' screen, select all the wells then press 'Well Types' and press 'Unknown' from the drop-down list. Check the boxes next to 'ROX' and 'FAM' and proceed to the 'Thermal Profile' tab.
3. Modify the default thermal profile for 'Hot Start' as follows: set 'Temp' to '25 °C', set 'Time' to '00:05', and set 'Cycle' to '1'. Modify the default thermal profile for 'Amplification' as follows: set 'Temp' (left) to '61 °C', set 'Time' (left) to '00:01', set 'Temp' (right) to '54 °C', set 'Time' (right) to '00:09' (data collection step), and set 'Cycle' to '35'. Proceed to initiate the run by pressing the 'Run Experiment' button at the top of the screen.
4. A message box opens asking you to save the experiment. Press 'OK'. Name 'New File Name' as "RNA STAR Complete [YYMMDD_Plate#]" and press 'Save'.
5. The instrument lid will begin its Warm-Up procedure (~2 minutes). Press the 'Pause' button when it is activated (green arrow). The 'Pause' button is active once the lid reaches 100 °C. Lift up the instrument door, pull the block lid out to unlock then lift the block lid and load the plate.

Note: Be cautious when loading the plate since the lid is heated. Position A1 is not defined in the instrument but load the plate so that position A1 on the 96-well plate is the top, left-most well.

Close and lock the block lid then close the instrument door. Press the 'Resume' button to now start the run.

6. Upon completion of the run, close the 'Run Data Plots' screen by selecting the "X". Remove the plate and discard the plate in a sealable waste bag or container.
7. Insert a USB drive into the USB port of the Agilent AriaMx instrument. On the 'Home' screen, press 'Saved Experiment' to open the 'Experiment Explorer' screen. Select the experiment and press the 'Copy' button. Select the USB folder from the list of folders, and press 'Paste' to complete the export. Press 'Cancel' to exit 'Experiment Explorer' screen.
8. Remove the plate and discard the plate in a sealable waste bag or container.

14.4 Analysis Instructions

1. From USB drive, load the saved experiment by double-clicking the experiment file. The Agilent AriaMx desktop software (version 1.71) launches and the 'Plate Setup' screen opens. Select all the wells and in the 'Properties' section on the right of the screen set 'Well Type' to 'Unknown', select the 'FAM' and 'ROX' boxes, and toggle the 'Target' arrow (across from 'Add Dyes'). Set the 'Target Name' for 'FAM' to "COVID" and 'ROX' to "IC".
2. Select just well 'A1' and re-set 'Well Type' to 'NTC' (No Template Control) from the drop-down menu. Click the 'Name' button next to and define the 'Well Name' as "NCM" (Negative Control Media). Select just well 'A12' and re-set 'Well Type' to 'Standard' from the drop-down menu. Click the 'Name' button next to and define the 'Well Name' as "PCM" (Positive Control Media). The other wells will remain 'Unknown' unless defined as Samples Names (optional).
3. Select 'Analysis Criteria' from the 'Experiment Area' panel on the left side of the software then select the appropriate wells for analysis using the plate map.
4. Select 'Graphical Displays' from the 'Experiment Area' panel on the left side of the screen, then confirm in the 'Amplification Plots' panel on the right side of the software that 'Fluorescence Term' is set to ' ΔR ' and 'Smoothing' is 'On'. Expand the section under 'Amplification Plots' by toggling the 'Arrow' icon (if needed) then click 'Adjust'. In the 'Baseline Correction' window, click 'Select All' to highlight all the wells to be analyzed. Set 'Start Cycle' to '5' and set 'End Cycle' to '8' then click 'Apply'. To exit the window, click 'OK'.
5. In the 'Amplification Plots' panel, change 'Graph Type' to 'Log'. Change automatically generated 'Threshold Fluorescence' values by defining 'COVID' as "120" then click the 'Unlock' icon immediately next to the newly defined threshold to lock the setting, additionally, define 'IC' as "100" then click the 'Unlock' icon immediately next to the newly defined threshold to lock the setting.
6. Select 'Generate Report' from the 'Experiment Area' panel on the left side of the screen, then confirm in the 'Report Configuration' panel that 'Definition' is set to 'Default'. The 'Report' type can be set to 'PDF' or 'PowerPoint'. In the 'Items' section, select the Pencil button next to the 'Tabular Results' line and set 'Include Target Information' to 'Yes'. Check mark the following results: 'Well', 'Well Type', 'Well Name', 'Dye', 'Target', 'Cq (ΔR)', and 'Final Call (ΔR)'. All other headings should be unchecked. Click 'OK' to exit the 'Tabular Results Properties' window. All other report features *can* remain unchanged. Click 'Generate Report', define 'File Name' and click 'Save'.
7. Select 'Export Data' from the 'Experiment Area' panel on the left side of the screen, then confirm in the 'Export Configuration' panel that 'Definition' is set to 'Default'. The file type can be set to 'Excel', 'Text', 'LIMS', 'RDML'. In the 'Items' section, select the Pencil button next to the 'Tabular Results' line and set 'Include Target Information' to 'Yes'. Check mark the following results: 'Well', 'Well Type', 'Well Name', 'Dye', 'Target', 'Cq (ΔR)', and 'Final Call (ΔR)'. All other headings should be unchecked. Click 'OK' to exit the 'Column Options' window then click 'Export Data' to generate the report.
8. To save the run, click 'File' from the menu and choose 'Save' from the drop-down menu.
9. Close the software.

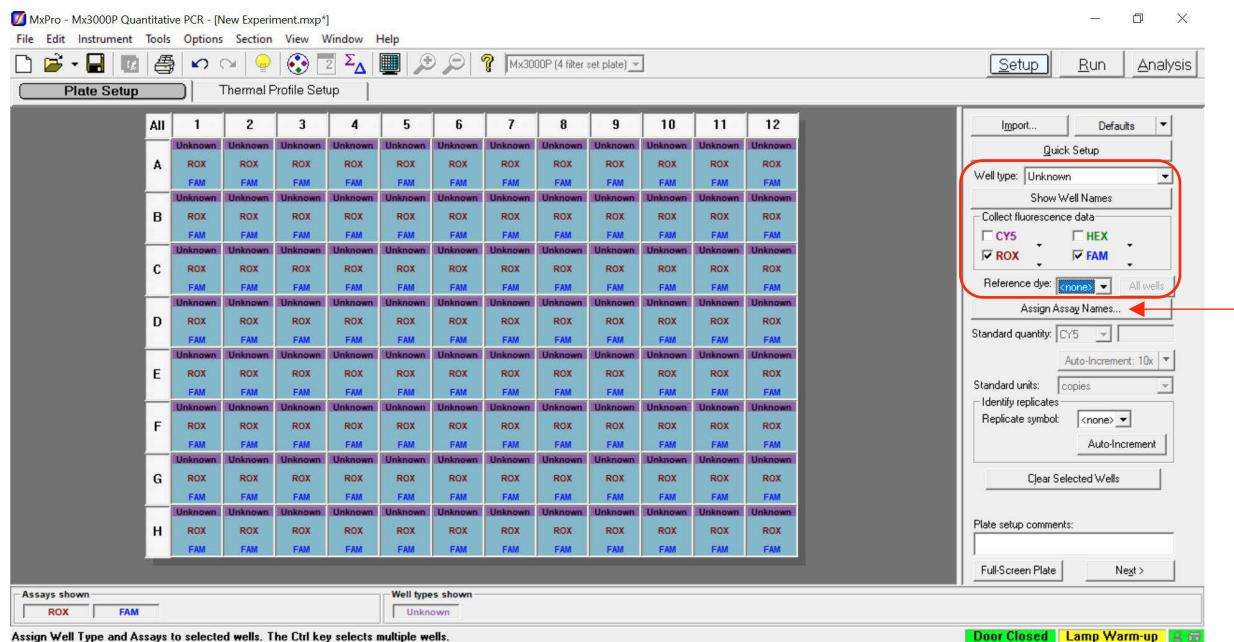
15 RNA STAR Complete Setup for Agilent™ Stratagene Mx3005P

Refer to "User Manual Part Number 70225 J.1" for additional information.

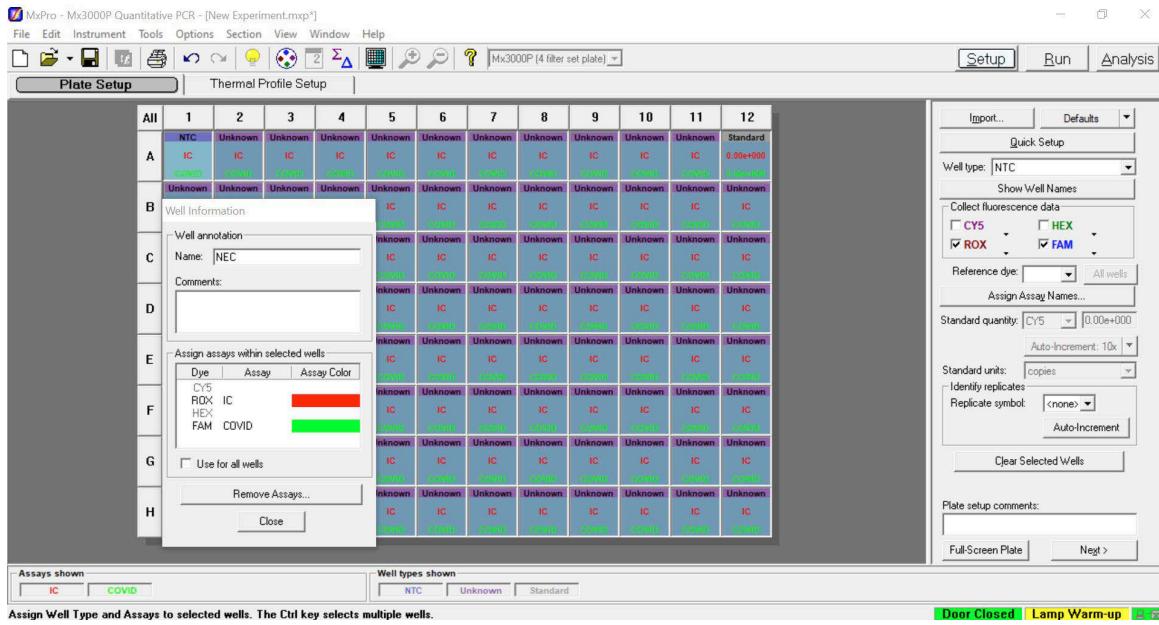
15.1 Programming Instructions for PC/Laptop-connected Instrument

It is recommended to setup the instrument up to step 6 before preparing the Reagent Mix as defined in the qSTAR Reagent Preparation section above. The 96-well plate should be recently centrifuged to guarantee all reagents are at the bottom of the plate and kept on a cold block. Use 96-well plates and seals recommended by the instrument manufacturer (see Option for PCR Instruments & Recommended Consumables table above).

