lumiraDx™
FastLab Solutions

SARS-CoV-2
RNA STAR Complete

Instructions for Use

REF L018180501096

IVD

SD-COM-ART-00103 Rev.2 2021/06

CE

Contents

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.

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.

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-19[1]. 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 days[2].

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.

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), Agilent Stratagene Mx3005P (software version 4.10) or the Analytik Jena qTOWER[3] (software version 4.1) 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	U.S. 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	U.S. Catalog #
Sodium Hypochlorite Solution (Bleach)	ThermoFisher Scientific	SS290-1
70% Isopropanol (or 70% Ethanol)	VWR	89499-420
DNAZapTM (or equivalent)	ThermoFisher Scientific	AM9890
RNaseZapTM (or equivalent)	ThermoFisher Scientific	AM9782
Molecular Biology Grade Water	Corning	46-000-CM
Compatible Transport Medias*	General lab supplier	n/a
Transport Medium	Corning	25-500-CM
Saline, 0.85%	Hardy Diagnostics	U157
PBS (pH 7.4), 1X	ThermoFisher Scientific	10010023

Equipment	Source	U.S Catalog #
-80ºC Laboratory Freezer	General lab supplier	n/a
-25ºC to -15ºC Laboratory Freezer	General lab supplier	n/a
2ºC to 8ºC 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

*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 swab matrix near LoD for each of the following and were tested: Corning Transport Medium, Saline, 0.85%, and 1X PBS (pH 7.4), with corresponding NTC in a NP matrix. Study results demonstrate that the evaluated specimen transport medias are comparable when performed with the LumiraDx SARS-CoV-2 RNA STAR Complete test.

Option for PCR Instruments2 & Consumables	Source	U.S 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	ThermoFisher Scientific	4346906
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
Bio-Rad CFX96 Touch System (software version 3.1)	BioRad	785BR09328 / CT003265
Eppendorf twin.tec Real-Time PCR Plate 96-Well Skirted	Eppendorf	951022003
Analytik Jena qTOWER3G (software version 4.1)	Analytik Jena	844-00554-4
Eppendorf twin.tec Real-Time PCR Plate 96-Well Semi-Skirted	Eppendorf	951022043
Universal Sealing Films*	General lab supplier	
ThermalSeal A Sealing Film	Research Product International	202545
Heat-Resistant Polypropylene Film for Raised-Rim Plates	VWR	89087-690
Utility Sealing Film		
VWR Adhesive Film for Microplates	VWR	60941-070

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

6. Warning and Precautions

- For *in vitro* Diagnostic Use (IVD).
- The Salt Mix and the Master Mix contains bovine serum albumin.
- 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.
- 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.
- For more information on the collection, storage and transport of samples, refer to the World Health Organization Laboratory biosafety guidance related to coronavirus disease (COVID-19): interim guidance, 28 January 2021. https://www.who.int/publications/i/item/WHO-WPE-GIH-2021.1
- 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 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 kit between - 15 °C to - 25 °C.
- The LumiraDx SARS-CoV-2 RNA STAR Complete Positive Control Media (Pos. Ctrl. Med.) may optionally be stored at < 8 °C after initial use.

- 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 Extraction Buffer, Internal Control/Primer Mix, and Master Mix must be always thawed and kept on a cold block 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.

8.1 Collecting Specimens

- 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 Corning transport media, 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.
- 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 (Corning Transport Media, 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.
- For more information on the collection, storage and transport of samples, refer to the World Health Organization Laboratory biosafety guidance related to coronavirus disease (COVID-19): interim guidance, 28 January 2021. https://www.who.int/publications/i/item/WHO-WPE-GIH-2021.1

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. Corning transport media, 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 -20°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 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 -20°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 -20°C or below.
- If specimens cannot be tested within 72 hours of collection, both dry-swab (expressed in Corning transport media, 0.85% saline solution, or PBS) and wet-swab specimens should be frozen at $\leq$ -20 °C until tested.

9. LumiraDx SARS-CoV-2 RNA STAR Complete Preparation

9.1 Quality Controls

- 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.
- 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.

9.2 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 (Corning Transport Media, 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.

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 µL Pos. Ctrl. Med. with 60.0 µL Neg. Ctrl. Med. in a pre-chilled microcentrifuge tube. To assemble the 1x NCM (Negative Control Media), always freshly pipette 80.0 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 Section 9.4. 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.

9.3 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.

9.4 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, Agilent Stratagene Mx3005P, or the Analytik Jena qTOWER^3) 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.
2. Transfer 24.0 µL of swab specimens prepared in Step 3 of Section 9.2 and transfer 24.0 µL of external controls prepared in Step 2 of Section 9.2 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 Reaction	100 Reactions	N Reactions
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. Invert the IC/P Mix and Master Mix to mix then centrifuge for 5 seconds to collect reagents at the bottom of the tube (do not vortex samples).
5. Vigorously vortex the Salt Mix for 20 seconds and centrifuge for 5 seconds to collect reagent at the bottom of tube.
6. 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.
7. 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.
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 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, Agilent Stratagene Mx3005P, or the Analytik Jena qTOWER^3) 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 (Corning Transport Media, 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. Before pipetting the Salt Mix, vigorously vortex the Salt Mix for 20 seconds and centrifuge for 5 seconds to collect reagent at the bottom of tube. Between each reagent, slowly mix by pipetting up and down 4 to 6 times without introducing bubbles and pulse centrifuge.

Reaction Mix	**1 Reaction**	**N Reactions**
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.

9.6 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, Agilent Stratagene Mx3005P, or the Analytik Jena qTOWER^3) 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 (Corning Transport Media, 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. Before pipetting the Salt Mix, vigorously vortex the Salt Mix for 20 seconds and centrifuge for 5 seconds to collect reagent at the bottom of tube. Between each reagent, slowly mix by pipetting up and down 4 to 6 times without introducing bubbles.

Reaction Mix	**100 Reactions**
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.

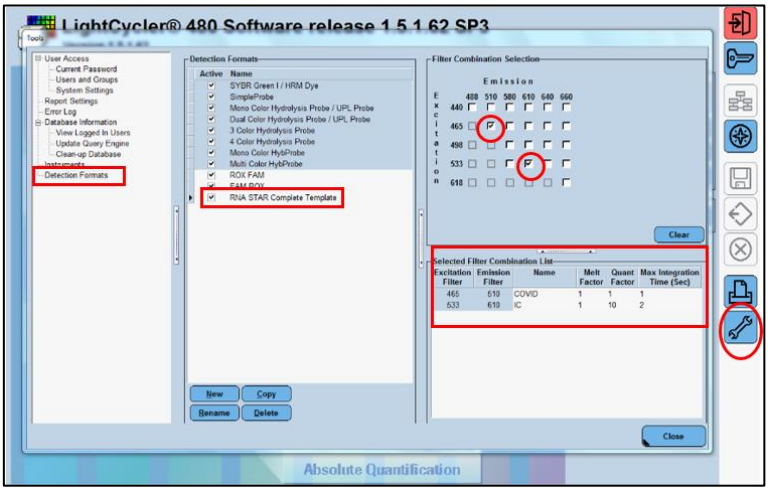
8. Place the 96-well plate in a validated thermocycler and follow instrument specific protocols and analysis procedures detailed below.

10. RNA STAR Complete Setup for Roche™ LightCycler 480 II

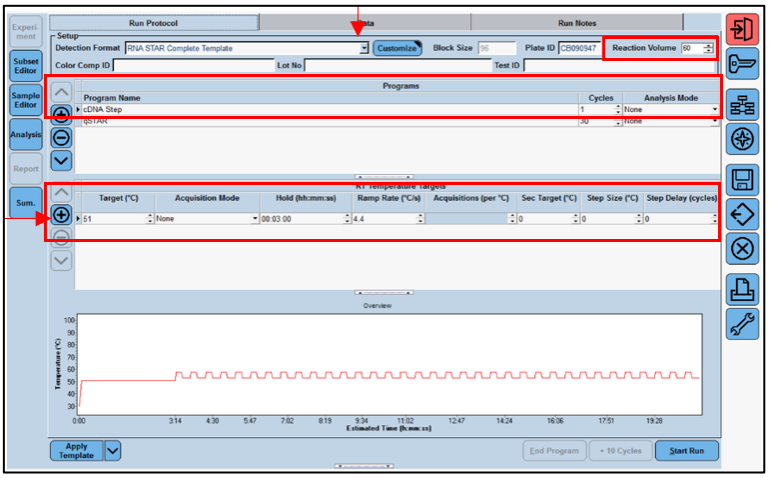
Refer to "User Manual Part Number 05152062001 0208" for additional information. The instrument programming instructions are intended for setup of an entire 96-well plate. If the entire plate is not used, please deselect wells as appropriate during analysis.

10.1 Programming the Run Template and Sample Template

1. Launch the LightCycler (LC) 480 desktop software (version SW 1.5.1.62). If prompted, 'Log On' to the 'Traceable Database'.
2. Click the tool icon 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 'Quant Factor' for '465-510' is set to '1' and for '533-610' is set to '10', and the 'Max Integration Time (Sec)' 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 1.5.1.62). After closing and reloading the software, in the 'Experiment Creation' section, select either 'White' or 'Clear Plates', and click 'New Experiment'.
4. From the 'Detection Format' drop-down menu select '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'.

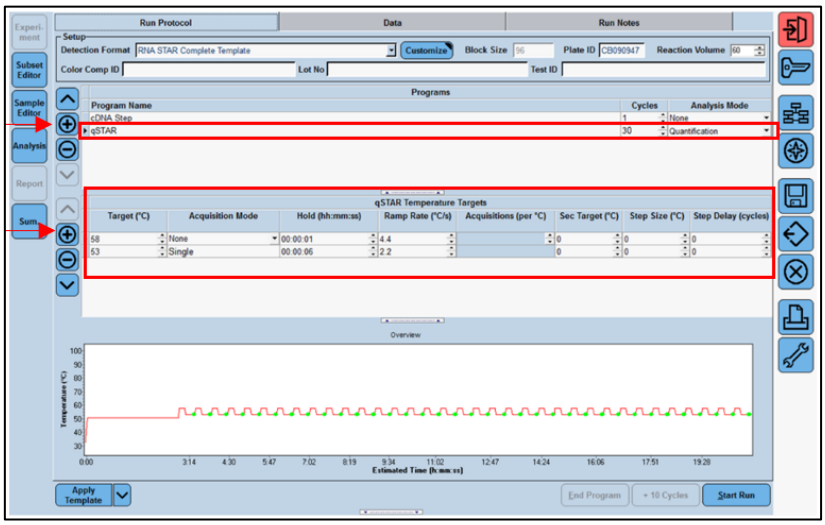


5. Click the "+" button on the upper left of the software to add another program. In the 'Program Name' section, type "qSTAR", set 'Cycles' to '30', and set 'Analysis Mode' to 'Quantification'. Change qSTAR 'Temperature Targets' as follows: set 'Target (°C)' to '58', set 'Acquisition Mode' to 'None', set 'Hold (hh:mm:ss)' to '00:00:01', set 'Ramp Rate (°C/s)' to '4.4', and set 'Sec

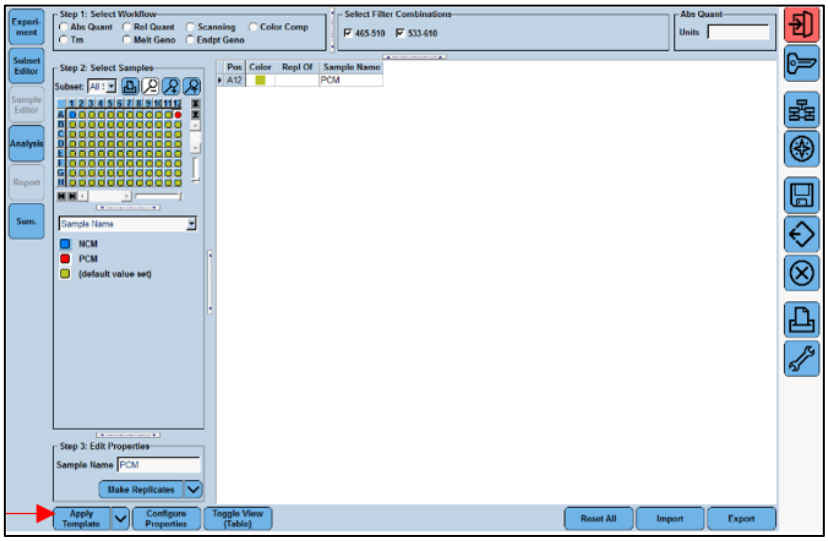
Target (°C)', 'Step Size (°C)', and 'Step Delay (cycles)' to '0'. Select the "+" button on the lower left of the software to add another 'Temperature Target'. Set 'Target (°C)' to '53', set 'Acquisition Mode' to 'Single', set 'Hold (hh:mm:ss)' to '00:00:06', set 'Ramp Rate (°C/s)' to '2.2', and set 'Sec Target (°C)', 'Step Size (°C)', and 'Step Delay (cycles)' to '0'.

NOTE: The instrument will display a run time that might not be accurate since the instrument does not accurately account for the 1 sec cycling 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 'Step 3: 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

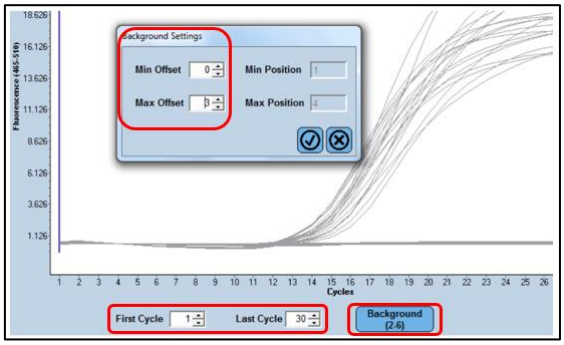
It is recommended to setup the instrument up to step 3 before preparing the Reagent Mix as defined in the qSTAR Reagent Preparation section of this document. The 96-well plate should be recently centrifuged to guarantee all reagents are at the bottom of the plate and kept on a cold block. Use 96-well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

NOTE: The analysis template is only established after the initial run has been completed.

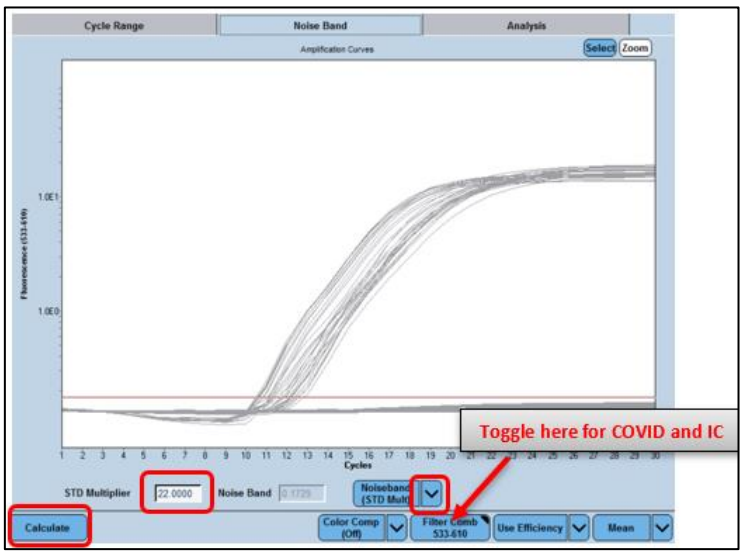
1. Launch the LightCycler (LC) 480 desktop software (version SW 1.5.1.62).
2. Click 'New Experiment from Template' and in the 'Run Templates' drop-down menu, select 'RNA STAR Complete Template' then click the 'Check' button to exit the 'Create Experiments from Template' window.
3. Proceed to the 'Sample Editor' tab on the left of the software. Select 'Apply Template' (do not select the drop-down menu next to the 'Apply Template' button) on the bottom left of the screen. Double-click the Templates folder then double-click the 'Sample Templates' folder and choose the 'RNA STAR Complete Sample Template'. Click the 'Check' button to exit the 'Apply Template' window. The NCM and PCM should be loaded into positions A1 and A12 of the plate. 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 "←→" button on the front of the instrument. The instrument drawer will automatically come out. Insert the 96-well plate onto the instrument drawer. Align the notch of the 96-well plate with the notch on the instrument drawer. Push the Load Plate "←→" button to close the plate holder.
5. In the LightCycler (LC) 480 desktop software, proceed to the 'Experiment' tab on the upper left of the software. Click 'Start Run' on the bottom right of the software. A 'Plate Sensor if off' warning may appear. Click the 'Check' button to exit this warning. In the 'Save LightCycler 480 Experiment' window that opens, double-click the 'Experiments' folder and save as "RNA STAR Complete [YYMMDD_Plate#]" then click the 'Check' button to exit this window.

10.3 Creating Analysis Template

1. 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.
2. 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.
3. 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'.



