



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 (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 (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), Applied Biosystems QuantStudio 7 Flex (software version 1.3), Applied Biosystems QuantStudio 7 Pro (software version 2.4.3), Bio-Rad CFX96 Touch System (software version 3.1), 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.)	SARS-CoV-2 Positive Control (ZeptoMetrix 50,000cp/mL)	500 µL	≤ 8°C
LumiraDx SARS-CoV-2 RNA STAR Complete Negative Control Media (Neg. Ctrl. Med.)	Negative Control (Molecular Biology Grade Water)	1.5 mL	-25°C to -15°C
LumiraDx SARS-CoV-2 RNA STAR Complete Salt Mix	Salt Mix	1 mL	-25°C to -15°C
LumiraDx SARS-CoV-2 RNA STAR Complete Extraction Buffer	Nucleic Acid Extraction Buffer	500 µL	-25°C to -15°C
LumiraDx SARS-CoV-2 RNA STAR Complete Internal Control & Primer Mix (IC/P Mix)	Internal Control & Primer Mix	120 µL	-25°C to -15°C
LumiraDx SARS-CoV-2 RNA STAR Complete Master Mix	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 Transport Medium	BD	220220
Corning	Corning	25-500-CM
Hardy Diagnostics	Hardy Diagnostics	R99
Hardy Diagnostics	Hardy Diagnostics	U157
ThermoFisher Scientific	ThermoFisher Scientific	10010023

Option for PCR Instruments ^{4,5} & Consumables	Source	Catalog #
Roche LightCycler 480 II (software version SW 1.5.1)	Roche Life Science	5015278001
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 MicroAmp Fast Optical		
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
Research Product International	Research Product International	202545
VWR	VWR	89087-690
VWR	VWR	60941-070

*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 5mL 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
IsoFreeze PCR Racks	Thomas Scientific	1148D61
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 assess 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.

⁴ Testing with RUO instruments require qualification prior to use with the LumiraDx SARS-CoV-2 RNA STAR Complete assay. Please refer to Appendix A for the required protocol and acceptance criteria.

⁵ Please refer to Appendix B of this IFU for EUO labelling that should be affixed to PCR instruments.

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 by FDA for emergency use 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.
- Reagents 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 Salt Mix, LumiraDx SARS-CoV-2 RNA STAR Complete Extraction Buffer, LumiraDx SARS-CoV-2 RNA STAR Complete Internal Control & Primer Mix (IC/P Mix), and LumiraDx SARS-CoV-2 RNA STAR Complete 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 Internal Control & Primer Mix (IC/P Mix).
- The Salt Mix, 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 assay 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 that 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.) are treated as patient specimens and both must be included as external controls in every plate. See Section 16.1 for more information about the LumiraDx SARS-CoV-2 RNA STAR Complete Controls.

NOTE: Please handle the Pos. Ctrl. Med. with care as it can cause false positives if accidentally spilled or handled 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 centrifuge for 5 seconds to collect reagents at the bottom of the tube.
2. To assemble the 1x PCM (Positive Control Media), freshly dilute 20.0 μ L Pos. Ctrl. Med. with 60.0 μ L Neg. Ctrl. Med. in a pre-chilled microcentrifuge tube. To assemble the 1x NCM (Negative Control Media), always freshly pipette 80.0 μ L Neg. Ctrl. Med into a pre-chilled microcentrifuge tube.
3. Upper respiratory specimens (24 μ L) will be added directly to the sample plate prepared in Section 9.3. If swab samples are provided dry, transfer the swab into a vial, such as a 5 mL tube or deep well plate, containing 1 mL compatible transport media and soak the swab for a minimum of 30 seconds. Swirl thoroughly by rotating the swab against the side of the tube 5 times then express the swab on the side of tube, outside of the liquid, prior to removing (beware of cross-contamination from splashing). Discard the swab in biohazard waste.

9.2 qSTAR Guidelines

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 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 Salt Mix, LumiraDx SARS-CoV-2 RNA STAR Complete Extraction Buffer, LumiraDx SARS-CoV-2 RNA STAR Complete IC/P Mix, and LumiraDx SARS-CoV-2 RNA STAR Complete 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 should be thawed and kept on a cold block equilibrated between 2 to 8 °C to maintain the integrity of the reagents. Furthermore, it is recommended to queue the validated thermocycler (i.e. Roche LC 480 II, ABI 7500 Fast Dx, ABI QS 5, ABI QS 7 Flex, ABI QS 7 Pro, Bio-Rad CFX96, Agilent AriaMx, or the Agilent Stratagene Mx3005P) prior to performing the instructions below to ensure the performance of this assay is maintained.

1. Thaw the LumiraDx SARS-CoV-2 RNA STAR Complete kit components in a cold block equilibrated between 2 to 8 °C; i.e. LumiraDx SARS CoV-2 RNA STAR Complete Salt Mix, LumiraDx SARS CoV-2 RNA STAR Complete Extraction Buffer, LumiraDx SARS CoV-2 RNA STAR Complete Internal Control & Primer Mix (IC/P Mix), and LumiraDx SARS CoV-2 RNA STAR Complete Master Mix. Invert each tube to mix then centrifuge for 5 seconds to collect reagents at the bottom of the tube (do not vortex samples).
2. Transfer 24.0 µL of swab specimens prepared in Step 3 of Section 8.1 and transfer 24.0 µL of external controls prepared in Step 2 of Section 8.1 into an appropriate, pre-chilled, 96-well plate. Add 4.8 µL Extraction Buffer, per well of specimen and external controls, and mix by slowly pipetting up and down 10 times while minimizing bubbles. The addition and mixing of Extraction Buffer can be simplified by using a multi-channel pipette and chilled reagent reservoir. If needed, seal and centrifuge the 96-well plate to collect the sample at the bottom of the well.
3. Determine the number of reactions (N) to be prepared per assay:

Reaction Mix Setup	1 Rxn	100 Rxns	N Rxns
Salt Mix	10.0 µL	1000 µL	N x 10.0 µL
IC/P Mix	1.2 µL	120 µL	N x 1.2 µL
Master Mix	20.0 µL	2000 µL	N x 20.0 µL
Total Volume	31.2 µL	3120 µL	N x 31.2 µL

4. Assuming one reaction is needed, perform the following to make the Reaction Mix:
 - a. Combine 10.0 µL Salt Mix and 1.2 µL IC/P Mix in a pre-chilled microcentrifuge tube, mix by slowly pipetting up and down 4 times without introducing bubbles, centrifuge briefly (do not vortex and do not spin down for an excessive amount of time), then place tube back on the cold block.
 - b. Add 20.0 µL Master Mix to finalize the Reaction Mix, mix by pipetting up and down 10 times without introducing bubbles, centrifuge briefly, then place tube back on the cold block.
5. Transfer 31.2 µL of Reaction Mix to each well with specimen and external controls. Mix by slowly pipetting up and down 10 times without introducing bubbles. The addition and mixing of Reaction Mix can be simplified by using a multi-channel pipette and chilled reagent reservoir. Seal the 96-well plate using an appropriate optically clear adhesive and centrifuge the plate at 2000 rpm for 10 seconds to collect contents at bottom of plate.
6. Place the 96-well plate in a validated thermocycler and follow instrument specific protocols and analysis procedures detailed below.

9.4 Stand-alone Instructions for 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, to improve performance, one (1) mL of buffer is recommended.

NOTE: It is recommended to read through the following instructions before attempting to set up the Reaction Mix. All components should be thawed and kept on a cold block equilibrated between 2 to 8 °C to maintain the integrity of the reagents. Additionally, it is recommended to queue the validated thermocycler (i.e. Roche LC 480 II, ABI 7500 Fast Dx, ABI QS 5, ABI QS 7 Flex, ABI QS 7 Pro, Bio-Rad CFX96, Agilent AriaMx, or the Agilent Stratagene Mx3005P) prior to performing the instructions below to ensure the performance of this assay is maintained.

1. Thaw the LumiraDx SARS-CoV-2 RNA STAR Complete kit components in a cold block equilibrated between 2 to 8 °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 Salt Mix, LumiraDx SARS CoV-2 RNA STAR Complete Extraction Buffer, LumiraDx SARS CoV-2 RNA STAR Complete Internal Control & Primer Mix (IC/P Mix), and LumiraDx SARS CoV-2 RNA STAR Complete Master Mix. Invert each tube to mix then 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 into a suitable tube (e.g. polypropylene microcentrifuge tube). Place and soak the swab for at least 30 seconds then swirl thoroughly by rotating the swab against the side of the tube up to 5 times. Express the swab on the side of tube, outside of the liquid, prior to removing (beware of cross-contamination from splashing). Discard the swab in biohazard waste. If swab specimen is provided wet, up to 3 mL of compatible transport media (VTM, 0.85% Saline, or PBS) is acceptable, but this higher volume may impact sensitivity.
3. Assemble fresh 1x PCM (Positive Control Media) by diluting 20.0 µL Pos. Ctrl. Med. with 60.0 µL Neg. Ctrl. Med. in a pre-chilled microcentrifuge tube. Assemble the 1x NCM (Negative Control Media) by transferring 80.0 µL Neg. Ctrl. Med. into a pre-chilled microcentrifuge tube.
4. Transfer 24.0 µL of swab specimen prepared in Step 2 and transfer 24.0 µL of external controls prepared in Step 3 using a single-channel pipette into an appropriate, pre-chilled, 96-well plate.
5. Add 4.8 µL of Extraction Buffer, per well, to the 96-well plate and mix by slowly pipetting up and down 10 times without introducing bubbles. The addition and mixing of Extraction Buffer can be simplified by using a multi-channel pipette and chilled reagent reservoir. If needed, seal and centrifuge the 96-well plate to collect the sample at the bottom of the well.
6. Determine the number of reactions (N) to be prepared per assay and prepare Reaction Mix in a suitable pre-chilled tube by following the order in the table below. Between each reagent, slowly mix by pipetting up and down 4 to 6 times without introducing bubbles and pulse centrifuge.

Reaction Mix	1 Rxn	N Rxns
Salt Mix	10.0 µL	N x 10.0 µL
IC/P Mix	1.2 µL	N x 1.2 µL
Master Mix	20.0 µL	N x 20.0 µL
Total Volume	31.2 µL	N x 31.2 µL

7. Transfer 31.2 µL of Reaction Mix to each well with sample and external controls. Mix by slowly pipetting up and down 10 times without introducing bubbles. Seal the 96-well plate using an appropriate optically clear adhesive and centrifuge the plate at 2000 rpm for 10 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 Stand-alone 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 between 2 to 8 °C to maintain the integrity of the reagents. Additionally, it is recommended to queue the validated thermocycler (i.e. Roche LC 480 II, ABI 7500 Fast Dx, ABI QS 5, ABI QS 7 Flex, ABI QS 7 Pro, Bio-Rad CFX96, Agilent AriaMx, or the Agilent Stratagene Mx3005P) prior to performing the instructions below to ensure the performance of this assay is maintained.

1. Thaw the LumiraDx SARS-CoV-2 RNA STAR Complete kit components in a cold block equilibrated between 2 to 8 °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 Salt Mix, LumiraDx SARS CoV-2 RNA STAR Complete Extraction Buffer, LumiraDx SARS CoV-2 RNA STAR Complete Internal Control & Primer Mix (IC/P Mix), and LumiraDx SARS CoV-2 RNA STAR Complete Master Mix. Invert each tube to mix then 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 multi-channel pipette. Leave two designated wells, A1 and A12, empty for the external controls assembled in Step 4.
3. Add a single dry swab specimen to each deepwell in use. Soak the swab for a minimum of 30 seconds then swirl thoroughly by rotating the swab against the side of the deepwell 5 times. Express the swab on the side of the well, outside of the liquid, prior to removing (beware of cross-contamination from splashing). Discard the swab in biohazard waste.
4. Assemble the 1x PCM (Positive Control Media) by diluting 200 µL Pos. Ctrl. Med. with 600 µL Neg. Ctrl. Med. in a pre-chilled microcentrifuge tube. Assemble the 1x NCM (Negative Control Media) by transferring 800 µL 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 and A12 should be loaded with the NCM and PCM, respectively.
5. Mix the specimen prepared in Step 3 and the external controls prepared in Step 4 by slowly pipetting up and down 4 to 6 times without introducing air bubbles then transfer 24.0 µL using a multi-channel pipette into an appropriate, pre-chilled, 96-well plate. Add 4.8 µL of Extraction Buffer, per well, and mix by slowly pipetting up and down 10 times while minimizing bubbles. If needed, seal and centrifuge the 96-well plate to collect the specimen at the bottom of the well.
6. 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 to 6 times without introducing bubbles.

Reaction Mix	100 Rxns
Salt Mix	1000µL
IC/P Mix	120µL
Master Mix	2x 1000µL
Total	3120µL

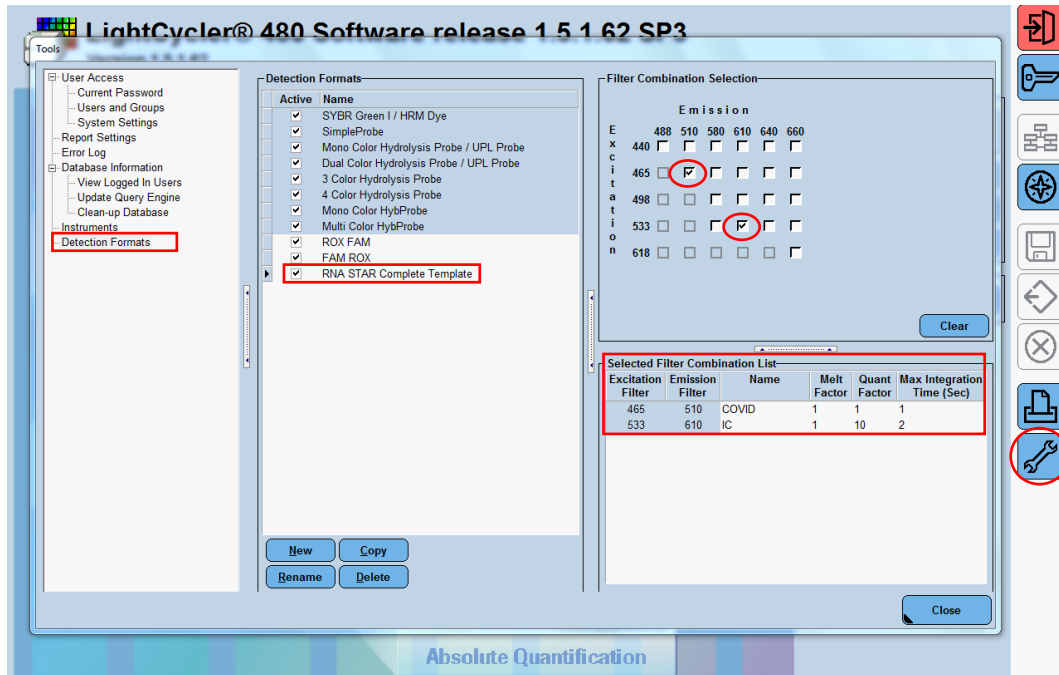
7. Transfer Reaction Mix to a pre-chilled reagent reservoir (for minimal dead volume) using a single channel pipette. Then, using a multi-channel pipette, transfer 31.2 µL of Reaction Mix to each well with specimen and external controls. Mix by slowly pipetting up and down 10 times without introducing bubbles. Seal the 96-well plate using an appropriate optically clear adhesive and centrifuge the plate at 2000 rpm for 20 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

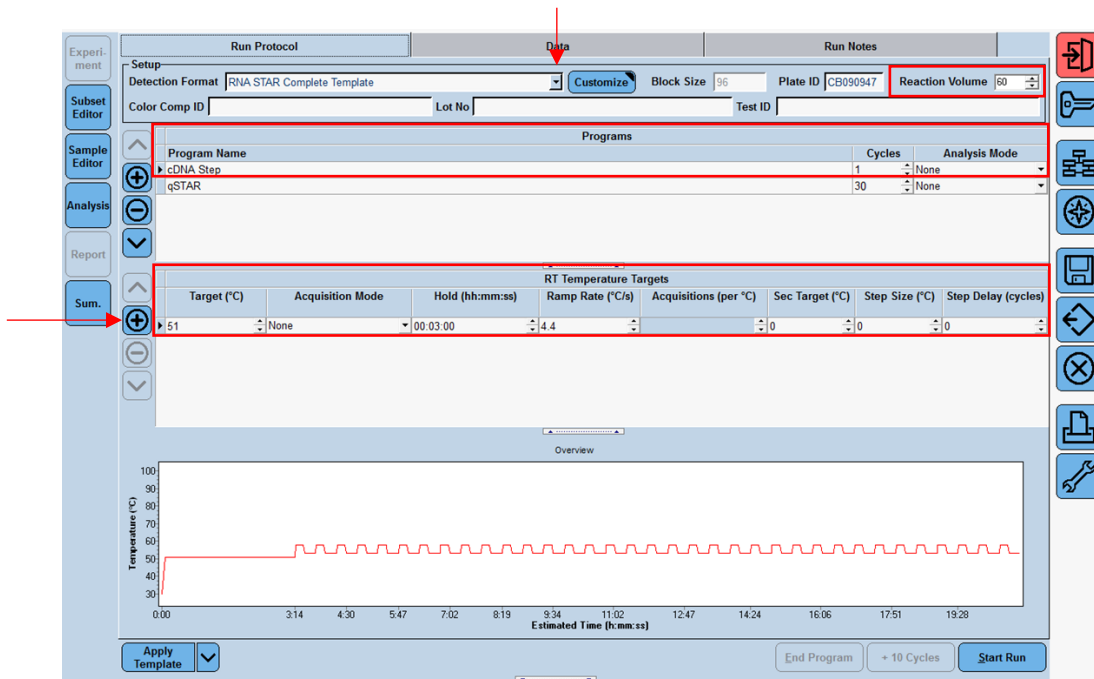
Refer to “User Manual Part Number 05152062001 0208” for additional information. The instrument programming instructions are intended for setup of an entire 96-well plate. If the entire plate is not used, please deselect wells as appropriate during analysis. It is required that the Roche LC 480 II instrument be appropriately qualified prior to use with the LumiraDx SARS-CoV-2 RNA STAR Complete assay using established testing protocols. Refer to the protocol provided in Appendix A.

10.1 Programming the Run Template and Sample Template

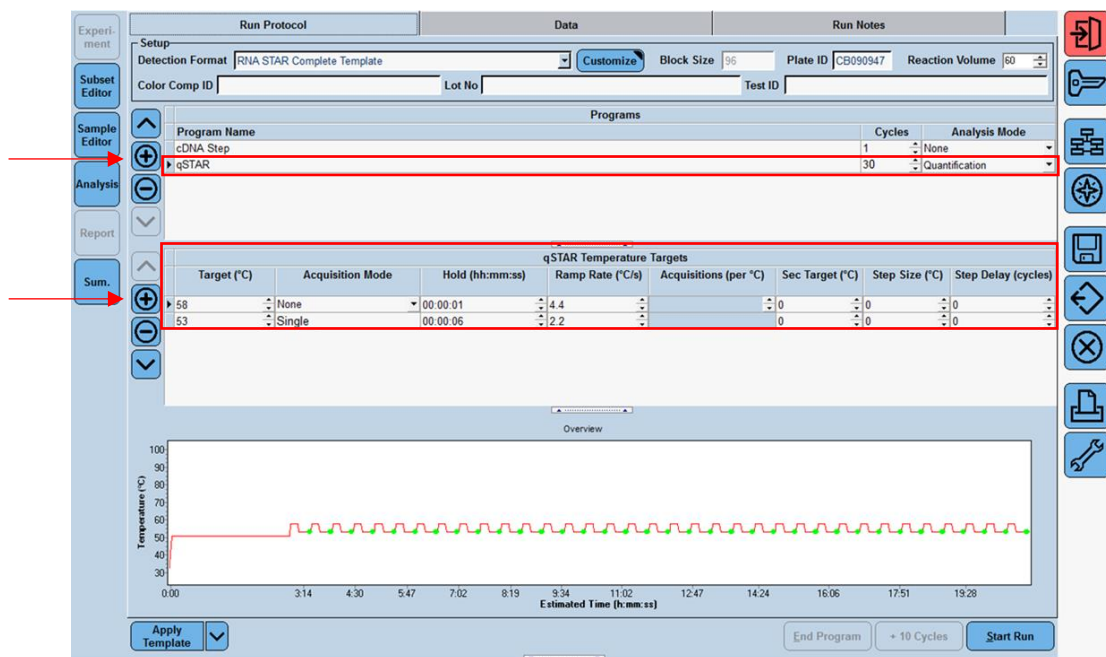
1. Launch the LightCycler (LC) 480 desktop software (version SW 1.5.1). If prompted, ‘Log On’ to the ‘Traceable Database’.



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’ (Excitation – Emission) 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’.



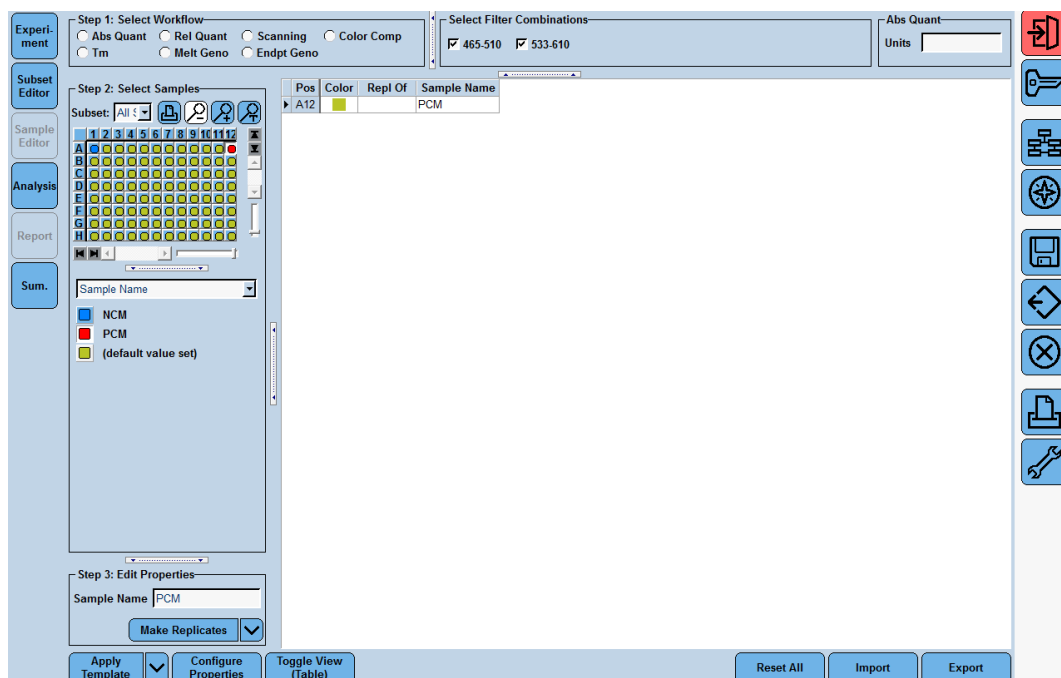
- Select from the 'Detection Format' drop-down menu "RNA STAR Complete Template". Set 'Reaction Volume' to '60' in the upper right of the software. In the 'Program Name' section type "cDNA Step", set 'Cycles' to '1', and set 'Analysis Mode' to 'None'. Set cDNA Step 'RT Temperature Targets' as follows: set 'Target (°C)' to '51', set 'Acquisition Mode' to 'None', set 'Hold (hh:mm:ss)' to '00:03:00', set 'Ramp Rate (°C/s)' to '4.4', and set 'Sec Target (°C)', 'Step Size (°C)', and 'Step Delay (cycles)' to '0' (see image above for guidance).