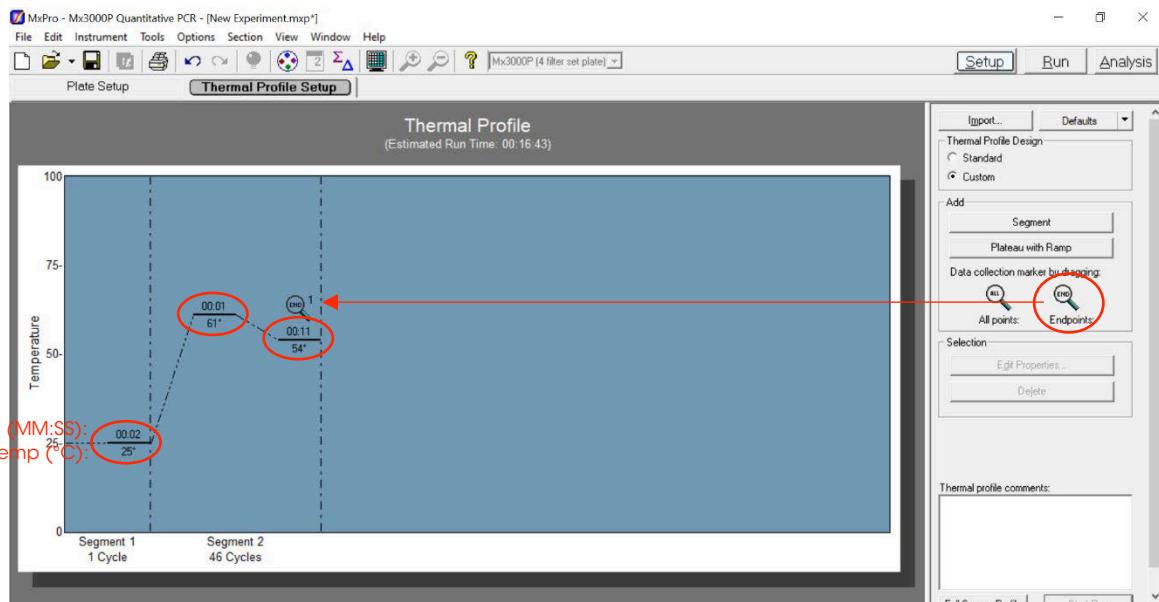
1. Launch the MxPro desktop software (version 4.10). Select 'Quantitative PCR (Multiple Standards)' in the 'New Options' window, check the box next to 'Turn lamp on for warm-up?', and click the 'OK' button. The lamp requires 20 minutes to properly warm-up.



2. On the 'Plate Setup' screen, select all the wells and, on the right of the software using the drop-down menu, set 'Well Type' to 'Unknown'. In the 'Collect Fluorescence Data' screen check the boxes next to 'ROX' and 'FAM'. From the 'Reference Dye' drop-down menu select '<none>'. Click the 'Assign Assay Names' button.
3. Select 'FAM' from the 'Assign assays within selected wells' section then define 'Assay' name as "COVID" from the drop-down menu and select green for the 'Assay Color'. Select 'ROX', define 'Assay' name as "IC" from the drop-down menu and select red for the 'Assay Color'. Click 'Close' to exit the 'Well Information' window.



4. Select just well 'A1' and, on the right of the software using the drop-down menu, re-define 'Well Type' to 'NTC' (No Template Control). Select just well 'A12' and re-define 'Well Type' to 'Standard'. Double-click well 'A1' and define the 'Name' as "NCM" (Negative Control Media). Click 'Close'. Double-click well 'A12' and define the 'Name' as "PCM" (Positive Control Media). Click 'Close'. Sample names can be added to wells using this method or can be imported from an Excel file (optional). Proceed to the 'Thermal Profile Setup' tab.



5. On the right of the screen in the 'Add' section, click 'Segment' to add a segment. Select the 'Segment 3' window and, on the right of the screen in the 'Selection' section, click 'Delete'. Select the new 'Segment 2' window then, on the right of the screen in the 'Add' section, select 'Plateau with Ramp'. Modify the default thermal profile for 'Segment 1' as follows: set 'Temp' (lower left) to '25°', set 'Time' (upper left) to '00:02', and set 'Cycle' to '1'. Modify the default thermal profile for 'Segment 2' as follows: set 'Temp' (lower middle) to '61°', set 'Time' (upper middle) to '00:01', set 'Temp' (lower right) to '54°', set 'Time' (upper right) to '00:111', set 'Cycle' to '46', and, from the right of screen in the 'Add' section, drag the 'Endpoints:' symbol to the lower right 'Temp' to add a data collection step.
6. Click 'Instrument' from the menu and choose 'Filter Set Gain Settings'. Verify the settings for 'ROX' and 'FAM' are set to 'x1' and 'x1', respectively. Click 'OK' to apply the changes and close the menu.

7. On the Agilent Stratagene Mx3005P instrument, lift the instrument door up, pull the block lid out to unlock then lift the block lid and load the plate.

Note: Be cautious when loading the plate since the lid is heated. Position A1 is not defined in the instrument but load the plate so that position A1 on the 96-well plate is the top, left-most well.

Close and lock the block lid then close the instrument door.

8. In the MxPro desktop software (version 4.10), click 'Run' on the top right of the screen then click 'Start' to initiate the run. 'Name' the experiment "RNA STAR Complete [YYMMDD_Plate#]" and click 'Save'. The run is started.
9. Upon completion of the run, the message "Turn off the lamp?" will appear. If running subsequent experiments, it is recommended to leave the lamp on. It is recommended to turn the lamp off at the end of each day.
10. Remove the plate and discard the plate in a sealable waste bag or container.

15.2 Analysis Instructions

1. Click 'Analysis Term Settings' in the 'Select data collection ramp/plateau' section on the right of the software in the 'Analysis Selection/Setup' tab. In the 'Active Settings' section select 'Adaptive Baseline' and check the box next to 'Use MX4000 v 1.00 to v 3.00 algorithm'. A message box will appear. Click 'Yes' then click 'OK' to exit the 'Analysis Term Settings' window. Proceed to 'Results' tab near the top of the software.
2. Verify 'Fluorescence' is set to 'dR' using the drop-down menu on the right of the software. In the 'Threshold Fluorescence' section, change the values for 'IC' and 'COVID' each to "1000". Right-click the Y-axis plot in the 'Amplification Plots' section and change 'Scale Y Axis' to 'Log'. In the 'Area to Analyze' section on the right of the software, select the 'Text Report'. Under the 'Column' section, select the boxes next to the following items to include in the final export file: 'Well', 'Well Name', 'Dye', 'Assay', 'Well Type', 'Ct (dR)', and 'Final Call (dR)'. Deselect all other column headings.
3. Export the results by right-clicking anywhere in the tabular text report. Choose 'Export Text Report' then click 'Export Text Report to Excel'.
4. From 'File' in the menu, choose 'Save' from the drop-down menu.
5. Exit the software

16 Interpretation of Results and Reporting

All test controls should be examined prior to interpretation of patient results. If the controls are not valid, the patient results cannot be interpreted.

16.1 LumiraDx SARS-CoV-2 RNA STAR Complete Controls

1. The LumiraDx SARS-CoV-2 RNA STAR Complete Positive Control Media (Pos. Ctrl. Med.) is an external control needed to ensure test reagents are properly detecting SARS-CoV-2 nucleic acids. It is comprised of a quantified NATtrol™ SARS-Related Coronavirus 2 (SARS-CoV-2) External Run Control (ZeptoMetrix Corporation; 50,000 copies / mL). The working concentration of the Pos. Ctrl. Med. is 500 copies per reaction⁴. The control is formulated in a proprietary matrix with purified, intact viral particles containing whole length genome. The virus particles have been chemically modified to render them non-infectious and refrigerator stable.
2. The LumiraDx SARS-CoV-2 RNA STAR Complete Negative Control Media (Neg. Ctrl. Med.) is an external control needed to ensure cross-contamination, or reagent contamination from the upper respiratory swab specimen does not occur and is comprised of a 0.85% Saline Solution (Hardy Diagnostics; Catalog #U157).
3. An Internal Control (a component in the LumiraDx SARS-CoV-2 RNA STAR Complete 50X IC/P Mix) consists of a 40-bp synthetic RNA – that the assay primers can bind and amplify from – with a unique probe region for molecular beacon detection in the ROX channel. The Internal Control serves as a control for detection of inhibitors present in the specimen, assures that adequate amplification has taken place, and that the enzymes and primers were not inadvertently damaged during production, shipment, and storage.