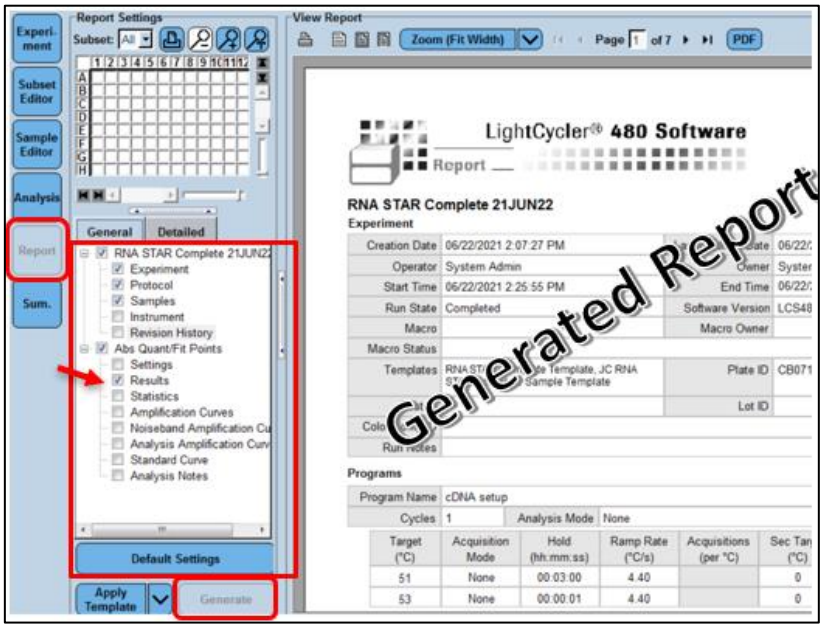
4. Proceed to the 'Noise Band' tab at the upper middle of the software. Locate the 'Noiseband (Auto)' button at the bottom of the software and select 'Noiseband (STD Mult)' from the drop-down menu. Click 'Filter Comb (456-510)' and select 'COVID'. Click the 'Check' button to exit the 'Filter Combination' window. Set 'STD Multiplier' to '22.0000'. Click 'Filter Comb (456-510)' and select 'IC'. The button should now read 'Filter Comb (533-610)'. Click the 'Check' button to exit the 'Filter Combination' window. Set 'STD Multiplier' to '22.0000'.



5. 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". Click the 'Check' button to exit the 'Save Template' window. Click 'Calculate' at the bottom of the software.

6. To create a report, select the 'Save' icon 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.
7. Proceed to the 'Report' tab on the left of the software. 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.



8. Remove the plate from the LightCycler 480 II instrument using the Load Plate "←→" button on the front of the instrument.
9. Remove the plate and discard the plate in a sealable waste bag or container.

10.4 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 1.5.1.62).
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.
3. Proceed to the 'Sample Editor' tab on the left of the software. Select 'Apply Template' (do not select the drop-down menu next to the 'Apply Template' button) on the bottom left of the screen. Double-click the Templates folder then double-click the 'Sample Templates' folder and choose the 'RNA STAR Complete Sample Template'. Click the 'Check' button to exit the 'Apply Template' window. The NCM and PCM should be loaded into positions A1 and A12 of the plate. If not already selected, select all wells. Individual 'Sample Names' can be entered in the 'Step 3: Edit Properties' section if desired.
4. On the LightCycler 480 II instrument, push the Load Plate "←→" button on the front of the instrument. The instrument drawer will automatically come out. Insert the 96-well plate onto the instrument drawer. Align the notch of the 96-well plate with the notch on the instrument drawer. Push the Load Plate "←→" button to close the plate holder.
5. In the LightCycler (LC) 480 desktop software proceed to the 'Experiment' tab on the upper left of the software. Click 'Start Run' on the bottom right of the software. A 'Plate Sensor if off' warning may appear. Click the 'Check' button to exit this warning. In the 'Save LightCycler 480 Experiment' window that opens, double-click the 'Experiments' folder and save under the 'Name' "RNA STAR Complete [YYMMDD_Plate#]" then click the 'Check' button to exit this window.
6. After the run has finished, proceed to the 'Analysis' tab on the left of the software. Choose 'Abs Quant/Fit Points' in the 'Create new analysis' section. Click the 'Check' button to exit the 'Create New Analysis' pop-up window.
7. 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.
9. Proceed to the 'Report' tab on the left of the software. 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 "←→" button on the front of the instrument.

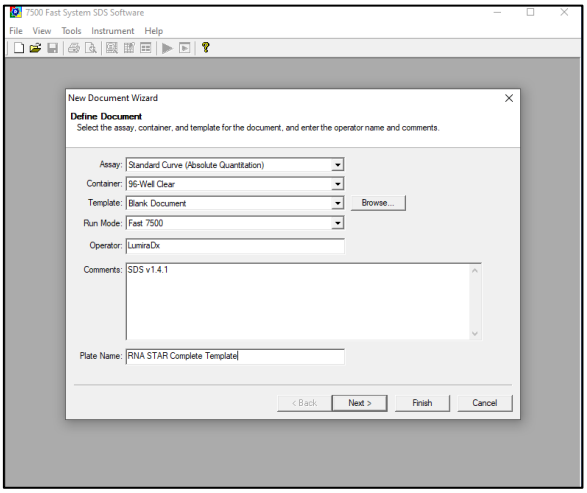
11. Remove the plate and discard the plate in a sealable waste bag or container.

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.

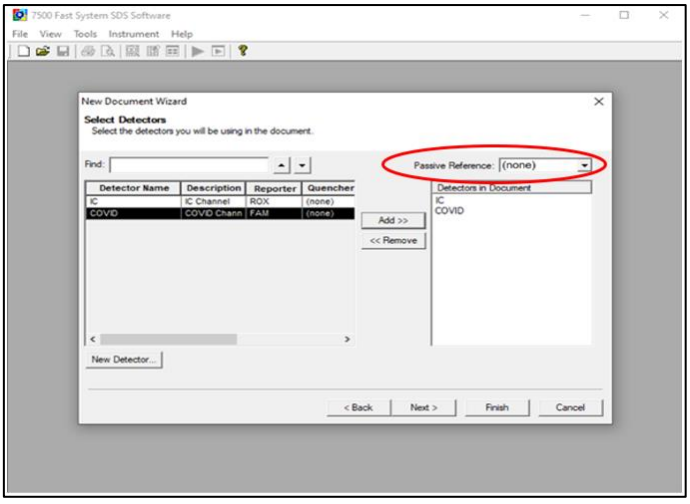
11.1 Programming the Run Template

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 Quantitation)', '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 define these settings: define 'Name' as "COVID", define 'Description' as "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'.

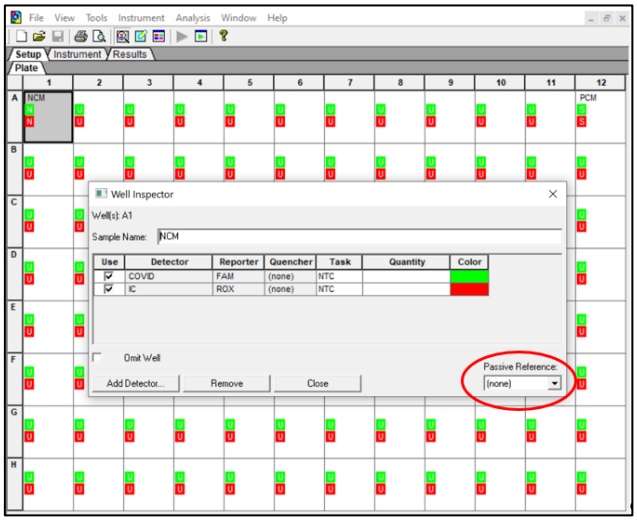
NOTE: It is important to make sure the 'Passive Reference' drop-down menu has '(none)' selected. If 'ROX' is not removed as a 'Passive Reference' dye the IC channel will be impacted and the run must be repeated.



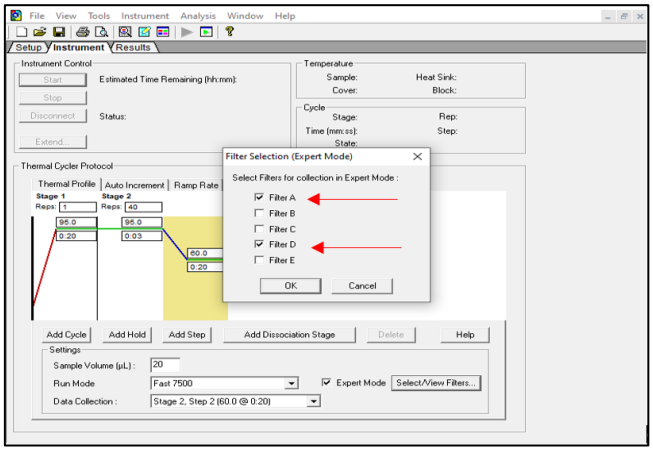
4. In the 'Set Up Sample Plate' window click the gray box between "A" and "1" to select all wells then select boxes under the 'Use' section to add the "COVID" and "IC" Detectors to all wells. Click 'Finish'. The wizard will close, and a screen will display

the ‘Setup’ tab and ‘Plate’ tab. This will show the sample plate that was set up during the quick start. For the initial set up, select location ‘A1’, right click and select ‘Well Inspector’. In the ‘Well Inspector’ window, define the ‘Sample Name’ as “NCM” (Negative Control Media). Select the ‘COVID’ Detector line, under ‘Task’ select from the drop-down menu ‘NTC’. Select the ‘IC’ Detector line, under ‘Task’ select from the drop-down menu ‘NTC’. Verify the ‘Passive Reference’ is set to ‘(none)’. Click ‘Close’.

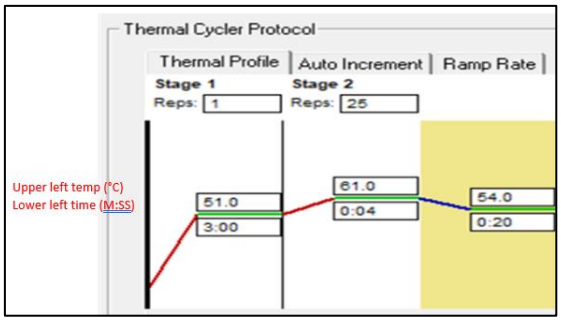
5. Select location ‘A12’, right click and select ‘Well Inspector’. In the ‘Well Inspector’ window, define the ‘Sample Name’ as “PCM” (Positive Control Media). Select the ‘COVID’ Detector line, under ‘Task’ select from the drop-down menu ‘Standard’. Select the ‘IC’ Detector line, under ‘Task’ select from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



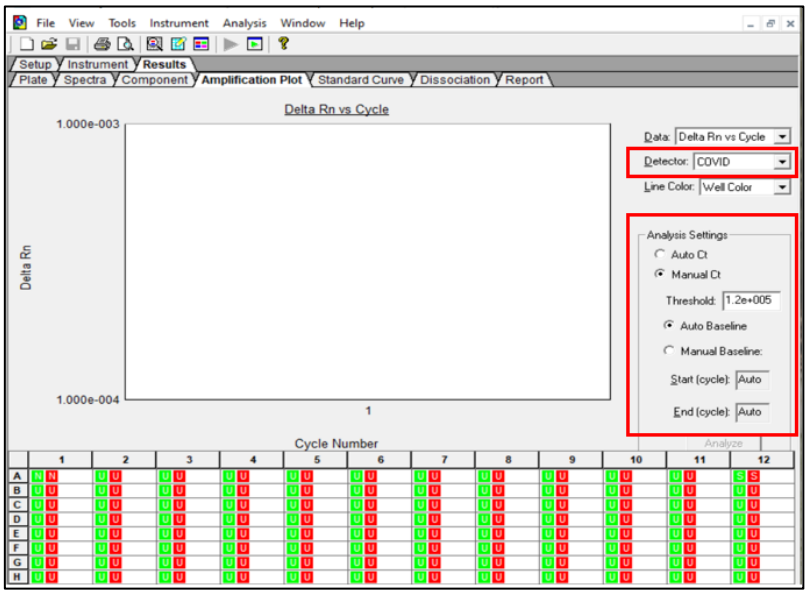
6. Select the ‘Instrument’ tab (do not select ‘Instrument’ from the menu), check the box next to ‘Expert Mode’ then click ‘Select/View Filters…’ and check only the boxes for ‘Filter A’ and ‘Filter D’. Click ‘OK’.



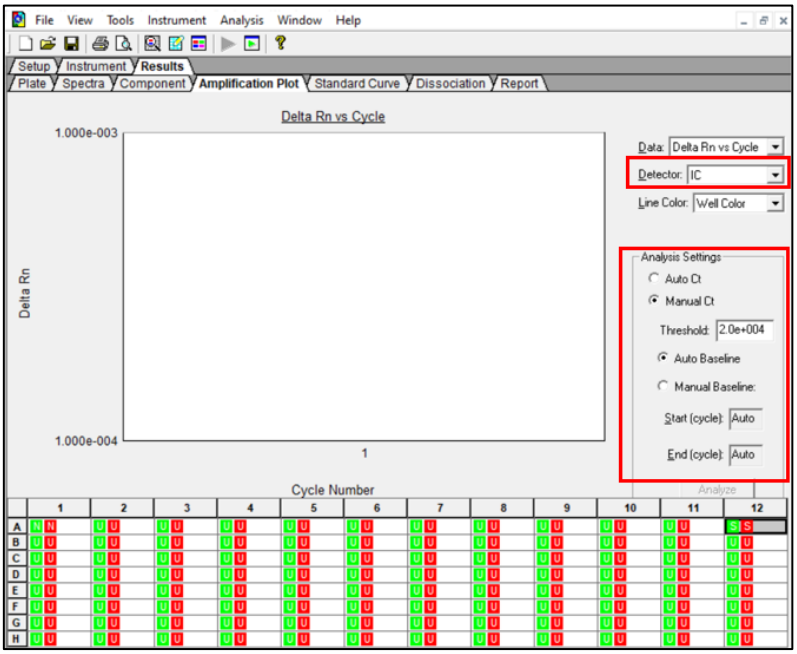
7. Modify the default thermal profile for ‘Stage 1’ as follows: set ‘Reps’ to ‘1’, set temp (upper left) to ’51.0’, and set time (lower left) to ‘3:00’. Modify the default thermal profile for ‘Stage 2’ as follows: set ‘Reps’ to ’25’, set time to (lower middle) ’0:04’, set temp (upper middle) to ’61.0’, set time (lower right) to ‘0:20, 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 ‘Fast 7500’, and confirm ‘Data Collection’ is set to ‘Stage 2, Step 2(54.0 @ 0:20)’.



8. Proceed to the ‘Results’ tab to set thresholds for each analyte. Select the ‘Amplification Plot’ tab under the ‘Results’. From the ‘Detector’ drop-down menu in the top right corner, select ‘COVID’. In the ‘Analysis Settings’ window, select the ‘Manual Ct’ and define ‘Threshold’ as “1.2e+005”. Confirm that the ‘Auto Baseline’ is selected.



9. From the ‘Detector’ drop-down menu again, select ‘IC’. In the ‘Analysis Settings’ section, select ‘Manual Ct’ and define ‘Threshold’ as “2.0e+004”. Confirm that the ‘Auto Baseline’ is selected.



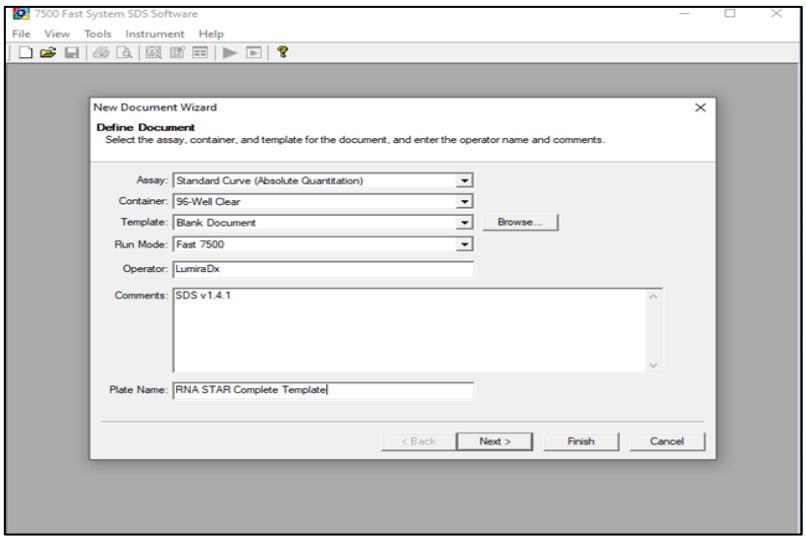
10. Select ‘File’ from the menu then click ‘Save As’. Save the template in file path “\Applied Biosystems\7500 Fast System\Template\”. Name the file as “RNA STAR Complete Template”. Change ‘File Type’ to ‘SDS Templates (*.sdt)’. Click ‘Save’.
11. Exit the software. A prompt may appear to ‘Save Changes 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 and Analysis Using a Template

If the instrument has been idle, performing a run using a blank plate may be needed to ensure the plate lid is heated when the run starts. 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).

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



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.sdt', '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 (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'.
5. Proceed to the 'Instrument' tab. 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 until 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'.
8. 'Save' the file. A window will open asking for the "Reason for the change". Type "Data Analysis" and click 'OK'.
9. To generate a report, click the 'Report' tab, then select the desired wells from the plate layout. From the top menu bar, click 'File', then select 'Export' then 'Results…' from the drop-down menu. Choose a file location to save the report, then click 'Save'. In the pop-up window that appears, check the box for 'Export only selected wells', then click 'OK'.
10. Remove the plate and discard the plate in a sealable waste bag or container.