- Click the "+" button on the upper left of the software to add another 'Programs'. In the 'Program Name' section type "qSTAR", set 'Cycles' to '30', 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 '00:00:01', set 'Ramp Rate (°C/s)' to '4.4', 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 'Single', set 'Hold (hh:mm:ss)' to '00:00:06', set 'Ramp Rate (°C/s)' to '2.2', 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.

6. 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.



7. 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.
8. In the lower left corner of the screen 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.
9. 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 compatible with the instrument manufacturer (see Option for PCR Instruments & Consumables table above).

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

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 " \leftrightarrow " 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 " \leftrightarrow " 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 '3' 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 '30'. 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 '22.0000'. Click 'Filter Comb (533-610)' and select 'IC'. Click the 'Check' button to exit the 'Filter Combination' window. Set 'STD Multiplier' to '22.0000'.
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 compatible with the instrument manufacturer (see Option for PCR Instruments & 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.

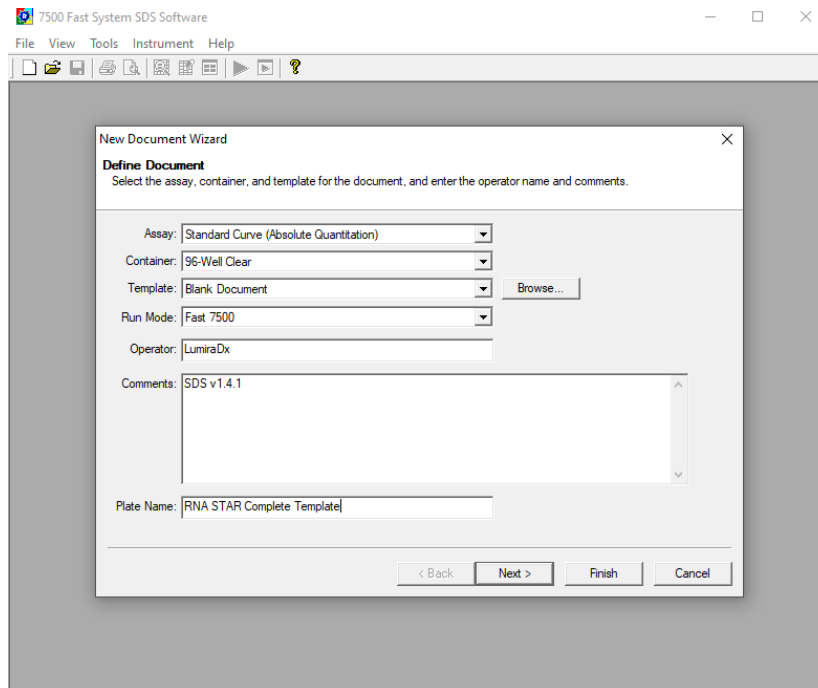
6. After the run has finished, proceed to the 'Analysis' tab on the left of the software. Choose 'Abs Quant/Fit Points' in the 'Create new analysis' section. Click the 'Check' button to exit the 'Create New Analysis' pop-up window.
7. In the lower left corner click 'Apply Template' (do not select the 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

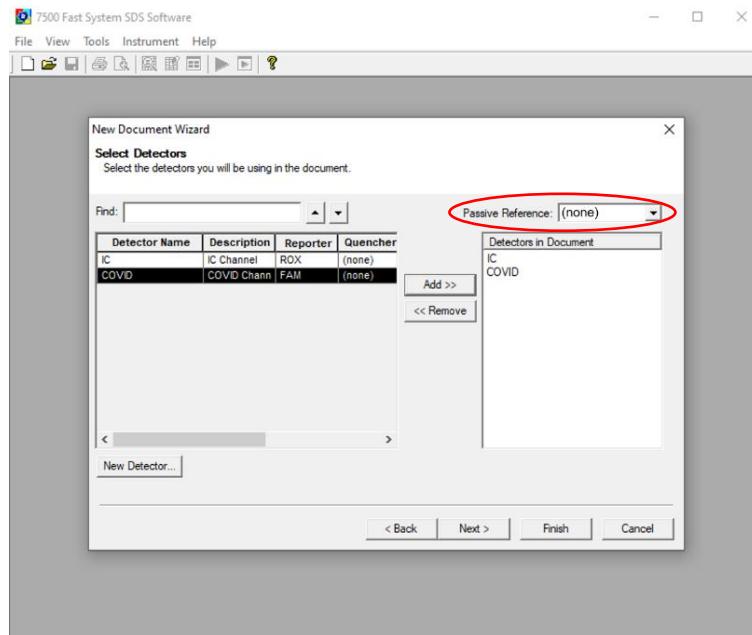
Refer to “User Manual Part Number 4406991” for additional information. The instrument programming instructions are intended for setup of an entire 96-well plate. If the entire plate is not used, please deselect wells as appropriate during analysis.

11.1 Programming the Run Template

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

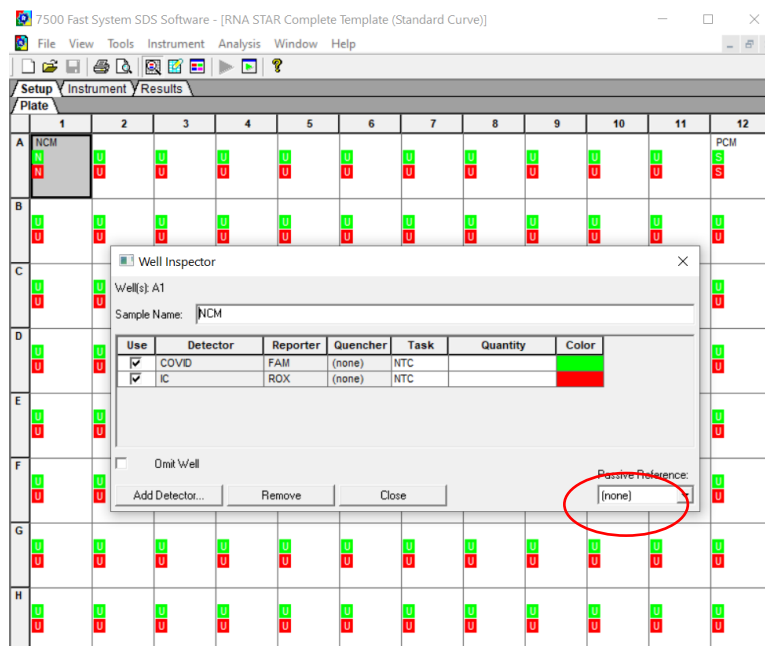


2. The ‘Quick Startup document’ window will open. Click ‘Create New Document’ to start the ‘New Document Wizard’. In the ‘Define Document’ window verify the default settings: ‘Assay’ is set to ‘Standard Curve (Absolute Quantification)’, ‘Container’ is set to ‘96-Well Clear’, ‘Template’ is set to ‘Blank 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’.



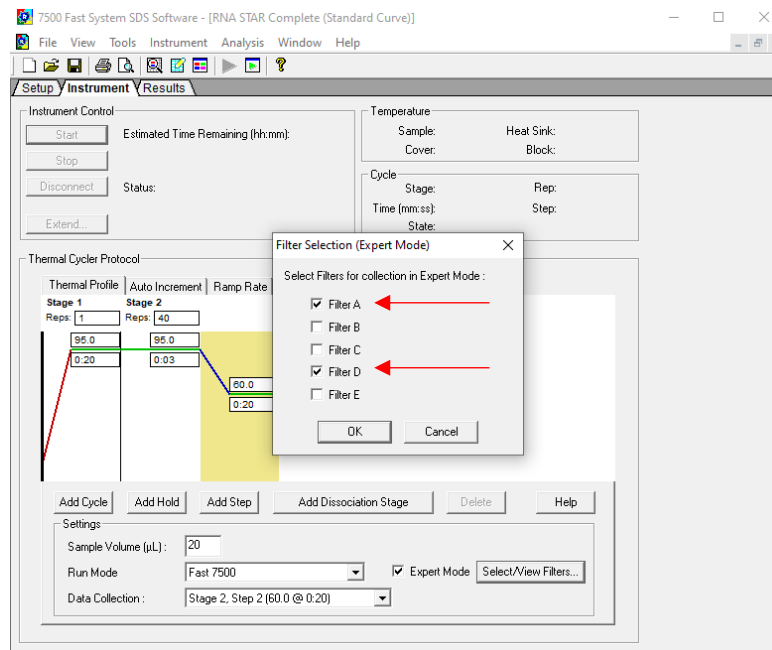
3. In the 'Select Detectors' window, click 'New Detector' to open the 'New Detector' pop-up window and define these settings: define 'Name' as "COVID", define 'Description' to "COVID Channel", set 'Reporter' dye to 'FAM', set 'Quencher' to '(none)', and set 'Color' to 'Green'. Select 'OK'. Click 'New 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.

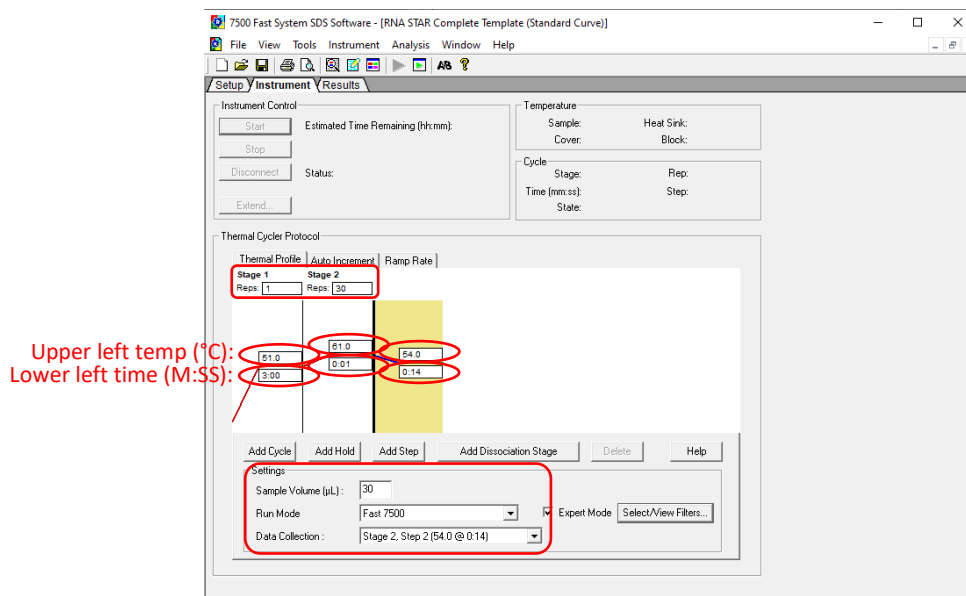


4. In the 'Set Up Sample Plate' window click the gray box between "A" and "1" to select all wells then select boxes under the 'Use' section to add the COVID and IC Detector to all wells. Click 'Finish'. The wizard will close, and a screen will display the 'Setup' tab and 'Plate' tab. This will show the 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'.

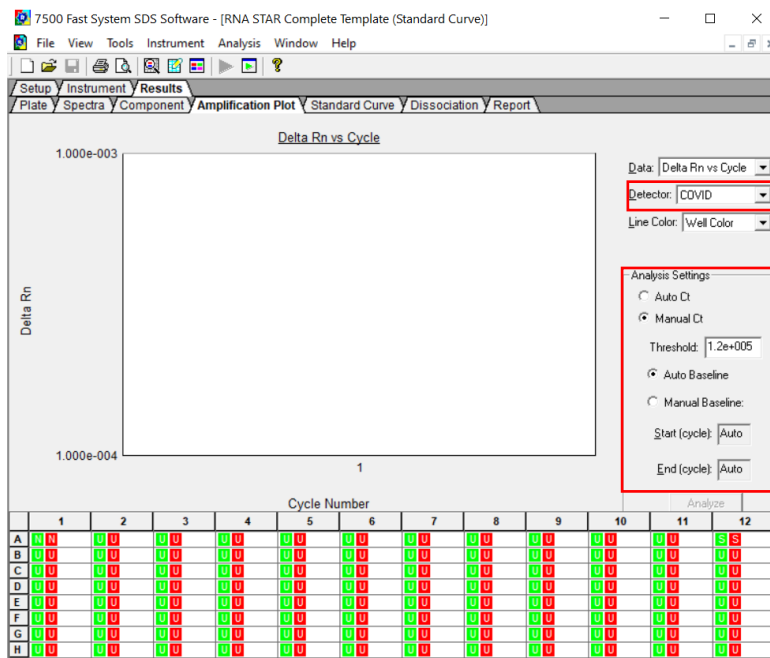
- Select location 'A12', right click and select 'Well Inspector'. In the 'Well Inspector' window, define the 'Sample Name' as "PCM" (Positive Control Media). Select the 'COVID' Detector line, under 'Task' select from the drop-down menu 'Standard'. Select the 'IC' Detector line, under 'Task' select from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



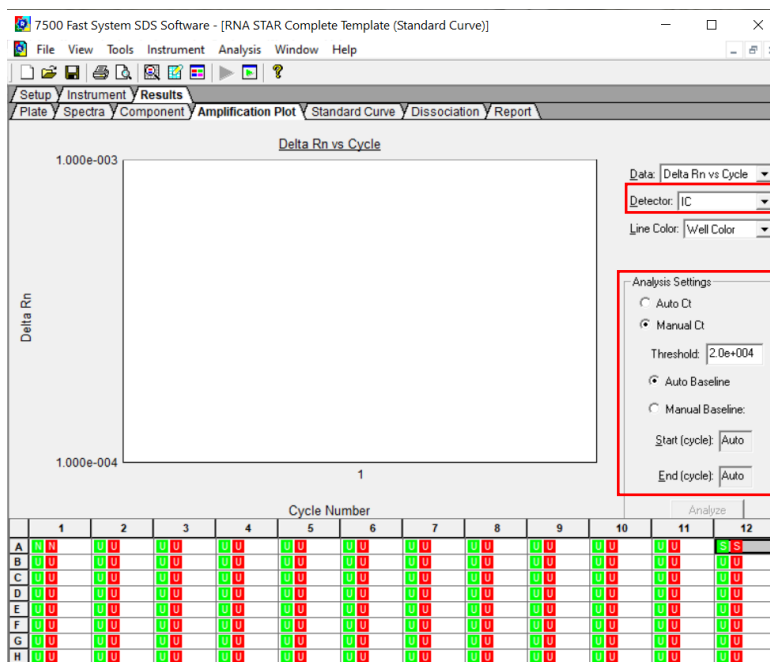
- Select the 'Instrument' tab (do not select 'Instrument' from the menu), check the box next to 'Expert Mode' then click 'Select/View Filters...' and check only the boxes for 'Filter A' and 'Filter D'. Click 'OK'.



- Modify the default thermal profile for 'Stage 1' as follows: set 'Reps' to '1', set temp (upper left) to '51.0', and set time (lower left) to '3:00'. Modify the default thermal profile for 'Stage 2' as follows: set 'Reps' to '30', set time to (lower middle) '0:01', set temp (upper middle) to '61.0', set time (lower right) to '0:14', and set temp (upper right) to '54.0'. Under the 'Settings' section perform the following: define 'Sample Volume (µL)' as "30" (the sample volume cannot be set higher than this), confirm 'Run Mode' is set to '7500 Fast' (default), and confirm 'Data Collection' is set to 'Stage 2, Step 2(54.0 @ 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 "1.2e+005". Confirm that the 'Auto Baseline' circle is selected.



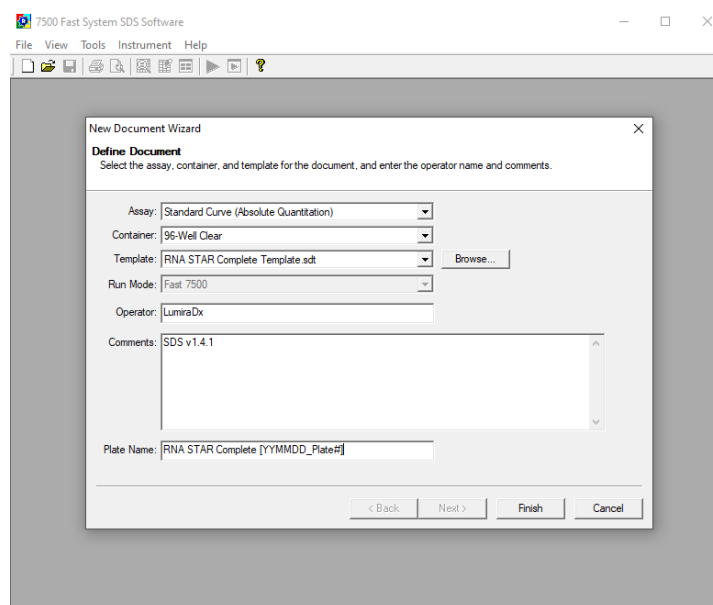
9. From the 'Detector' drop-down menu again, select 'IC' from the 'Detector'. In the 'Analysis Settings' section, select the 'Manual Ct' circle and define 'Threshold' as "2.0e+004". Confirm that the 'Auto Baseline' circle is selected.
10. Select 'File' from the menu then click 'Save As'. Save the template 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'.

NOTE: Defining the Analysis Settings for COVID and IC here actually define the settings that will be used for analysis post run.

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 compatible with the instrument manufacturer (see Option for PCR Instruments & 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. Ct values can be exported to Excel, if needed. Remove the plate and discard the plate in a sealable waste bag or container.

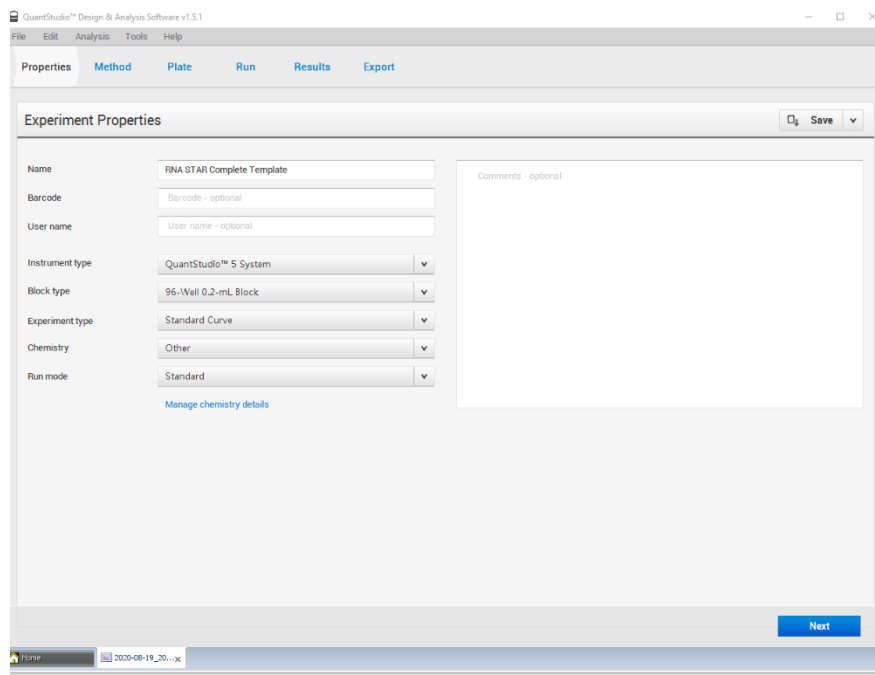
12 RNA STAR Complete Setup for Applied Biosystems™ QuantStudio 5

Refer to “User Manual Part Number MAN0010407” for additional information. 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.

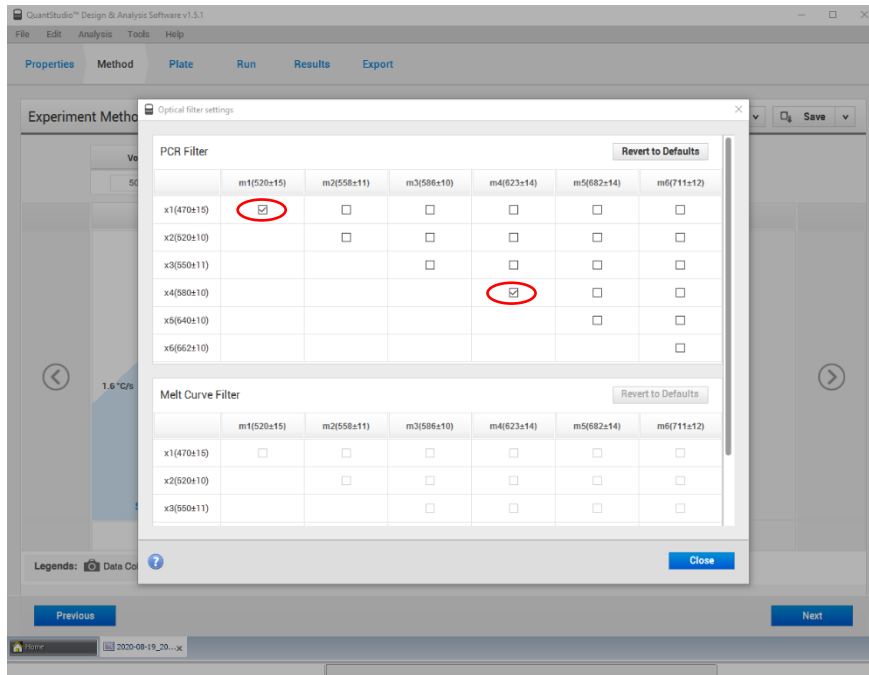
12.1 Programming Instructions for PC/Laptop-connected Instrument

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 compatible with the instrument manufacturer (see Option for PCR Instruments & Consumables table above).