The testing algorithm for all instruments is based on the standard practice of determining background fluorescence and calling a sample well positive if the change in fluorescent signal exceeds an established threshold. Background fluorescence levels are calculated from cycles 1-6 for the Roche LightCycler 480 II and the Applied Biosystems QuantStudio 5. The Applied Biosystems 7500 Fast Dx uses cycles 2-6, the Applied Biosystems QuantStudio 7 Flex uses cycles 1-3, the Agilent AriaMx uses cycles 1-4, and the Agilent Stratagene Mx3005p uses the Adaptive Baseline settings. A threshold (further described starting in section 11, for each instrument) is applied to the run. Positive and negative results are based on a sample well exceeding this threshold. No Cycle Threshold (Ct) cutoff is used for this testing algorithm, and it is expected that all reactions should occur between a Ct value of 5 to 35, with the exception that the reaction for Applied Biosystems QuantStudio 7 Flex should occur between a Ct value of 3 to 30.

Failure of either the Positive (Pos. Ctrl. Med.) or Negative (Neg. Ctrl. Med.) Controls Media invalidates the qSTAR run and results should not be reported. The qSTAR assay should be repeated with the aliquot of external controls and specimens first. If the results continue to be invalid, test another aliquot of the external controls and specimen or obtain another sample from the patient and retest. If the Internal Control (IC) fails to amplify (in the absence of a positive signal), the qSTAR assay should be repeated as described above.

Table 1. Expected Results from External Controls (on Roche LightCycler 480 II, Applied Biosystems 7500 Fast Dx, Applied Biosystems QuantStudio 5, Agilent Aria Mx, and Agilent Stratagene Mx3005P).

Control Type/Name	Used to Monitor	SARS-CoV-2 (FAM)	Expected Ct Values	Internal Control (ROX)	Expected Ct Values
Pos. Control Media	Substantial reagent failure including primer and probe integrity	+ (Positive)	5.0 ≤ Ct ≤ 35.0	+ or - (Can be positive or negative)	5.0 ≤ Ct ≤ 35.0*
Neg. Control Media	Reagent and/or environmental contamination	- (Negative)	None detected	+ (Positive)	5.0 ≤ Ct ≤ 35.0

*The Internal Control is not required to amplify for the Pos. Ctrl. Med. to be deemed positive.

Table 2. Expected Results from External Controls on Applied Biosystems QuantStudio 7 Flex.

Control Type/Name	Used to Monitor	SARS-CoV-2 (FAM)	Expected Ct Values	Internal Control (ROX)	Expected Ct Values
Pos. Control Media	Substantial reagent failure including primer and probe integrity	+ (Positive)	3.0 ≤ Ct ≤ 30.0	+ or - (Can be positive or negative)	3.0 ≤ Ct ≤ 30.0*
Neg. Control Media	Reagent and/or environmental contamination	- (Negative)	None detected	+ (Positive)	3.0 ≤ Ct ≤ 30.0

*The Internal Control is not required to amplify for the Pos. Ctrl. Med. to be deemed positive.

⁴ A two-fold dilution of the stock is made using 0.85% saline (Neg. Ctrl. Med.) and 20 µL is added directly to the reaction.

16.2 Interpretation of Patient Specimen Results

Assessment of a clinical specimen test result should be performed after the Positive and Negative Controls have been examined and determined to be valid. If the Controls are not valid, the patient results cannot be interpreted.

Table 3. Interpretation of LumiraDx SARS-CoV-2 RNA STAR Complete (on Roche LightCycler 480 II, Applied Biosystems 7500 Fast Dx, Applied Biosystems QuantStudio 5, Agilent AriaMx, and the Agilent Stratagene Mx3005P).

Assay Results	SARS-CoV-2 (FAM)	Internal Control (ROX)	Interpretation of Results	Notes
SARS-CoV-2 Positive ("+")	$5.0 \leq Ct \leq 35.0$	$5.0 \leq Ct \leq 35.0^*$	SARS-CoV-2 Viral RNA detected; IC possibly detected	Report results
Negative ("-")	No Ct detected	$5.0 \leq Ct \leq 35.0$	No SARS-CoV-2 Viral RNA detected; IC detected	Report results. Consider testing for other respiratory pathogens.
Invalid	No Ct detected	No Ct detected	No SARS-CoV-2 Viral RNA detected; No IC detected	Invalid test. Retest the same processed sample. If the second test is also invalid obtain a new specimen and retest.

*The Internal Control is not required to amplify.

Table 4. Interpretation of LumiraDx SARS-CoV-2 RNA STAR Complete on Applied Biosystems QuantStudio 7 Flex.

Assay Results	SARS-CoV-2 (FAM)	Internal Control (ROX)	Interpretation of Results	Notes
SARS-CoV-2 Positive ("+")	$3.0 \leq Ct \leq 30.0$	$3.0 \leq Ct \leq 30.0^*$	SARS-CoV-2 Viral RNA detected; IC possibly detected	Report results
Negative ("-")	No Ct detected	$3.0 \leq Ct \leq 30.0$	No SARS-CoV-2 Viral RNA detected; IC detected	Report results. Consider testing for other respiratory pathogens.
Invalid	No Ct detected	No Ct detected	No SARS-CoV-2 Viral RNA detected; No IC detected	Invalid test. Retest the same processed sample. If the second test is also invalid obtain a new specimen and retest.

*The Internal Control is not required to amplify.

17 Quality Control

- Quality control requirements must be performed in conformance with local, state, and federal regulations or accreditation requirements and the user's laboratory standard quality control procedures. For further guidance on appropriate quality control practices, refer to 42 CFR 493.1200.
- Quality control procedures are intended to monitor reagent and assay performance.
- Test all Positive and Negative Controls Media when running diagnostic samples and with each new lot of the LumiraDx SARS-CoV-2 RNA STAR Complete kit to ensure all reagents and kit components are working properly.
- Good laboratory practice (GLP) recommends running a Positive Control (Pos. Ctrl. Med.) and Negative Control (Neg. Ctrl. Med.) in each amplification reaction.
- All samples include an Internal Control for validation of enzyme, primer, and probe stability.

18 Limitations

- This assay is for *in vitro* diagnostic use under FDA Emergency Use Authorization only.
- Do not use reagents past their expiration date.
- This Test cannot rule out diseases caused by other bacterial or viral pathogens.
- Negative results do not preclude SARS-CoV-2 infection and should not be the sole basis for treatment of patient management decisions. Optimum specimen types and timing for peak viral levels during infections caused by 2019-nCoV have not been determined.
- The performance of LumiraDx SARS-CoV-2 RNA STAR Complete was assessed using nasal and nasopharyngeal swab specimens. Other upper respiratory specimens (such as mid-turbinate, and oropharyngeal swab specimens) are also considered acceptable specimen types for use with LumiraDx SARS-CoV-2 RNA STAR Complete. However, performance has not been established.
- Samples must be collected, transported, and stored using appropriate procedures and conditions. Improper collection, transport, or storage of specimens may hinder the ability of this Test to detect the target sequences.
- If the virus mutates in the qSTAR target region, SARS-CoV-2 virus may not be detected or may be detected less predictably.
- Inhibitors present in the specimen and/or errors in following the Test procedure may lead to false negative results.
- Excessive levels of mucin, greater than 0.625mg/ml, may inhibit the Test and lead to false negative or invalid results.
- A trained health care professional should interpret assay results in conjunction with the patient's medical history, clinical signs and symptoms, and the results of other diagnostic tests.
- Analyte targets (viral sequences) may persist *in vivo*, independent of virus viability. Detection of analyte target(s) does not imply that the corresponding virus(es) are infectious, nor that they are the causative agents for clinical symptoms.
- There is a risk of false positive values resulting from cross-contamination by target organisms, their nucleic acids or amplified product, or from non-specific signals in this Test.
- There is a risk of false negative values due to the presence of sequence variants in the viral targets of this Test.
- This Test performance was not established in immunocompromised patients.
- The clinical performance of this test 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.

19 Conditions of Authorization for the Laboratory

The "LumiraDx SARS-CoV-2 RNA STAR Complete Letter of Authorization", along with the authorized "Fact Sheet for Healthcare Providers", the authorized "Fact Sheet for Patients", and other authorized labeling are available on the FDA website: <https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/vitro-diagnostics-euas>. However, to assist clinical laboratories using LumiraDx SARS-CoV-2 RNA STAR Complete, the relevant Conditions of Authorization are listed below:

1. Authorized laboratories⁵ using LumiraDx SARS-CoV-2 RNA STAR Complete 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.
2. Authorized laboratories using LumiraDx SARS-CoV-2 RNA STAR Complete must use the product as outlined in the authorized labeling. Deviations from the authorized procedures, including the authorized instruments, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to use this product are not permitted.
3. Authorized laboratories that receive the LumiraDx SARS-CoV-2 RNA STAR Complete test must notify the relevant public health authorities of their intent to run this product prior to initiating testing.
4. Authorized laboratories using LumiraDx SARS-CoV-2 RNA STAR Complete must have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.
5. Authorized laboratories must collect information on the performance of LumiraDx SARS-CoV-2 RNA STAR Complete and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and LumiraDx (via email: customerservices.US@lumiradx.com) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of your product of which they become aware.
6. All laboratory personnel using LumiraDx SARS-CoV-2 RNA STAR Complete must be appropriately trained in nucleic acid amplification techniques and use appropriate laboratory and personal protective equipment when handling this Test and use this product in accordance with the authorized labeling.
7. LumiraDx, authorized distributors, and authorized laboratories using LumiraDx SARS-CoV-2 RNA STAR Complete must ensure that any records associated with this EUA are maintained until otherwise notified by the FDA. Such records will be made available to the FDA for inspection upon request.

⁵ 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."