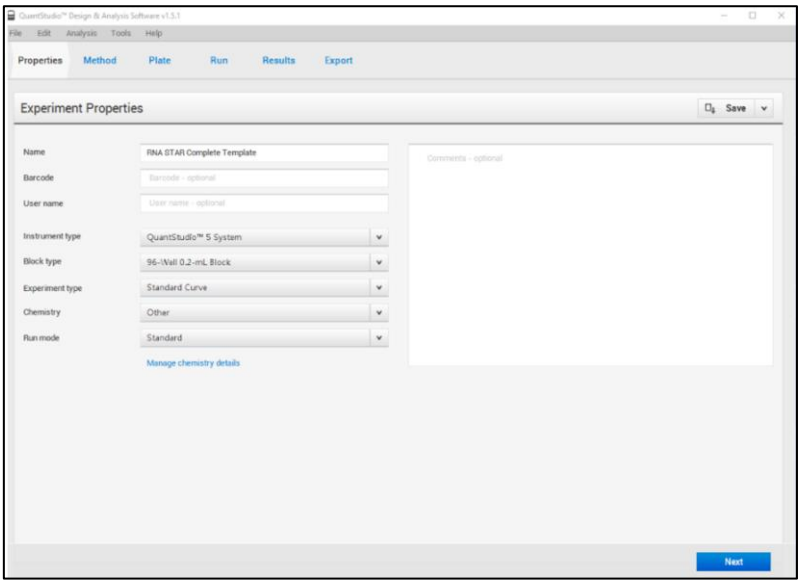
12. RNA STAR Complete Setup for Applied Biosystems™ QuantStudio 5

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.

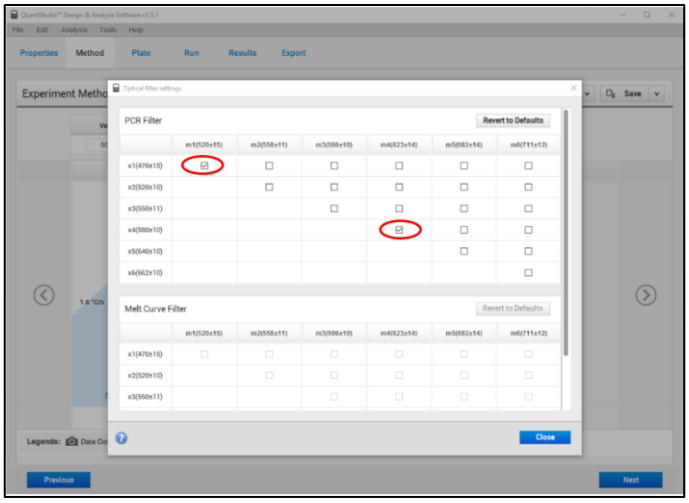
12.1 Programming Instructions for PC/Laptop-connected Instrument

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).

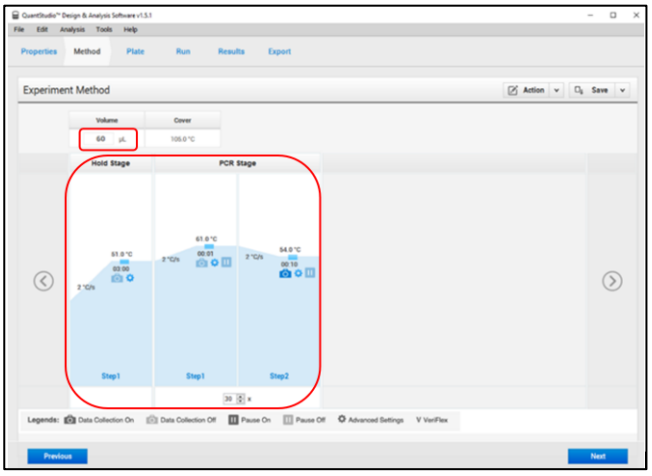
1. If the instrument touchscreen is in sleep mode (dark screen), touch the screen anywhere to activate the instrument. Sleep mode lowers the temperature of the heated cover. It is important that the heated cover idle at 105°C before loading a plate.
2. Launch the 'QuantStudio Design & Analysis' Desktop 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'.



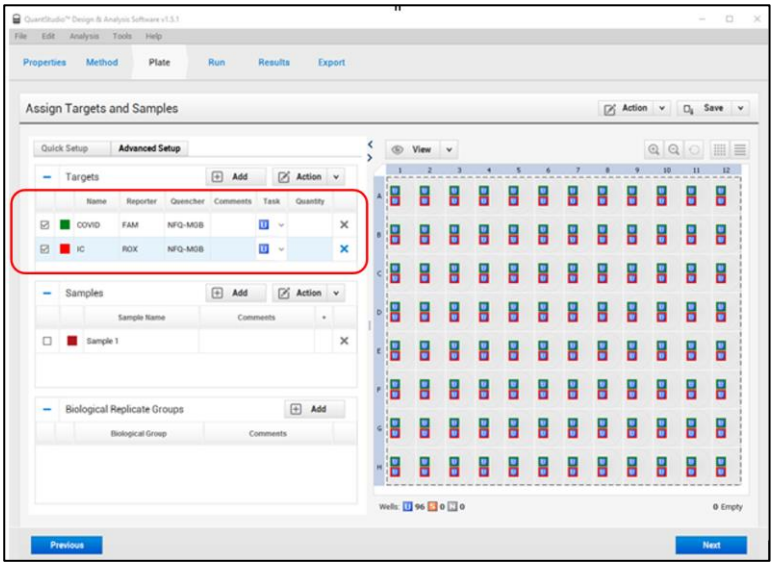
4. Proceed to the ‘Method’ tab. Click ‘Action’ and select ‘Optical filter settings’ from the drop-down menu. In the ‘PCR Filter’ table, uncheck all filter combinations except the following: x1(470±15), m1(520±15), x4(580±10), m4(623±14). Click ‘Close’ to return to the ‘Method’ tab.



5. 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’. Ensure that the last step, the data collection step, displays the camera icon. At the bottom of the ‘PCR Stage’ section, set ‘Cycles’ to ‘30’.



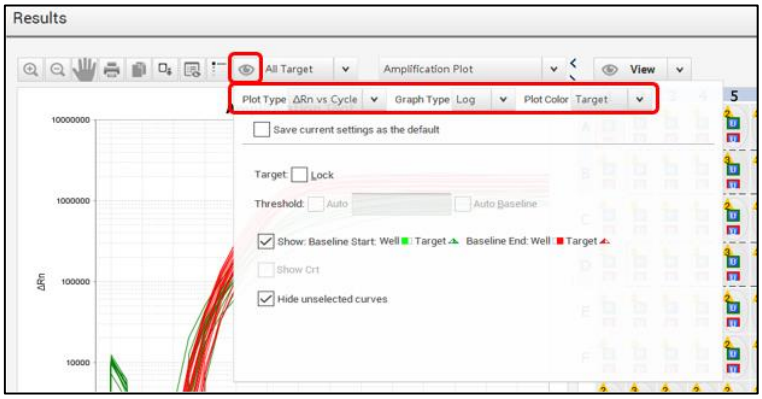
6. Proceed to the ‘Plate’ tab. Change ‘Passive Reference’ to ‘None’ in the ‘Plate Attributes’ window. Select all the wells from the plate layout.
7. Proceed to the ‘Advanced Setup’ tab. Under the ‘Targets’ section, rename ‘Target 1’ to ‘COVID’, change the default color to green, select ‘FAM’ as the ‘Reporter’ (the ‘Quencher’ should automatically be 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’ should be 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.



9. 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’.
10. Load the plate by pressing the eject icon on the QuantStudio 5 instrument touchscreen at the top right of the screen. 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 eject icon on the instrument touchscreen to close the drawer.
11. Return to the desktop software ‘Run’ tab and click the ‘START RUN’ button. When instrument connection is made, the instrument serial number will appear in a drop-down menu below the ‘START RUN’ button. Select the instrument serial number to initiate the run. When prompted to save, change the ‘File Name’ to “LumiraDx SARS-CoV-2 RNA STAR Complete [YYMMDD_Plate#]” and click ‘Save’.
12. Following the run, the instrument touchscreen indicates when the run is ‘Complete’. Dismiss the run screen by pressing ‘Done’. Remove and discard the plate in a sealable waste bag or container.

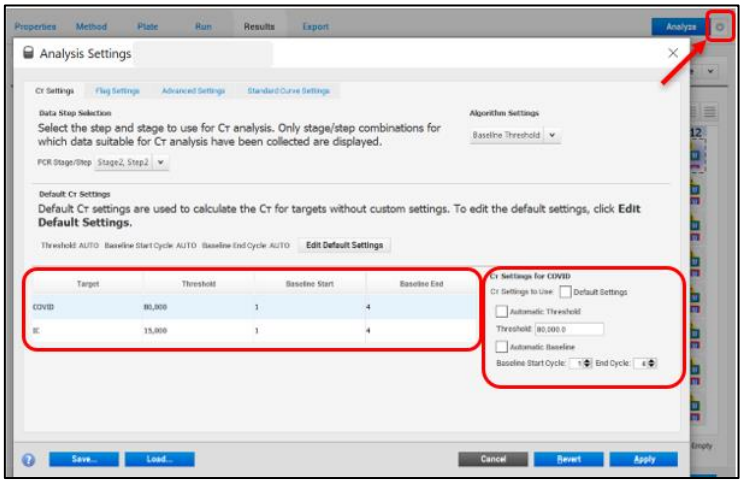
12.2 Analysis Instructions

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. Click on the Eye symbol above the ‘Amplification Plot’ to configure the plot as follows: set ‘Plot Type’ to ‘ΔRn vs Cycle’, set ‘Graph Type’ to ‘Log’, and set ‘Plot Color’ to ‘Target’. All other content remains unchanged. Click out of the window to accept changes.



2. Click the gear icon to the right of the ‘Analyze’ button in the top left of the software to open the ‘Analysis Settings’ window. In the ‘Ct settings’ tab, uncheck the ‘Default settings’ box under the ‘Ct settings for COVID’ section, uncheck the ‘Automatic Threshold’ box, and uncheck the ‘Automatic Baseline’ box. Enter “80,000” for the ‘Threshold’, set ‘Baseline Start Cycle’ to

'1' and set 'End Cycle' to '4'. Select 'IC' 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'.



3. Proceed to the 'Export' tab to the 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. Go to the "Results" tab and 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.

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

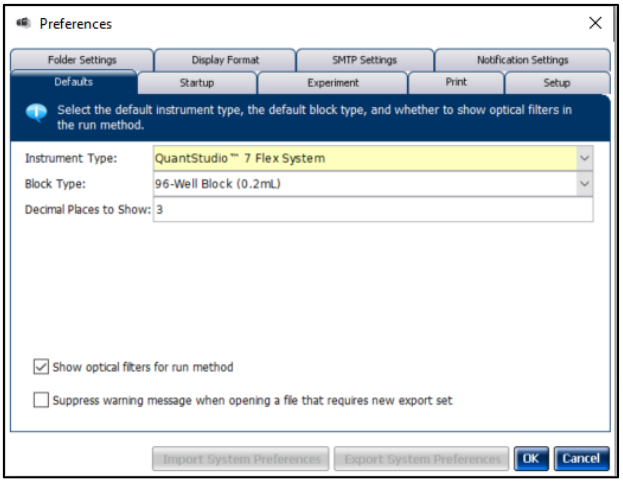
Refer to "User Manual Part Number 4489821" for additional information. The instrument programming instructions are intended for setup of an entire 96-well plate. If the entire plate is not used, please deselect wells as appropriate during analysis.

13.1 Programming Instructions for PC/Laptop-connected Instrument

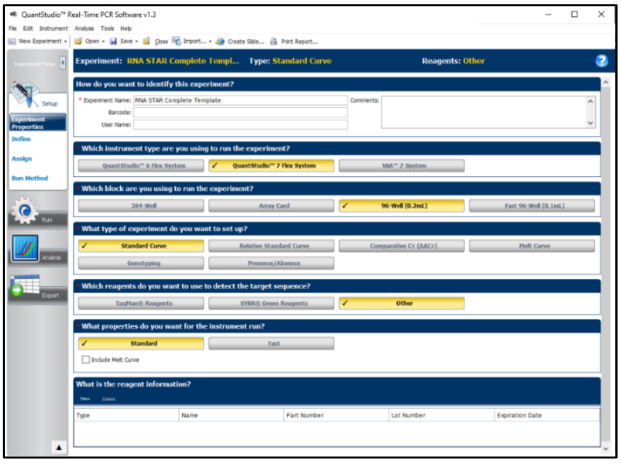
It is recommended to setup the instrument up to step 11 before preparing the Reagent Mix as defined in the qSTAR Reagent Preparation section above. The 96-well plate should be recently centrifuged to guarantee all reagents are at the bottom of the plate and kept on a cold block. Use 96-well plates and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

1. If the instrument touchscreen is in sleep mode (dark screen), wake it by touching anywhere on the screen. Touch the power button to turn on 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 'Preferences' to open 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'.

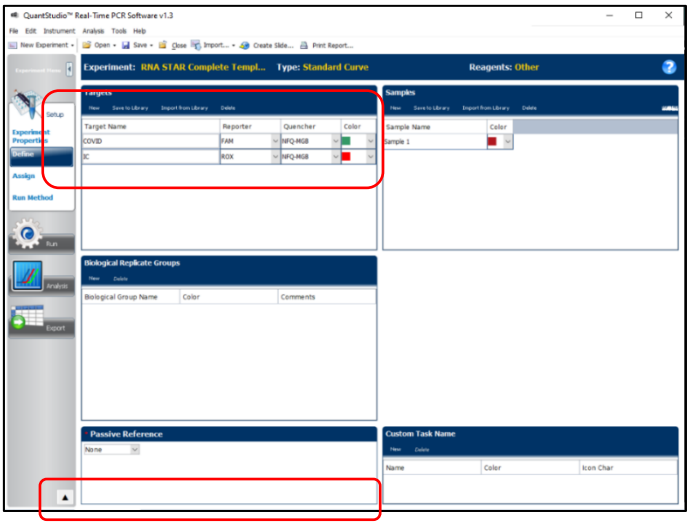
4. Select ‘Create New Experiment’. A window will open with the ‘Experiment Properties’ tab selected.



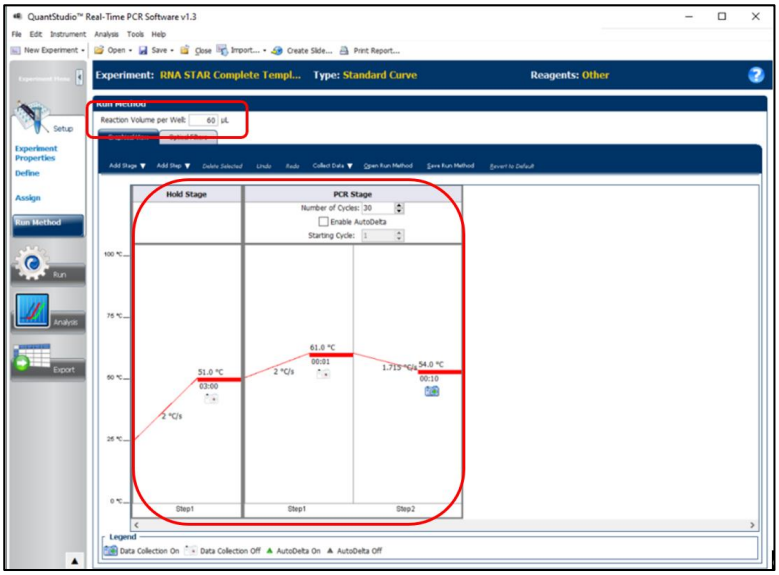
5. On the left of the screen, the ‘Experiment Properties’ tab will be selected. Enter the ‘Experiment Properties' as follows: define the ‘Experiment Name’ as “RNA STAR Complete Template”, select the instrument type as ‘QuantStudio™ 7 Flex System’, select the block being used for the run as ‘96-Well (0.2mL)’,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



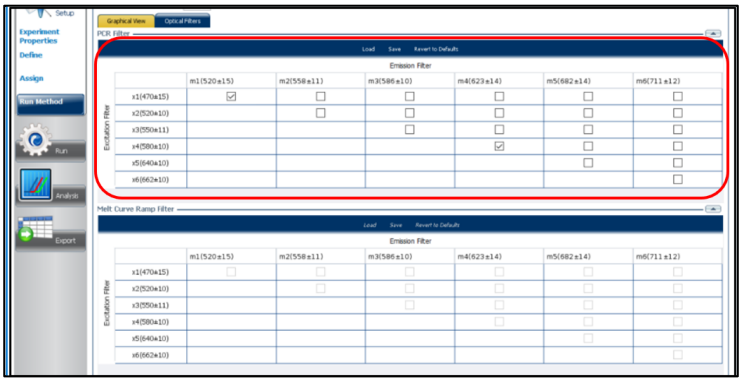
6. On the left of the screen, proceed to the ‘Define’ tab. Under the ‘Targets’ section: define the ‘Target Name’ for ‘Target 1’ as “COVID”, select ‘FAM’ as the ‘Reporter’, change the ‘Color’ to green, and confirm the ‘Quencher’ is automatically entered as ‘NFQ-MGB’. Add a second target by clicking ‘New’ (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’.
7. (Optional) Under the ‘Samples’ section, the ‘Sample Name’ can be added individually.