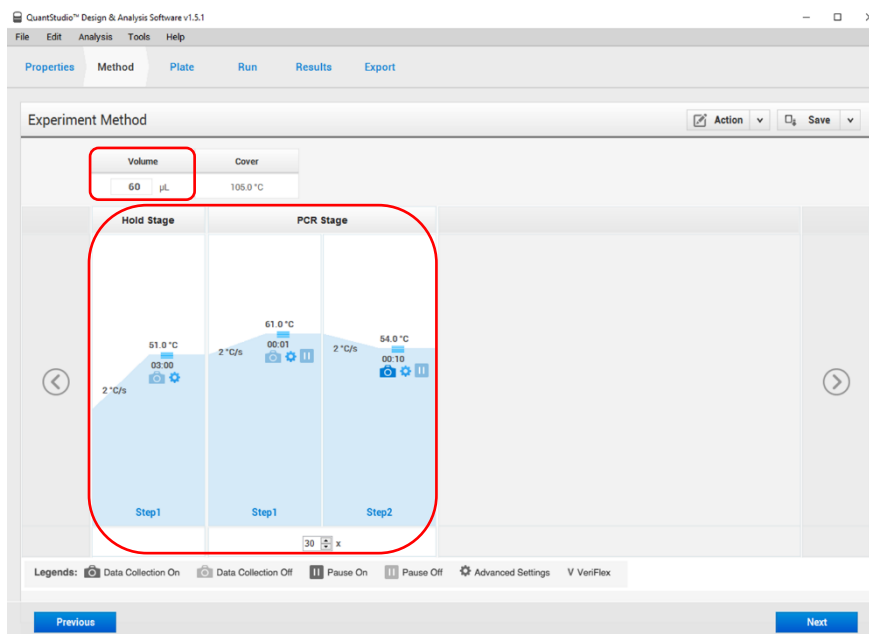
1. If the instrument touchscreen is in sleep mode (dark screen), touch the screen anywhere to activate the instrument. Sleep mode lowers the temperature of the heated cover. It is important that the heated cover idle at 105°C before loading a 96-well plate.



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.

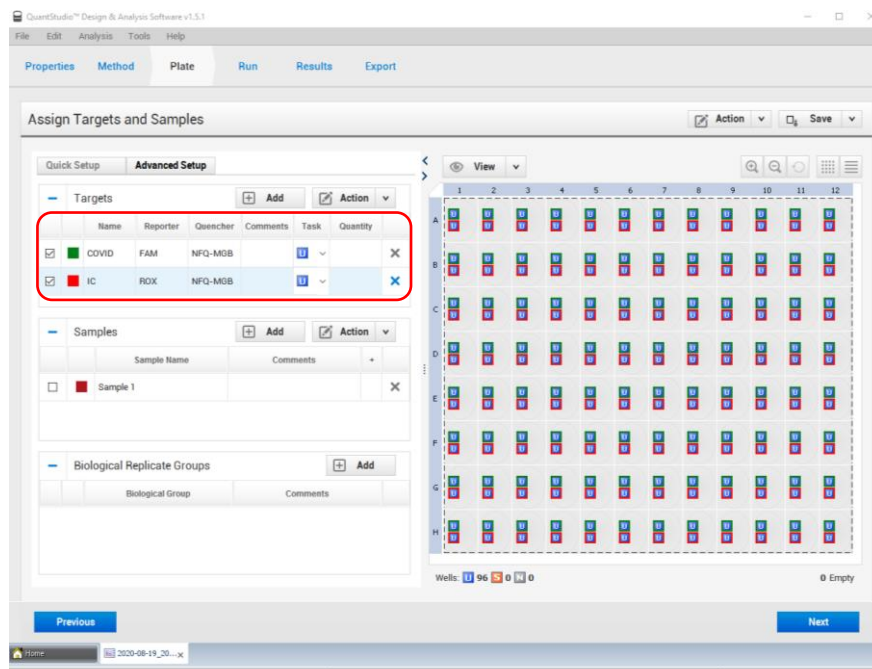


- Click 'Action' and select 'Optical filter settings' from the drop-down menu. In the 'PCR Filter' table, uncheck all filter combinations except the following: x1(470±15), m1(520±15), x4(580±10), m4(623±14)



- Click 'Close' to return to the 'Method' tab. Set the 'Volume' to '60 µL'. In the 'Hold Stage' section, hover over 'Step 2' and click the '-' button that appears just above the wording to delete this plateau. In the 'Hold Stage' section, change 'Step 1' settings as follows: set ramp rate to '2 °C/s', set temp to '51 °C', and set time to '03:00'. In the 'PCR Stage' section, change 'Step 1' settings as follows: set ramp rate to '2 °C/s', set temp to '61 °C', and set time to '00:01'. In the 'PCR Stage' section, change 'Step 2' settings as follows: set ramp rate to '2 °C/s', set temp to '54 °C', and set time to '00:10' (data collection step). At the bottom of the 'PCR Stage' section, set 'Cycles' to '30'. Then proceed to the 'Plate' tab.

- 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.



- 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.
- (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.
- 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'.
- 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.
- 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 Complete [YYMMDD_Plate#]" and click 'Save'.
- 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.

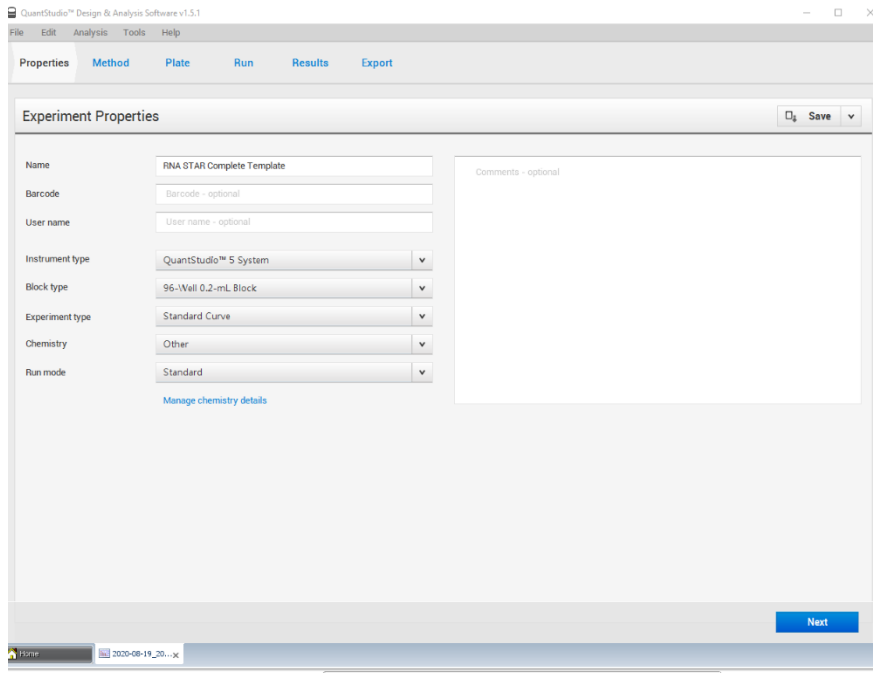
12.2 Analysis Instructions

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 ' ΔR_n 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 "80,000" for the 'Threshold', set 'Baseline Start Cycle' to '1' and set 'End Cycle' to '4'. Select the 'IC' line then uncheck the 'Default settings' box, the 'Automatic Threshold' box, and the 'Automatic Baseline' box. Enter "15,000" for the 'Threshold', set 'Baseline Start Cycle' to '1' and set 'End Cycle' to '4'. 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.

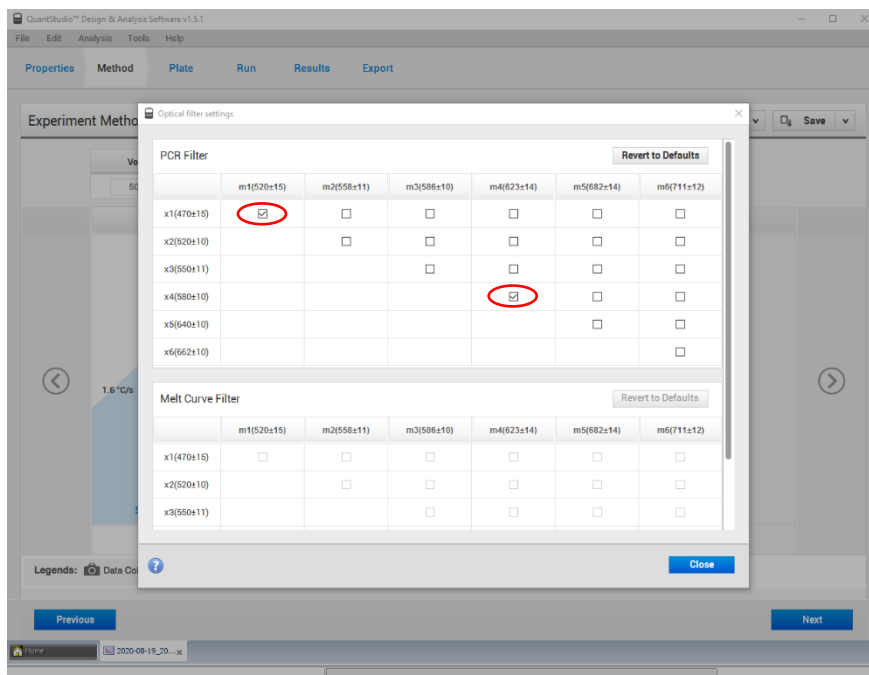
12.3 Programming Instructions for Stand-Alone Instrument

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 & Consumables table above).

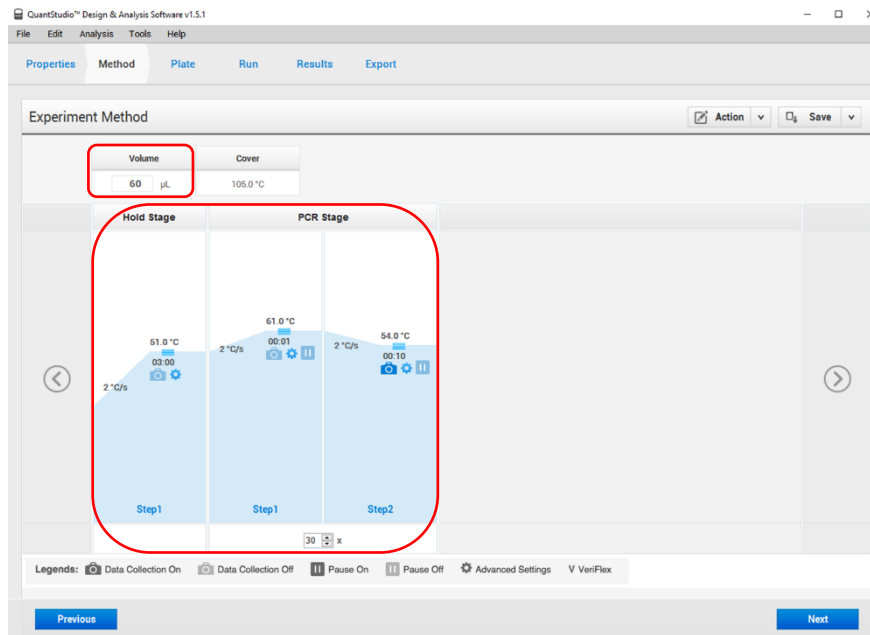
1. If the instrument touchscreen is in sleep mode (dark screen), touch the screen anywhere to activate the instrument. Sleep mode lowers the temperature of the heated cover. It is important that the heated cover idle at 105°C before loading a 96-well plate.



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 '60 µL'. In the 'Hold Stage' section, hover over 'Step 2' and click the '-' button that appears just above the wording to delete this plateau. In the 'Hold Stage' section, change 'Step 1' settings as follows: set ramp rate to '2 °C/s', set temp to '51 °C', and set time to '03:00'. In the 'PCR Stage' section, change 'Step 1' settings as follows: set ramp rate to '2 °C/s', set temp to '61 °C', and set time to '00:01'. In the 'PCR Stage' section, change 'Step 2' settings as follows: set ramp rate to '2 °C/s', set temp to '54 °C', and set time to '00:10' (data collection step). At the bottom of the 'PCR Stage' section, set 'Cycles' to '30'. 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 [YMMDD_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 ' ΔR_n 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 "80,000" for the 'Threshold', set 'Baseline Start Cycle' to '1' and set 'End Cycle' to '4'. Select the 'IC' line then uncheck the 'Default settings' box, the 'Automatic Threshold' box, and the 'Automatic Baseline' box. Enter "15,000" for the 'Threshold', set 'Baseline Start Cycle' to '1' and set 'End Cycle' to '4'. 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.

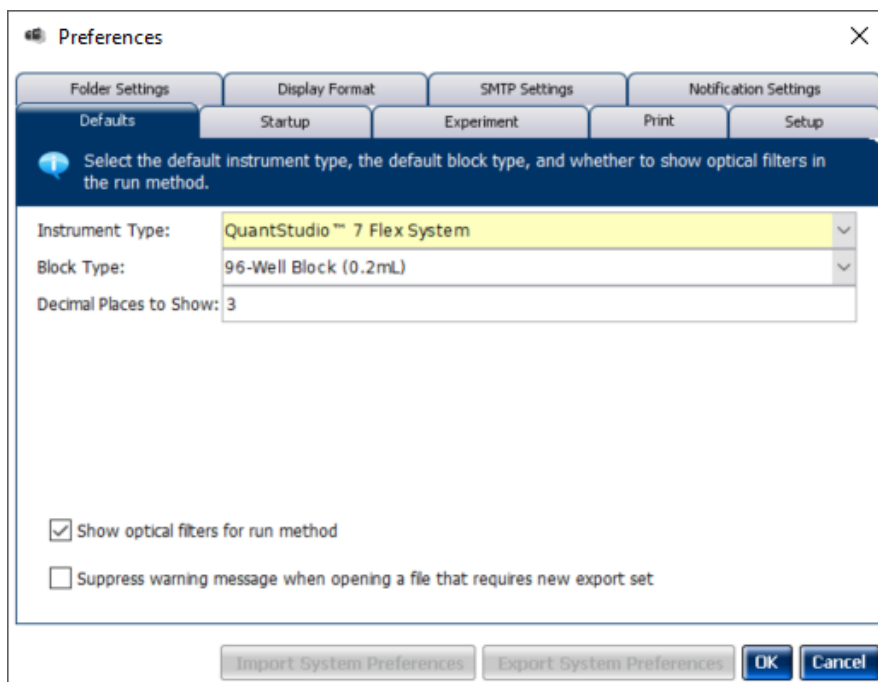
13 RNA STAR Complete Setup for Applied Biosystems™ QuantStudio 7 Flex

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.

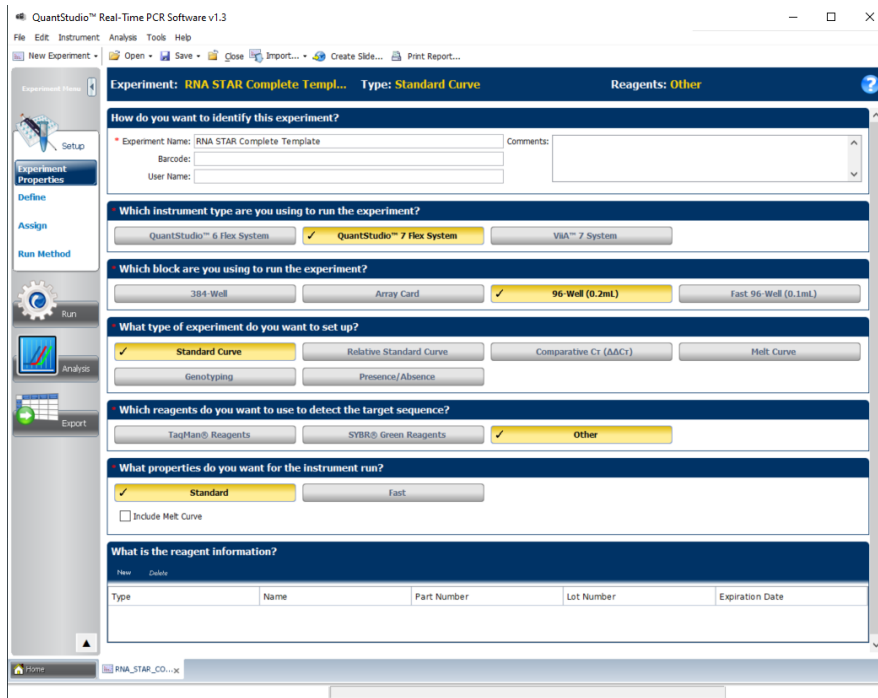
13.1 Programming Instructions for PC/Laptop-connected Instrument

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).

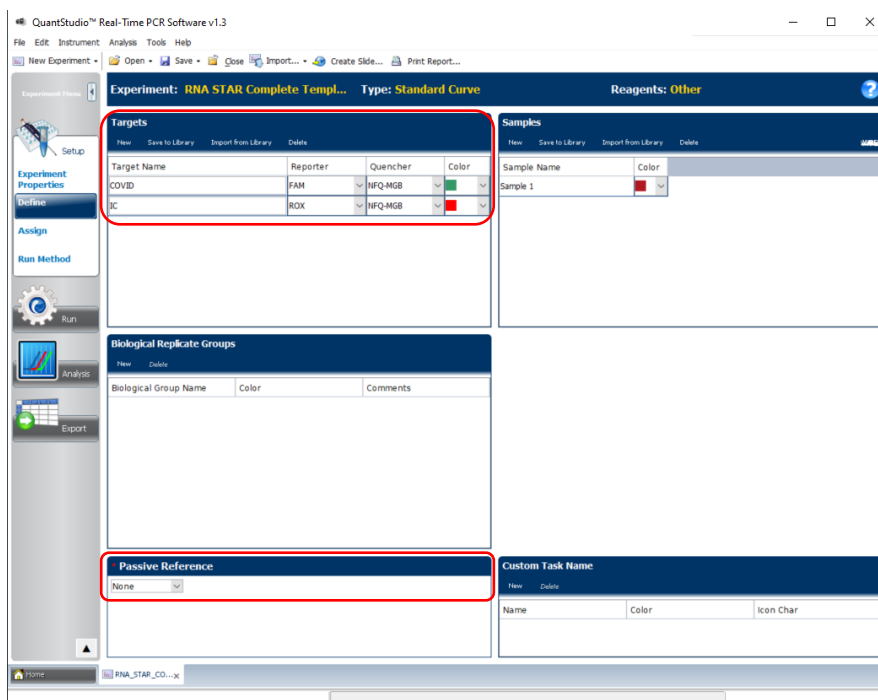
1. If the instrument touchscreen is in sleep mode (dark screen), touch the screen anywhere to activate the instrument. Sleep mode lowers the temperature of the heated cover. It is important that the heated cover idle at 105°C before loading a 96-well plate.
2. Launch the ‘QuantStudio Real-Time PCR Software’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



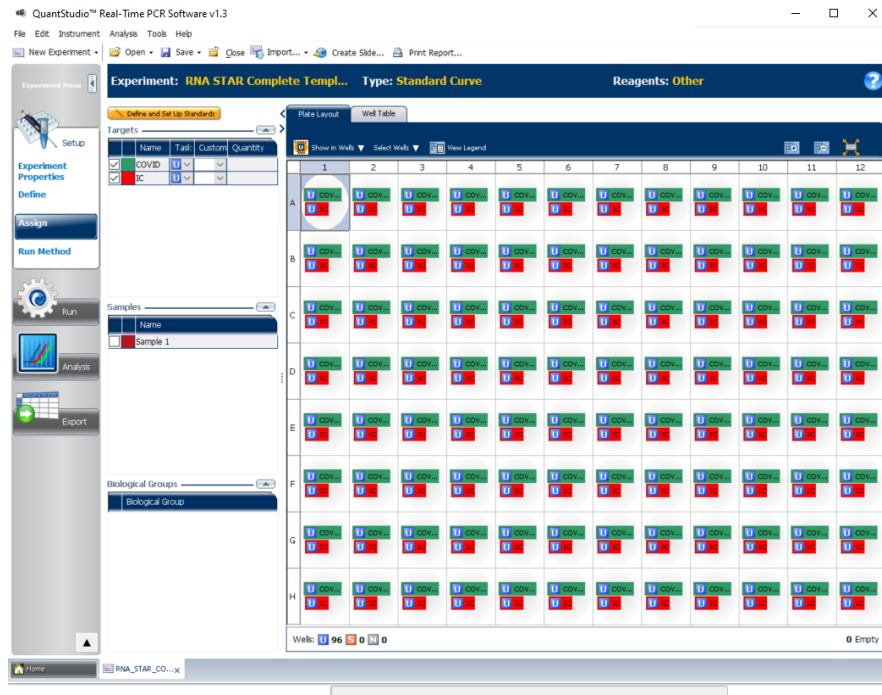
3. Confirm that the ‘Instrument Type:’ is set to ‘QuantStudio™ 7 Flex System’, the ‘Block Type:’ is set to ‘96-Well Block (0.2mL)’, and that the ‘Decimal Places to Show:’ is set to ‘3’. Click the box next to ‘Show optical filters for run method’ to select this feature. Click on ‘OK’.
4. Select ‘Create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



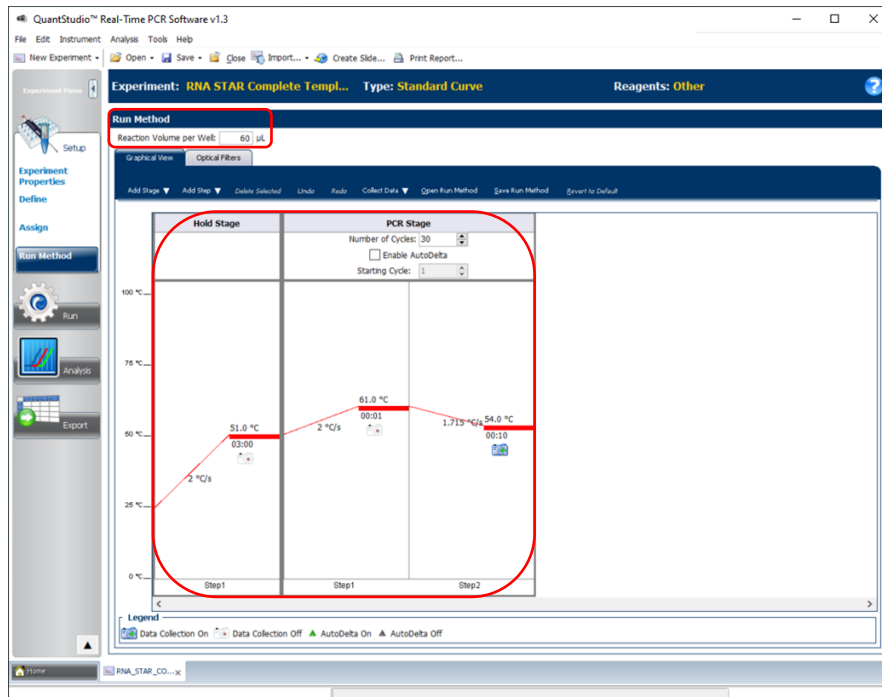
- On the left of the screen, the 'Experiment Properties' tab will be selected. Enter the 'Experiment Properties' as follows: define the 'Experiment Name' as "RNA STAR Complete Template", select the instrument type as 'QuantStudio™ 7 Flex System', select the block being used for the run as '96-Well (0.2mL)', 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.