20 Performance Characteristics

20.1 Limit of Detection (LoD)

20.1.1 Purpose

To establish the lowest SARS-CoV-2 viral copy number that can be detected by LumiraDx SARS-CoV-2 RNA STAR at least 95% of the time.

20.1.2 Procedure

Sample matrix pools were prepared from nasopharyngeal (NP) and Nasal (NS) swab samples and spiked with a preparation of heat-inactivated Severe Acute Respiratory Syndrome Related Coronavirus 2 (SARS-CoV-2) strain 2019nCoV/USA/WA1/2020 (ATCC® VR1986HK™). The heat-inactivated virions were spiked into the NP and NS swab sample matrix pools at 0, 1875, 3750, 7500, and 15000 copies/mL. A 20 µL input volume was transferred into a 50 µL final reaction volume for LumiraDx SARS-CoV-2 RNA STAR Complete per the standard assay protocol on the Agilent Stratagene Mx3005P (software version 4.10) RT-PCR Instrument.

20.1.3 Results and Conclusions

The results are shown in Table 5 for the preliminary LoD studies. The preliminary LoD was expected to be 3,750 copies/mL.

Table 5. Preliminary LoD study.

Concentration (Copies/mL)	Concentration (Copies/Rxn)	Observed Properties	
		Replicates Detected	Mean Ct Value
0	0.00	0/5	n/a
1,875	35.5	2/5	17.5
3,750	75.0	5/5	17.8
7,500	150	5/5	18.0
15,000	300	5/5	16.6

The wet swab LoD was confirmed by processing and testing 20 replicates at 3,750 and 7,500 copies/mL. A 20 µL input volume was transferred into a 50 µL final reaction volume for LumiraDx SARS-CoV-2 RNA STAR Complete per the standard assay protocol on the Agilent Stratagene Mx3005P (software version 4.10) RT-PCR Instrument. Based on the full extraction process from contrived clinical samples, the LoD is 7,500 copies/mL, as shown in Table 6.

Table 6. Wet Swab LoD confirmation study.

Concentration (Copies/mL)	Observed Properties	
	Replicates Detected	Mean Ct Value
0	0/3	n/a
3,750	17/20	18.8
7,500	20/20	18.5

The dry swab LoD was confirmed by depositing 20 µL nasopharyngeal (NP) matrix containing either 3,750 or 7,500 copies and allowing the swab to air dry for 30 minutes. The dried NP swabs were then expressed in 1 mL of medium and a 20 µL input volume was transferred into a 50 µL final reaction volume for LumiraDx SARS-CoV-2 RNA STAR Complete per the standard assay protocol on the Agilent Stratagene Mx3005P (software version 4.10) RT-PCR Instrument. The LoD is 7,500 copies/mL, as shown in Table 7.

Table 7. Dry Swab (expressed in 1mL medium) LoD confirmation study.

Concentration (Copies/mL)	Observed Properties	
	Replicates Detected	Mean Ct Value
0	0/2	n/a
3,750	15/20	19.6
7,500	19/20	17.8

20.2 RT-PCR Instrument Validation

20.2.1 Purpose

To demonstrate consistent results between different laboratory RT-PCR instruments.

20.2.2 Procedure

Sample matrix pools were prepared from nasopharyngeal (NP) and Nasal (NS) swab samples and spiked with a preparation of heat-inactivated Severe Acute Respiratory Syndrome Related Coronavirus 2 (SARS-CoV-2) strain 2019nCoV/USAWA1/2020 (ATCC® VR1986HK™). The heat-inactivated virions were spiked into the sample matrix pools at 0, 0.5x LoD, and 1x LoD. A 20 µL input volume was transferred into a 50 µL final reaction volume for LumiraDx SARS-CoV-2 RNA STAR Complete per the standard assay protocol on each RT-PCR Instrument.

20.2.3 Results and Conclusions

All zero copy replicates showed internal control amplification and no target amplification. All other results are reported in Table 8. The replicates for each instrument and target level are shown. Study results demonstrated that LumiraDx SARS-CoV-2 RNA STAR Complete generated comparable results when performed on the Roche LightCycler 480 II, Applied Biosystems 7500 Fast Dx, Applied Biosystems QuantStudio 5, Applied Biosystems QuantStudio 7 Flex, Agilent AriaMx and Stratagene Mx3005P.

Table 8. LumiraDx SARS-CoV-2 RNA STAR Complete on different RT-PCR Instruments.

Concentration (Copies/mL)	Roche LightCycler 480 II	Applied Biosystems 7500 Fast Dx	Applied Biosystems QuantStudio 5	Applied Biosystems* QuantStudio 7 Flex	Agilent AriaMx	Agilent Stratagene Mx3005P
0	FAM (COVID)	0/5	0/5	0/5	0/20	0/5
	ROX (IC)	5/5	5/5	5/5	20/20	5/5
0.5x	FAM (COVID)	4/5	3/5	2/5	18/20	4/5
	ROX (IC)	3/5	5/5	5/5	20/20	5/5
1x	FAM (COVID)	5/5	5/5	5/5	20/20	5/5
	ROX (IC)	5/5	5/5	5/5	20/20	5/5

*Heat inactivated virus was spiked into transport media.

20.3 Inclusivity (Analytical Sensitivity)

20.3.1 Purpose

To demonstrate inclusivity for all SARS-CoV-2 sequences in the GISAID database⁶ through *in silico* analysis of the LumiraDx SARS-CoV-2 RNA STAR Complete.

19.3.2 Procedure

LumiraDx SARS-CoV-2 RNA STAR Complete detects ORF1a of SARS-CoV-2. To ensure the performance of this test design, an *in silico* analysis was performed to confirm test inclusivity. A multisequence alignment was generated with complete high coverage SARS-CoV-2 sequences maintained in the GISAID database submitted between the dates of December 24, 2019, and August 26, 2020 (n = 89,126).

⁶ GISAID (Global Initiative on Sharing All Influenza Data). Initiative. GISAID. <https://www.epicov.org/epi3/frontend#5c74af>. Accessed August 26, 2020.

20.3.3 Results & Conclusions

An alignment was performed on August 26, 2020 with the oligonucleotide primer and probe sequences of the LumiraDx SARS-CoV-2 RNA STAR Complete with 89,126 publicly available SARS-CoV-2 sequences maintained in the GISAID database to demonstrate the predicted inclusivity of the assay. All the alignments show over 99% identity of the assay to the SARS-CoV-2 sequences. The 1% of sequences that did not match fell into two categories. Approximately 0.62% of the sequences that did not match had a single base mismatch, of the remaining unmatched sequences, approximately 0.36%, had full homology for the bases that had been sequenced, however the remaining bases in the genomes were low sequence coverage and ambiguous and were represented by an "N" or shifted. Based on the high percentage of analyzed sequences having no mismatches, and of the very few sequences with mismatches (only had a single mismatched base), the likelihood of a false negative is very low.

20.4 Cross Reactivity (Analytical Specificity)

20.4.1 Purpose

To demonstrate, through *in silico* analysis, the cross-reactivity of the LumiraDx SARS-CoV-2 RNA STAR Complete against sequences of pathogens potentially present in respiratory specimens and/or with genetic similarities to SARS-CoV-2.

20.4.2 Procedure

To ensure the performance of this test, *in silico* analysis was performed to confirm test exclusivity. The primer and probe sequences of the LumiraDx SARS-CoV-2 RNA STAR Complete are identical to the ones used in the LumiraDx SARS-CoV-2 RNA STAR test. The cross-reactivity of the LumiraDx SARS-CoV-2 RNA STAR test has been evaluated under an EUA202169. The potential cross-reactivity was analyzed *in silico* organisms listed in Table 9.

20.4.3 Results and Conclusions

In-silico analysis for the ORF1a primer/probe set of the LumiraDx SARS-CoV-2 RNA STAR Complete test was conducted to access cross-reactivity against sequences of pathogens potentially present in respiratory specimens and/or with genetic similarities to SARS-CoV-2. *In-silico* cross-reactivity is defined as greater than 80% homology between 'primer and probe set' and any sequence present in the targeted microorganism. The conditions under which cross-reaction can occur are at least established as being capable of producing an amplicon (<500 bp) and are limited to more than 80% homology of all the oligos that bind to the microorganism.

In silico analysis confirmed organisms listed in Table 9 maintain less than 80% homology with the SARS-CoV-2 qSTAR primers and probes.

Table 9. *In silico* homology for potential cross reactivity with LumiraDx SARS-CoV-2 RNA STAR Complete.