8. 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.
9. 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’.



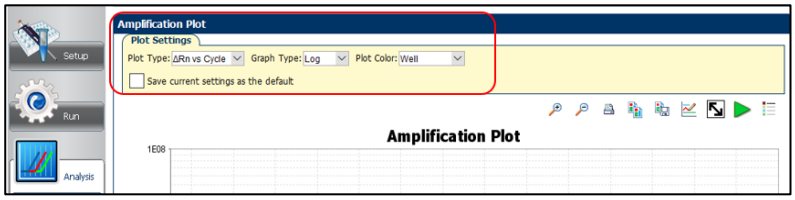
10. Near the top of the window next to the ‘Graphical View’ tab select ‘Optical Filters’. Just below in the ‘PCR Filter’ section, uncheck all ‘Emission Filter’ combinations except the following: x1(470±15), m1(520±15), and x4(580±10), m4(623±14).



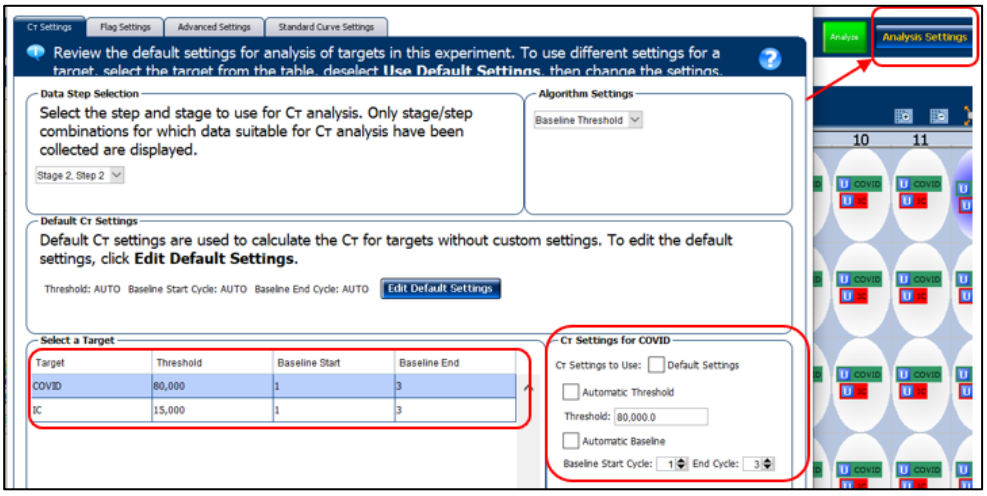
11. Save the experiment as a template for subsequent runs by clicking the ‘Save’ drop-down menu and selecting ‘Save As Template’. Name the template “RNA STAR Complete Template” and click ‘Save’. The experiment must be saved again to connect to the instrument. Click the drop-down menu next to ‘Save’ and 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 serial 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 under '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.

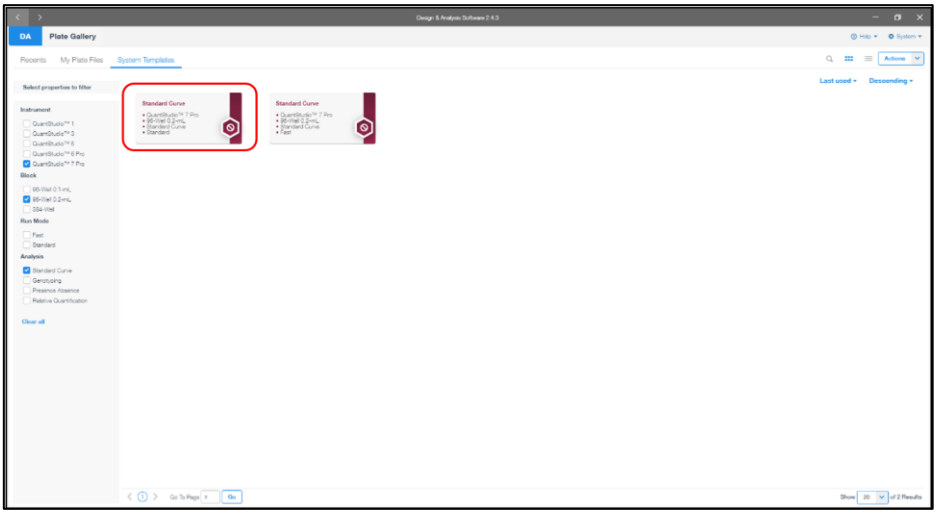
14. RNA STAR Complete Setup for Applied Biosystems™ QuantStudio 7 Pro

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.

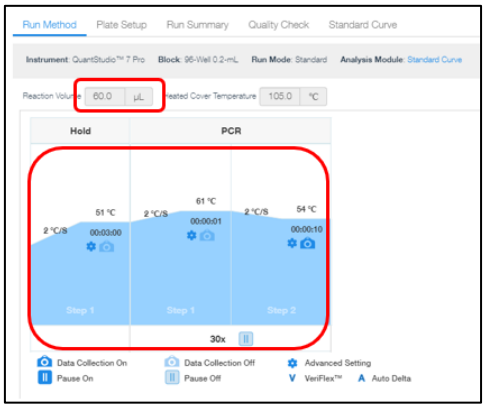
14.1 Programming Instructions for PC/Laptop-connected Instrument

It is recommended to setup the instrument up to step 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).

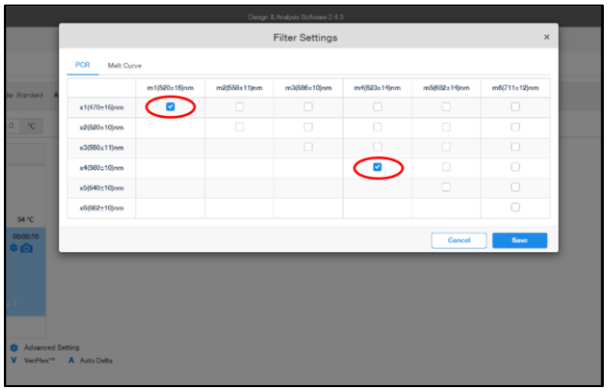
1. Launch the ‘Design & Analysis’ Desktop Software (version v2.4.3) and click ‘Set Up Plate’. In the new screen, click on ‘Standard Curve, QuantStudio™ 7 Pro, 96-Well 0.2mL, Standard Curve, Standard’ to begin setup.



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’.

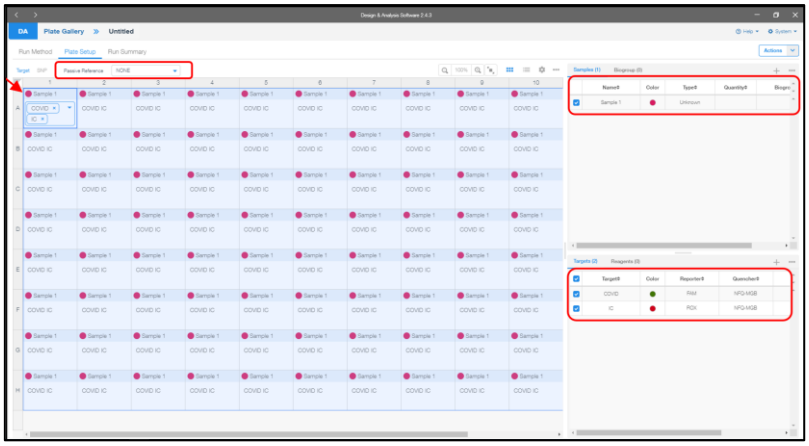


3. Set the filter settings by clicking on the three dots at the top right of the screen and clicking ‘Filter Settings...’ Ensure that only the following combination is selected: ‘x1(470±15)nm’, ‘m1(520±15)nm’; and ‘x4(580±10n)m’, ‘m4(623±14)nm’. Click ‘Save’ to dismiss the window.

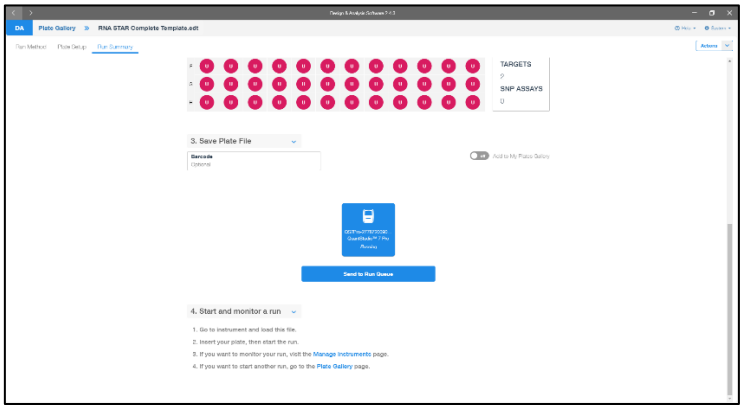


4. Proceed to the ‘Plate Setup’ tab. Select the whole plate by clicking the box between ‘A’ and ‘1’ and confirm just below the ‘Plate Setup’ tab that ‘Passive Reference’ is set to ‘NONE’. On the far top right of the screen, click the ‘+’ icon in the ‘Samples’ gray-shaded section. Edit the new line that 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.



5. 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.

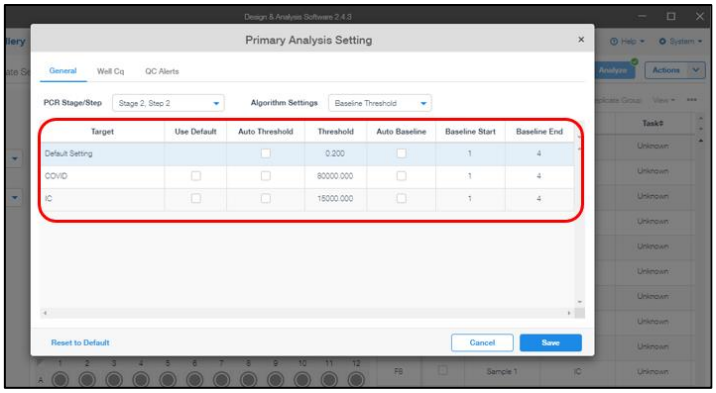


6. On the home instrument touch screen, press ‘Set up run’. In new screen go to ‘Run Queue’, press ‘RNA STAR Complete Template’ in the ‘Plate file’ section. In the ‘Data file properties’ screen, re-name the ‘Data file name’ as “RNA STAR Complete [YYMMDD_Plate#]”. Press ‘Enter’ to confirm ‘Data file name’.
7. 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.
8. At the bottom of the screen, press ‘Start run’. A message might display asking if a plate has been inserted. Dismiss by confirming which will initialize the run.
9. 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’.
10. 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.
11. Remove the plate and discard the plate in a sealable waste bag or container.

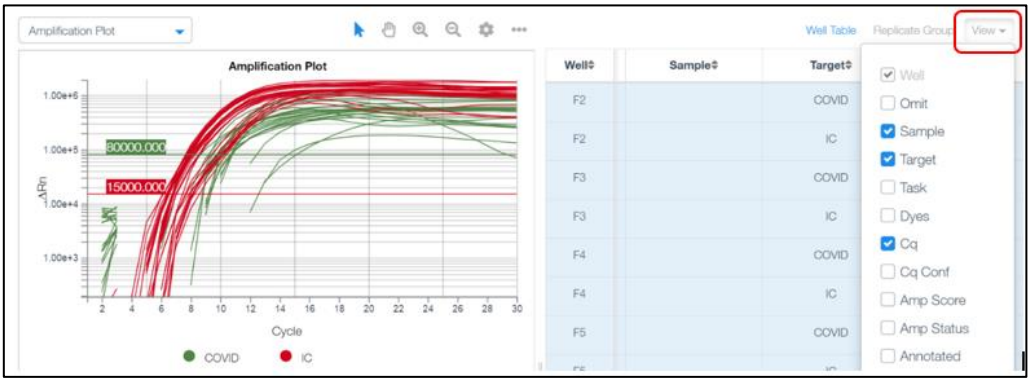
14.2 Analysis Instructions

1. 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’.

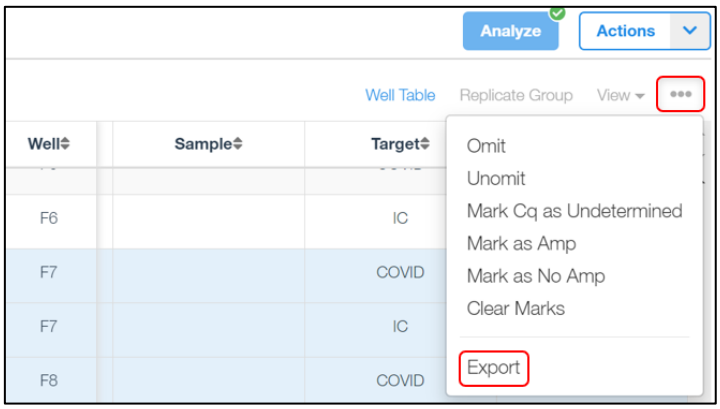
2. On the far top right of the screen, select the drop-down menu next to 'Actions' and select 'Primary Analysis Setting…' A pop-up window will display the default threshold and baseline settings. In the 'General' tab, deselect the checkboxes in the 'Use Default' column for 'COVID' and 'IC' to the right 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.



3. 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.



4. 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'.



5. Close the software.

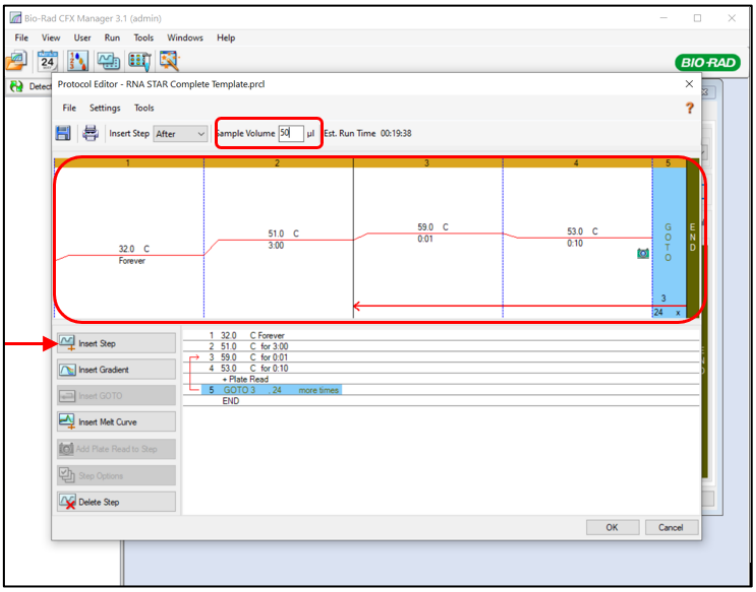
15. RNA STAR Complete Setup for Bio-Rad CFX96 Touch System

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.

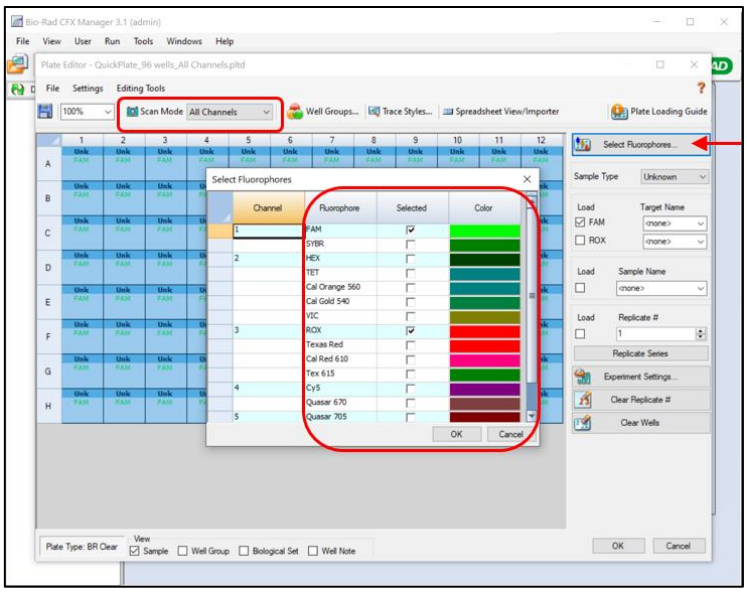
15.1 Programming Instructions for PC/Laptop-connected instrument

It is recommended to setup the instrument up to step 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).

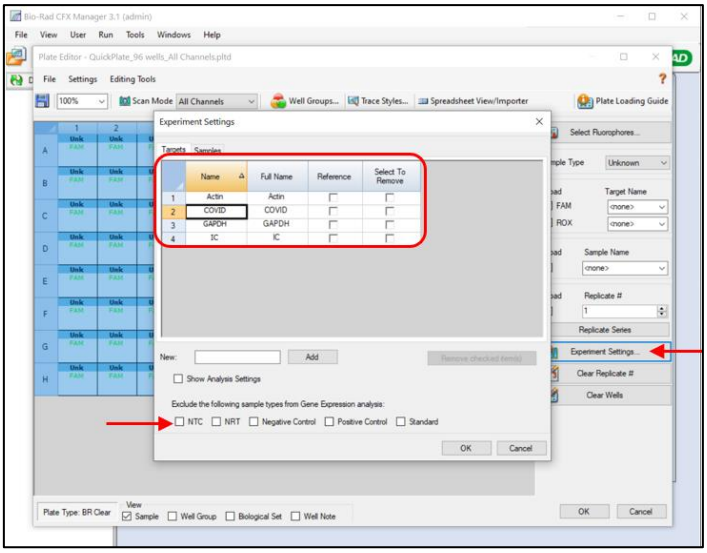
1. Launch the 'Bio-Rad CFX Manager' Desktop Software (version v3.1). In the 'Startup Wizard' pop-up window, select 'User-defined' as the run type then, in the 'Run Setup' pop-up window, select 'Edit Selected…' on the right of the window under the 'Protocol' tab to begin setup.
2. The 'Protocol Editor' 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.prcl" in the appropriate file path and select 'Save'.