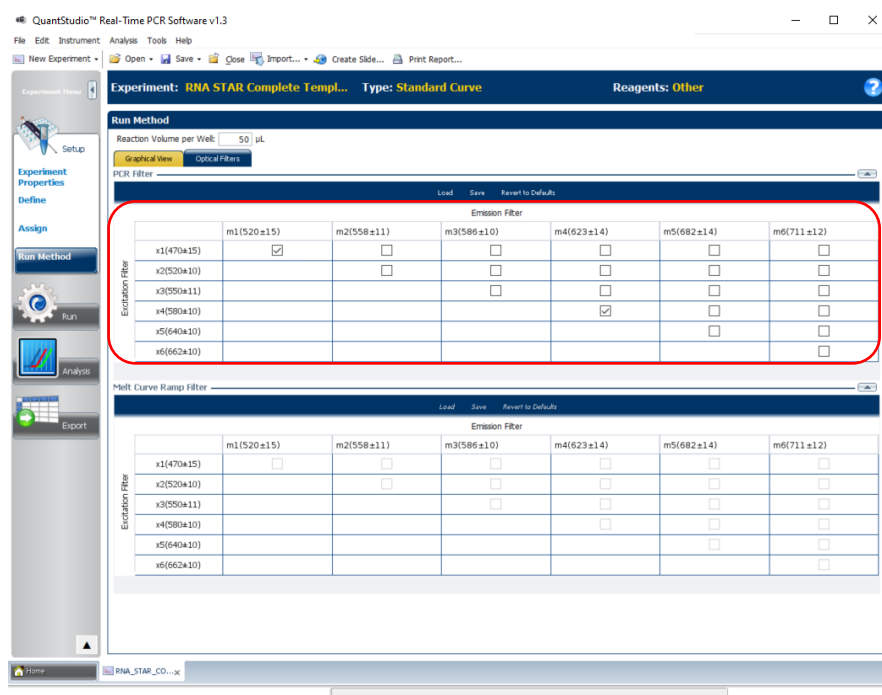
- Under the 'Targets' section: define the 'Target Name' for 'Target 1' as "COVID", select 'FAM' as the 'Reporter', change the 'Color' to green, and confirm the 'Quencher' is automatically entered as 'NFQ-MGB'. Add a second target by clicking 'New' (just above 'Target Name') then rename to 'IC', change 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'.
- (Optional) Under the 'Samples' section, the 'Sample Name' can be added individually.



- On the left of the screen, proceed to the 'Assign' tab. Select all wells from the plate layout by clicking the box between 'A' and '1'. 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.



- Under the 'Run Method' section, define the 'Reaction Volume per Well' to "60" (µL). In the 'Graphical View' section, select the 'Hold Stage' and delete one step by right-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 next to the 'Graphical View' tab 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)
11. Save the experiment as a template for subsequent runs by clicking the 'Save' drop-down menu and selecting 'Save As Template'. Name the template "RNA STAR Complete Template" and click 'Save'. The experiment must be saved again to connect to the instrument. Click the drop-down menu next to 'Save' and select 'Save As'. Save the 'File Name' as "LumiraDx SARS-CoV-2 RNA STAR Complete [YYMMDD_Plate#]" and click 'Save'.
12. 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.
13. Return to the desktop software 'Run' tab and click the 'START RUN' button. The instrument number will appear in a drop-down menu below the 'START RUN' button. Select the instrument number to initiate the run. A pop-up window may appear indicating the ramp rate in Stage 1 and 2 is above the maximum. Select 'Yes' to proceed.
14. 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.

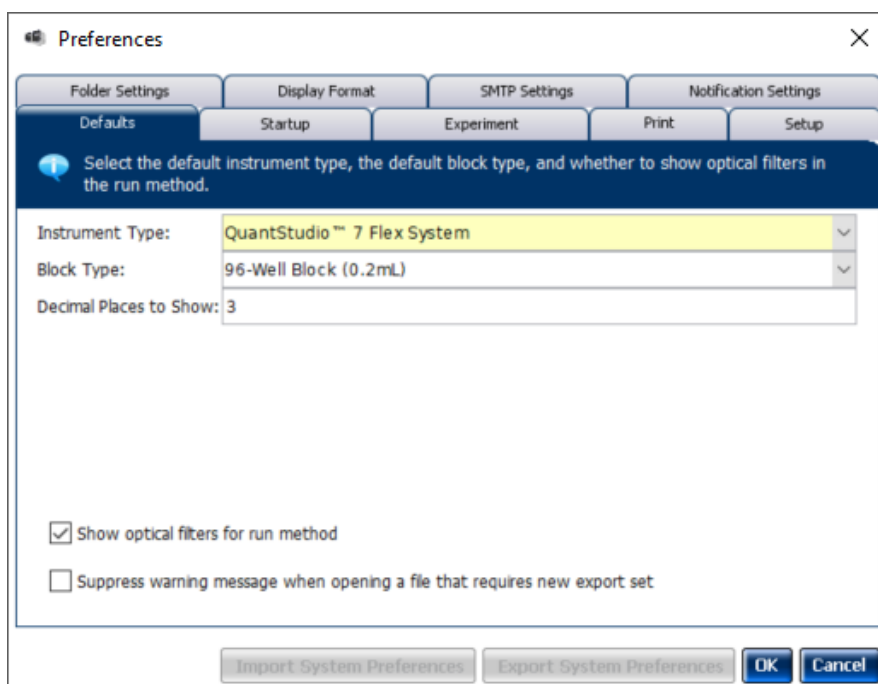
13.2 Analysis Instructions

1. Modify the analysis settings using the 'Analysis' 'Amplification Plot' 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' window on the left as follows: set 'Plot Type' to 'ΔRn vs Cycle', set 'Graph Type' to 'Log', and set 'Plot Color' to 'Target'.
2. On the top right of the software to the right of the 'Analyze' button, click the 'Analysis Settings' to open the 'Analysis Settings' window. In the 'Ct settings' tab in the bottom right window for 'CT Settings for COVID', uncheck the 'CT Settings to Use' box next to 'Default Settings'. This will toggle the 'Automatic Threshold' and 'Automatic Baseline' boxes to be checked. Uncheck the boxes to the left of 'Automatic Threshold' and 'Automatic Baseline', and define the 'Threshold' as "80,000", set the 'Baseline Start Cycle' to '1' and set 'End Cycle' to '3'.
3. On the bottom left in the 'Select a Target' window, select the 'IC' line then, in the 'CT Settings for IC' uncheck the boxes to the left of 'Default settings', 'Automatic Threshold', and 'Automatic Baseline'. Define the 'Threshold' as "15,000", set 'Baseline Start Cycle' to '1' and set 'End Cycle' to '3'. On the bottom of the window, select 'Apply Analysis Settings' to close out of the 'Analysis Settings' pop-up window.
4. On the left of the screen, proceed to the 'Export' tab. Define the 'Export File Name' as "RNA STAR Complete [YYMMDD_Plate#]", choose the 'File Type' (default is QuantStudio as an .xls file), choose the 'Export File Location' to export the file.
5. Select the desired tabs below 'Export File Location' to determine what is exported. The box next to the 'Results' tab needs to be checked to export CT values. Within the 'Results' tab, select only 'Well', 'Well Position', 'Sample Name', 'Target Name', 'Task', 'Reporter', 'Quencher', and 'CT'. Click 'Start Export' at the bottom of the screen to generate the export data file. The exported file will include a 'Results' section which contains the sample Ct values.
6. Click 'Save' to save the modified settings.
7. Close the software.

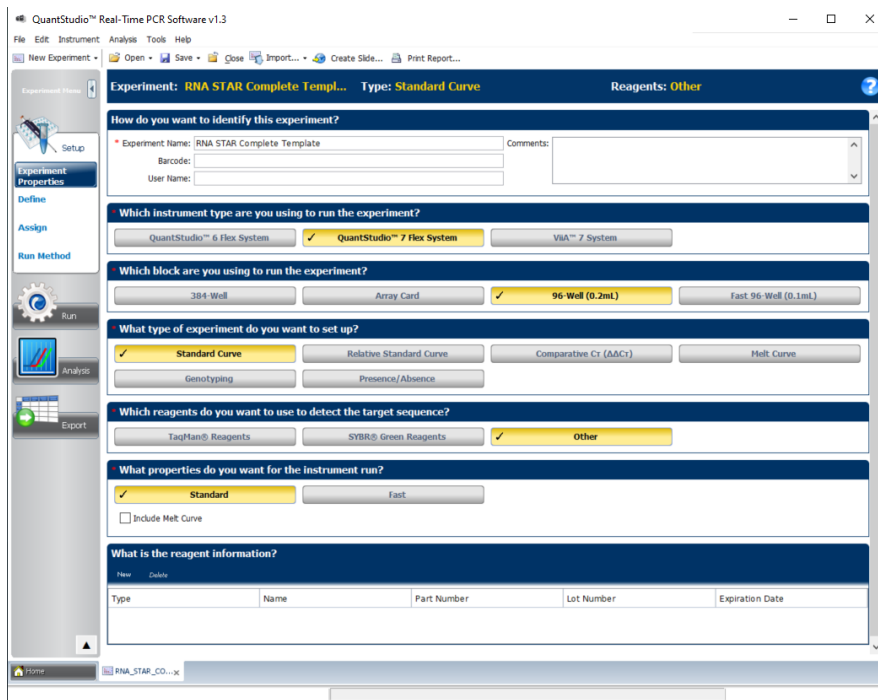
13.3 Programming Instructions for Stand-Alone Instrument

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).

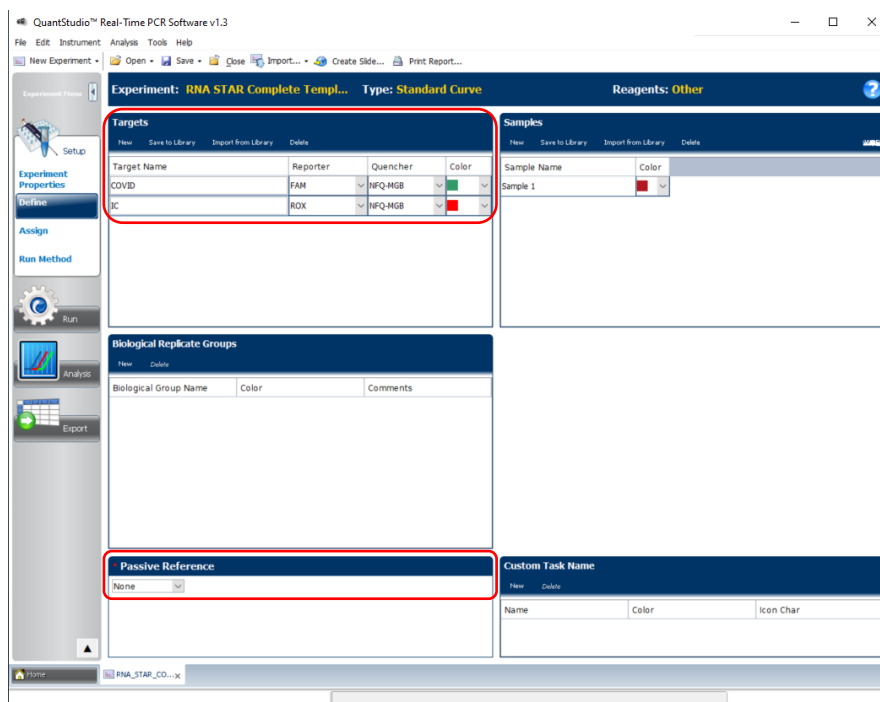
1. If the instrument touchscreen is in sleep mode (dark screen), touch the screen anywhere to activate the instrument. Sleep mode lowers the temperature of the heated cover. It is important that the heated cover idle at 105°C before loading a 96-well plate.
2. Launch the 'QuantStudio Real-Time PCR Software'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



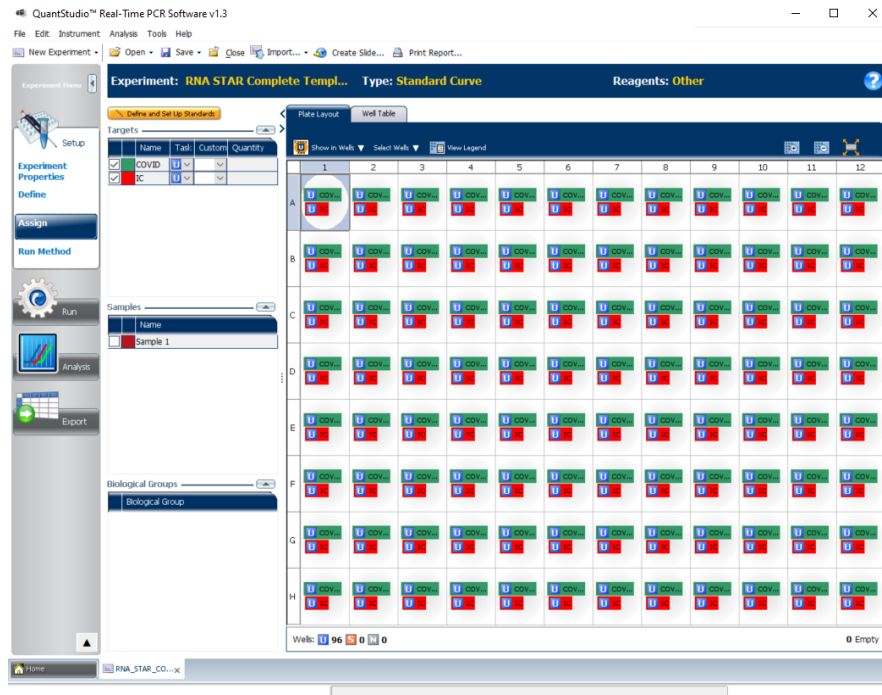
3. Confirm that the 'Instrument Type:' is set to 'QuantStudio™ 7 Flex System', the 'Block Type:' is set to '96-Well Block (0.2mL)', and that the 'Decimal Places to Show:' is set to '3'. Click the box next to 'Show optical filters for run method' to select this feature. Click 'OK'.
4. On the toolbar,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



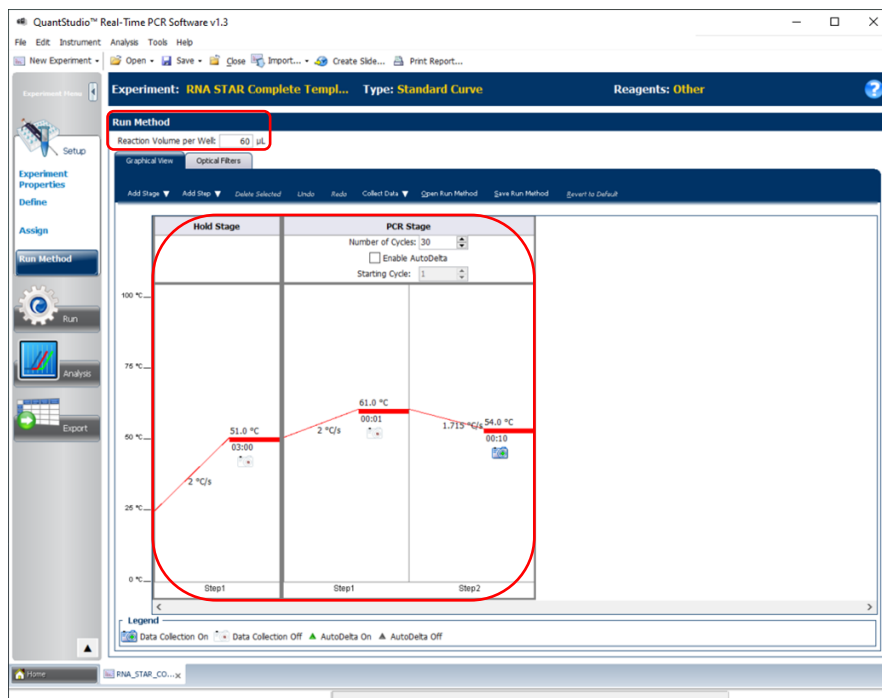
- On the left of the screen, the 'Experiment Properties' tab will be selected. Enter the 'Experiment Properties' as follows: define the 'Experiment Name' as "RNA STAR Complete Template", select the instrument type as 'QuantStudio™ 7 Flex System', select the block being used for the run as '96-Well (0.2mL)', 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.



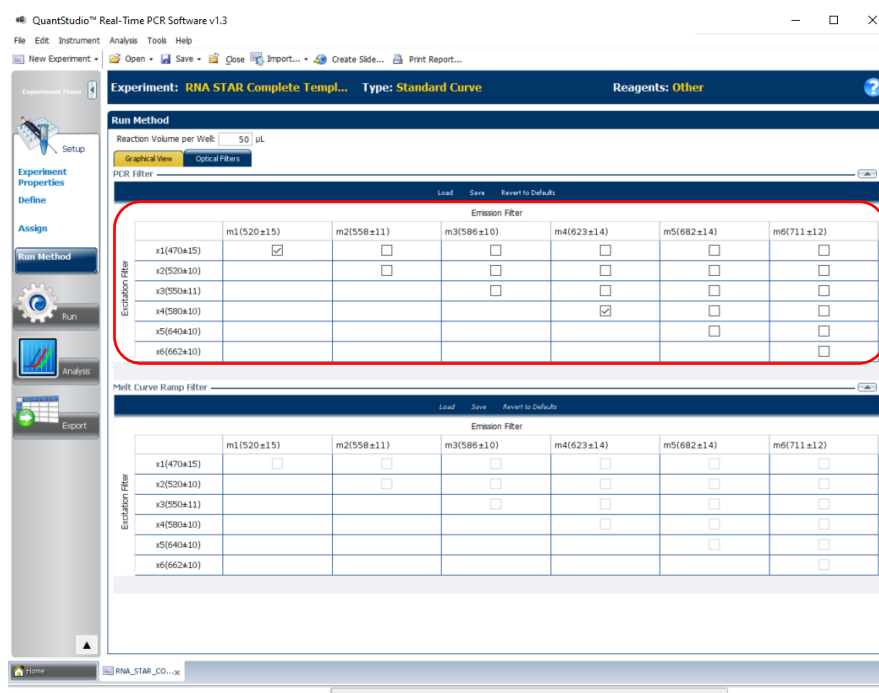
- Under the 'Targets' section: define the 'Target Name' for 'Target 1' as "COVID", select 'FAM' as the 'Reporter', change the 'Color' to green, and confirm the 'Quencher' is automatically entered as 'NFQ-MGB'. Add a second target by clicking 'New' (just above 'Target Name') then rename to 'IC', select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



- On the left of the screen, proceed to the 'Assign' tab. Select all wells from the plate layout by clicking 'A' and '1'. 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.



- Under the 'Run Method' section, define the 'Reaction Volume per Well' to "60" (µL). In the 'Graphical View' section, select the 'Hold Stage' and delete one step by right-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 next to the 'Graphical View' 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 Template'. Name the template "RNA STAR Complete Template" and click 'Save'. Transfer this file onto a USB drive.
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, press the appropriate file, and press 'Start Run' to import it.
14. Change the 'Experiment' name to "RNA STAR Complete [YYMMDD_Plate#]" then press 'Start Run Now'. A notification may appear that the 'Required ramp rates may not be achievable on this instrument.' Press "OK" to continue with the run.
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'. Select the appropriate file from the USB to analyze.
2. Modify the analysis settings using the 'Analysis' 'Amplification Plot' tab in the 'QuantStudio Real-Time PCR Software' Desktop Software (version v1.3). Select the desired wells for analysis in the 'Plate Layout' window on the right then configure the 'Amplification Plot' window on the left as follows: set 'Plot Type' to 'ΔRn vs Cycle', set 'Graph Type' to 'Log', and set 'Plot Color' to 'Target'.
3. On the top right of the software to the right of the 'Analyze' button, click the 'Analysis Settings' open the 'Analysis Settings' window. In the 'Ct settings' tab in the bottom right window of 'CT Settings for COVID', uncheck the 'CT Settings to Use' box next to 'Default Settings'. This will toggle the 'Automatic Threshold' and 'Automatic Baseline' boxes to be checked. Uncheck the boxes to the left of 'Automatic Threshold', and 'Automatic Baseline', and define the 'Threshold' as "80,000", set 'Baseline Start Cycle' to '1' and set 'End Cycle' to '3'.
4. On the bottom left in the 'Select a Target' window, select the 'IC' line then, in the 'CT Settings for IC' uncheck the boxes to the left of 'Default settings', 'Automatic Threshold', and the 'Automatic Baseline'. Define the 'Threshold' as "15,000", set 'Baseline Start Cycle' to '1' and set 'End Cycle' to '3'. On the bottom of the window, select 'Apply Analysis Settings' to close out of the 'Analysis Settings' pop-up window.
5. On the left of the screen, proceed to the 'Export' tab. Define the 'Export File Name' as "RNA STAR Complete [YYMMDD_Plate#]", choose the 'File Type' (default is QuantStudio as an .xls file), and choose the 'Export File Location' to export the file.