Pathogen	Strain	GenBank Accession #
Human coronavirus 229E	Seattle/USNSC9724/2018	MN369046.1
Human coronavirus OC43	MDS12	MK303623.1
Human coronavirus HKU1	SI17244	MH940245.1
Human coronavirus NL63	Seattle/USNSC0768/2019	MN306040.1
SARS CoV-1	Urbani isolate icSARS-C7-MA	MK062184.1
MERS CoV	Hu/Riyadh-KSA1801382/2018	MN723544.1
Adenovirus C1	SG09/HAdvC1/2016	MN513345.1
Human Metapneumovirus (hMPV)	HMPV/Seattle/USA/SC0380/2019	KC562241.1
Parainfluenza virus 1	HPIV1/USA/38078 A/2011	KF530203.1
Parainfluenza virus 2	HPIV2/Los Angeles/USA/C HLA18/2016	MK167027.1
Parainfluenza virus 3	HPIV3/Seattle/USA/SC9406/2019	MN306052.1
Parainfluenza virus 4	HPIV4b/Seattle/USA/SC9597/2019	MN306058.1
Influenza A	H3N2 A/Kitakyushu/ 159/93 PB1	AF037418.1
Influenza B	B/Iowa/14/2017	CY236691.1
Enterovirus EV68	USA/CA/2014-RGDS-1025	MK681490.1
Respiratory syncytial virus	RSV-A/US/BID-V8392/2003	MG027862.1
Rhinovirus	Rhinovirus 59 strain 16-J2	KY629935.1
<i>Chlamydia pneumoniae</i>	AR39	AE002161.1
<i>Haemophilus influenzae</i>	10P129H1	CP029620.1
<i>Legionella pneumophila</i>	Albuquerque 1 (D-7474)	CP021286.1
<i>Mycobacterium tuberculosis</i>	TCDC11	CP046728
<i>Mycobacterium tuberculosis</i>	H37Rv	NC_000962
<i>Streptococcus pneumoniae</i>	R6CIB17	CP038808.1
<i>Streptococcus pyogenes</i>	1085	CP047120.1
<i>Bordetella pertussis</i>	A340	CP033420.1
<i>Mycoplasma pneumoniae</i>	M129	U00089.2
<i>Candida albicans</i>	TIMM1768	CP032012.1
<i>Pseudomonas aeruginosa</i>	VIT PC9	CP048791.1
<i>Staphylococcus epidermidis</i>	1457	CP020463.1
<i>Streptococcus salivarius</i>	57.1	CP002888.1

20.5 Endogenous Interference Substances Studies

20.5.1 Purpose

To demonstrate that interfering substances that could potentially be found in the upper respiratory tract do not cross-react or degrade the performance of LumiraDx SARS-CoV-2 RNA STAR Complete.

20.5.2 Procedure

Eleven (11) potential interfering substances listed in Table 10 were tested in the absence or presence of SARS-CoV-2 in sample matrix pools prepared from nasopharyngeal (NP) and Nasal (NS) swab samples.

20.5.3 Results and Conclusions

None of the eleven (11) potential interfering substances listed in Table 10 tested demonstrated interference except Mucin, which required further dilution.

Table 10. Endogenous Interference Substances Studies for LumiraDx SARS-CoV-2 RNA STAR Complete.

Potential Interferent	Active Ingredient	Final Concentration	Target	% Agreement with Expected Results
Vick's VapoCool (throat lozenge)	Benzocaine, Menthol	5%	Positive SARS-CoV-2 (3X LoD)	100% (3/3)
			Negative	100% (3/3)
Human Blood	Blood	5%	Positive SARS-CoV-2 (3X LoD)	100% (3/3)
			Negative	100% (3/3)
Flonase	Fluticasone	5%	Positive SARS-CoV-2 (3X LoD)	100% (3/3)
			Negative	100% (3/3)
Halls Relief (Cherry)	Menthol	0.8 g/mL	Positive SARS-CoV-2 (3X LoD)	100% (3/3)
			Negative	100% (3/3)
Mucin, Type II (from porcine stomach) ¹	Purified mucin protein	2.5 mg/mL	Positive SARS-CoV-2 (3X LoD)	0% (0/3)
			Negative	0% (0/3)
Mucin, Type II (from porcine stomach) ¹	Purified mucin protein	250 ng/mL	Positive SARS-CoV-2 (3X LoD)	100% (3/3)
			Negative	100% (3/3)
Rhinocort	Budesonide (Glucocorticoid)	5%	Positive SARS-CoV-2 (3X LoD)	100% (3/3)
			Negative	100% (3/3)
Nasal spray	Oxymetazoline	5%	Positive SARS-CoV-2 (3X LoD)	100% (3/3)
			Negative	100% (3/3)
Nasal spray	Sodium Chloride	15%	Positive SARS-CoV-2 (3X LoD)	100% (3/3)
			Negative	100% (3/3)
Tobramycin (antibacterial)	Tobramycin	1.25 mg/mL	Positive SARS-CoV-2 (3X LoD)	100% (3/3)
			Negative	100% (3/3)
Zanamivir	Zanamivir	282 ng/mL	Positive SARS-CoV-2 (3X LoD)	100% (3/3)
			Negative	100% (3/3)
Zicam Cold Remedy	Galphimia glauca, Luffa operculata, Sabadilla	5%	Positive SARS-CoV-2 (3X LoD)	100% (3/3)
			Negative	100% (3/3)

¹Mucin inhibited both target and IC RNA at 2.5 mg/mL. Mucin was diluted to 250 ng/mL and showed 3 positives out of 3 attempts (100%).

20.6 Clinical Evaluation

20.6.1 Purpose

To demonstrate positive and negative percent agreements between the LumiraDx SARS-CoV-2 RNA STAR Complete test when compared to an approved FDA EUA RT-PCR test using at least 30 negative and 30 positive nasopharyngeal (NP) and Nasal (N) swab clinical specimens.

20.6.2 Procedure

A selected panel of 65 negative and 45 positive nasopharyngeal and nasal swab samples were prepared and tested in a randomized blind study. Sixteen of the nasopharyngeal swab samples were collected in 3 mL VTM and the remaining ninety-four swab samples were “dry swabs” and were expressed in 1 mL of Corning VTM. Each sample was processed with the LumiraDx SARS-CoV-2 RNA STAR Complete test using the Stratagene Mx3005P (software version 4.10) and the FDA EUA RT-PCR test.

20.6.3 Results and Conclusions

Positive and negative percent agreements between the LumiraDx SARS-CoV-2 RNA STAR Complete test when compared to an authorized FDA EUA RT-PCR test is shown below in Table 11, the data stratified by dry versus wet swab is shown below in Table 12. Confidence limits at the 95% level are also included. LumiraDx SARS-CoV-2 RNA STAR Complete overall performed with 97.78% Positive Agreement and 98.46% Negative Agreement for 45 positive and 65 negative samples when compared to the FDA EUA RT-PCR test. It should be noted that samples with an invalid/indeterminate result from the reference test results were removed from the study prior to blinding. The negative invalid result upon three subsequent runs were all negative.

Table 11. Clinical evaluation of LumiraDx SARS-CoV-2 RNA STAR Complete.

FDA EUA RT-PCR Test (Comparator)			
	Positive	Negative	Total
LumiraDx SARS-CoV-2 RNA STAR Complete	Positive	44	1 *
	Negative	1	64
	Total	45	65
Percent Agreement	97.78% (Pos.)		98.46% (Neg.)
95% Confidence Interval	88.23-99.94%		91.72-99.96%

*The result was invalid for the Test and marked as missed.

Table 12. Stratified Clinical evaluation of LumiraDx SARS-CoV-2 RNA STAR Complete.

Dry Swabs (expressed in 1mL medium)	FDA EUA RT-PCR Test (Comparator)		
	Positive	Negative	Total
LumiraDx SARS-CoV-2 RNA STAR Complete	Positive	29	1 *
	Negative	0	64
	Total	29	65
Percent Agreement	100% (Pos.)		98.46% (Neg.)
95% Confidence Interval	88.06-100%		91.72-99.96%

*The result was invalid for the Test and marked as missed.

Wet Swabs	FDA EUA RT-PCR Test (Comparator)		
	Positive	Negative	Total
LumiraDx SARS-CoV-2 RNA STAR Complete	Positive	15	-
	Negative	1	-
	Total	16	65
Percent Agreement	93.75% (Pos.)		N/A (Neg.)
95% Confidence Interval	69.77-99.84%		N/A

*The result was invalid for the Test and marked as missed.

21 Contact Information, Ordering, and Product Support

For ordering, contact LumiraDx at:

Website: www.lumiradx.com.
Email (US): customerservices.US@lumiradx.com.
Email (International): customerservices@lumiradx.com.

For product information, contact LumiraDx at:

Email: customerservices.US@lumiradx.com. Include "LumiraDx SARS-CoV-2 RNA STAR Complete" in the subject line.
Phone: 1-888-586-4721.

For technical support, contact LumiraDx at:

Email: technicalservices@lumiradx.com. Include "LumiraDx SARS-CoV-2 RNA STAR Complete" in the subject line.

For return policy, contact LumiraDx at:

If there is a problem with LumiraDx SARS-CoV-2 RNA STAR Complete you may be asked to return the item. Before returning Tests please obtain a return authorization number from LumiraDx Customer Services (customerservices.US@lumiradx.com). This return authorization number must be on the shipping carton for return. For ordinary returns following purchase, please contact LumiraDx Customer Services for terms and conditions.

Intellectual property

The LumiraDx Test and all provided LumiraDx documentation ('Products') are protected by law. The Intellectual Property of the LumiraDx Products remains at LumiraDx. Details of relevant Intellectual Property regarding our products can be found at lumiradx.com/IP.

Legal notices

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Manufacturer information

LumiraDx UK Ltd, 3 More London Riverside, London, SE1 2AQ, UK. Company Number 09206123.

LumiraDx US Office

221 Crescent St, Suite 502, Waltham, MA 02453. Telephone: 1-617-621-9775.



lumiraDx™ SARS-CoV-2 RNA STAR Complete

Materials required (But not provided)

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PCR Instruments & Related Consumables	Source	Catalog #
Roche LightCycler 480 II	Roche Life Science	5015278001
VWR Adhesive Film for Microplates	VWR	60941-070
LightCycler 480 Multiwell Plate 96, Clear	Roche Life Science	5102413001
Applied Biosystems 7500 Fast Dx Real-Time PCR Instrument	ThermoFisher Scientific	4406984
Applied Biosystems QuantStudio 5	ThermoFisher Scientific	A28574
Applied Biosystems QuantStudio 7 Flex (software version 1.3)	ThermoFisher Scientific	4485698
Applied Biosystems Optical Adhesive Covers	ThermoFisher Scientific	4360954
Applied Biosystems MicroAmp Optical 96-Well Plate	ThermoFisher Scientific	4306737
Agilent Stratagene Mx3005P	Agilent	401511
Applied Biosystems MicroAmp Clear Adhesive Film	ThermoFisher Scientific	4306311
Eppendorf twin.tec Real-Time PCR Plate 96-Well Semi-Skirted	Eppendorf	951022043
Agilent Aria Mx	Agilent Technologies	G8830A
AriaMx 96 Adhesive Seals	Agilent Technologies	401492
AriaMx 96 Well Plates, Skirted, LP	Agilent Technologies	401490
Universal Sealing Films *	General lab supplier	n/a
ThermalSeal A Sealing Film	Research Product Int	202545
Heat-Resistant Polypropylene Film for Raised-Rim Plates	VWR	89087-690

*These universal sealing films have been found to be compatible with all instruments listed above.