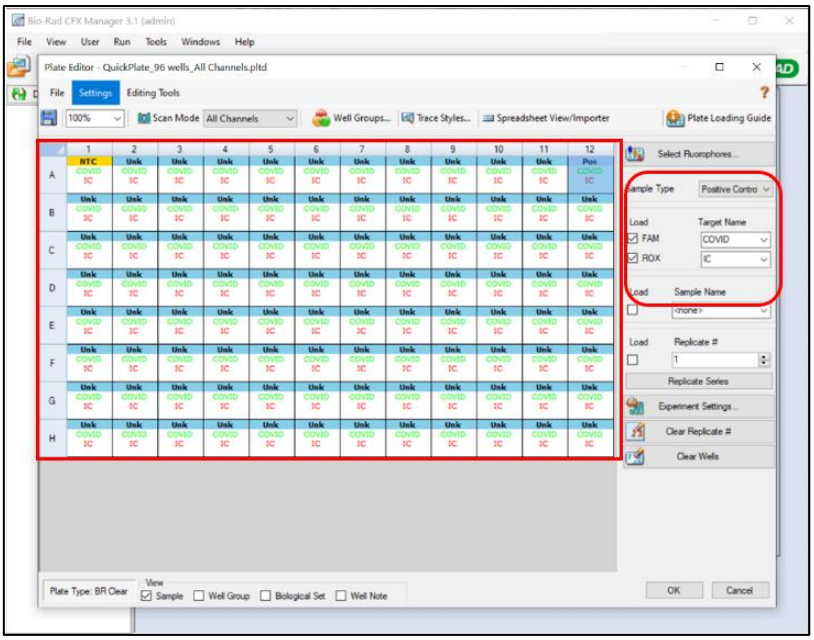
3. Proceed to the 'Plate' tab by selecting the 'Next' button on the bottom right of the 'Protocol Editor' window. Select 'Edit Selected…' on the right of the window then select the entire plate by selecting the gray box between position 'A' and '1' on the plate. Ensure that 'Scan Mode' is set to 'All 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.



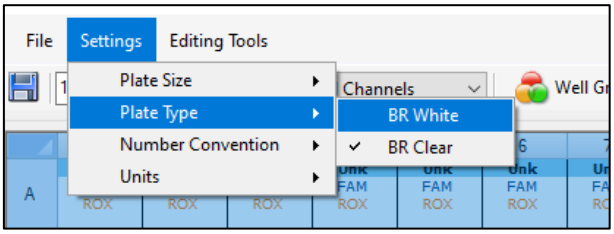
4. Select ‘Experiment Settings…’ near the bottom right of the window to open the ‘Experiment Settings’ pop-up window. Under the ‘Targets’ tab, add a new target by defining ‘New’ as “COVID” and selecting ‘Add’. Add a second target and define ‘New’ as “IC” then select ‘Add’. Under the ‘Exclude the following 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.



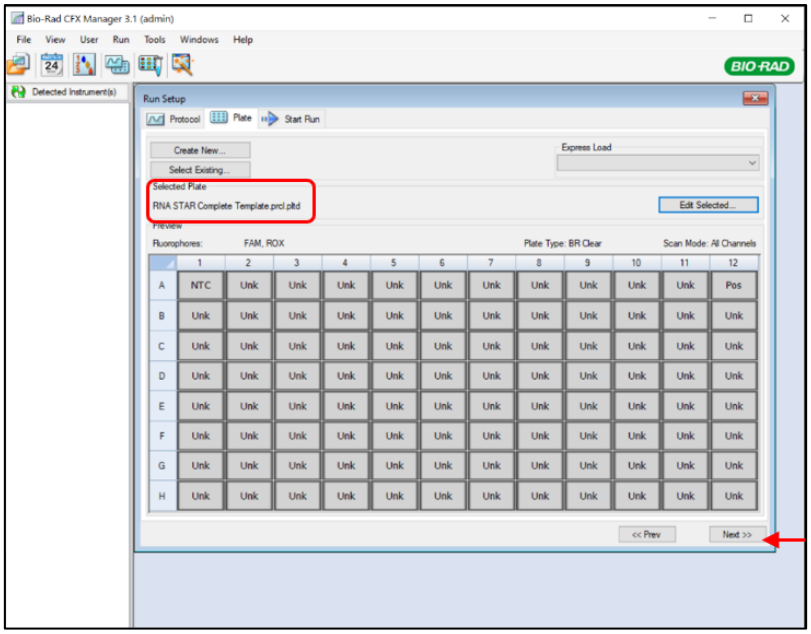
5. Select the entire plate again by selecting the blue box between position ‘A’ and ‘1’ on the plate and ensure the check boxes to the left of the ‘Load’ selection are checked for both ‘FAM’ and ‘ROX’. Using the drop-down menu under ‘Target Name’ define the ‘Target Name’ for ‘FAM’ as ‘COVID’ and the ‘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’.



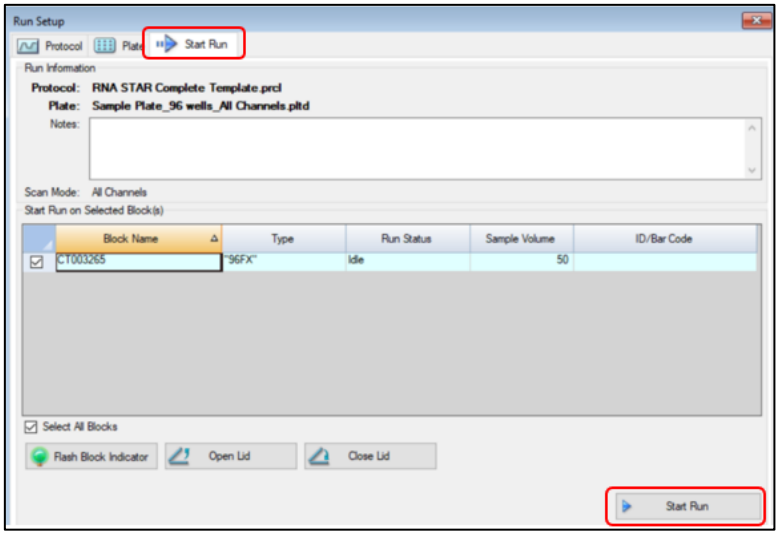
6. To select a plate type, click ‘Settings’ from ‘Plate Editor’ menu bar, and select ‘Plate Type’ then ‘BR White’ from the drop-down list.



7. 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.
8. 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.

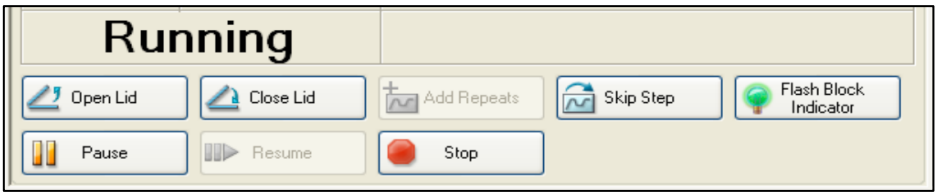


9. On the 'Start Run' tab, click 'Start Run' and save the experiment by defining the ‘File name’ as “RNA STAR Complete [YYMMDD_Plate#].pcrd” in the appropriate file path then selecting ‘Save’. The run will begin initializing and lid will begin preheating.



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 if preheated, proceed to the next step to add the sample plate to the amplification block and begin the run.

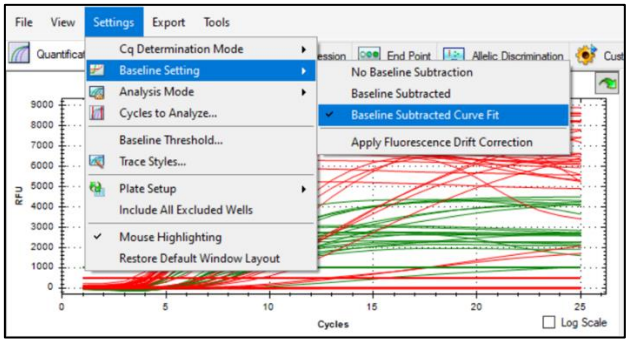
10. At the bottom left of the screen, select ‘Open Lid’. A pop-up window may appear indicating the run will pause to open the lid. Click ‘OK’ to proceed. The lid will automatically open, place the sample plate on the amplification block, then select ‘Close Lid’ to close the lid. Once the lid has finished closing, the ‘Skip Step’ button will become available. Select ‘Skip Step’ to initiate the run.



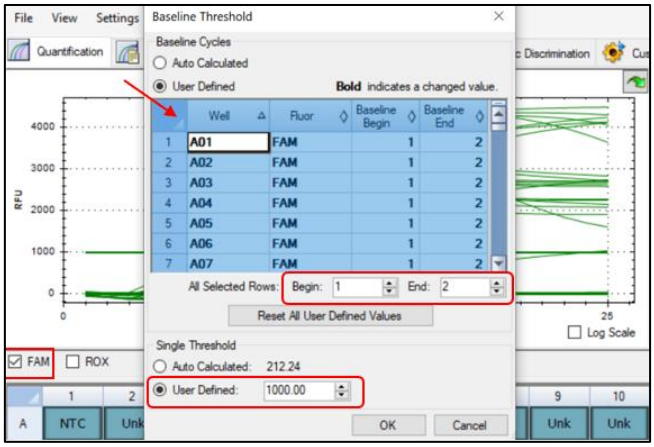
11. When the run finishes, the instrument will become idle and the 'Data Analysis' window will pop up.
12. Refer to the 'Bio-Rad CFX Manager' Desktop Software (version v3.1) and select 'Open Lid'. The lid will automatically open. Remove the plate and discard the plate in a sealable waste bag or container. 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 followings 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 followings 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 chose 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.

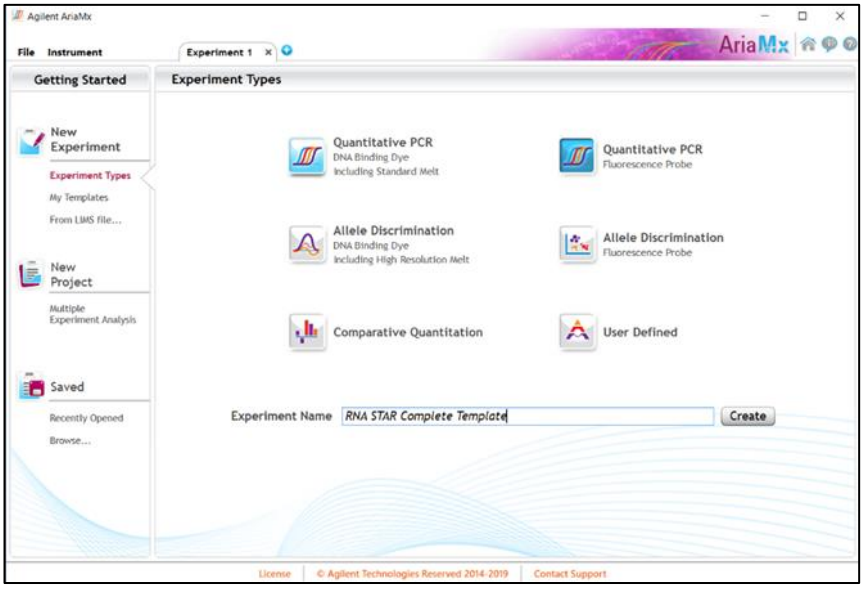
16. RNA STAR Complete Setup for Agilent™ AriaMx

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.

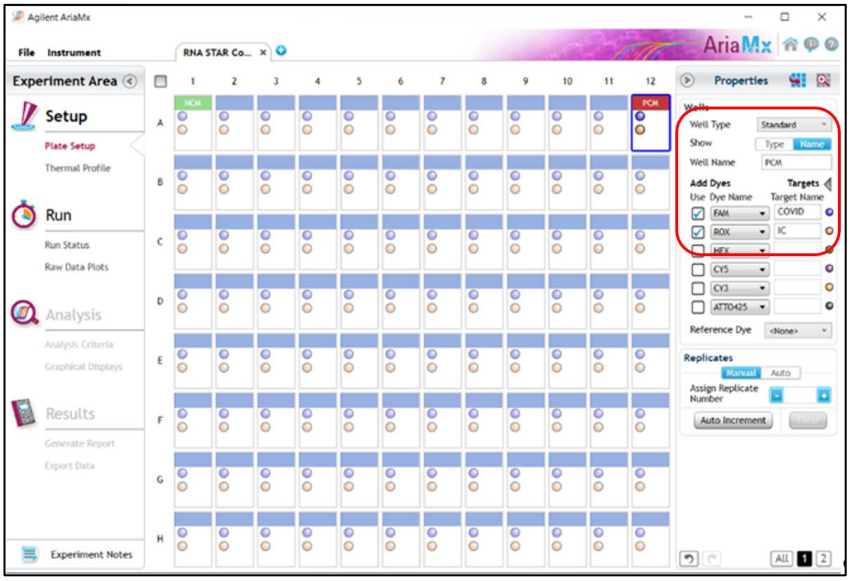
16.1 Programming Instructions for PC/Laptop-connected Instrument

It is recommended to setup the instrument up to step 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).

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'.

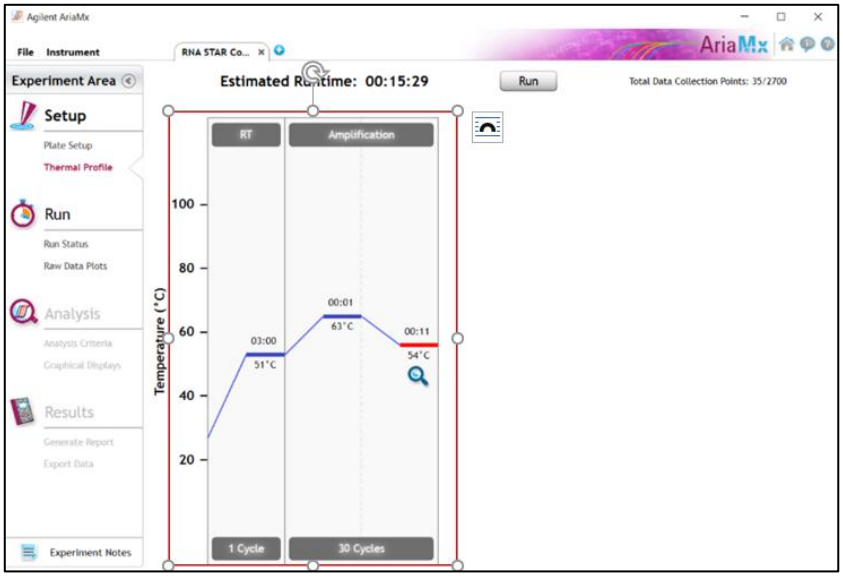


2. Select all the wells and in the 'Properties' section on the right of the screen set 'Well Type' to 'Unknown' using the drop-down menu, select the 'FAM' and 'ROX' boxes, and toggle the 'Target' arrow (across from 'Add Dyes'). Set the 'Target Name' for 'FAM' to "COVID" and 'ROX' to "IC".
3. Select just 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'. Ensure that 'Well Type' is set to 'Unknown' using the drop-down menu. This is to ensure proper analysis. 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).



4. 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'.

5. Proceed to save the file by clicking ‘File’ from the menu and choose ‘Save As Template’ from the drop-down menu. Name the file “RNA STAR Complete Template” and click ‘Save’.
6. Click ‘Run’ and, once instrument connection is made, click ‘Send Config’. The software will prompt you to save the experiment again. Click ‘Save’ and define ‘File Name’ as “RNA STAR Complete [YYMMDD_Plate#]” and click ‘Save’.



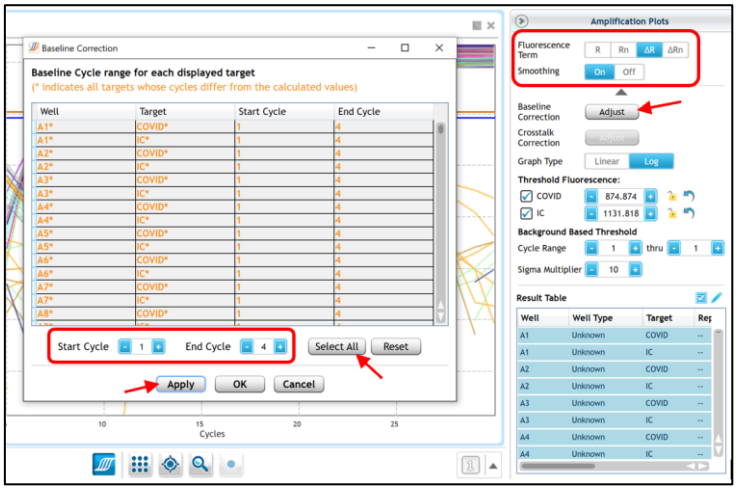
7. On the Agilent AriaMx instrument touchscreen, locate and press the ‘Monitor’ icon () at the bottom of the touch screen. Press ‘Open Primed Experiment’ from pop-up menu. Press the ‘Thermal Profile’ tab on the left side of the touch screen then press ‘Run Experiment’ to initiate the run. The instrument lid will begin its Warm-Up procedure (~2 minutes). Press the ‘Pause’ button when it is activated (green arrow). The ‘Pause’ button is active once the lid reaches 100 °C.
8. 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.