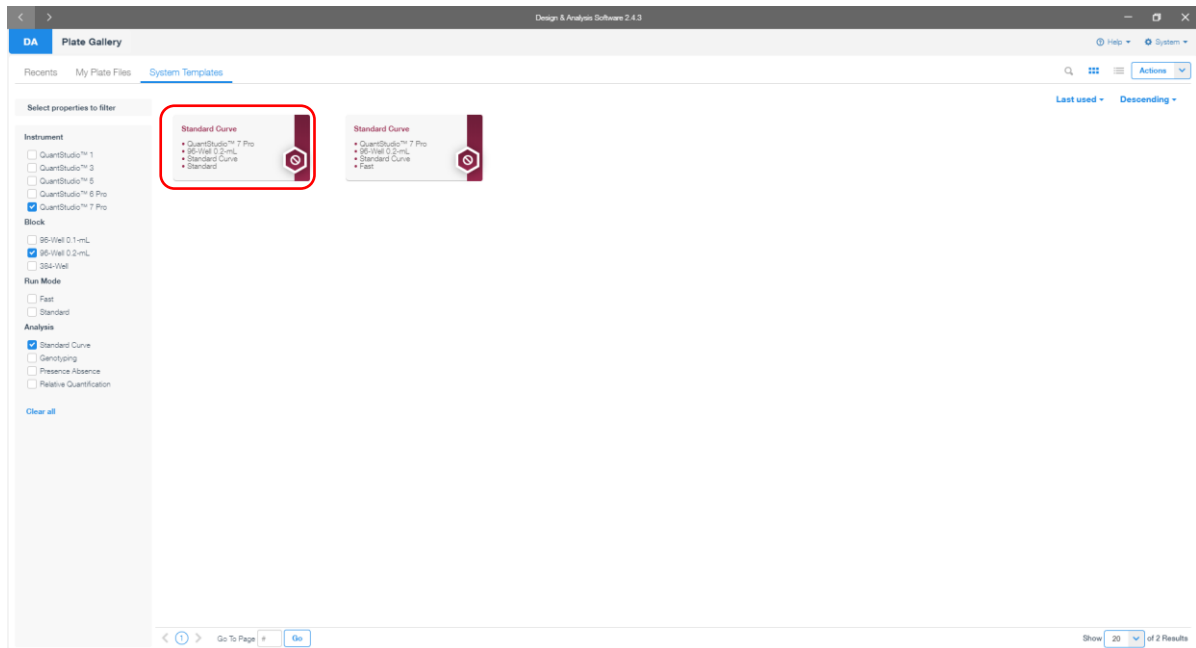
6. Select the desired tabs below 'Export File Location' to determine what is exported. The box next to the 'Results' tab needs to be checked to export CT values. Within the 'Results' tab, select only 'Well', 'Well Position', 'Sample Name', 'Target Name', 'Task', 'Reporter', 'Quencher', and 'CT'. Click 'Start Export' at the bottom of the screen to generate the export data file. The exported file will include a 'Results' section which contains the sample Ct values.
7. Click 'Save' to save the modified settings.
8. Close the software.

14 RNA STAR Complete Setup for Applied Biosystems™ QuantStudio 7 Pro

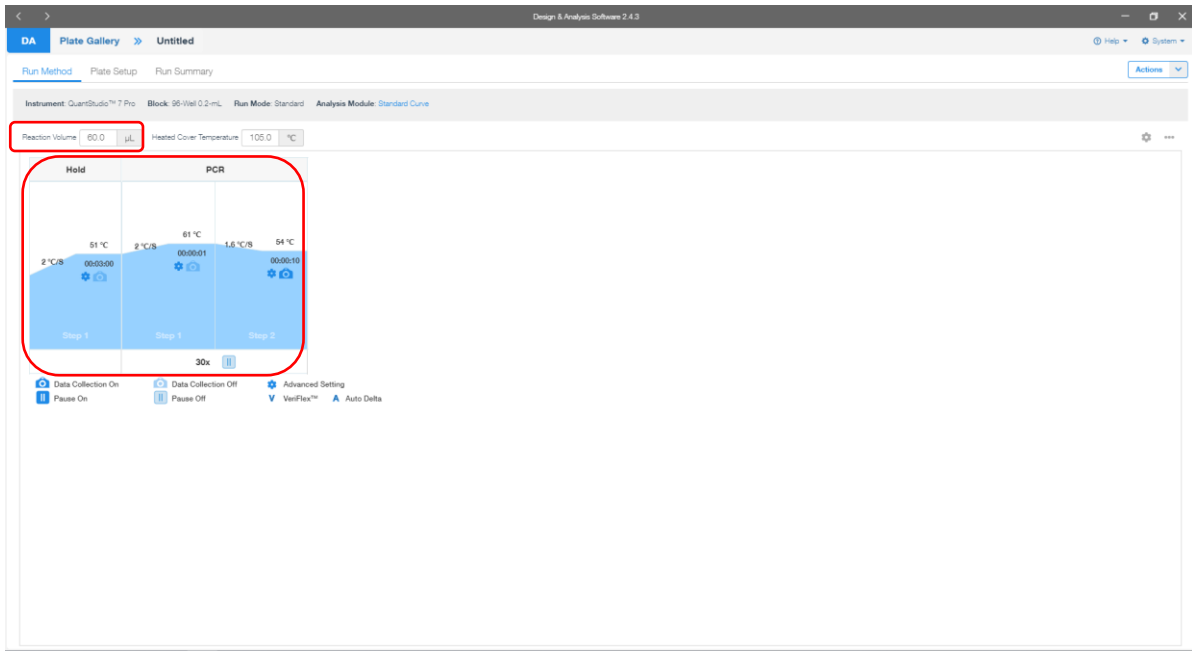
Refer to “User Manual Part Number MAN0018045” for additional information. The instrument programming instructions are intended for setup of an entire 96-well plate. If the entire plate is not used, please deselect wells as appropriate during analysis.

14.1 Programming Instructions for PC/Laptop-connected Instrument

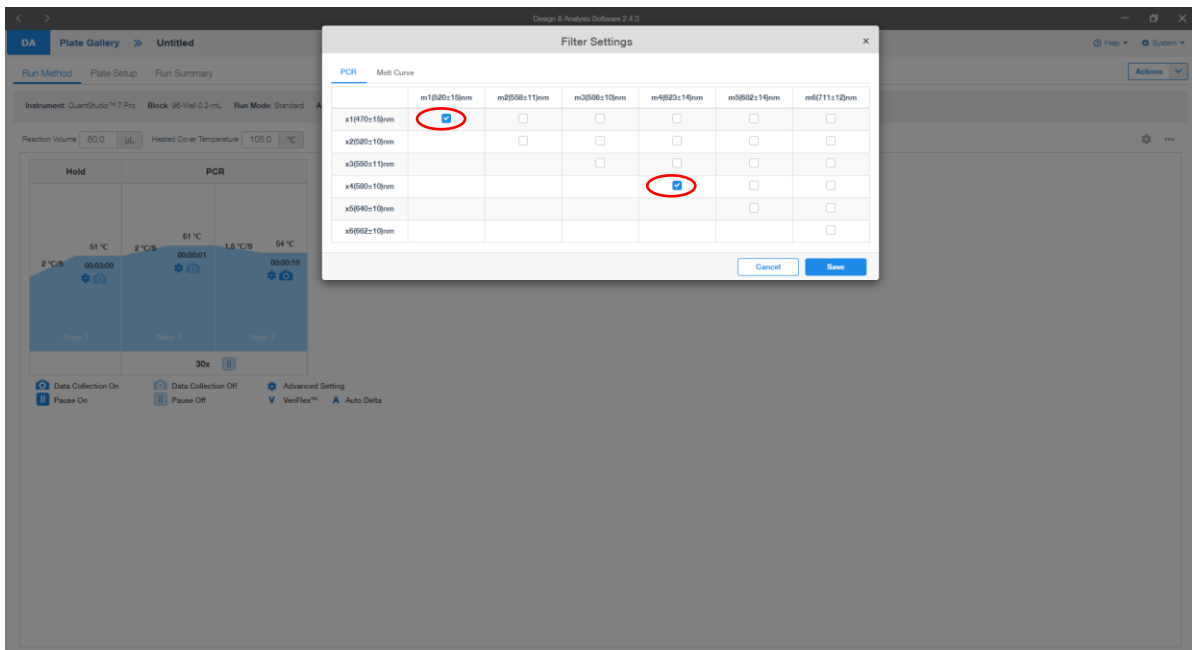
It is recommended to setup the instrument up to step 7 before preparing the Reagent Mix as defined in the qSTAR Reagent Preparation section above. The 96-well plate should be recently centrifuged to guarantee all reagents are at the bottom of the plate and kept on a cold block. Use 96-well plates and seals compatible with the instrument manufacturer (see Option for PCR Instruments & Consumables table above).



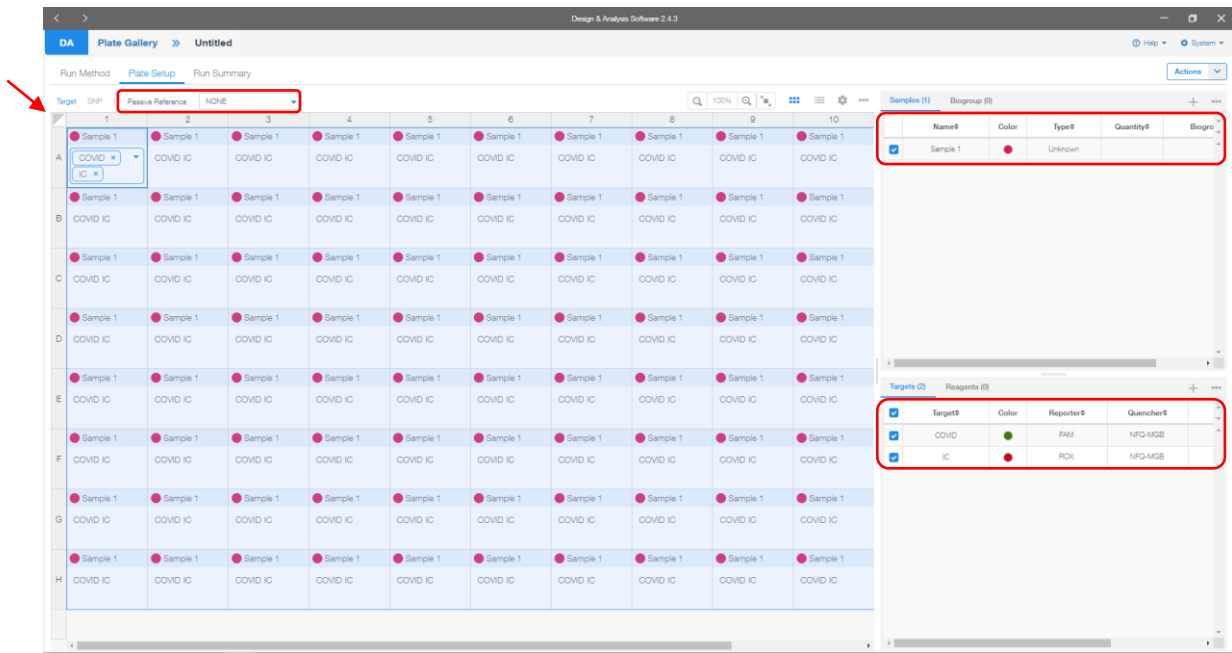
1. Launch the 'Design & Analysis' Desktop Software (version v2.4.3) and click 'Set Up Plate'. In the new screen, click on 'Standard Curve, QuantStudio™ 7 Pro, 96-Well 0.2mL, Standard Curve, Standard' to begin setup.



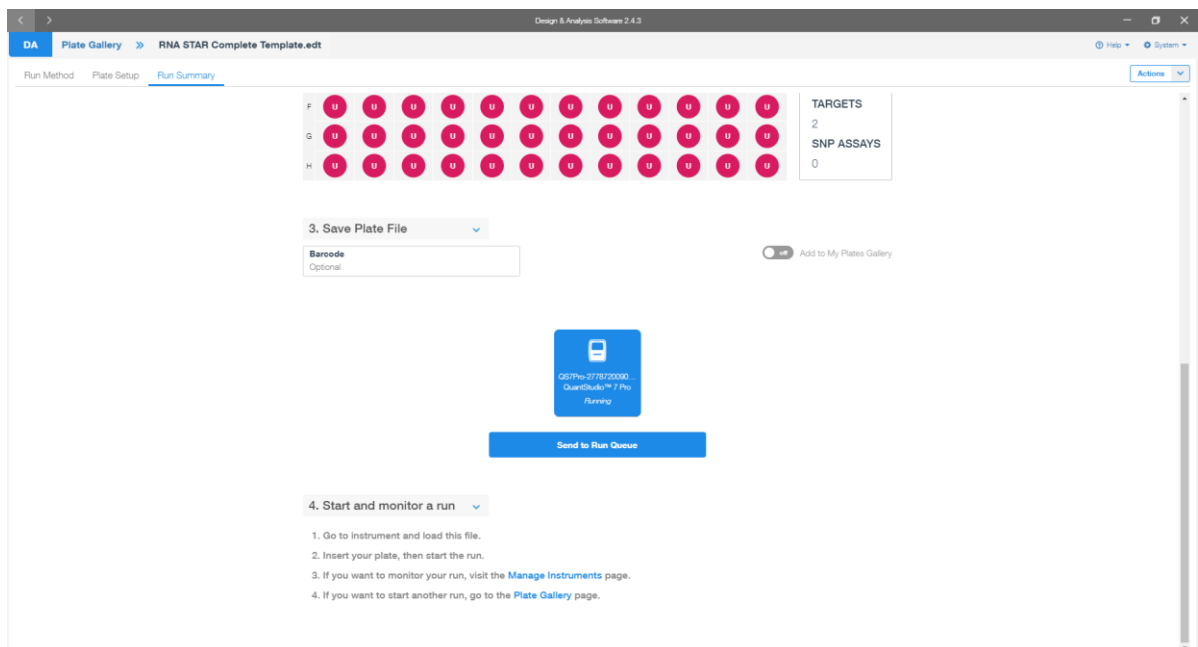
2. A new window will open with the 'Run Method' tab selected. Set the 'Reaction Volume' to '60.0 µL'. In the 'Hold Stage' section, hover over 'Step 2' and click the "-" button that appears just above the wording to delete this plateau. In the 'Hold Stage' section, change 'Step 1' settings as follows: set ramp rate to '2 °C/s', set temp to '51 °C', and set time to '00:03:00'. In the 'PCR Stage' section, change 'Step 1' settings as follows: set ramp rate to '2 °C/s', set temp to '61 °C', and set time to '00:00:01'. In the 'PCR Stage' section, change 'Step 2' settings as follows: set ramp rate to '2 °C/s', set temp to '54 °C', and set time to '00:00:10' (data collection step). At the bottom of the 'PCR Stage' section, set cycles to '30x'.




3. Set the filter settings by clicking on the three dots at the top right of the screen and clicking 'Filter Settings...'. Ensure that only the following combination is selected: 'x1(470±15)nm', 'm1(520±15)nm'; and 'x4(580±10)nm', 'm4(623±14)nm'. Click 'Save' to dismiss the window. Then proceed to the 'Plate Setup' tab.



- Proceed to the 'Plate Setup' tab. Select the whole plate by clicking the box between 'A' and '1' and confirm just below the 'Plate Setup' tab that 'Passive Reference' is set to 'NONE'. On the far top right of the screen, click the '+' icon in the 'Samples' gray-shaded section. Edit the new line that appears as follows: select the box on the left of 'Sample 1', confirm the 'Color' is set to red, confirm the 'Type' is set to 'Unknown' and leave 'Quantity' undefined. In the bottom right of the screen, click the '+' icon in the 'Targets' gray-shaded section. Edit the new line that appears as follows: select the box on the left of 'Target 1', rename 'Target 1' to "COVID", change the 'Color' to green by clicking the color dot, confirm the 'Reporter' is set to 'FAM', confirm the 'Quencher' is set to 'NFQ-MGB', and leave 'Task' and 'Quantity' undefined. Click the '+' icon in the bottom right 'Targets' gray-shaded section again. Edit the new line that appears as follows: select the box on the left of the new 'Target 1', rename 'Target 1' to "IC", change the 'Color' to red, change the 'Reporter' using the drop-down menu to 'ROX', confirm the 'Quencher' is set to 'NFQ-MGB', and leave 'Task' and 'Quantity' unknown. Then proceed to the 'Run Summary' tab.

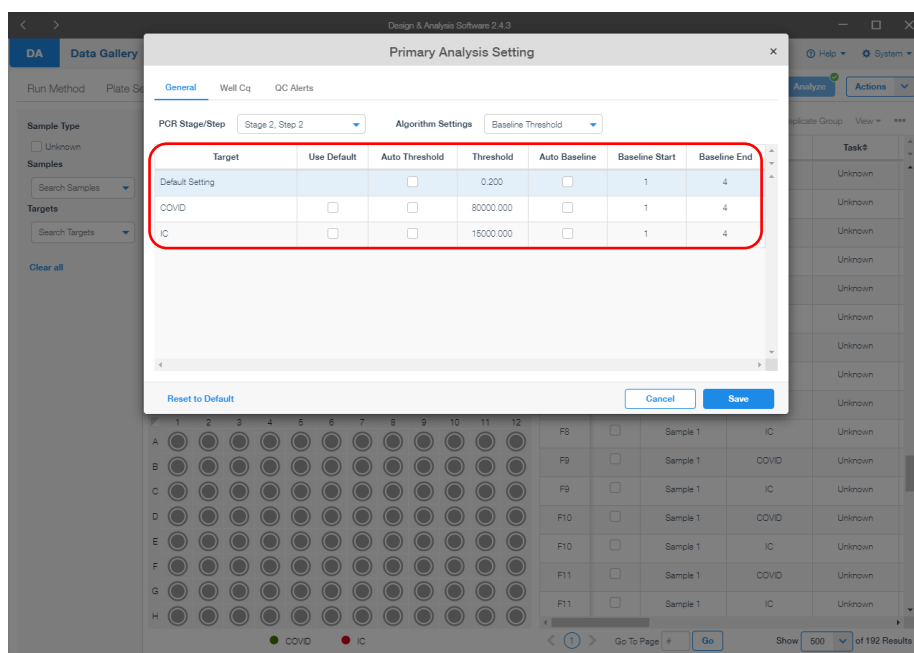


- Scroll down to the '3. Save Plate File' section and click 'QuantStudio™ 7 Pro' instrument icon. In the top right corner click 'Actions' and using the drop-down menu click 'Save As...'. Name the 'File Name' as 'RNA STAR Complete Template.edt' (.edt identifies this file as a template). Chose a 'Folder' path then click 'Save'. Back in the 'Run Summary' tab under the '3. Save Plate File' section, click 'Send to Run Queue'. A notification should appear indicating the run was sent to the instrument chosen.

- On the home instrument touch screen, press 'Load Plate File'. In new screen in the 'Run Queue' folder, press 'RNA STAR Complete Template' in the 'Plate file' section. In the 'Data file properties' screen, re-name the 'Data file name' as "RNA STAR Complete [YYMMDD_Plate#]". Press 'Enter' to confirm 'Data file name'.
- At the top right of the screen, press the tray button, , 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 the same tray button to close the instrument drawer.
- At the bottom of the screen, press 'Start run' to start the run. A 'Did you remember to insert your plate?' screen will occur and press 'Start run' again to start the run.
- Following the run, the instrument touchscreen indicates 'Run Completed'. Press 'Transfer file', insert a USB drive, press 'USB drive', and press 'Done' to export the file to the USB drive. Remove the USB drive from the instrument and press 'Close'.
- 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.
- Remove the plate and discard the plate in a sealable waste bag or container.

14.2 Analysis Instructions

- Plug the USB into the laptop and open the exported file. The 'Design & Analysis' Desktop Software (version v2.4.3) and proceed to the 'Quality Check' tab. In the upper right-hand corner, click 'Analyze'. Change the data plot to display the 'Amplification Plot' by using the drop-down arrow just above the data plot and selecting 'Amplification Plot'. By default, this should show a graph with ' ΔRn vs Cycle' with the y-axis in logarithmic format. If not, select the gear icon just above the data plot on the right and in the 'General' tab change the 'Y Scale' to using the drop-down menu 'Log'.



- On the far top right of the screen, select the drop-down menu next to 'Actions' and select 'Primary Analysis Setting...'. A pop-up window will display the default threshold and baseline settings. In the 'General' tab, deselect the checkboxes in the 'Use Default' column for 'COVID' and 'IC' to the right of the 'Target' column. The checkboxes in the 'Auto Threshold' column will become selected; deselect the checkboxes in the 'Auto Threshold' column for 'Default Setting', 'COVID' and 'IC'. The checkboxes in the 'Auto Baseline' column will become selected; deselect the checkboxes in the 'Auto Baseline' column for 'Default Setting', 'COVID' and 'IC'. Define the 'Threshold' values for 'COVID' and 'IC' to "80000.000" and "15000.000", respectively. Define the 'Baseline Start' and 'Baseline End' for 'COVID' and 'IC' to "1" and "4". Click 'Save' to dismiss the window and apply changes to the samples.
- On the far top right of the screen, click 'View' and deselect all the checkboxes but for 'Well', 'Target' and 'Cq' then click out of the drop-down menu.
- To export the results in an Excel friendly format, in the far top right corner, click on the three dots and click 'Export'. Choose the desired folder path, define the 'File Name' and click 'Save'.
- Close the software.