Consumables	Source	Catalog #
Appropriate Personal Protective Equipment	General lab supplier	n/a
Aerosol Barrier Pipette Tips with Filters	General lab supplier	n/a
ART 20 SoftFit L Low Retention Pipette Tips (Filtered, Sterile)	ThermoFisher Scientific	2749-05-HR
ART 200 SoftFit L Low Retention Pipette Tips (Filtered, Sterile)	ThermoFisher Scientific	2769-05-HR
ART 1000 SoftFit L Low Retention Pipette Tips (Filtered, Sterile)	ThermoFisher Scientific	2779-05-HR
Microcentrifuge Tubes (DNase/RNase free)	General lab supplier	n/a
Screw Cap w/ O-Ring	ThermoFisher Scientific	3471
1.5mL Microcentrifuge Tubes	ThermoFisher Scientific	3466
2.0mL Microcentrifuge Tubes	ThermoFisher Scientific	3490
5mL Self Standing Screw Cap Transport Tube	Corning	SCT-5ML-S
0.6mL Maxymum Recovery Snaplock Microcentrifuge Tube	Corning	MCT-060-L-C
1.7mL Maxymum Recovery Snaplock Microcentrifuge Tube	Corning	MCT-175-L-C
2.0mL Maxymum Recovery Snaplock Microcentrifuge Tube	Corning	MCT-200-L-C
Powder-Free Nitrile Glove	General lab supplier	n/a
Fisherbrand Small Powder-Free Nitrile Exam Gloves	ThermoFisher Scientific	19-130-1597B
Fisherbrand Medium Powder-Free Nitrile Exam Gloves	ThermoFisher Scientific	19-130-1597C
Fisherbrand Large Powder-Free Nitrile Exam Gloves	ThermoFisher Scientific	19-130-1597D
Sealable Waster Bag or Container	General lab supplier	n/a
Nalgene LDPE Sample Bags	ThermoFisher Scientific	6255-0913
Poxygrid Bag Holder for Biohazard Bags	Bel-Art	131931000
Deep Well 96-Well Plates (U-bottom)	General lab supplier	n/a
96 Deep-Well Plate, Sterile, 2mL	Thomson Instrument	931130-S
Reagent Reservoirs (for minimal dead volume)	General lab supplier	n/a
10mL Reagent Reservoir Sample Pack	Integra Biosciences	4370
100mL Disposable Reagent Reservoirs	VistaLab Technologies	3054-1000
Kimwipes	General lab supplier	n/a
KimWipe Delicate Task Wipes (2-ply, wipe size 11.8" x 11.8")	Kimco	34705
KimWipe Delicate Task Wipes (single ply, wipe size 4.4" x 8.4")	Kimco	34155

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

Customer Service: If the LumiraDx SARS-CoV-2 RNA STAR Complete does not perform as expected, contact LumiraDx Customer Services 1-888-586-4721 or customerservices.us@lumiradx.com.

Manufacturer Information: LumiraDx UK Ltd, 3 More London Riverside, London, SE1 2AQ, UK. Company Number 09206123.

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lumiraDx™ SARS-CoV-2 RNA STAR Complete

Materials required (But not provided)

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Reagents	Source	Catalog #
Sodium Hypochlorite Solution (Bleach)	ThermoFisher Scientific	SS290-1
70% Isopropanol (or 70% Ethanol)	VWR	89499-420
DNAZap™ (or equivalent)	ThermoFisher Scientific	AM9890
RNaseZap™ (or equivalent)	ThermoFisher Scientific	AM9782
Molecular Biology Grade Water	Corning	46-000-CM
Compatible Transport Medias	General lab supplier	n/a
345TK-2 Viral Transport Medium with Flocked Swab	Ruhof Corporation	345TK-2
Copan Universal Transport Media	Copan	3C047N
Puritan UniTranz-RT Transport System	Puritan Medical Product	UT-316
BD Universal Viral Transport	BD	220220
Transport Medium	Corning	25-500-CM
Viral Transport Medium	Hardy Diagnostics	R99
Saline, 0.85%	Hardy Diagnostics	U157
PBS (pH 7.4), 1X	ThermoFisher Scientific	10010023

Equipment	Source	Catalog #
-80°C Laboratory Freezer	General lab supplier	n/a
Stirling Ultracold	Stirling	SU780XLE
-25°C to -15°C Laboratory Freezer	General lab supplier	n/a
-20°C Laboratory Freezer Futura Silver Series	LABRep Co	LHP-14-FM
2°C to 8°C Laboratory Refrigerator	General lab supplier	n/a
Isotemp General Purpose Laboratory Refrigerator	Fisherbrand	FBG12RPGA
Adjustable Multi-Channel Pipettes	General lab supplier	n/a
Pipet-Lite XLS+ 12-Channel Pipette 2-20 μ L Pipette	Rainin	17013808
Pipet-Lite XLS+ 12-Channel Pipette 20-200 μ L Pipette	Rainin	17013810
Pipet-Lite XLS+ 8-Channel Pipette 100-1200 μ L Pipette	Rainin	17014496
Adjustable Micropipettes	General lab supplier	n/a
Pipet-Lite XLS+ 0.5-10 μ L Manual Single-Channel Pipette	Rainin	17014388
Pipet-Lite XLS+ 2-20 μ L Manual Single-Channel Pipette	Rainin	17014392
Pipet-Lite XLS+ 20-200 μ L Manual Single-Channel Pipette	Rainin	17014391
Pipet-Lite XLS+ 100-1000 μ L Manual Single-Channel Pipette	Rainin	17014382
Centrifuges / Rotors	General lab supplier	n/a
Flexifuge Microcentrifuge	Cole-Parmer	EW-22999-50
Eppendorf Microcentrifuge (needs rotor)	Eppendorf	22620560
Two Bucket Rotor (Holds PCR Plates)	Eppendorf	A-2-MTP
0.2 to 0.6mL Tube Rotor	Eppendorf	FA-45-24-11-Kit
1.5 to 2mL Tube Rotor	Eppendorf	S-24-11-AT
PCR Hood	General lab supplier	n/a
AirClean600 PCR Workstation	AirClean Systems	AC648LFUVC
Vortexer	General lab supplier	n/a
Vortex-Genie 2	Scientific Industries	SI-0236
Cold Blocks	General lab supplier	n/a
96-Well PCR Plate Block	ThermoFisher Scientific	88871107
IsoFreeze PCR Racks	Thomas Scientific	1148D61
CoolRack M96, Holds 96 x 1.5 or 2mL Microcentrifuge Tubes	VWR	75779-900
CoolRack XT 5mL, Holds 12 x 5mL Microfuge Tubes	VWR	75779-746
XT Cooling Core	Corning	432081
Racks for Microcentrifuge Tubes	General lab supplier	n/a
96-Well Work Racks (w/ Lids)	RPI Research Products	145921
Interlocking Cube Racks	RPI Research Products	248876
USB Flash Drive	General lab supplier	n/a
16GB USB 2.0 Flash Drive	Best Buy	SDCZ60-016G-A46



LumiraDx™ SARS-CoV-2 RNA STAR Complete

Quick Reference Instructions for Single Swab Format

For Emergency Use Authorization (EUA). For in vitro Diagnostic Use. For Rx Use Only.

LumiraDx SARS-CoV-2 RNA STAR Complete kit components

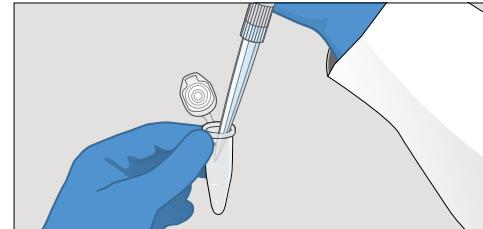


1. Thaw reagents

Thaw the LumiraDx SARS-CoV-2 RNA STAR Complete kit components in cold block at 4 °C; i.e. Positive Control Media (Pos. Ctrl. Med.), Negative Control Media (Neg. Ctrl. Med.), 20X Salt Mix, 10X Extraction Buffer, 50X Internal Control & Primer (IC/P) Mix, and 2.5X Master Mix. Once thawed, invert to mix then pulse centrifuge for 5 seconds.

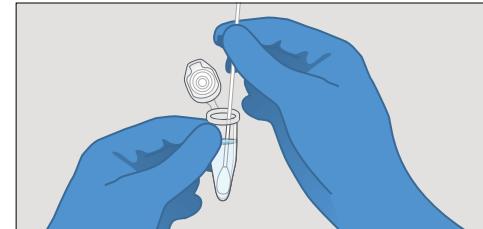
Preparing the sample - single swab format

Collect a dry swab sample before following the steps below.



2. Prepare single tube

If swab is provided dry, transfer 1 mL of a compatible transport media (VTM, 0.85% Saline, or PBS) using a single channel pipette into a suitable tube (e.g. microcentrifuge tube). Swabs (previously) collected in up to 3 mL of compatible transport media are acceptable.



3. Soak swab

Place and soak the swab for 30 seconds then swirl thoroughly by rotating the swab against the side of the tube up to 5 times (beware of cross-contamination from splashing). Discard the swab in biohazard waste.