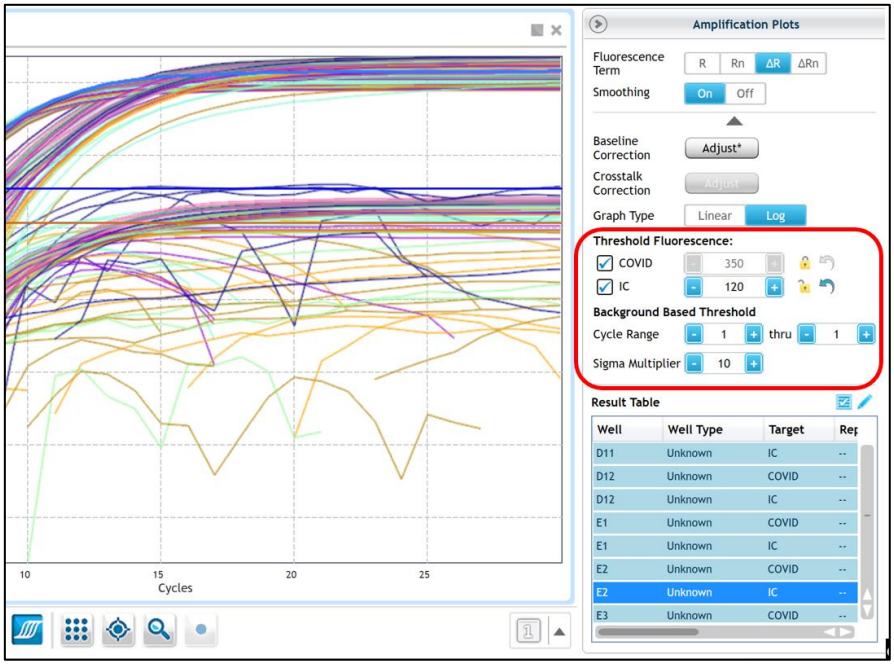
9. Close and lock the block lid then close the instrument door. Press the ‘Resume’ button to now start the run.
10. Upon completion of the run, close the ‘Run Data Plots’ screen by selecting the “X”. Remove the plate and discard the plate in a sealable waste bag or container.

16.2 Analysis Instructions

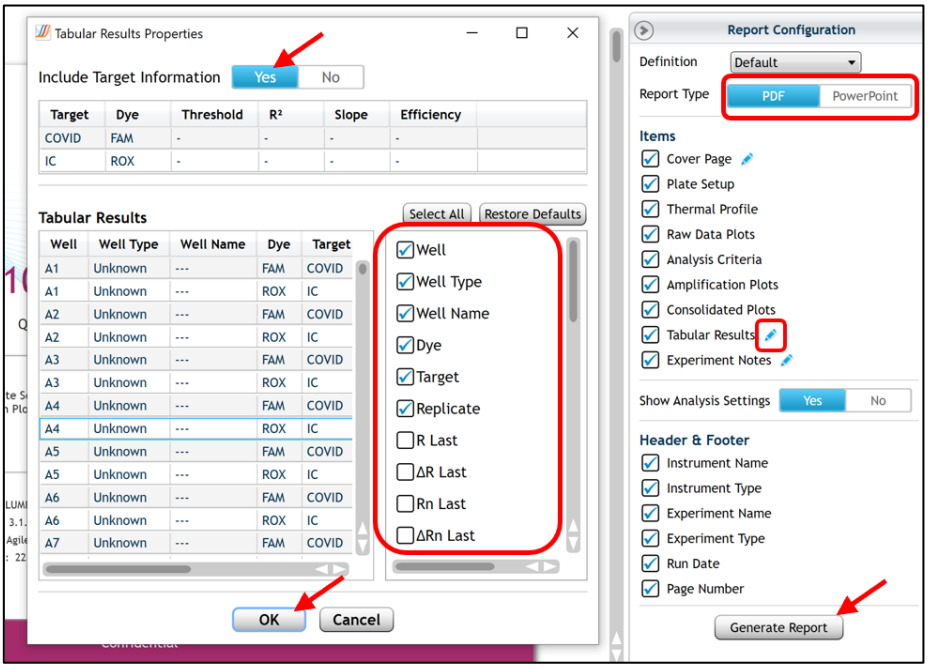
1. In the Agilent AriaMx desktop software (version 1.71), select ‘Analysis Criteria’ from the ‘Experiment Area’ panel on the left side of the software then select the appropriate wells for analysis using the plate map.
2. Select ‘Graphical Displays’ from the ‘Experiment Area’ panel on the left side of the screen, then confirm in the ‘Amplification Plots’ panel on the right side of the software that ‘Fluorescence Term’ is set to ‘ΔR’ and ‘Smoothing’ is ‘On’. Expand the section under ‘Amplification Plots’ by toggling the ‘Arrow’ icon (if needed) then click ‘Adjust’. In the ‘Baseline Correction’ window, click ‘Select All’ to highlight all the wells to be analyzed. Set ‘Start Cycle’ to ‘1’ and set ‘End Cycle’ to ‘4’ then click ‘Select All’ again to confirm the baseline. Click ‘Apply’ then click ‘OK’ to exit the window.



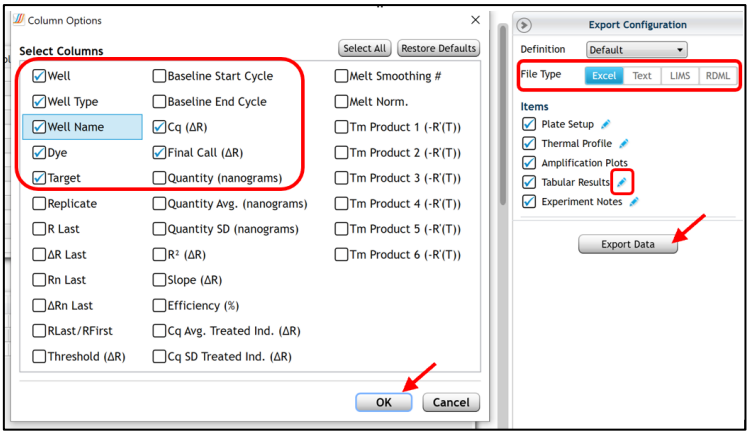
3. In the ‘Amplification Plots’ panel, change ‘Graph Type’ to ‘Log’. Change automatically generated ‘Threshold Fluorescence’ values by defining ‘COVID’ as “350” then click the ‘Unlock’ icon immediately next to the newly defined threshold to lock the setting, additionally, define ‘IC’ as “120” then click 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’. 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.

17. RNA STAR Complete Setup for Agilent™ Stratagene Mx3005P

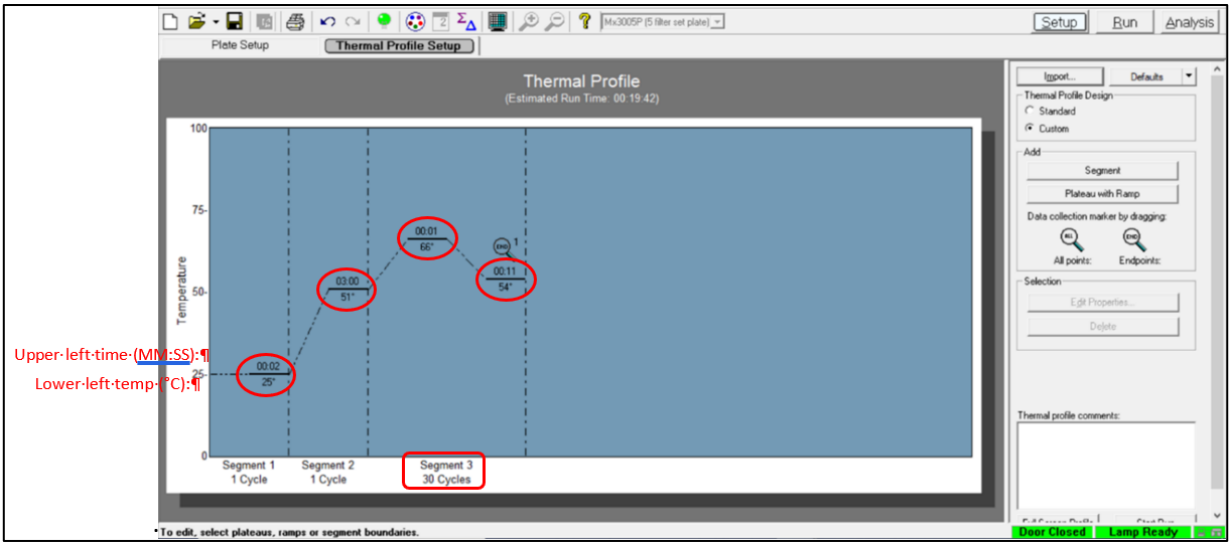
Refer to “User Manual Part Number 70225 J.1” for additional information. The instrument programming instructions are intended for setup of an entire 96-well plate. If the entire plate is not used, please deselect wells as appropriate during analysis. It is required that the Agilent Stratagene Mx3005P 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>’.
3. Click the ‘Assign Assay Names’ button. 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).

5. Proceed to the ‘Thermal Profile Setup’ tab. 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’.



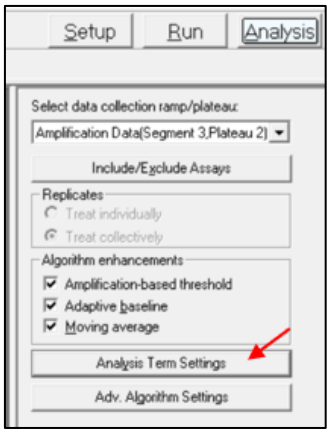
6. Click ‘Instrument’ from the menu and choose ‘Filter Set Gain Settings’. Verify the settings for ‘ROX’ and ‘FAM’ are set to ‘x1’ and ‘x1’, respectively. Click ‘OK’ to apply the changes and close the menu.
7. On the Agilent Stratagene Mx3005P instrument, lift the instrument door up, pull the block lid out to unlock then lift the block lid and load the plate.

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

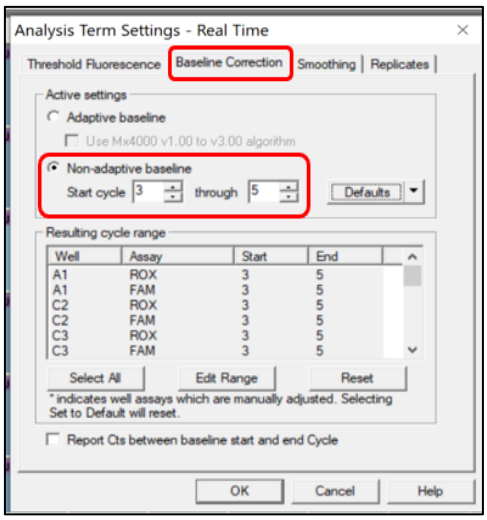
8. Close and lock the block lid then close the instrument door.
9. In the MxPro desktop software (version 4.10), click ‘Run’ on the top right of the screen then click ‘Start’ to initiate the run. ‘Name’ the experiment “RNA STAR Complete [YYMMDD_Plate#]” and click ‘Save’. The run is started.
10. Upon 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.
11. Remove the plate and discard the plate in a sealable waste bag or container.

17.2 Analysis Instructions

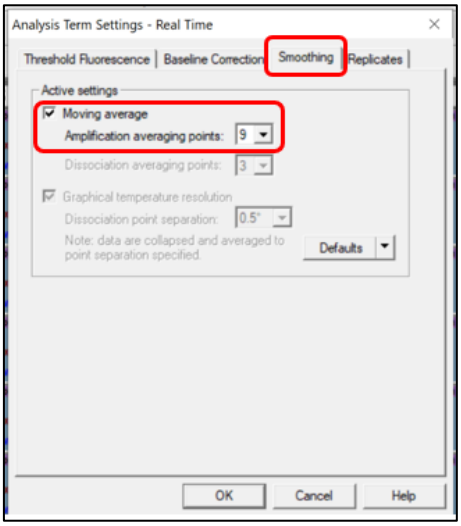
1. Select the ‘Analysis Selection/Setup’ tab. Click ‘Analysis Term Settings’ in the ‘Select data collection ramp/plateau’ section.



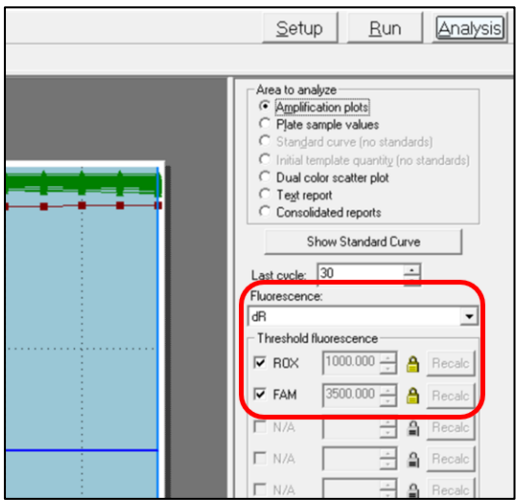
2. Under ‘Active Settings’, select ‘Non-adaptive Baseline’ and set the ‘Start cycle’ to ‘3’ and ‘through’ to ‘5’.



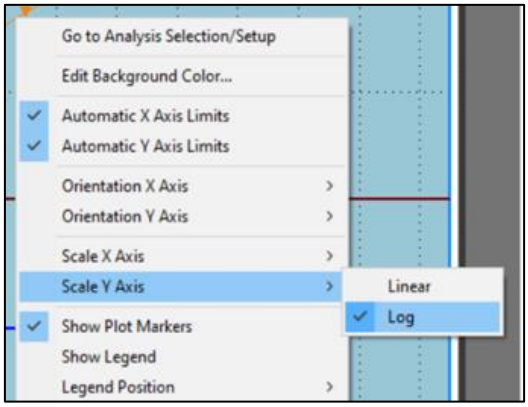
3. Click on the ‘Smoothing’ tab and under ‘Active settings’ click on the ‘Moving average’ box and select ‘9’ from the drop-down menu. Click ‘OK’ to exit the ‘Analysis Term Settings’ window.



4. Proceed to ‘Results’ tab near the top of the software. 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 “3500” then click the ‘Unlock’ icon immediately next to the newly defined threshold to lock the setting.



5. Right-clock the Y-axis plot in the ‘Amplification Plots’ section and change ‘Scale Y Axis’ to ‘Log’.



6. 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.
7. Export the results by right-clicking anywhere in the tabular text report. Choose ‘Export Text Report’ then click ‘Export Text Report to Excel’.
8. From ‘File’ in the menu, choose ‘Save’ from the drop-down menu.
9. Exit the software.

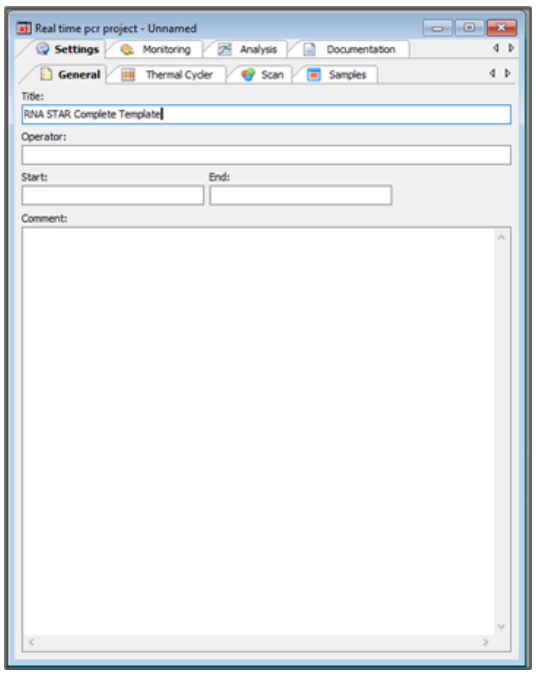
18. RNA STAR Complete Setup for Analytic Jena qTOWER^3 G

Refer to “User Manual Part Number 10-3107-012-23” for additional information regarding the instrument software. Refer to “User Manual Part Number 10-3107-002-23” for additional information regarding the instrument. 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.