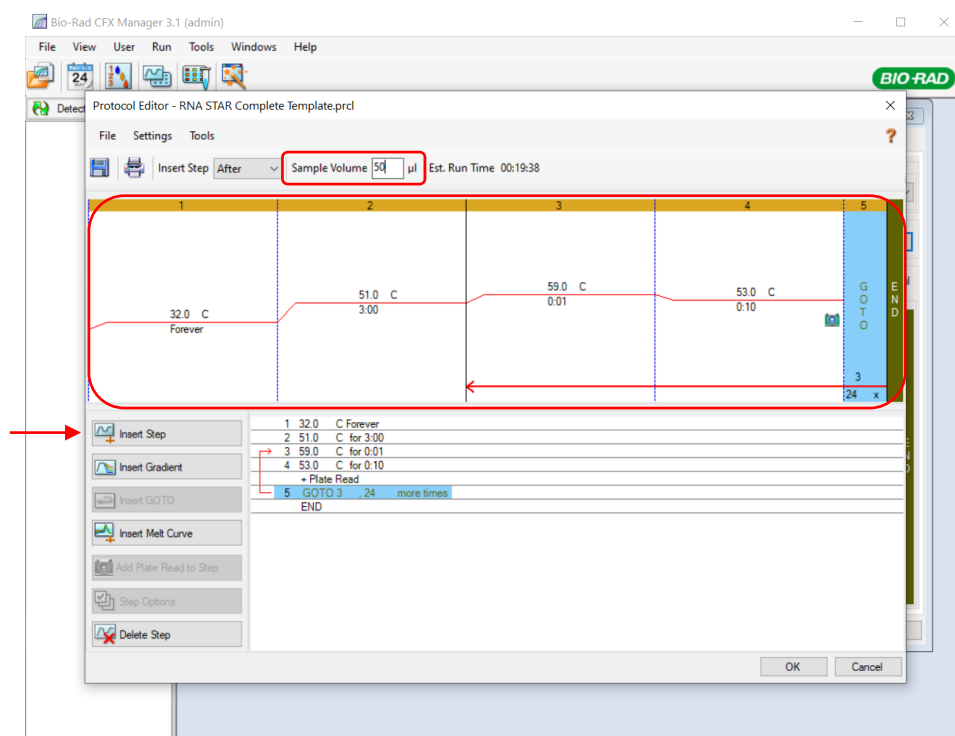
15 RNA STAR Complete Setup for Bio-Rad CFX96 Touch System

Refer to “User Manual Part Number 10021418” 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. It is required that the Bio-Rad CFX96 instrument be appropriately qualified prior to use with the LumiraDx SARS-CoV-2 RNA STAR Complete assay using established testing protocols. Refer to the protocol provided in Appendix A.

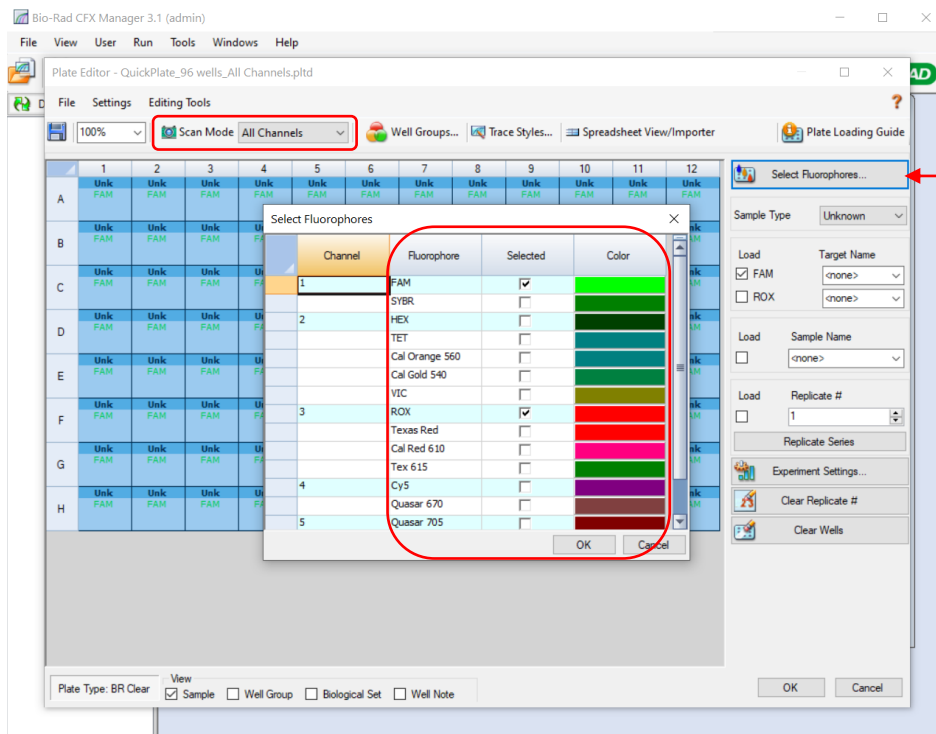
15.1 Programming Instructions for PC/Laptop-connected instrument

It is recommended to setup the instrument up to step 7 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 compatible with the instrument manufacturer (see Option for PCR Instruments & Consumables table above).

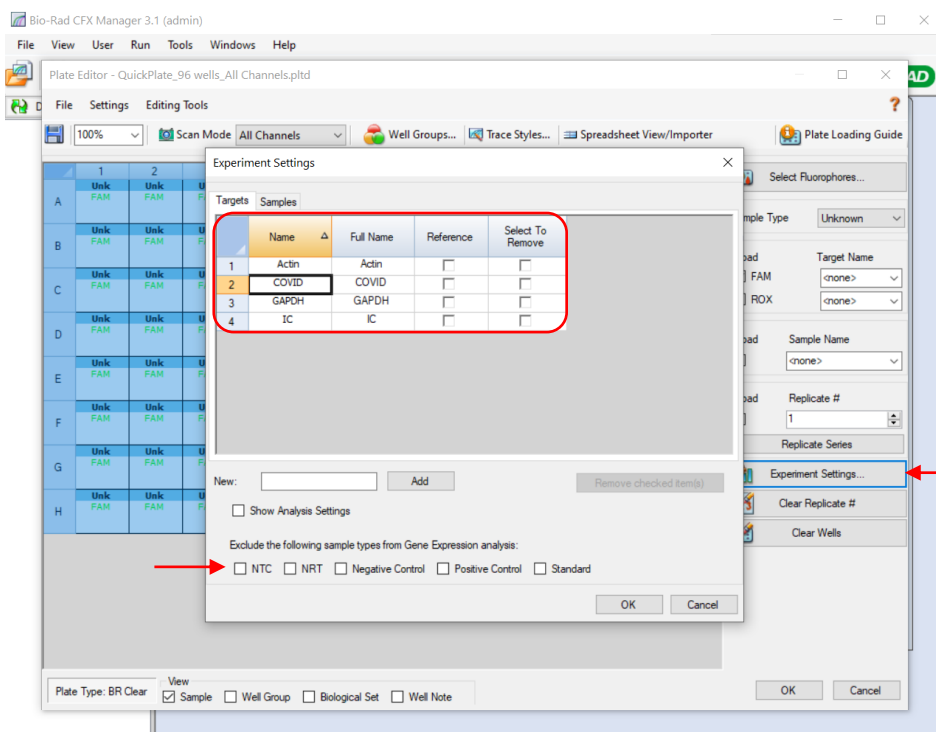
1. Launch the ‘Bio-Rad CFX Manager’ Desktop Software (version v3.1). In the ‘Startup Wizard’ pop-up window, select ‘User-defined’ as the run type then, in the ‘Run Setup’ pop-up window, select ‘Edit Selected...’ on the right of the window under the ‘Protocol’ tab to begin setup.



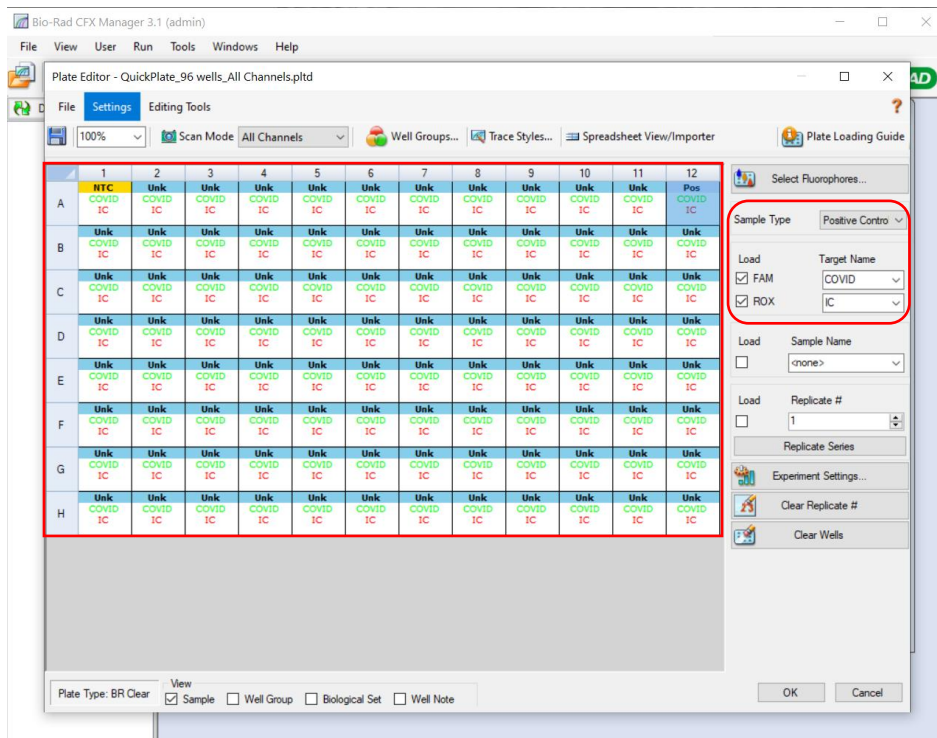
2. The ‘Protocol Editor’ pop-up window will open. Set the ‘Sample Volume’ to “50” µL (the sample volume should not be set higher than this). Click ‘Insert Step’ to add a total of five steps to the protocol. Edit steps ‘1’ through ‘5’ as follows: set step ‘1’ temp to ‘32.0’ C, set ‘1’ time to ‘0:00’ (this sets the time to ‘Forever’), set step ‘2’ temp to ‘51.0’ C, set step ‘2’ time to ‘3:00’, set step ‘3’ temp to ‘59.0’ C, set ‘3’ time to ‘0:01’, set step ‘4’ temp to ‘53.0’ C, set ‘4’ time to ‘0:10’, and set step ‘5’ GO TO’ to ‘3’ and ‘24’ x (this defines that step 3 through step 4 will be repeated a total of 25 times). Select ‘OK’ and then select ‘Yes’ to save the changes to the protocol file. Define the ‘File name’ as “RNA STAR Complete Template.prc1” in the appropriate file path and select ‘Save’.



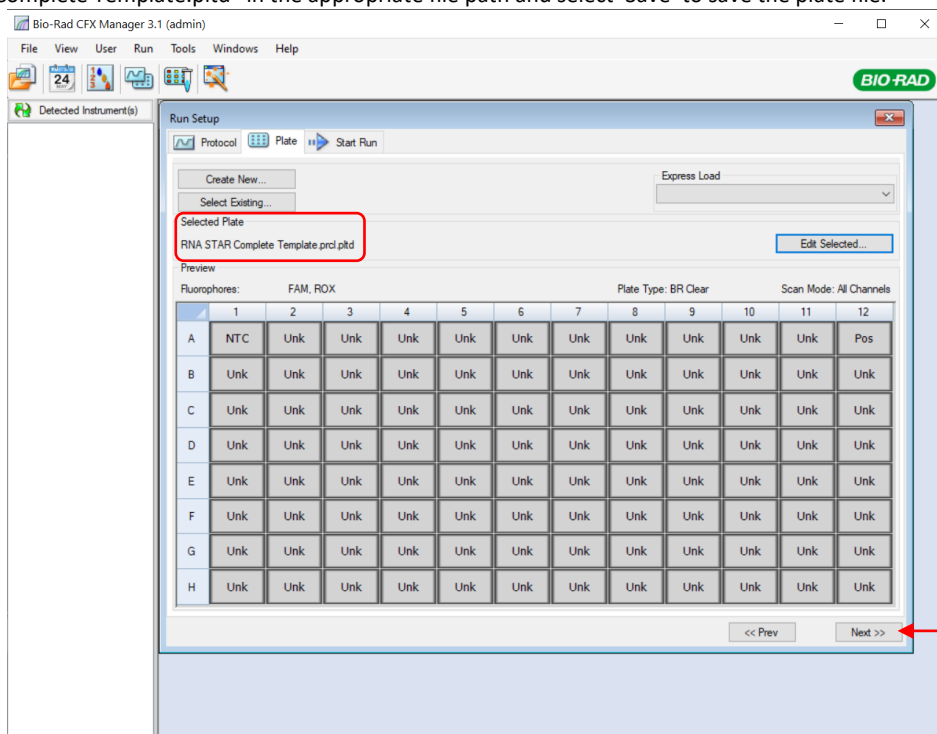
- Proceed to the 'Plate' tab by selecting the 'Next' button on the bottom right of the 'Protocol Editor' window. Select 'Edit Selected...' on the right of the window then select the entire plate by selecting the gray box between position 'A' and '1' on the plate. Ensure that 'Scan Mode' is set to 'All Channels' by using the drop-down menu then select 'Select Fluorophores...' on the far top right of the window. Ensure the only fluorophores selected are 'FAM' and 'ROX'. Change the 'Color' for 'FAM' to green and the 'Color' for 'ROX' to red. Select 'OK' to close out of the pop-up window.



- Select 'Experiment Settings...' near the bottom right of the window to open the 'Experiment Settings' pop-up window. Under the 'Targets' tab, add a new target by defining 'New' as "COVID" and selecting 'Add'. Add a second target and define 'New' as "IC" then select 'Add'. Under the 'Exclude the following sample types from Gene Expression analysis' at the bottom of the pop-up window, deselect all checkboxes and select 'OK' to close out the pop-up window.



5. Select the entire plate again by selecting the gray box between position 'A' and '1' on the plate and ensure the check boxes to the left of the 'Load' selection are checked for both 'FAM' and 'ROX'. Using the drop-down menu under 'Target Name' define the 'Target Name' for 'FAM' as 'COVID' and the 'Target Name' for 'ROX' as 'IC'. Select just well 'A1' in the depiction of the plate and use the drop-down menu on the right of 'Sample Type' to set this well as 'NTC'. Select just well 'A12' and set the 'Sample Type' using the drop-down menu to 'Positive Control'. Select 'OK' and save the changes by clicking 'Yes'. Define the 'File name' as "RNA STAR Complete Template.pltd" in the appropriate file path and select 'Save' to save the plate file.



6. The 'Plate Editor' pop-up window will close and the new plate will be loaded in the 'Plate' tab of the 'Run Setup' window. Proceed to the 'Start Run' tab by selecting 'Next >>' on the bottom right of the window.

7. On the 'Start Run' tab, click 'Start Run' and save the experiment by defining the 'File name' as "RNA STAR Complete [YYMMDD_Plate#].pcrd" in the appropriate file path then selecting 'Save'. The run will begin initializing and lid will begin preheating.

NOTE: The run will stay paused on Step 1 until the 'Skip Step' button is clicked. When the sample plate is ready and the lid is preheated, proceed to the next step to add the sample plate to the amplification block and begin the run.

8. At the bottom left of the screen, select 'Open Lid'. A pop-up window may appear indicating the run will pause to open the lid. Click 'OK' to proceed. The lid will automatically open, place the sample plate on the amplification block, then select 'Close Lid' to close the lid. Once the lid has finished closing, the 'Skip Step' button will become available. Select 'Skip Step' to initiate the run.
9. When the run finishes, the instrument will become idle and the 'Data Analysis' window will pop up.
10. Refer to the 'Bio-Rad CFX Manager' Desktop Software (version v3.1) and select 'Open Lid'. The lid will automatically open. Remove the plate and discard the plate in a sealable waste bag or container. Select 'Close Lid' to close the lid.

15.2 Analysis Instructions

1. In the 'Data Analysis' pop-up window under the 'Quantification' tab, select 'Settings' in the main menu then select 'Baseline Setting' and then 'Baseline Subtracted Curve Fit'.
2. Edit the baseline and cycle threshold settings for COVID by performing the following steps: Under the 'Amplification' curves, uncheck the box on the left of 'ROX'. From the main menu, select 'Settings' then select 'Baseline Threshold...' to open the 'Baseline Threshold' pop-up window. Under the 'Baseline Cycles' section, select 'User Defined' then, immediately below this, click the square between 'Well' and '1' to select all the wells. Edit 'All Selected Rows: Begin' to "1" and 'End:' to "2". In the 'Single Threshold' section, select 'User Defined:' and set this to "1000.00". Click 'OK' to proceed.
3. Edit the baseline and cycle threshold settings for IC by performing the following steps: Under the 'Amplification' curves, uncheck the box on the left of 'FAM' and check the box on the left of 'ROX'. From the main menu, select 'Settings' then select 'Baseline Threshold...' to open the 'Baseline Threshold' pop-up window. Under the 'Baseline Cycles' section, select 'User Defined' then, immediately below this, click the square between 'Well' and '1' to select all the wells. Edit 'All Selected Rows: Begin' to "1" and 'End:' to "2". In the 'Single Threshold' section, select 'User Defined:' and set this to "500.00". Click 'OK' to proceed.
4. After defining baseline and cycle threshold settings for COVID and IC, check both boxes on the left of 'FAM' and 'ROX' then proceed to the 'Quantification Data' tab. Right-click over the 'Results' table, and choose the desired method for exporting the data (e.g. 'Export to Excel...').
5. Save the report by defining the 'File name' as "RNA STAR Complete Cq Results [YYMMDD_Plate#].xlsx" in the appropriate file path then selecting 'Save'.
6. Close out the software. If prompted, save the changes to the experiment.

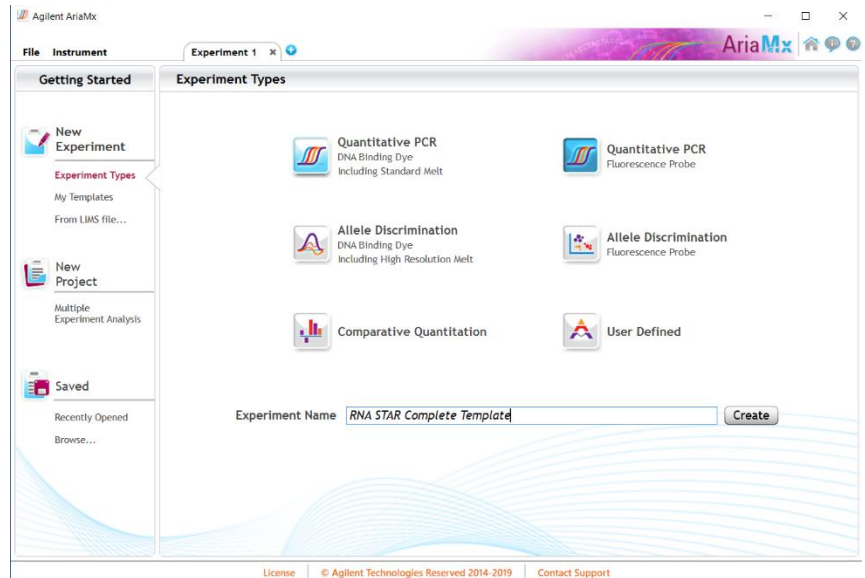
16 RNA STAR Complete Setup for Agilent™ AriaMx

Refer to “User Manual Part Number G8830A” 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. It is required that the Agilent AriaMx instrument be appropriately qualified prior to use with the LumiraDx SARS-CoV-2 RNA STAR Complete assay using established testing protocols. Refer to the protocol provided in Appendix A.

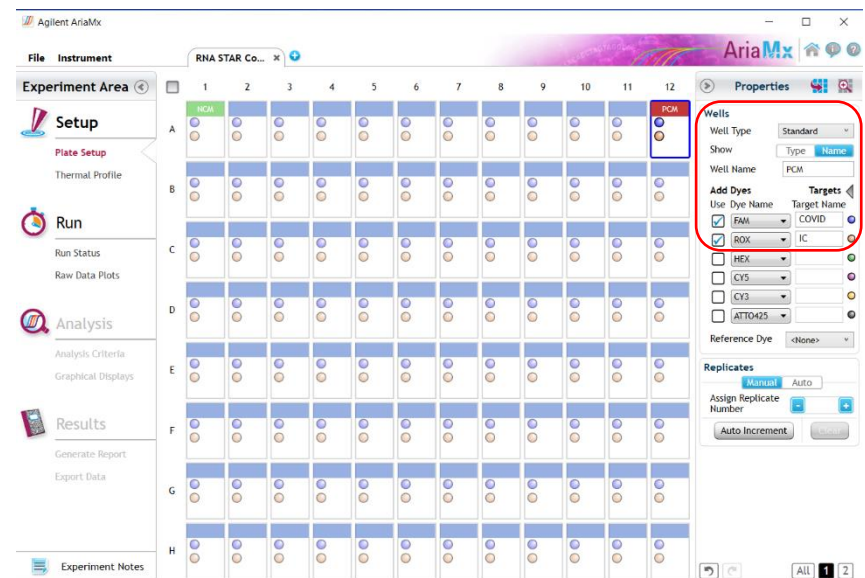
16.1 Programming Instructions for PC/Laptop-connected Instrument

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 compatible with the instrument manufacturer (see Option for PCR Instruments & Consumables table above).

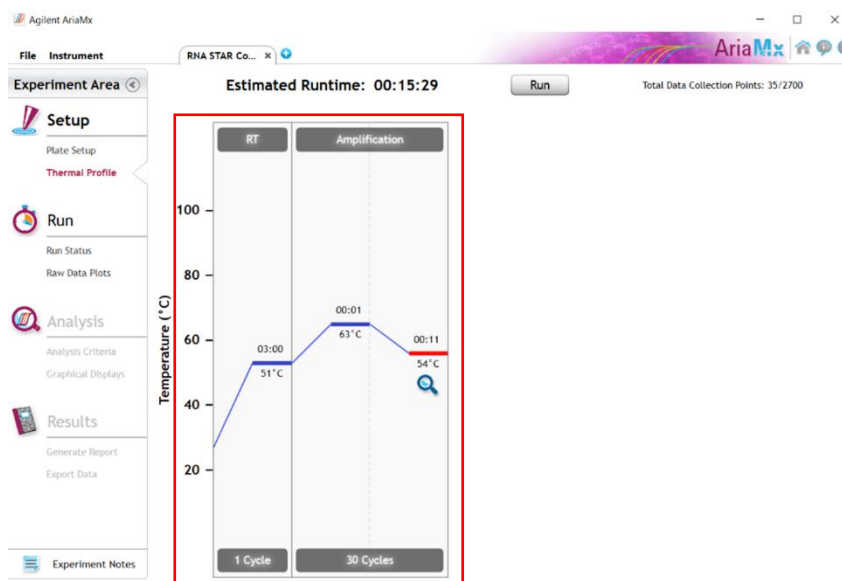
1. Launch the Agilent AriaMx desktop software (version 1.71). Select ‘Quantitative PCR Fluorescence Probe’ in the ‘Experiment Types’ section (do not double click). Define the ‘Experiment Name’ as “RNA STAR Complete Template” and click ‘Create’.