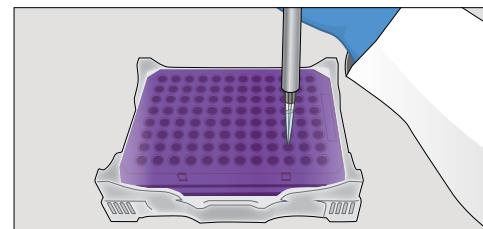


4. Prepare external controls

Assemble fresh 1x PCM (Positive Control Media) by diluting 40 µL Pos. Ctrl. Med. with 40 µL Neg. Ctrl. Med. in a pre-chilled microcentrifuge tube. Assemble the 1x NCM (Negative Control Media) by transferring 80 µL Neg. Ctrl. Med. into a pre-chilled microcentrifuge tube. Excess 1x PCM and 1x NCM should be thrown out.

qSTAR reagent preparation

All components should be kept cold to maintain the integrity of the reagents.



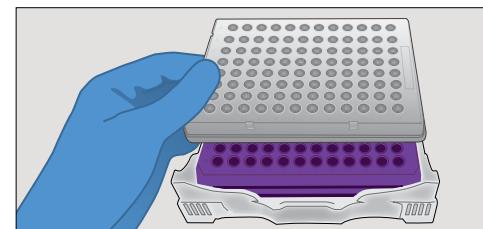
5. Prepare reaction mix

Determine the number of reactions (N) to be prepared per assay and prepare Reaction Mix in a suitable pre-chilled tube (i.e. microcentrifuge tube or 5 mL tube) by following the order in the table below. Between each reagent, slowly mix by pipetting up and down 4 times without introducing bubbles and pulse centrifuge.

Reaction Mix	1 Rxn	N Rxns
Molecular Biology Grade Water	1.5µL	N x 1.5µL
20X Salt Mix	2.5µL	N x 2.5µL
10X Extraction Buffer	5.0µL	N x 5.0µL
50X IC/P Mix	1.0µL	N x 1.0µL
2.5X Master Mix	20µL	N x 20µL
Total	30µL	N x 30µL

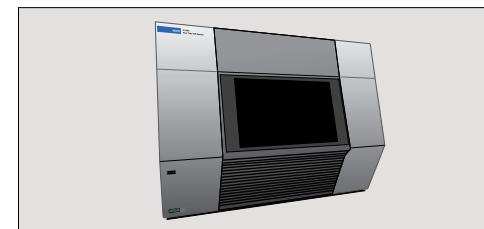
Final setup

Setup for the validated thermocyclers (i.e. Roche LC 480 II, ABI 7500 Fast Dx, ABI QS 5, ABI QS 7 Flex, Agilent AriaMx, or the Agilent Stratagene Mx3005P) is described in the IFU.



6 . Prepare sample plate

Transfer 30 µL Reaction Mix per well into an appropriate pre-chilled, 96-well plate. If there is a delay before adding specimen, this plate should be kept cold (i.e. by using a 96-well PCR plate block, an IsoFreeze PCR rack and/or by storing the plate at 4 °C) and temporarily sealed.



7 . Add sample to plate

Transfer 20 µL, per well, of sample prepared in Step 3 and the external controls prepared in Step 4 to the 96-well plate from Step 6 and mix by slowly pipetting up and down 3 times without introducing bubbles. Seal the 96-well plate using an appropriate optically clear adhesive and centrifuge the plate at 2000 rpm for 5 seconds to collect contents at bottom of plate.

8. Run reaction

Place the 96-well plate in a validated thermocycler and follow instrument specific protocols and analysis procedures detailed in the LumiraDx SARS-CoV-2 RNA STAR Complete IFU.

Precautions: This test has not been FDA cleared or approved but has been authorized by FDA for emergency use under an EUA for use by authorized laboratories; this test has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens; and the emergency use of this test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug, and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated or authorization is revoked sooner. **Customer Service:** If the LumiraDx SARS-CoV-2 RNA STAR Complete does not perform as expected, contact LumiraDx Customer Services 1-888-586-4721 or customerservices.us@lumiradx.com. **Manufacturer Information:** LumiraDx UK Ltd, 3 More London Riverside, London, SE1 2AQ, UK. Company Number 09206123.



lumiraDx™ SARS-CoV-2 RNA STAR Complete

Quick Reference Instructions for Deepwell/Dry Swab Format

For Emergency Use Authorization (EUA). For in vitro Diagnostic Use. For Rx Use Only.

LumiraDx SARS-CoV-2 RNA STAR Complete kit components

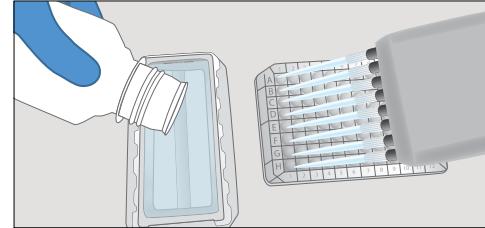


1. Thaw reagents

Thaw the LumiraDx SARS-CoV-2 RNA STAR Complete kit components in cold block at 4 °C; i.e. Positive Control Media (Pos. Ctrl. Med.), Negative Control Media (Neg. Ctrl. Med.), 20x Salt Mix, 10x Extraction Buffer, 50x Internal Control & Primer (IC/P) Mix, and 2.5x Master Mix. Once thawed, invert to mix then pulse centrifuge for 5 seconds.

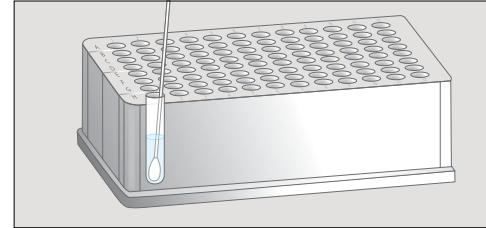
Preparing the sample - deepwell format

Collect a dry swab sample before following the steps below.



2. Prepare deepwell

Pour 100 mL of a compatible transport media (VTM, 0.85 % Saline, or PBS) into a suitable reagent reservoir. Transfer 1 mL to each deepwell using a multichannel pipette. Leave two designated wells empty for the external controls assembled in Step 4.



3. Soak swab

Add the material from a single dry swab specimen to each deepwell in use. Soak the swab for 30 seconds then swirl thoroughly by rotating the swab against the side of the deepwell 5 times (beware of cross-contamination from splashing). Discard the swab in biohazard waste.

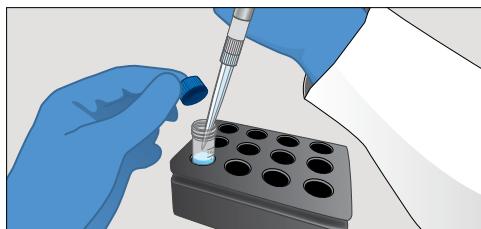


4. Prepare external controls

Assemble the 1x PCM (Positive Control Media) by diluting 500 µL Pos. Ctrl. Med. with 500 µL Neg. Ctrl. Med. in a pre-chilled microcentrifuge tube. Assemble the 1x NCM (Negative Control Media) by transferring 1 mL Neg. Ctrl. Med. into a pre-chilled microcentrifuge tube. Alternatively, the PCM and NCM can be prepared in the deepwell.

qSTAR reagent preparation

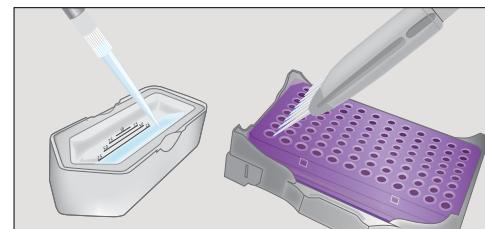
All components should be kept cold to maintain the integrity of the reagents.



5. Prepare reaction mix

Assuming 96 reactions are needed, prepare Reaction Mix in a pre-chilled 5 mL tube by following the order in the table below. Between each reagent, slowly mix by pipetting up and down 4 times without introducing bubbles and pulse centrifuge.

Reaction Mix	100 Rxns
Molecular Biology Grade Water	150µL
20X Salt Mix	250µL
10X Extraction Buffer	500µL
50X IC/P Mix	100µL
2.5X Master Mix	2x 1000 µL
Total	3000µL

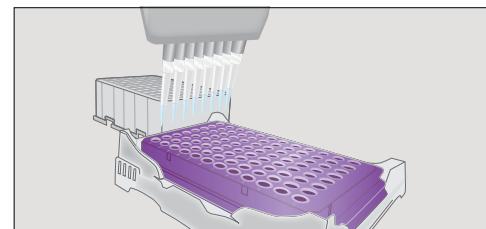


6 . Prepare sample plate

Transfer 3 mL Reaction Mix to a pre-chilled reagent reservoir (for minimal dead volume) using a single channel pipette. Then, using a multichannel pipette, transfer 30 µL Reaction Mix per well into an appropriate, pre-chilled, 96-well plate. If there is a delay before adding specimen, this plate should be kept cold and temporarily sealed.

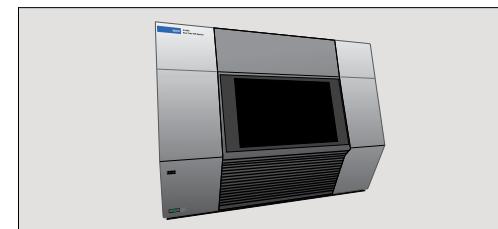
Final setup

Setup for the validated thermocyclers (i.e. Roche LC 480 II, ABI 7500 Fast Dx, ABI QS 5, ABI QS 7 Flex, Agilent AriaMx, or the Agilent Stratagene Mx3005P) is described in the IFU.