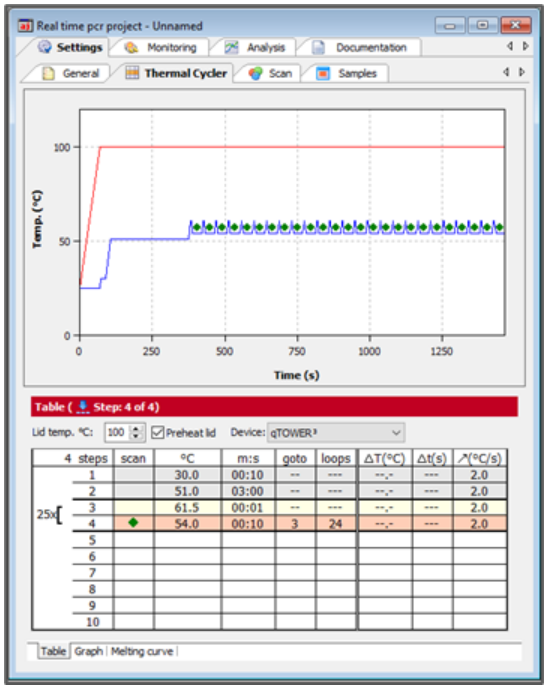
18.1 Programming Instructions for PC/Laptop-connected Instrument

It is recommended to setup the instrument up to step 8 before preparing the Reagent Mix as defined in the qSTAR Reagent Preparation section above. The 96-well plate should be recently centrifuged to guarantee all reagents are at the bottom of the plate and kept on a cold block. Use 96-well plates and seals recommended by the instrument manufacturer (see Option for PCR Instruments & Recommended Consumables table above).

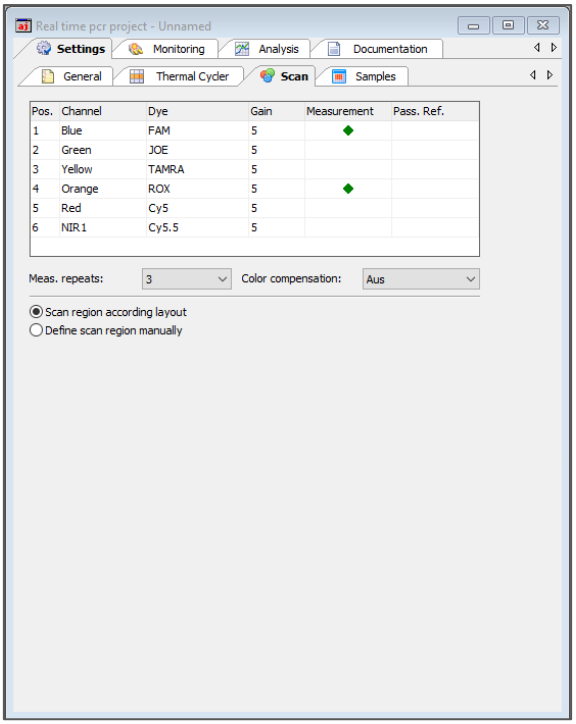
1. Launch the ‘qPCRsoft’ (version v4.1) software. On the toolbar select ‘File’ then ‘New’. This opens the ‘Real time pcr project – Unnamed’ window which opens to the ‘General’ tab.
2. In the ‘General’ tab, define the ‘Title’ as “RNA STAR Complete Template”.



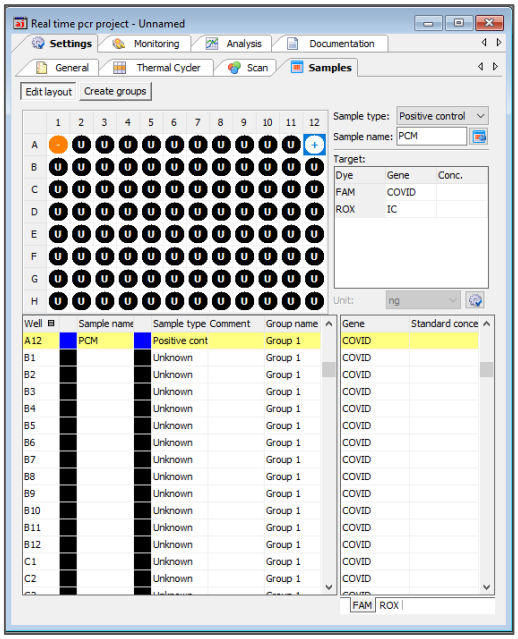
3. Select the ‘Thermal Cycler’ tab, confirm ‘Lid temp. ºC’ is set to ‘100’. Confirm the ‘Preheat lid’ check box is checked. Select your instrument from the ‘Device’ drop-down menu. Setup the thermal profile in the table provided. Change step ‘1’ settings as follows: confirm ‘scan’ is blank, set ‘°C’ to ‘30’, set ‘m:s’ to ’00:10’, confirm ‘goto’ is set to ‘--’, confirm ‘loops’ is set to ‘---’, confirm ‘ΔT(°C)’ is set to ‘--.-’, confirm ‘Δt(s)’ is set to ‘---’and set ‘↗(°C/s)’to ‘2’. Change step ‘2’ settings as follows: confirm ‘scan’ is blank, set ‘°C’ to ‘51’, set ‘m:s’ to ’03:00’, confirm ‘goto’ is set to ‘--’, confirm ‘loops’ is set to ‘---’, confirm ‘ΔT(°C)’ is set to ‘--.-’, confirm ‘Δt(s)’ is set to ‘---’and set ‘↗(°C/s)’to ‘2’. Change step ‘3’ settings as follows: confirm ‘scan’ is blank, set ‘°C’ to ‘61.5’, set ‘m:s’ to ’00:01’, confirm ‘goto’ is set to ‘--’, confirm ‘loops’ is set to ‘---’, confirm ‘ΔT(°C)’ is set to ‘--.-’, confirm ‘Δt(s)’ is set to ‘---’and set ‘↗(°C/s)’to ‘2’. Change step ‘4’ settings as follows: set ‘scan’ to ‘◊’by clicking in the empty box, set ‘°C’ to ’54’, set ‘m:s’ to ’00:10’, confirm ‘goto’ is set to ‘3’, confirm ‘loops’ is set to ‘24’, confirm ‘ΔT(°C)’ is set to ‘--.-’, confirm ‘Δt(s)’ is set to ‘---’and set ‘↗(°C/s)’to ‘2’.



4. Select the ‘Scan’ tab. Change ‘Pos.’ ‘1’ settings as follows: confirm ‘Channel’ is set to ‘Blue’, confirm ‘Dye’ is set to ‘FAM’, set ‘Gain’ to ‘5’, set ‘Measurement’ to ‘◊’ by clicking in the empty box, and confirm ‘Pass. Ref.’ is left blank. Change ‘Pos.’ ‘4’ settings as follows: confirm ‘Channel’ is set to ‘Orange’, confirm ‘Dye’ is set to ‘ROX’, set ‘Gain’ to ‘5’, set ‘Measurement’ to ‘◊’ by clicking in the empty box, and confirm ‘Pass. Ref.’ is left blank. Confirm ‘Meas. Repeats:’ is set to ‘3’. Confirm ‘Color compensation’ is set to ‘Aus’ (Aus = German for OFF). Confirm ‘Scan region according layout’ is selected.

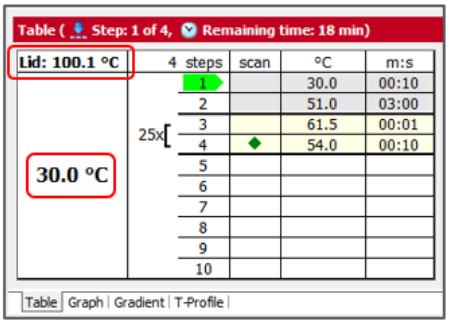


5. Select the ‘Samples’ tab. From the plate layout, select all 96 wells. Add sample details as follows: change ‘Sample type:’ to ‘Unknown’ from the drop-down menu, in the ‘Target:’ table under ‘Gene’ enter “COVID” in the empty box to the right of the ‘FAM’ ‘Dye’, enter “IC” in the empty box to the right of the ‘ROX’ ‘Dye’, and click on the execute icon ( ) to apply changes to all 96 samples. From the plate layout, select position ‘A1’, change ‘Sample type:’ to ‘Negative Control’ from the drop-down menu, enter “NCM” in the empty box next to ‘Sample name:’, and click on the execute icon ( ) to apply changes to the sample. From the plate layout, select position ‘A12’, change ‘Sample type:’ to ‘Positive Control’ from the drop-down menu, enter “PCM” in the empty box next to ‘Sample name:’, and click on the execute icon ( ) to apply changes to the sample.



6. Save the template for subsequent runs by clicking on ‘File’ from the top menu bar and selecting ‘Save Template’ from the drop-down menu. In the ‘Save As’ pop-up window next to ‘File Name’, name the template “RNA STAR Complete Template” and click ‘Save’.
7. When starting the instrument thermal profile, it is critical that the lid is pre-heated prior to loading the sample plate. To pre-heat the lid, select the ‘Monitoring’ tab then click on the ‘play arrow’ icon ( ) from the top menu bar. The instrument lid will begin its warm-up procedure (~2 minutes).

8. Locate the status bar below the real-time plot to monitor the lid and block temperatures. Press the ‘pause’ icon ( ) when the lid reaches ‘100 °C’ and the block temperature reaches ‘30 °C’. A pop-up window will read ‘Pause PCR Run?’, click ‘Yes’.



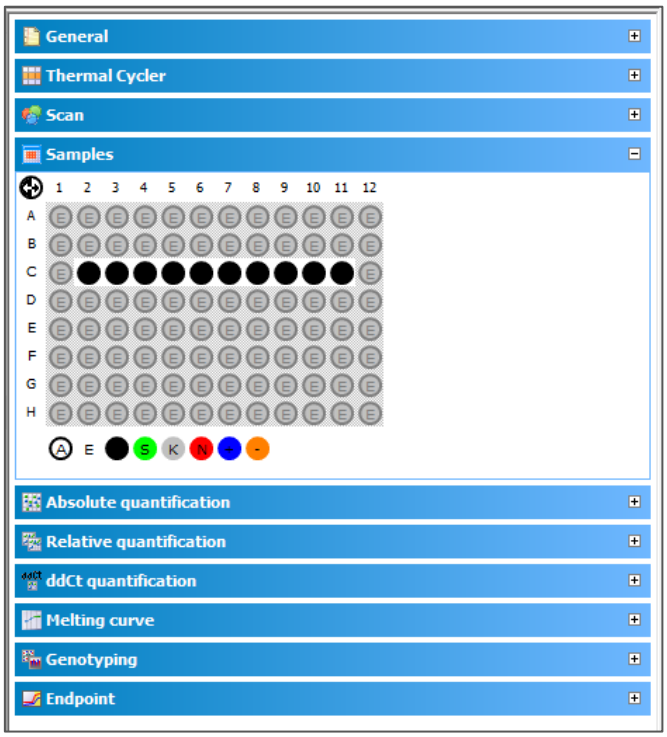
9. Lift the instrument lid and load the plate.

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.

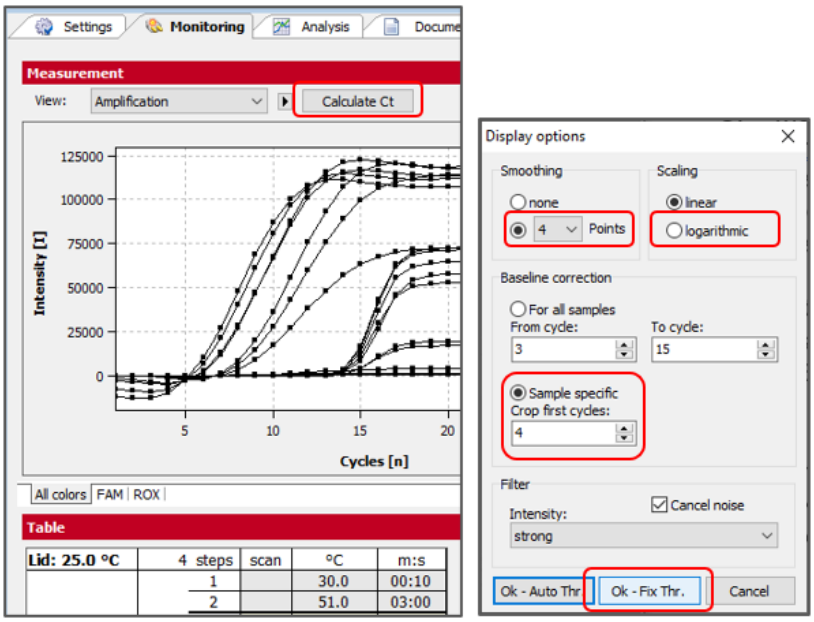
10. Close and lock the lid. Press the ‘pause’ icon ( ) to resume the run.
11. Following the run, a pop-up window will read ‘PCR run finished successfully’. To save the run file, click ‘Save’ and define the ‘File Name’ as “RNA STAR Complete [YYMMDD_Plate#]” and click ‘Save’. Remove the plate and discard the plate in a sealable waste bag or container.

18.2 Analysis Instructions

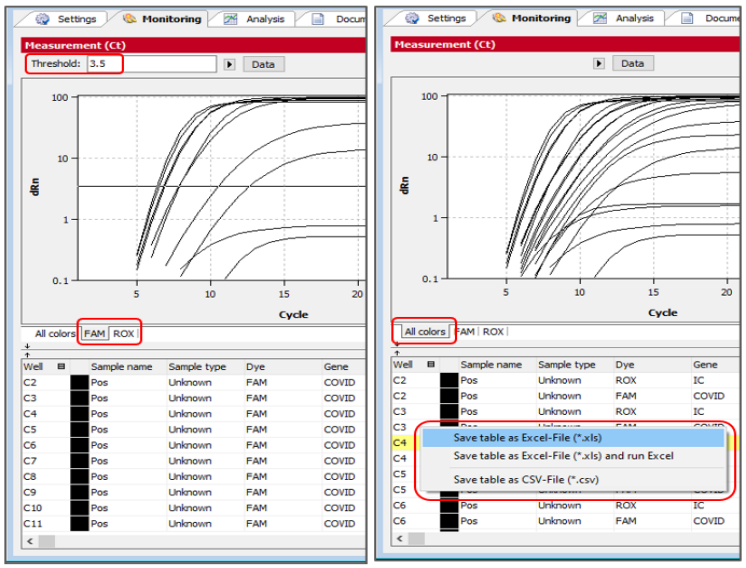
1. Select the desired wells for analysis using the menu option on the far left titled ‘Samples’. Open the option by clicking on the ‘plus’ icon (+). A plate layout will appear where the desired wells can be selected.



2. Modify the analysis settings using the ‘Monitoring’ tab. Under ‘Measurement’, click on the ‘Calculate Ct’ button which will open the ‘Measurement (Ct)’ window. Click on ‘Monitoring’ in top menu bar, and select ‘Display options’ from the drop-down menu. A Pop-up window titled ‘Display options’ will appear. Change the options as follows: under ‘Smoothing’ change the value to ‘4’ next to ‘Points’, under ‘Scaling, select ‘logarithmic’, under ‘Baseline correction’ select ‘Sample specific Crop first cycles:’, change the corresponding value to ‘4’, confirm ‘Intensity’ under ‘Filter’ is set to ‘strong’, and click ‘Ok – Fix Thr.’.



3. Apply thresholds to the data set as follows: in the 'Monitoring' tab under 'Measurement (Ct)' select the 'FAM' tab below the plot and change the 'Threshold' to '3.5', then select the 'ROX' tab and change the 'Threshold' to '1'. Save the analysis by clicking on 'File' from the top menu bar and selecting 'Save project' from the drop-down menu.
4. Export Ct values as follows: in the 'Monitoring' tab under 'Measurement (Ct)' select the 'All colors' tab below the plot, right click on the table below the plot, and save the data in one of the options provided (Save table as Excel-File, Save table as Excel-File and run Excel, Save table as CSV-File). In the 'Save As' pop-up window define the 'File name:' for the data set, then click 'Save'.



19. 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.

19.1 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 reaction. 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.
   Note: 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.
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, Analytik Jena qTower^3 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 (or 3 to 25 for instruments programmed with 25 cycles).

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 QuantStudio 5, Applied Biosystems QuantStudio 7 Flex, Applied Biosystems QuantStudio 7 Pro, Agilent Aria Mx, and the 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 ≤ Ct ≤ 30.0	+ or – (Can be positive or negative)	3.0 ≤ Ct ≤ 30.0*
Neg. Control Media	Reagent and/or environmental contamination	- (Negative)	None detected	+ (Positive)	3.0 ≤ Ct ≤ 30.0

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

Table 2. Expected Results from External Controls on Applied Biosystems 7500, Bio-Rad CFX96, and Analytik Jena qTOWER3.

Control Type/Name	Used to Monitor	SARS-CoV-2 (FAM)	Expected Ct Values	Internal Control (ROX)	Expected Ct Values
Pos. Control Media	Substantial reagent failure including primer and probe integrity	+ (Positive)	3.0 ≤ Ct ≤ 25.0	+ or – (Can be positive or negative)	3.0 ≤ Ct ≤ 25.0*
Neg. Control Media	Reagent and/or environmental contamination	- (Negative)	None detected	+ (Positive)	3.0 ≤ Ct ≤ 25.0

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

19.2 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 QuantStudio 5, Applied Biosystems QuantStudio 7 Flex, Applied Biosystems QuantStudio 7 Pro, and the Agilent AriaMx, Agilent Stratagene Mx3005P).