2. Select all the wells and in the ‘Properties’ section on the right of the screen set ‘Well Type’ to ‘Unknown’ using the drop-down menu, 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’ using the drop-down menu. Click the ‘Name’ button just below and define the ‘Well Name’ as “NCM”(Negative Control Media). Select just well ‘A12’ and re-set ‘Well Type’ to ‘Standard’ using the drop-down menu. Click the ‘Name’ button just below and define the ‘Well Name’ as “PCM” (Positive Control Media). The other wells will remain ‘Unknown’ unless defined as Samples Names (optional).



- Proceed to the 'Thermal Profile' tab on the left of the software. Right-click in the 'Hot Start' section and click 'Remove Segment' to delete this section. Hover over the 'Amplification' section and click the left "+" icon near the bottom of the section. In the 'New Segment' section that appears click 'RT'. Modify the default thermal profile for 'RT' as follows: set temp (lower left) to '51 °C', set time to (upper left) '03:00' and confirm 'Cycle' is automatically set to '1'. Modify the default thermal profile for 'Amplification' as follows: set temp (lower middle) to '63 °C', set time (upper middle) to '00:01', set temp (lower right) to '54 °C', set time (upper right) to '00:11' (this is the data collection step), and set 'Cycles' to '30'.



- 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'.
- 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'.
- 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.

- 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.

16.2 Analysis Instructions

- 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.
- 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 '1' and set 'End Cycle' to '4' then click 'Apply'. To exit the window, click 'OK'.
- In the 'Amplification Plots' panel, change 'Graph Type' to 'Log'. Change automatically generated 'Threshold Fluorescence' values by defining 'COVID' as "350" then click the 'Unlock' icon immediately next to the newly defined threshold to lock the setting, additionally, define 'IC' as "120" 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. 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'.
5. 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.
6. To save the run, click 'File' from the menu and choose 'Save' from the drop-down menu.
7. Close the software.

16.3 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 compatible with the instrument manufacturer (see Option for PCR Instruments & 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' using the drop-down list. Check the boxes next to 'ROX' and 'FAM' and proceed to the 'Thermal Profile' tab.
3. Press 'Hold Start' and press the 'X' that appears next to this to delete this section. In the 'Amplification' section, press the left "+" icon near the bottom of the section. In the 'New' section that appears click 'RT'. Modify the default thermal profile for 'RT' as follows: set temp (lower left) to '51 °C', set time to (upper left) '03:00' and confirm 'Cycle' is automatically set to '1'. Modify the default thermal profile for 'Amplification' as follows: set temp (lower middle) to '63 °C', set time (upper middle) to '00:01', set temp (lower right) to '54 °C', set time (upper right) to '00:11' (this is the data collection step), and set 'Cycles' to '30'. 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.

16.4 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 '1' and set 'End Cycle' to '4' 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 "350" then click the 'Unlock' icon immediately next to the newly defined threshold to lock the setting, additionally, define 'IC' as "120" 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.

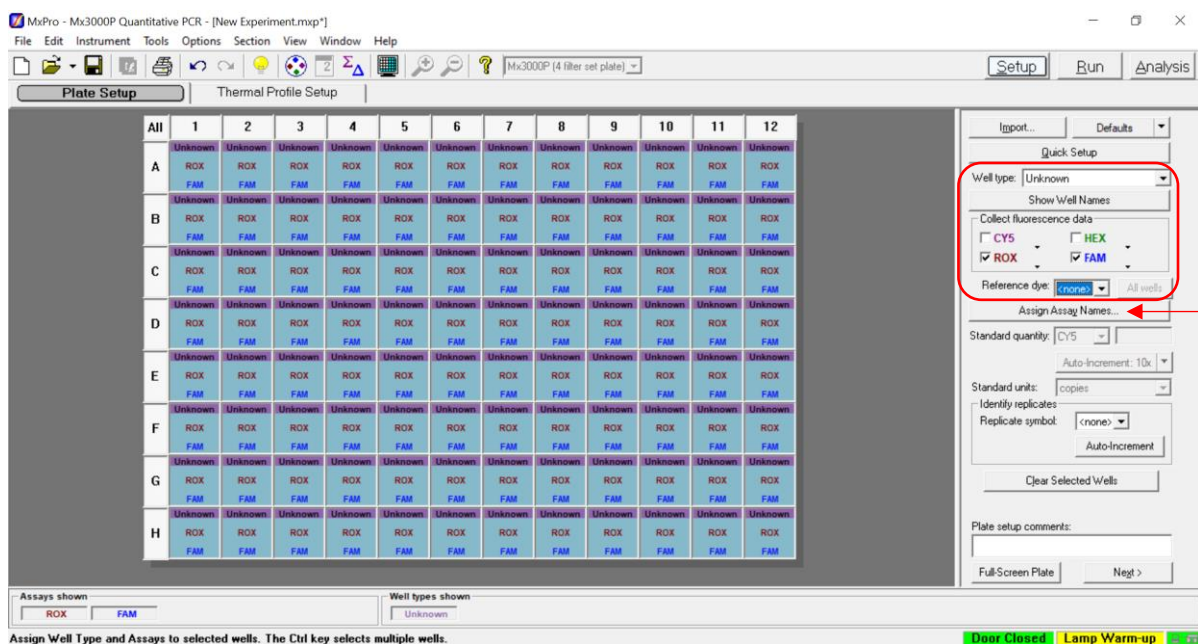
17 RNA STAR Complete Setup for Agilent™ Stratagene Mx3005P

Refer to “User Manual Part Number 70225 J.1” 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. It is required that the Agilent Stratagene Mx3005P instrument be appropriately qualified prior to use with the LumiraDx SARS-CoV-2 RNA STAR Complete assay using established testing protocols. Refer to the protocol provided in Appendix A.

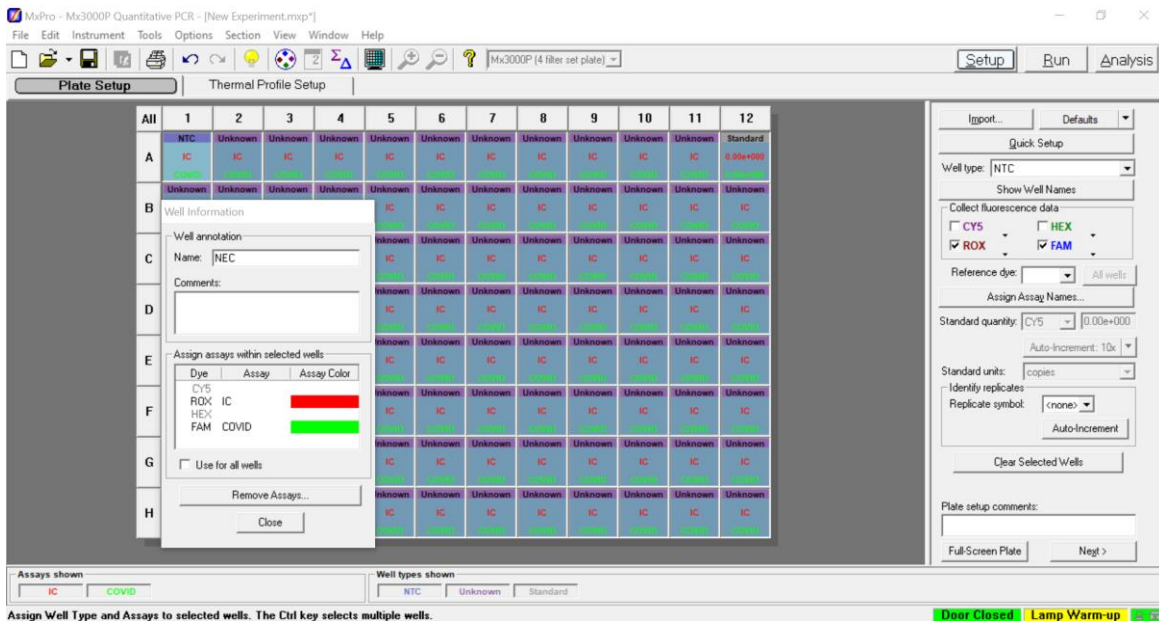
17.1 Programming Instructions for PC/Laptop-connected Instrument

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 compatible with the instrument manufacturer (see Option for PCR Instruments & Consumables table above).

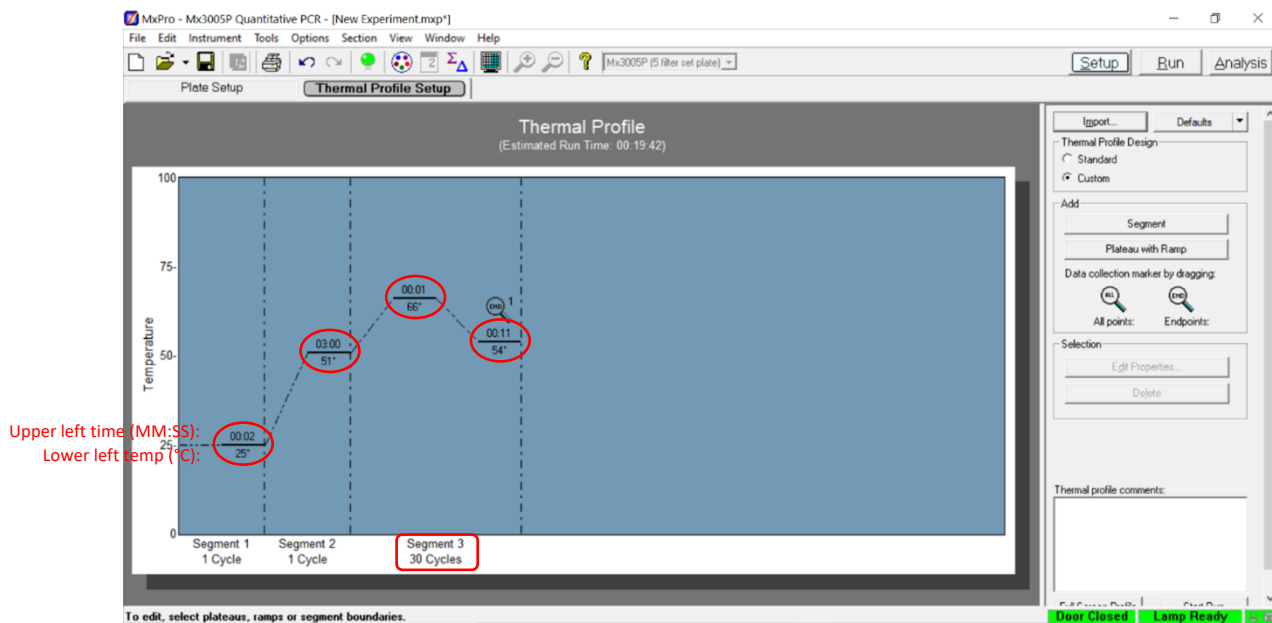
1. Launch the MxPro desktop software (version 4.10). Select ‘Quantitative PCR (Multiple Standards)’ in the ‘New Options’ window, check the box next to ‘Turn lamp on for warm-up?’, and click the ‘OK’ button. The lamp requires 20 minutes to properly warm-up.



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. A new 'Segment 1' should appear in the 'Thermal Profile'. In the 'Segment 3' window, select the third plateau line then, on the right of the screen in the 'Selection' section, click 'Delete' to delete the third plateau. Modify the default thermal profile for 'Segment 1' as follows: set 'Temp' (lower left) to '25°', set 'Time' (upper left) to '00:02', and confirm 'Cycle' is set to '1'. Modify the default thermal profile for 'Segment 2' as follows: set 'Temp' (lower left) to '51°', set 'Time' (upper left) to '03:00', and confirm 'Cycle' is set to '1'. Modify the default thermal profile for 'Segment 3' as follows: set 'Temp' (lower left) to '66°', set 'Time' (upper middle) to '00:01', set 'Temp' (lower right) to '54°', set 'Time' (upper right) to '00:11', set 'Cycles' to '30'. 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.

6. 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.

7. 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.
8. 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.
9. Remove the plate and discard the plate in a sealable waste bag or container.

17.2 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 'Non-adaptive Baseline' and set the 'Start cycle' window to '1' and the 'through' window to '4'. Click on the 'Smoothing' tab and under 'Active settings' uncheck the 'Moving average' box. 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 "1000" then click the 'Unlock' icon immediately next to the newly defined threshold to lock the setting, and then change the value for 'COVID' to "1475" then click the 'Unlock' icon immediately next to the newly defined threshold to lock the setting. 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

18 Interpretation of Results and Reporting

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

18.1 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 300 copies per reaction⁶. 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, negative, control needed to ensure cross-contamination, or reagent contamination from the upper respiratory swab specimen does not occur and is comprised of Molecular Biology Grade Water (Corning; Catalog #46-000-CM).
3. An Internal Control (a component in the LumiraDx SARS-CoV-2 RNA STAR Complete Internal Control and Primer 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 to 4 for the Roche LightCycler 480 II, Agilent Aria Mx, Applied Biosystems QuantStudio 5, Applied Biosystems QuantStudio 7 Pro, and the Agilent Stratagene Mx3005p. The Applied Biosystems 7500 Fast Dx uses auto baseline for background, Bio-Rad CFX96 uses cycles 1-2, and the Applied Biosystems QuantStudio 7 Flex uses cycles 1-3. A threshold (further described starting in section 9, 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 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, Applied Biosystems QuantStudio 7 Flex, Applied Biosystems QuantStudio 7 Pro, 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)	$3.0 \leq Ct \leq 30.0$	+ or – (Can be positive or negative)	$3.0 \leq Ct \leq 30.0^*$
Neg. Control Media	Reagent and/or environmental contamination	- (Negative)	None detected	+ (Positive)	$3.0 \leq Ct \leq 30.0$

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

⁶ A four-fold dilution of the stock is made using Molecular Biology Grade Water (Neg. Ctrl. Med.) and 24 µL is added directly to the reaction.

Table 2. Expected Results from External Controls on Bio-Rad CFX96.

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)	$3.0 \leq Ct \leq 25.0$	+ or – (Can be positive or negative)	$3.0 \leq Ct \leq 25.0^*$
Neg. Control Media	Reagent and/or environmental contamination	- (Negative)	None detected	+ (Positive)	$3.0 \leq Ct \leq 25.0$

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

18.2 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, Applied Biosystems QuantStudio 7 Flex, Applied Biosystems QuantStudio 7 Pro, Agilent AriaMx, and the Agilent Stratagene Mx3005P).

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.

Table 4. Interpretation of LumiraDx SARS-CoV-2 RNA STAR Complete on Bio-Rad CFX96.

Assay Results	SARS-CoV-2 (FAM)	Internal Control (ROX)	Interpretation of Results	Notes
SARS-CoV-2 Positive ("+")	$3.0 \leq Ct \leq 25.0$	$3.0 \leq Ct \leq 25.0^*$	SARS-CoV-2 Viral RNA detected; IC possibly detected	Report results
Negative ("-")	No Ct detected	$3.0 \leq Ct \leq 25.0$	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.

19 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.

20 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 anterior 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.

21 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 laboratories⁷ 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.

⁷ 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.”

22 Performance Characteristics

22.1 Limit of Detection (LoD)

22.1.1 Purpose

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

22.1.2 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/USAWA1/2020 (ATCC® VR1986HK™). The heat-inactivated virions were spiked into the NP and NS swab sample matrix pools at 0, 469, 938, 1875, 3750, and 7500 copies/mL. A 24 µL input volume was transferred into a 60 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.

22.1.3 Results and Conclusions

The results are shown in Table 5 for the preliminary LoD studies. The preliminary LoD was expected to be 1,875 copies/mL.

Table 5. Preliminary LoD study.

Concentration (Copies/mL)	Observed Properties	
	Replicates Detected	Mean Ct Value
0	0/5	n/a
469	2/5	13.7
938	2/5	12.9
1,875	5/5	11.4
3,750	5/5	10.6
7,500	5/5	9.54

The wet swab LoD was confirmed by processing and testing a minimum of 20 replicates at 0, 938, 1,875, 3,750 and 7,500 copies/mL. A 24 µL input volume was transferred into a 60 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 results of this study, the LoD is 1,875 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	0/21	n/a
938	17/20	12.0
1,875	20/20	11.0
3,750	20/20	11.0
7,500	20/20	10.0

The dry swab LoD was confirmed by depositing 50 µL nasopharyngeal (NP) matrix containing either 0, 938, or 1,875 copies/mL virus and allowing the swab to air dry for 30 minutes. The dried NP swabs were then expressed in 1 mL of medium and a 24 µL input volume was transferred into a 60 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 1,875 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	0/20	n/a
938	16/20	13.0
1,875	20/20	11.0

22.2 RT-PCR Instrument Validation

22.2.1 Purpose

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

22.2.2 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, 938, 1,875, and 3,750 copies/mL. A 24 µL input volume was transferred into a 60 µL final reaction volume for LumiraDx SARS-CoV-2 RNA STAR Complete per the standard assay protocol on each RT-PCR Instrument. A total of 5 replicates were tested on each instrument system.

22.2.3 Results and Conclusions

All zero copy replicates showed internal control amplification and no target amplification. Six replicates of Positive Control Media and Negative Control Media were run on all instruments; all replicates amplified for the Positive Control Media and no replicates amplified for the Negative Control Media across all instruments (data not shown). 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 on the Stratagene Mx3005P as demonstrated in the pre-LoD and LoD replicate data. The following table highlights the performance on the Roche LC 480 II, ABI 7500 Fast Dx, ABI QS 5, ABI QS 7 Flex, ABI QS 7 Pro, Bio-Rad CFX96 and Agilent AriaMx.

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

Concentration (Copies/mL)		Roche LC 480 II	ABI 7500 Fast Dx	ABI QS 5	ABI QS 7 Flex	ABI QS 7 Pro	Bio-Rad CFX96	Agilent AriaMx
0	FAM (COVID)	0/5	0/5	0/5	0/5	0/5	0/5	0/5
	ROX (IC)	5/5	5/5	5/5	5/5	5/5	5/5	5/5
938	FAM (COVID)	4/5	5/5	4/5	4/5	4/5	4/5	2/5
	ROX (IC)	5/5	5/5	5/5	5/5	5/5	5/5	5/5
1,875	FAM (COVID)	5/5	5/5	5/5	5/5	5/5	5/5	5/5
	ROX (IC)	5/5	5/5	5/5	5/5	5/5	5/5	5/5
3,750	FAM (COVID)	5/5	5/5	5/5	5/5	5/5	5/5	5/5
	ROX (IC)	5/5	5/5	5/5	5/5	5/5	5/5	5/5

22.3 Inclusivity (Analytical Sensitivity)

22.3.1 Purpose

To demonstrate inclusivity for all SARS-CoV-2 sequences in the GISAID database⁸ through *in silico* analysis of the LumiraDx SARS-CoV-2 RNA STAR Complete.

22.3.2 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 January 8, 2021 (n = 322,744).

22.3.3 Results & Conclusions

An alignment was performed on January 8, 2021 with the oligonucleotide primer and probe sequences of the LumiraDx SARS-CoV-2 RNA STAR Complete with 322,744 publicly available SARS-CoV-2 sequences maintained in the GISAID database to demonstrate the predicted inclusivity of the assay. 99.7% of sequences showed perfect identity or a single mismatch of the assay to the SARS-CoV-2 target region. Of the remaining unmatched sequences, approximately 0.3%, 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.

Table 9. Number and Percent of strains in the databases that showed less than 100% homology.

Primer 1		
Mismatches	Number of Sequences	Percent of Sequences
0	321435	99.59%
1	555	0.17%
2	22	0.01%
3	18	0.01%
4	526	0.16%
5+	188	0.06%
Primer 2		
Mismatches	Number of Sequences	Percent of Sequences
0	314790	97.54%
1	6998	2.17%
2	80	0.02%
3	17	0.01%
4	785	0.24%
5+	74	0.02%
Primer 3		
Mismatches	Number of Sequences	Percent of Sequences
0	319455	98.98%
1	2390	0.74%
2	37	0.01%
3	8	0.00%
4	12	0.00%
5+	842	0.27%

Any sequence that has 3 or more mismatches could result in a reduction in detection due to the decreased melting temperature of the primers and probes, reducing sensitivity and total fluorescence. The increase in mismatches more than 3 could cause a detrimental loss of binding resulting in loss of detection. An *in silico* analysis of the SARS-CoV-2 primers and probe sets was performed against the variant UK, South Africa, Brazil, and California strains. The *in silico* analysis predicts that assay performance is unlikely to be impacted by the currently circulating escape variants.

⁸ GISAID (Global Initiative on Sharing All Influenza Data). Initiative. GISAID. <https://www.epicov.org/epi3/frontend#5c74af>. Accessed August 26, 2020.

22.4 Cross Reactivity (Analytical Specificity)

22.4.1 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.

22.4.2 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.

22.4.3 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 assess 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 10. *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

22.5 Endogenous Interference Substances Studies

22.5.1 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.

22.5.2 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.

22.5.3 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 11. 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%).

22.6 Clinical Evaluation

22.6.1 Purpose

To demonstrate positive and negative percent agreement 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.

22.6.2 Procedure

A selected panel of 34 SARS-CoV-2 negative and 60 SARS-CoV-2 positive nasopharyngeal (NP) and anterior nasal (N) swab samples were prepared and tested in a randomized blind study. The samples were prepared by expression in 1 or 3ml of compatible media. Each sample was processed with the LumiraDx SARS-CoV-2 RNA STAR Complete test using the Agilent AriaMx (software version 1.71).

22.6.3 Results and Conclusions

Positive and negative percent agreement between the LumiraDx SARS-CoV-2 RNA STAR Complete test when compared to a highly sensitive authorized FDA EUA RT-PCR test is shown below in Table 11. Confidence limits at the 95% level are also included. LumiraDx SARS-CoV-2 RNA STAR Complete overall performed with 95% Positive Agreement and 100% Negative Agreement for 60 positive and 34 negative samples when compared to the FDA EUA RT-PCR test.

Table 12. 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	57	0	57
	Negative	3	34	37
	Total	60	34	94
Percent Agreement		95.00% (Pos.)	100% (Neg.)	
95% Confidence Interval		86.3-100%	89.9-100%	

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

23 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.