7 . Add sample to plate

Transfer 20 µL, per well, of samples from the deepwell (Step 3) and the external control tubes (Step 4) to the 96-well plate from Step 6 and mix by slowly pipetting up and down 3 times without introducing bubbles. Seal the 96 -well plate using an appropriate optically clear adhesive and centrifuge the plate at 2000 rpm for 5 seconds to collect contents at bottom of plate.



8. Run reaction

Place the 96-well plate in a validated thermocycler and follow instrument specific protocols and analysis procedures detailed in the LumiraDx SARS-CoV-2 RNA STAR Complete IFU.

Precautions: This test has not been FDA cleared or approved but has been authorized by FDA for emergency use under an EUA for use by authorized laboratories; this test has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens; and the emergency use of this test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug, and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated or authorization is revoked sooner. **Customer Service:** If the LumiraDx SARS-CoV-2 RNA STAR Complete does not perform as expected, contact LumiraDx Customer Services 1-888-586-4721 or customerservices.us@lumiradx.com. **Manufacturer Information:** LumiraDx UK Ltd, 3 More London Riverside, London, SE1 2AQ, UK. Company Number 09206123.



lumiraDx SARS-CoV-2 RNA STAR Complete

Product Insert SPEC-30078 Rev1 ART-00004 Rev8 Rev Date 2021/02 **IVD**
 For *in vitro* diagnostic use Emergency Use Authorization (EUA) only Rx Only

Intended use

LumiraDx SARS-CoV-2 RNA STAR Complete is a rapid, non-isothermal nucleic acid amplification qSTAR (Selective Temperature Amplification Reaction) method intended for the qualitative detection of nucleic acid from SARS-CoV-2 in upper respiratory specimens (such as anterior nasal, mid-turbinate, nasopharyngeal and oropharyngeal swabs) collected from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to laboratories - certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 USC, §263a that 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 patient history and other diagnostic information is necessary to determine patient 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.

LumiraDx SARS-CoV-2 RNA STAR Complete 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. LumiraDx SARS-CoV-2 RNA STAR Complete is only for use under the Food and Drug Administration's Emergency Use Authorization.

Procedure/interpretation/limitations

Users should refer to the LumiraDx SARS-CoV-2 RNA STAR Complete Instructions for Use posted on the FDA website for all IVD products used under Emergency Use Authorization, <http://www.fda.gov/MedicalDevices/Safety/EmergencySituations/ucm161496.htm>.

Quick reference instructions

Read the full Instructions for Use available at <https://www.lumiradx.com/us-en/what-we-do/diagnostics/fast-lab-solutions/rna-star-complete> thoroughly for LumiraDx SARS-CoV-2 RNA STAR Complete before using the quick setup described below in this product insert. This Product Insert is not a complete set of instructions. A free paper copy of the full Instructions for Use can be obtained by using the contact info under Reagent complaints/questions below, if needed.

Package contents

Component	Description	Amount	Storage
LumiraDx SARS-CoV-2 RNA STAR Complete Positive Control Media (Pos. Ctrl. Med.)	COVID-19 Positive Control (ZeptoMetrix 50,000cp/mL)	500µL	≤ 8°C
LumiraDx SARS-CoV-2 RNA STAR Complete Negative Control Media (Neg. Ctrl. Med.)	0.85% Saline Solution	1.5mL	-25°C to -15°C
LumiraDx SARS-CoV-2 RNA STAR Complete 20X Salt Mix	COVID-19 Salt Mix	250µL	-25°C to -15°C
LumiraDx SARS-CoV-2 RNA STAR Complete 10X Extraction Buffer	Nucleic Acid Extraction Buffer	500µL	-25°C to -15°C
LumiraDx SARS-CoV-2 RNA STAR Complete 50X Internal Control & Primer Mix (50X IC/P Mix)	COVID-19 Internal Control & Primer Mix	100µL	-25°C to -15°C
LumiraDx SARS-CoV-2 RNA STAR Complete 2.5X Master Mix	COVID-19 Master Mix	2x 1mL	-25°C to -15°C

Storage instructions

Upon receipt, store Pos. Ctrl. Med. at ≤ 8 °C. Store Neg. Ctrl. Med., 20X Salt Mix, 10X Extraction Buffer, 50X IC/P Mix, and 2.5X Master Mix at ≤ -15 °C. Refer to the LumiraDx SARS-CoV-2 RNA STAR Complete Instructions for Use before opening and preparing reagents.

Precautions

The Controls should be handled in an approved BSL-2 handling area to avoid contamination of laboratory equipment and reagents that could cause false positive results. This Test is non-infectious. However, this Test should be handled in accordance with Good Laboratory Practices.

This test has not been FDA cleared or approved but has been authorized by FDA for emergency use under an EUA for use by authorized laboratories;.

This test has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.

The emergency use of this test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of *in vitro* diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug, and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated or authorization is revoked sooner .

For return policy

If there is a problem with the LumiraDx SARS-CoV-2 RNA STAR Complete Test you may be asked to return the item. Before returning Tests please obtain a return authorization number from LumiraDx Customer Services (customerservices.US@lumiradx.com). This return authorization number must be on the shipping carton for return. For ordinary returns following purchase, please contact LumiraDx Customer Services for terms and conditions.

Limited warranty

LumiraDx SARS-CoV-2 RNA STAR Complete – As per shelf life. Unused Tests must be stored according to the required storage conditions as printed in this product insert and they can be used only up to the expiry date printed on packaging. For the applicable warranty period, LumiraDx warrants that each product shall be (i) of good quality and free of material defects, (ii) function in accordance with the material specifications referenced in the product insert, and (iii) approved by the proper governmental agencies required for the sale of products for their intended use (the “limited warranty”). If the product fails to meet the requirements of the limited warranty, then as customer’s sole remedy, LumiraDx shall either repair or replace, at LumiraDx’s discretion, the Test. Except for the limited warranty stated in this section, LumiraDx disclaims any and all warranties, express or implied, including but not limited to, any warranty of merchantability, fitness for a particular purpose and non-infringement regarding the product. LumiraDx’s maximum liability with any customer claim shall not exceed the net product price paid by the customer. Neither party shall be liable to the other party for special, incidental or consequential damages, including, without limitation, loss of business, profits, data or revenue, even if a party receives notice in advance that these kinds of damages might result. The Limited Warranty above shall not apply if the customer has subjected LumiraDx SARS-CoV-2 RNA STAR Complete to physical abuse, misuse, abnormal use, use inconsistent with product insert provided, fraud, tampering, unusual physical stress, negligence or accidents. Any warranty claim by customer pursuant to the Limited Warranty shall be made in writing within the applicable Limited Warranty period.

Intellectual property

The LumiraDx Test and all provided LumiraDx documentation ('Products') are protected by law. The Intellectual Property of the LumiraDx Products remains at LumiraDx. Details of relevant Intellectual Property regarding our products can be found at lumiradx.com/IP.

Legal notices

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Manufacturer information

LumiraDx UK Ltd, 3 More London Riverside, London, SE1 2AQ, UK. Company Number 09206123.

Reagent complaints/questions

If you have a question/comment about this product, please contact LumiraDx by telephone at 1-888-586-4721 or by email at customerservices.US@lumiradx.com. Please include "LumiraDx SARS-CoV-2 RNA STAR Complete" in the subject line of the email.

Symbols glossary

	Temperature limitation		Use-by Date – The date after which the unopened IVD cannot be used.
	Manufacturer		Refer to instructions for use.
	<i>In Vitro Diagnostic Product</i>	100 Rxns	Contains sufficient reaction for 100 Tests
	Catalog Reference Number	Rx Only	Prescription Use Only
	Lot Number/Batch Code		

Specimen preparation

1. Thaw Pos. Ctrl. Med. and Neg. Ctrl. Med. on a cold block, vortex for 5 seconds then pulse centrifuge for 5 seconds to collect reagents at the bottom of the tube.
2. Assuming 94 dry swabs are being used, assemble the 1x PCM (Positive Control Media) by diluting 500 µL Pos. Ctrl. Med. with 500 µL Neg. Ctrl. Med. in a pre-chilled microcentrifuge tube. Assemble the 1x NCM (Negative Control Media) by transferring 1 mL Neg. Ctrl. Med into a pre-chilled microcentrifuge tube.
3. Upper respiratory specimens (20 µL) will be added directly to the sample plate prepared in the qSTAR reagent preparation. If swab is provided dry, transfer swab into a vial, such as a 5mL tube or deepwell, containing 1 mL compatible transport media (VTM, 0.85% Saline, or PBS) and soak the swab for 30 seconds then swirl thoroughly by rotating the swab against the side of the deepwell 5 times.

qSTAR reagent preparation

1. Thaw 20X Salt Mix, 10X Extraction Buffer, 50X IC/P Mix and 2.5X Master Mix on a cold block. Once thawed, invert each tube to mix (do not vortex) then pulse centrifuge for 5 seconds to collect reagents at the bottom of the tube.
2. Assuming 96 reactions are needed, prepare the Reaction Mix in a pre-chilled 5mL tube by combining 150 µL Molecular Biology Grade Water, 250 µL 20X Salt Mix, 500 µL 10X Extraction Buffer, 100 µL 50X IC/P Mix and 2 mL 2.5X Master Mix. Between each reagent, slowly mix by pipetting up and down 4 times without introducing bubbles.
3. Transfer 30 µL Reaction Mix into an appropriate, pre-chilled, 96-well plate. Add 20 µL, per well, samples or external controls into the 96-well plate then mix by slowly pipetting up and down 3 times without introducing bubbles. Seal the 96-well plate using an appropriate optically clear adhesive and centrifuge for 5 seconds.
4. Place the 96-well plate in a validated thermocycler and follow instrument specific protocols and analysis procedures detailed in the IFU.