Assay Results	SARS-CoV-2 (FAM)	Internal Control (ROX)	Interpretation of Results	Notes
SARS-CoV-2 Positive ("+")	3.0 ≤ Ct ≤ 30.0	3.0 ≤ Ct ≤ 30.0*	SARS-CoV-2 Viral RNA detected; IC possibly detected	Report results
Negative ("-")	No Ct detected	3.0 ≤ Ct ≤ 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 Applied Biosystems 7500, Bio-Rad CFX96, and Analytik Jena qTOWER3.

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

20. Limitations

- 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 Cold&Flu Relief Cough Syrup , greater than 2.5% (v/v) , Benzocaine, greater than 7.5 mg/mL and Advil Liqui-Gels, greater than 0.5% (v/v), 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. Performance Characteristics

21.1 Limit of Detection (LoD)

21.1.1 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.

21.1.2 Procedure

Sample matrix pools were prepared from nasopharyngeal (NP) 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 at 0 and 1875 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 Applied Biosystems 7500 Fast Dx (software version 1.4.1) RT-PCR Instrument.

21.1.3 Results and Conclusions

Based on the results of this study, the LoD is 1,875 copies/mL, as shown in Table 5.

Table 5. LumiraDx SARS-CoV-2 RNA STAR Complete Limit of Detection

Concentration	**Observed Properties**		
(Copies/mL)	**Replicates Detected**	**Mean Ct Value (FAM)**	**Mean Ct Value (ROX)**
0	0/120 (0%)	undetermined	6.2
1875	119/120 (99%)	8.4	6.1

21.2 RT-PCR Instrument Validation

21.2.1 Purpose

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

21.2.2 Procedure

Sample matrix pools were prepared from nasopharyngeal (NP) 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, and 1,875 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 20 replicates were tested on each instrument system.

21.2.3 Results and Conclusions

All zero copy replicates showed internal control amplification and no target amplification. 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 6. 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 Applied Biosystems 7500 Fast Dx as demonstrated in the LoD replicate data. The following table highlights the performance on the Roche LC 480 II, Stratagene Mx3005P, ABI QS 5, ABI QS 7 Flex, ABI QS 7 Pro, Bio-Rad CFX96 Agilent AriaMx, and the Analytik Jena qTOWER[3].

Table 6. LumiraDx SARS-CoV-2 RNA STAR Complete on different RT-PCR Instruments.

	Replicates Detected	
Concentration (copies/mL)	**0**	**1875**
Agilent Aria Mx	0/20	20/20
Roche LC480 II	0/20	20/20
Bio-Rad CFX96 Touch System	0/20	20/20
Applied Biosystems QuantStudio 5	0/20	19/20
Applied Biosystems QuantStudio 7 Flex	0/20	20/20
Applied Biosystems QuantStudio 7 Pro	0/20	20/20
Analytik Jena qTOWER[3]	0/20	20/20
Agilent Stratagene Mx3005P	0/20	19/20

21.3 Inclusivity (Analytical Sensitivity)

21.3.1 Purpose

To demonstrate inclusivity for all SARS-CoV-2 sequences in the GISAID database[3] through *in silico* analysis of the LumiraDx SARS-CoV-2 RNA STAR Complete.

21.3.2 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 all SARS-CoV-2 sequences maintained in the GISAID database on June 11, 2021 (n = 1,750,156).

21.3.3 Results & Conclusions

An alignment was performed on June 11, 2021 with the oligonucleotide primer and probe sequences of the LumiraDx SARS-CoV-2 RNA STAR Complete with 1750156 publicly available SARS-CoV-2 sequences maintained in the GISAID database to demonstrate the predicted inclusivity of the assay. 99.5% 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 0.5%, 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 7. Number and Percent of strains in the databases that showed less than 100% homology.

Primer 1		
Mismatches	Number of Sequences	Percent of Sequences
0	1737295	99.27%
1	4607	0.26%
2	103	0.01%
3	58	0.00%
4	1011	0.06%
5+	7082	0.40%
Primer 2		
Mismatches	Number of Sequences	Percent of Sequences
0	1718491	98.19%
1	22983	1.31%
2	122	0.01%
3	27	0.00%
4	1475	0.08%
5+	7058	0.40%
Primer 3		
Mismatches	Number of Sequences	Percent of Sequences
0	1731252	98.92%
1	10229	0.58%
2	101	0.01%
3	37	0.00%
4	44	0.00%
5+	8493	0.49%

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.

21.4 Exclusivity / Cross-Reactivity:

21.4.1 Purpose

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

21.4.2 Procedure

Twenty-nine (29) organisms were selected to test the exclusivity and cross-reactivity of the LumiraDx SARS-CoV-2 Test. The test panel represents related pathogens, high prevalence disease agents, and normal or pathogenic flora including various microorganisms and viruses and negative matrix that are reasonably likely to be encountered in a respiratory clinical sample. Each organism was tested in the absence or presence of heat Inactivated SARS-CoV-2 at 3X LoD.

21.4.3 Results and Conclusions

No cross-reactivity or interference was observed for each organism at the concentrations listed in Table 8.

Table 8. LumiraDx SARS-CoV-2 RNA STAR Complete Cross-Reactivity

Microorganism	**Source**	**Concentration**	**Cross-reactivity/Interference (replicates detected)**	
			Negative	**3X LoD**
Human coronavirus 229E	Zeptometrix	1.78E+04 $TCID_{50}$/mL	0/3	3/3
Human coronavirus OC43	Zeptometrix	1.58E+05 $TCID_{50}$/mL	0/3	3/3
Human coronavirus NL63	Zeptometrix	5.85E+03 $TCID_{50}$/mL	0/3	3/3
MERS coronavirus	Zeptometrix	2.09E+04 $TCID_{50}$/mL	0/3	3/3
Adenovirus Type 5	Zeptometrix	5.10E+06 $TCID_{50}$/mL	0/3	3/3
Human Metapneumovirus (hMPV)	Zeptometrix	2.04E+06 $TCID_{50}$/mL	0/3	3/3
Parainfluenza virus Type 1	Zeptometrix	6.30E+04 $TCID_{50}$/mL	0/3	3/3
Parainfluenza virus Type 2	Zeptometrix	7.55E+04 $TCID_{50}$/mL	0/3	3/3

Microorganism	Source	Concentration	Cross-reactivity/Interference (replicates detected)	
			Negative	3X LoD
Parainfluenza virus Type 3	Zeptometrix	6.90E+05 $TCID_{50}$/mL	0/3	3/3
Parainfluenza virus Type 4a	Zeptometrix	2.81E+03 $TCID_{50}$/mL	0/3	3/3
Influenza A H3N2 (Wisconsin/67/05)	Zeptometrix	7.05E+03 $TCID_{50}$/mL	0/3	3/3
Influenza A H1N1	Zeptometrix	2.29E+05 $TCID_{50}$/mL	0/3	3/3
Influenza B (Malaysia/2506/04)	Zeptometrix	1.90E+05 $TCID_{50}$/mL	0/3	3/3
Enterovirus Type 68	Zeptometrix	6.30E+04 $TCID_{50}$/mL	0/3	3/3
Respiratory syncytial virus	Zeptometrix	1.78E+04 $TCID_{50}$/mL	0/3	3/3
Rhinovirus Type 1A	Zeptometrix	8.50E+03 $TCID_{50}$/mL	0/3	3/3
Haemophilus influenzae	*Zeptometrix*	2.81E+07 CFU/mL	0/3	3/3
Streptococcus pneumoniae	*Zeptometrix*	1.80E+07 CFU/mL	0/3	3/3
Streptococcus pyogenes	*Zeptometrix*	1.94E+08 CFU/mL	0/3	3/3
Candida albicanns	*Zeptometrix*	6.3E+06 CFU/mL	0/3	3/3
Bordetella pertussis	*Zeptometrix*	1.68E+08 CFU/mL	0/3	3/3
Mycoplasma pneumoniae	*Zeptometrix*	1.35E+07 CFU/mL	0/3	3/3
Chlamydia pneumoniae	*Zeptometrix*	2.11E+06 IFU/mL	0/3	3/3
Legionella pneumophila	*Zeptometrix*	9.55E+08 CFU/mL	0/3	3/3
Mycobacterium tuberculosis	*Zeptometrix*	1.15E+07 CFU/mL	0/3	3/3
Pneumocystis jirovecii	*Zeptometrix*	3.17E+07 CFU/mL	0/3	3/3
Pseudomonas Aeruginosa	*Zeptometrix*	7.15E+06 CFU/mL	0/3	3/3
Staphylococcus Epidermidis	*Zeptometrix*	1.24E+08 CFU/mL	0/3	3/3
Streptococcus Salivarius	*Zeptometrix*	2.26E+07 CFU/mL	0/3	3/3

21.5 Endogenous Interference Substances Studies

21.5.1 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.

21.5.2 Procedure

Twenty-one (21) potential interfering substances listed in Table 9 were tested in the absence or presence of SARS-CoV-2 at 3X LoD in sample matrix pools prepared from nasopharyngeal (NP) swab samples.

21.5.3 Results and Conclusions

None of the potential interfering substances listed in Table 9 demonstrated interference at the concentration reported.

Table 9. LumiraDx SARS-CoV-2 RNA STAR Complete Interfering Substances

Potential Interferent	Active Ingredient	Final Concentration	Target	% Agreement with Expected Results
Whole Blood (Human)	Whole Blood	5% (v/v)	Negative	100% (3/3)
			Positive SARS-CoV-2 (3X LoD)	100% (3/3)
Chloroseptic Max	Phenol 1.5%, Glycerin 33%	5% (v/v)	Negative	100% (3/3)
			Positive SARS-CoV-2 (3X LoD)	100% (3/3)
Cold&Flu Relief Cough Syrup	Acetaminophen, Dextromethorphan, Guaifenesin, Phenylephrine	2.5% (v/v)*	Negative	100% (3/3)
			Positive SARS-CoV-2 (3X LoD)	100% (3/3)
Halls	Menthol	20 mg/mL	Negative	100% (3/3)
			Positive SARS-CoV-2 (3X LoD)	100% (3/3)

Potential Interferent	Active Ingredient	Final Concentration	Target	% Agreement with Expected Results
Cepacol	Benzocaine	7.5 mg/mL**	Negative	100% (3/3)
			Positive SARS-CoV-2 (3X LoD)	100% (3/3)
Advil Liqui-Gels	Ibuprofen	0.5% (v/v) ***	Negative	100% (3/3)
			Positive SARS-CoV-2 (3X LoD)	100% (3/3)
Cephalexin	Cephalexin	0.0126 mg/mL	Negative	100% (3/3)
			Positive SARS-CoV-2 (3X LoD)	100% (3/3)
Mucin, Type II (from porcine stomach)	Mucin, Type II (from porcine stomach)	0.25 mg/mL	Negative	100% (3/3)
			Positive SARS-CoV-2 (3X LoD)	100% (3/3)
Tobramycin (antibacterial)	Tobramycin (antibacterial)	1.25 mg/mL	Negative	100% (3/3)
			Positive SARS-CoV-2 (3X LoD)	100% (3/3)
Phenylephrine Nasal Spray	Phenylephrine HCl	5% (v/v)	Negative	100% (3/3)
			Positive SARS-CoV-2 (3X LoD)	100% (3/3)
Oxymetazoline Nasal Spray	Oxymetazoline HCl	5% (v/v)	Negative	100% (3/3)
			Positive SARS-CoV-2 (3X LoD)	100% (3/3)
Ocean Saline Nasal Spray	Sodium chloride	15% (v/v)	Negative	100% (3/3)
			Positive SARS-CoV-2 (3X LoD)	100% (3/3)
Nasacort (triamcinolone, nasal corticosteroid)	Triamcinolone acetonide	5% (v/v)	Negative	100% (3/3)
			Positive SARS-CoV-2 (3X LoD)	100% (3/3)
Zicam (Nasal gel, homeopathic allergy relief)	Galphimia glauca, Luffa operculate, Sabadilla	5% (v/v)	Negative	100% (3/3)
			Positive SARS-CoV-2 (3X LoD)	100% (3/3)
Beclomethasone	Beclomethasone	0.100 mg/mL	Negative	100% (3/3)
			Positive SARS-CoV-2 (3X LoD)	100% (3/3)
Budesonide	Budesonide	0.10 mg/mL	Negative	100% (3/3)
			Positive SARS-CoV-2 (3X LoD)	100% (3/3)
Flonase	Flonase	5% (v/v)	Negative	100% (3/3)
			Positive SARS-CoV-2 (3X LoD)	100% (3/3)
Zanamivir (anti-viral drug, e.g. Relenza)	Zanamivir	0.75 mg/mL	Negative	100% (3/3)
			Positive SARS-CoV-2 (3X LoD)	100% (3/3)
No Substance	No Substance	N/A	Negative	100% (3/3)
			Positive SARS-CoV-2 (3X LoD)	100% (3/3)

*Cold&Flu Relief Cough Syrup produced all invalid results at 5% (v/v). The substance was further diluted to 2.5% (v/v) and produced valid results with 100% agreement with expected results.

**Ibuprofen was initially tested at 5% (v/v) and produced invalid results. The substance was further diluted to 1% (v/v) and still produced invalid results. A final dilution at 0.5% (v/v) produced valid results with 100% agreement with expected results.

***Benzocaine (active ingredient of Cepacol) produced one false negative at 15 mg/mL. This substance was retested at 7.5 mg/mL and produced 100% agreement with expected results.

21.5 Reproducibility

21.5.1 Purpose

To demonstrate the reproducibility of the LumiraDx SARS-CoV-2 RNA STAR Complete kit across three sites based in the United States.

21.5.2 Procedure

Three sites were selected to test the reproducibility of the LumiraDx SARS-CoV-2 Test. A three-member test panel including negative, low positive (1.5X LoD), and moderate positive (3X LoD) samples were evaluated. Negative samples were prepared from

nasopharyngeal (NP) samples and pooled to create a negative sample matrix. Positive samples were prepared by spiking heat-inactivated SARS-CoV-2 virus into a negative sample matrix. Samples were blinded and randomized to the testing operator.

Testing was performed over six days, with two operators per site, three replicates per sample type, to yield a total of one-hundred eight (108) observations per sample type (3 sites x 2 operators x 6 days x 3 replicates = 108 observations).

21.5.3 Results and Conclusions

Agreement of observed with expected results was 100% for all samples tested across all operators, sites, and days as reported in Table 10. There were no significant differences observed within run (replicates tested by operator), between runs (over six days), between the three sites, or between operators.

Table 10. LumiraDx SARS-CoV-2 RNA STAR Complete Reproducibility

	Site 1		Site 2		Site 3		Overall % Agreement (95% CI)		
Target	**Observed/ Expected**	**% Agreement**	**Observed/ Expected**	**% Agreement**	**Observed/ Expected**	**% Agreement**	**Overall**	**Lower**	**Upper**
Negative	36/36	100	36/36	100	36/36	100	100%	96.57%	100.00%
1.5X LoD	36/36	100	36/36	100	36/36	100	100%	96.57%	100.00%
3 X LoD	36/36	100	36/36	100	36/36	100	100%	96.57%	100.00%

21.6 Clinical Evaluation

21.6.1 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 353 nasopharyngeal (NP) and nasal (N) swab clinical specimens.

21.6.2 Procedure

A panel of 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.

21.6.3 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 95.45% Positive Agreement and 98.31% Negative Agreement when compared to the FDA EUA RT-PCR test.

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

		FDA EUA RT-PCR Test (Comparator)		
		Positive	Negative	Total
LumiraDx SARS-CoV-2 RNA STAR Complete	Positive	168	3	171
	Negative	8	174	182
	Total	176	177	353
Percent Agreement		95.45% (Pos.)	98.31% (Neg.)	
95% Confidence Interval		91.24 - 98.02%	95.13 - 99.65%	

22. Contact Information, Ordering, and Product Support

22.1 Ordering

For ordering, contact LumiraDx at:
Website: www.lumiradx.com.
Email (US): customerservices.US@lumiradx.com.
Email (International): customerservices@lumiradx.com.

22.2 Product Information

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.

22.3 Technical Support

For technical support, contact LumiraDx at:
Email: technicalservices@lumiradx.com. Include "LumiraDx SARS-CoV-2 RNA STAR Complete" in the subject line.
Phone: 1-888-586-4721.

22.4 Return Policy

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.

22.5 Limited Warranty

LumiraDx RNA STAR Complete SARS-CoV-2 – As per shelf life.
Reagents must be stored according to the required storage conditions as printed in this Instructions for use and they can be used only up to the expiry date printed on the kit box. 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. 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 the LumiraDx RNA STAR Complete SARS-CoV-2 Kit to physical abuse, misuse, abnormal use, use inconsistent with the LumiraDx instructions for use, 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.

22.6 Intellectual Property

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.

22.7 Legal Notices

Legal notices

23. References

[1] World Health Organization: www.who.int.

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

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

24. Glossary of Symbols

	Temperature limitation
	In vitro diagnostic medical device
	Catalog Reference Number
	Lot Number/Batch Code
	CE Mark of Conformity
	Authorized representative in the European Community
 www.lumiradx.com	Refer to www.lumiradx.com for the electronic form of the instructions for use

	Manufacturer
	Use-by Date – The date after which the unopened reagent cannot be used.
	Contains sufficient reagent for 'n' reactions
	Positive Control Media
	Negative Control Media
	Uncontaminated recycled content-packaging, kit box, Instructions for Use is recyclable if it can be collected, separated, or otherwise recovered from the waste stream through an established recycling program.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