Appendix A: Instrument Qualification

A.1 Purpose

This appendix is intended to provide a qualification procedure describing how to prepare a panel of mock specimens for use in verifying performance of the below RUO instruments with the LumiraDx SARS-CoV-2 RNA STAR Complete test by the end user. Qualification of these instruments with the LumiraDx SARS-CoV-2 RNA STAR Complete test must be achieved prior to usage for diagnostic testing.

- Roche LightCycler 480 II (software version SW 1.5.1),
- Bio-Rad CFX96 Touch System (software version 3.1),
- Agilent AriaMx (software version 1.71),
- Agilent Stratagene Mx3005P (software version 4.10) RT-PCR Instruments

A.2 Required Materials

Item	Quantity
AccuPlex™ SARS-CoV-2 Molecular Controls Kit – Full Genome (Material # 0505-0519)	1 Tube
LumiraDx SARS-CoV-2 RNA STAR Complete Kit	1 Kit
Molecular Biology Grade Water	N/A

A.3 Precautions

The AccuPlex™ SARS-CoV-2 Molecular Controls Kit Full Genome is non-infectious and replication deficient, enabling safe handling of the positive control. The Control Kit should be stored between 2 to 8 °C and should be handled in accordance with Good Laboratory Practices. LumiraDx SARS-CoV-2 RNA STAR Complete Kit components must be stored and handled in accordance with the conditions described in the Instruction for Use (IFU).

A.4 Procedure and Expected Results

If test results fail to meet expected results, contact LumiraDx Technical Support by telephone at 1-888-586-4721 or by email at technicalservices@lumiradx.com. The material should be stored between 2 to 8°C per manufacturers recommendations. A vial of the Positive reference material from the AccuPlex™ SARS-CoV-2 Molecular Controls Kit-Full Genome should be considered as single use only. A new Positive vial should be used with each instrument run. The qualification run will consist of running 20 replicates of positive reference material and 20 replicates of no target control. The steps below will provide instructions on how to prepare the 24 µL of Positive or Negative Sample to be used in each RNA STAR Complete Reaction.

1. Obtain a Positive vial from the AccuPlex™ SARS-CoV-2 Molecular Controls Kit -Full Genome.
2. Thaw the LumiraDx SARS-CoV-2 RNA STAR Complete Negative Control Media (Neg. Ctrl. Med.) in a cold block equilibrated to 2 to 8°C, vortex the Neg. Ctrl. Med. and the Positive vial for 5 seconds then pulse centrifuge for 5 seconds to collect reagents at the bottom of the tube.
3. Dilute 188ul of the Positive vial in 312ul of Molecular Biology Grade Water to get 500 µL at 1,875 copies/mL (1x LoD of the SARS-CoV-2 RNA STAR Complete Kit) in a pre-chilled microcentrifuge tube labelled "Positive".
4. Depending on the thermocycler, pipette into a pre-chilled microcentrifuge tube labelled "Negative" either 500µL Neg. Ctrl. Med. Or 500µL Molecular Biology Grade Water.
 - The Roche LC 480 II, Agilent Aria Mx, and Bio-Rad CFX96 Touch thermocyclers require Neg. Ctrl. Med. To be used as the negative sample.
 - The Agilent Stratagene Mx3005P thermocyclers require Molecular Biology Grade Water to be used as the negative sample.
5. Follow instructions listed in Section 9.2 and 9.3 of the IFU to prepare the qSTAR reagents, using 24 µL of "Positive" or "Negative" samples, in replicates of 20, as the specimen. No external controls are needed.
6. Run the positive and negative samples on the intended thermocycler and follow instrument specific protocols and analysis procedures detailed in the RNA STAR Complete Instrument Setup Sections above. Fill in Table 13 below and determine if results meet acceptable criteria.

Table 13. Results from Instrument Qualification Procedure (on Roche LightCycler 480 II, Bio-Rad CFX96 Touch System, Agilent Aria Mx, and Agilent Stratagene Mx3005P).

Controls Name	SARS-CoV-2 (FAM) Acceptable Ct Values	IC (ROX) Acceptable Ct Values	Acceptance Criteria	Instrument Results
Positive	$5.0 \leq Ct \leq 28.0$	$5.0 \leq Ct \leq 28.0^*$	19/20 (95%)	
Negative	None detected	$5.0 \leq Ct \leq 28.0$	0/20 (0%)	

*The Internal Control is not required to amplify for the Positive Sample to be deemed positive.

Appendix B: Additional Label

For Roche LightCycler 480 II (software version SW 1.5.1), Bio-Rad CFX96 Touch System (software version 3.1), Agilent AriaMx (software version 1.71), or the Agilent Stratagene Mx3005P (software version 4.10) RT-PCR Instruments.

Please print and place this label on the front panel of the above instruments. If the instruments include labeling indicating “For Research Use Only”, please cover with the below “Emergency Use Only” labeling. The instruments should retain this labeling throughout the EUA use of the LumiraDx SARS-CoV-2 RNA STAR Complete test.

* Refer to Appendix A: Instrument Qualification for instructions

Emergency Use Only

This instrument is authorized for use with the LumiraDx
SARS-CoV-2 RNA STAR Complete test.



LumiraDx SARS-CoV-2 RNA STAR Complete

Product Insert SPEC-30078 Rev1 ART-00004 Rev10 Rev Date 2021/03 **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/ma-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.)	SARS-CoV-2 Positive Control (ZeptoMetrix 50,000cp/mL)	500µL	≤ 8°C
LumiraDx SARS-CoV-2 RNA STAR Complete Negative Control Media (Neg. Ctrl. Med.)	Negative Control (Molecular Biology Grade Water)	1.5mL	-25°C to -15°C
LumiraDx SARS-CoV-2 RNA STAR Complete Salt Mix	Salt Mix	1mL	-25°C to -15°C
LumiraDx SARS-CoV-2 RNA STAR Complete Extraction Buffer	Nucleic Acid Extraction Buffer	500µL	-25°C to -15°C
LumiraDx SARS-CoV-2 RNA STAR Complete Internal Control & Primer Mix (IC/P Mix)	Internal Control & Primer Mix	120µL	-25°C to -15°C
LumiraDx SARS-CoV-2 RNA STAR Complete Master Mix	Master Mix	2x 1mL	-25°C to -15°C

Storage instructions

Upon receipt, store Pos. Ctrl. Med. at ≤ 8 °C. Store Neg. Ctrl. Med., Salt Mix, Extraction Buffer, IC/P Mix, and 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; this test 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* diagnostic tests 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.

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

Specimen preparation

1. Thaw Pos. Ctrl. Med. and Neg. Ctrl. Med. on a cold block, vortex for 5 seconds then 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 200 µL Pos. Ctrl. Med. with 600 µL Neg. Ctrl. Med. in a pre-chilled microcentrifuge tube. Assemble the 1x NCM (Negative Control Media) by transferring 800 µL Neg. Ctrl. Med into a pre-chilled microcentrifuge tube.
3. Upper respiratory specimens (24 µL) will be added directly to the sample plate prepared in the qSTAR reagent preparation. If swab is provided dry, transfer the 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 a minimum of 30 seconds. Swirl thoroughly by rotating the swab against the side of the deepwell 5 times. Express the swab on the side of the well, outside of the liquid, prior to removing (beware of cross-contamination from splashing). Discard the swab in biohazard waste.

qSTAR reagent preparation

1. Thaw Salt Mix, Extraction Buffer, IC/P Mix and Master Mix on a cold block. Once thawed, invert each tube to mix (do not vortex) then centrifuge for 5 seconds to collect reagents at the bottom of the tube.
2. Transfer 24 µL of specimen and 24 µL of external controls into an appropriate, pre-chilled 96-well plate. Add 4.8 µL of Extraction Buffer, per well, and mix by slowly pipetting up and down 10 times while minimizing bubbles. If needed, seal and centrifuge the 96-well plate to collect the specimen at the bottom of the well.
3. Assuming 96 reactions are needed, prepare the Reaction Mix in a pre-chilled 5mL tube by combining 1000 µL Salt Mix, 120 µL IC/P Mix and 2 mL Master Mix. Between each reagent, slowly mix by pipetting up and down 4 to 6 times without introducing bubbles.
4. Transfer 31.2 µL of Reaction Mix, per well, into an appropriate, pre-chilled, 96-well plate. Mix by slowly pipetting up and down 10 times without introducing bubbles. Seal the 96-well plate using an appropriate optically clear adhesive and centrifuge the plate at 2000 rpm for 20 seconds to collect contents at bottom of the plate.
5. Place the 96-well plate in a validated thermocycler and follow instrument specific protocols and analysis procedures detailed in the IFU.

LumiraDx™ SARS-CoV-2 RNA STAR Complete

Quick Reference Instructions for Single Swab Format

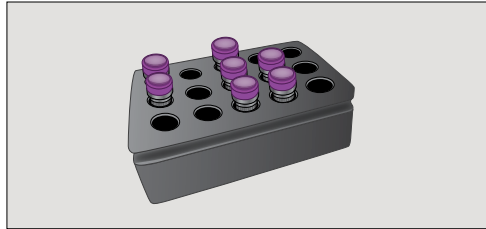
For Emergency Use Authorization (EUA) only. For in vitro Diagnostic Use. For Rx Use Only.

SD-QMS-SPEC-30176 R1 · SD-COM-ART-00047 R7 · Rev Date 2021/03

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 purification or extraction.

Study the Product Insert and Instructions for Use (IFU) thoroughly before using this Procedure Card. This Procedure Card is not a complete set of instructions. Refer to the Materials Required (But Not Provided) document for specific vendors and catalog numbers for materials used below that are not provided in the LumiraDx SARS-CoV-2 RNA STAR Complete kit.

LumiraDx SARS-CoV-2 RNA STAR Complete kit components

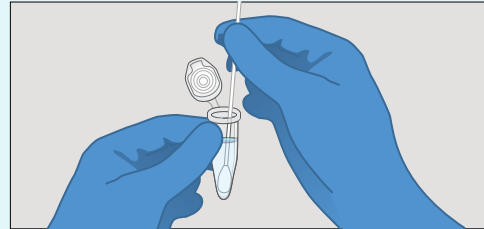


1. Thaw reagents

Thaw the LumiraDx SARS-CoV-2 RNA STAR Complete kit components in a cold block equilibrated between 2 to 8 °C; i.e. Positive Control Media (Pos. Ctrl. Med.), Negative Control Media (Neg. Ctrl. Med.), Salt Mix, Extraction Buffer, Internal Control & Primer (IC/P) Mix, and Master Mix. Once thawed, invert to mix then centrifuge for 5 seconds.

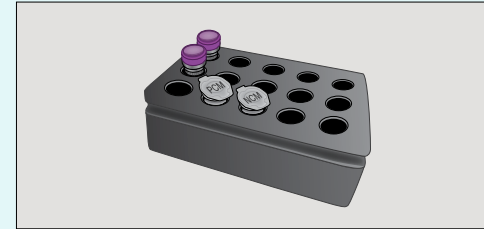
Preparing the specimen – single swab format

Collect specimens (can be a dry swab specimen or a wet swab specimen) before following steps 2 to 4.



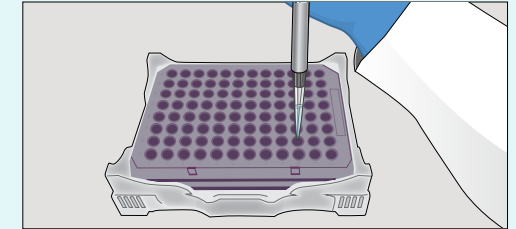
2. Prepare single tube

If swab is provided dry, transfer one (1) mL of a compatible transport media into a suitable tube (e.g. polypropylene microcentrifuge tube). Place and soak the swab for at least 30 seconds then swirl thoroughly by rotating the swab against the side of the tube up to 5 times. Express the swab on the side of tube, outside of the liquid, prior to removing (beware of cross-contamination from splashing). Discard the swab in biohazard waste. If swab specimen is provided wet, up to 3 mL of compatible transport media (VTM, 0.85% Saline, or PBS) is acceptable, but this higher volume may impact sensitivity.



3. Prepare external controls

Assemble fresh 1x PCM (Positive Control Media) by diluting 20.0 µL Pos. Ctrl. Med. with 60.0 µL Neg. Ctrl. Med. in a pre-chilled microcentrifuge tube. Assemble the 1x NCM (Negative Control Media) by transferring 80.0 µL Neg. Ctrl. Med. into a pre-chilled microcentrifuge tube.

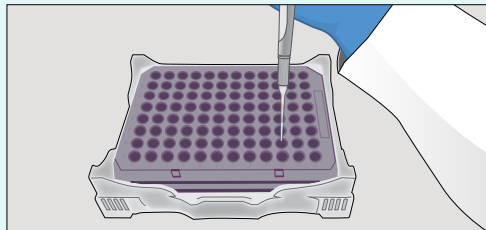


4. Prepare sample plate

Transfer 24.0 µL of swab specimen prepared in Step 2 and transfer 24.0 µL of external controls prepared in Step 3 using a single-channel pipette into an appropriate, pre-chilled, 96-well plate.

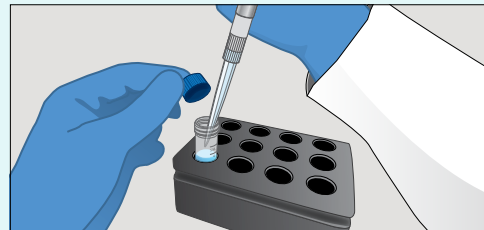
qSTAR reagent preparation

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



5. Prepare sample plate

Add 4.8 µL of Extraction Buffer, per well, to the 96-well plate and mix by slowly pipetting up and down 10 times without introducing bubbles. The addition and mixing of Extraction Buffer can be simplified by using a multi-channel pipette and chilled reagent reservoir. If needed, seal and centrifuge the 96-well plate to collect the sample at the bottom of the well.



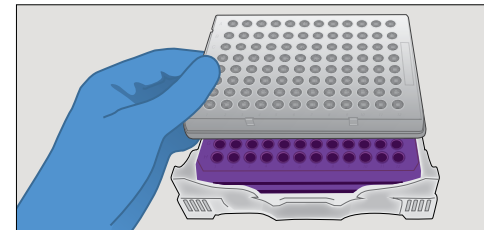
6. Prepare reaction mix

Determine the number of reactions (N) to be prepared per assay and prepare Reaction Mix in a suitable pre-chilled tube by following the order in the table below. Between each reagent, slowly mix by pipetting up and down 4 to 6 times without introducing bubbles and pulse centrifuge.

Reaction Mix	1 Rxn	N Rxns
Salt Mix	10.0 µL	N x 10.0 µL
IC/P Mix	1.2 µL	N x 1.2 µL
Master Mix	20.0 µL	N x 20.0 µL
Total Volume	31.2 µL	N x 31.2 µL

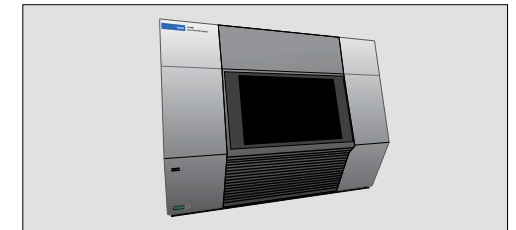
Final setup

Setup for validated thermocyclers (i.e. Roche LC 480 II, ABI 7500 Fast Dx, ABI QS 5, ABI QS 7 Flex, ABI QS 7 Pro, Bio-Rad CFX96, Agilent AriaMx, or the Agilent Stratagene Mx3005P) is described in the IFU.



7. Add reaction mix to plate

Transfer 31.2 µL of Reaction Mix to each well with sample and external controls. Mix by slowly pipetting up and down 10 times without introducing bubbles. Seal the 96-well plate using an appropriate optically clear adhesive and centrifuge the plate at 2000 rpm for 10 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.

LumiraDx™ SARS-CoV-2 RNA STAR Complete

Quick Reference Instructions for Deepwell/Dry Swab Format

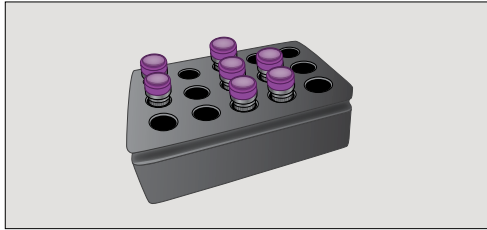
For Emergency Use Authorization (EUA) only. For in vitro Diagnostic Use. For Rx Use Only.

SD-QMS-SPEC-30176 R1 · SD-COM-ART-00047 R7 · Rev Date 2021/03

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 purification or extraction.

Study the Product Insert and Instructions for Use (IFU) thoroughly before using this Procedure Card. This Procedure Card is not a complete set of instructions. Refer to the Materials Required (But Not Provided) document for specific vendors and catalog numbers for materials used below that are not provided in the LumiraDx SARS-CoV-2 RNA STAR Complete kit.

LumiraDx SARS-CoV-2 RNA STAR Complete kit components

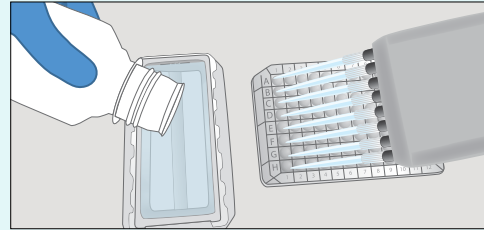


1. Thaw reagents

Thaw the LumiraDx SARS-CoV-2 RNA STAR Complete kit components in a cold block equilibrated between 2 to 8 °C; i.e. Positive Control Media (Pos. Ctrl. Med.), Negative Control Media (Neg. Ctrl. Med.), Salt Mix, Extraction Buffer, Internal Control & Primer (IC/P) Mix, and Master Mix. Once thawed, invert to mix then centrifuge for 5 seconds.

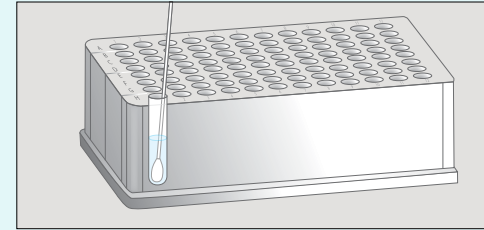
Preparing the specimen – deepwell/dry swab format

Collect a dry swab specimen before following the steps below.



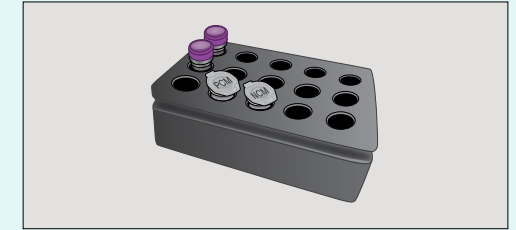
2. Prepare deepwell

Pour 100 mL of a compatible transport media (VTM, 0.85% Saline, or PBS) into a suitable reagent reservoir. Transfer one (1) mL to each deepwell using a multi-channel pipette. Leave two designated wells, A1 and A12, empty for the external controls assembled in Step 4.



3. Soak swab

Add a single dry swab specimen to each deepwell in use. Soak the swab for a minimum of 30 seconds then swirl thoroughly by rotating the swab against the side of the deepwell 5 times. Express the swab on the side of the well, outside of the liquid, prior to removing (beware of cross-contamination from splashing). Discard the swab in biohazard waste.

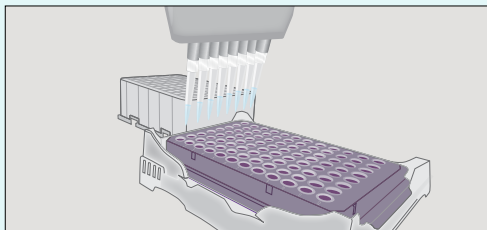


4. Prepare external controls

Assemble the 1x PCM (Positive Control Media) by diluting 200 µL Pos. Ctrl. Med. with 600 µL Neg. Ctrl. Med. in a pre-chilled microcentrifuge tube. Assemble the 1x NCM (Negative Control Media) by transferring 800 µL 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 and A12 should be loaded with the NCM and PCM, respectively.

qSTAR reagent preparation

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



5. Prepare sample plate

Mix the specimen prepared in Step 3 and the external controls prepared in Step 4 by slowly pipetting up and down 4 to 6 times without introducing air bubbles then transfer 24.0 µL using a multi-channel pipette into an appropriate, pre-chilled, 96-well plate. Add 4.8 µL of Extraction Buffer, per well, and mix by slowly pipetting up and down 10 times while minimizing bubbles. If needed, seal and centrifuge the 96-well plate to collect the specimen at the bottom of the well.



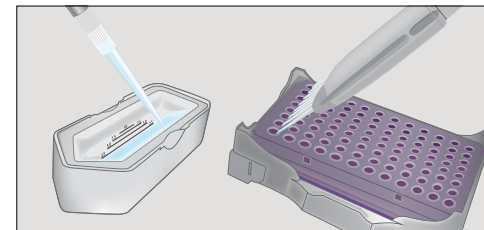
6. Prepare reaction mix

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 to 6 times without introducing bubbles.

Reaction Mix	100 Rxns
Salt Mix	1000 µL
IC/P Mix	120 µL
Master Mix	2x 1000 µL
Total Volume	3120 µL

Final setup

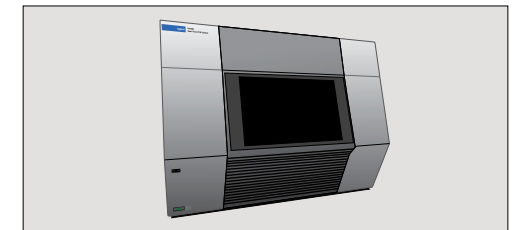
Setup for validated thermocyclers (i.e. Roche LC 480 II, ABI 7500 Fast Dx, ABI QS 5, ABI QS 7 Flex, ABI QS 7 Pro, Bio-Rad CFX96, Agilent AriaMx, or the Agilent Stratagene Mx3005P) is described in the IFU.



7. Add reaction mix to plate

Transfer Reaction Mix to a pre-chilled reagent reservoir (for minimal dead volume) using a single channel pipette. Then, using a multi-channel pipette, transfer 31.2 µL of Reaction Mix to each well with specimen and external controls. Mix by slowly pipetting up and down 10 times without introducing bubbles. Seal the 96-well plate using an appropriate optically clear adhesive and centrifuge the plate at 2000 rpm for 20 seconds to collect contents at bottom of plate.

Precautions: This Test has not been FDA cleared or approved; This Test has been authorized by FDA under an EUA for use by authorized laboratories; This Test has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens; and 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.



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.