



SARS-CoV-2 RNA STAR Complete

Instructions for Use

For Research Use Only - Not For Use in Diagnostic Procedures

Catalog #L018180330096

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1 Summary and Explanation

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 sample 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 samples (such as nasal, mid-turbinate, nasopharyngeal and oropharyngeal swabs) and saliva samples. LumiraDx SARS-CoV-2 RNA STAR Complete reagents are for research use only and not for use in diagnostic procedures.

2 Principles of the Procedure

In a single reaction, SARS-CoV-2 virus can be lysed and amplified from upper respiratory samples (such as nasal, mid-turbinate, nasopharyngeal and oropharyngeal swabs) and saliva samples. The SARS-CoV-2 virions are lysed by the presence of detergents found in the LumiraDx SARS-CoV-2 RNA STAR Complete Extraction Buffer. The nucleic acids present in the lysed swab samples 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 viral nucleic acid without performing extraction within minutes. Generated products are specifically detected with molecular beacons designed to anneal to the target amplicon by any of the following instruments: Roche LightCycler 480 II (software version SW 1.5.1), Applied Biosystems 7500 Fast Dx (software version 1.4.1), Applied Biosystems QuantStudio 5 (software version 1.5.1), Applied Biosystems QuantStudio 7 Flex (software version 1.3), Applied Biosystems QuantStudio 7 Pro (software version 2.4.3), Bio-Rad CFX96 Touch System (software version 3.1), Agilent AriaMx (software version 1.71), or the Agilent Stratagene Mx3005P (software version 4.10) RT-PCR Instruments.

3 Materials 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 Saliva Reagent	Saliva Reagent	120 µL	-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

4 Materials Required (But Not Provided)

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

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

Option for PCR Instruments & Consumables	Source	Catalog #
Roche LightCycler 480 II (software version SW 1.5.1)	Roche Life Science	5015278001
VWR Adhesive Film for Microplates	VWR	60941-070
LightCycler 480 Multiwell Plate 96, Clear	Roche Life Science	5102413001
Applied Biosystems 7500 Fast Dx (software version 1.4.1)	ThermoFisher Scientific	4406984
Applied Biosystems QuantStudio 5 (software version 1.5.1)	ThermoFisher Scientific	A28574
Applied Biosystems QuantStudio 7 Flex (software version 1.3)	ThermoFisher Scientific	4485698
Applied Biosystems QuantStudio 7 Pro (software version 2.4.3)	ThermoFisher Scientific	A43183
Applied Biosystems Optical Adhesive Covers	ThermoFisher Scientific	4360954
Applied Biosystems MicroAmp Optical 96-Well Plate	ThermoFisher Scientific	4306737
Bio-Rad CFX96 Touch System (software version 3.1)	Bio-Rad	1855195
Eppendorf twin.tec Real-Time PCR Plate 96-Well Semi-Skirted	Eppendorf	951022043
Agilent Aria Mx (software version 1.71)	Agilent Technologies	G8830A
AriaMx 96 Adhesive Seals	Agilent Technologies	401492
AriaMx 96 Well Plates, Skirted, LP	Agilent Technologies	401490
Agilent Stratagene Mx3005P (software version 4.10)	Agilent	401511
Applied Biosystems MicroAmp Clear Adhesive Film	ThermoFisher Scientific	4306311
Eppendorf twin.tec Real-Time PCR Plate 96-Well Semi-Skirted	Eppendorf	951022043
Universal Sealing Film and Plate*	General lab supplier	n/a
ThermalSeal A Sealing Film	Research Product International	202545
Heat-Resistant Polypropylene Film for Raised-Rim Plates	VWR	89087-690

*This universal sealing film and plate have been found to be compatible with all instruments listed above (except Bio-Rad CFX96).

Equipment	Source	Catalog #
-80°C Laboratory Freezer	General lab supplier	n/a
-25°C to -15°C Laboratory Freezer	General lab supplier	n/a
2°C to 8°C Laboratory Refrigerator	General lab supplier	n/a
Adjustable Multi-Channel Pipettes (2-20µL, 20-200µL)	General lab supplier	n/a
Adjustable Micropipettes (0.5-10µL, 2-20µL, 20-200µL, 100-1000µL)	General lab supplier	n/a
Centrifuges (for 0.6 to 5mL tubes and 96-well plates)	General lab supplier	n/a
PCR Hood	General lab supplier	n/a
Vortex	General lab supplier	n/a
Cold Blocks	General lab supplier	n/a
IsoFreeze PCR Racks	Thomas Scientific	1148D61
Racks for Microcentrifuge Tubes	General lab supplier	n/a
USB Flash Drive	General lab supplier	n/a

5 Warnings and Precautions

- For research use only - not for use in diagnostic procedures.
- Follow standard precautions. All samples and positive controls should be considered potentially infectious and handled accordingly.
- Do not eat, drink, smoke, apply cosmetics or handle contact lenses in areas where reagents and samples are handled.
- Use personal protective equipment such as, but not limited to, gloves and lab coats when handling kit reagents while performing this assay and handling materials including samples, reagents, pipettes, and other equipment and reagents.
- Dispose of unused kit reagents and samples according to institutional, local, and national regulations.
- Reagents used with this assay described in section 8 contain guanidinium thiocyanate or 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 assay.
- Before collection of the saliva sample, ensure that the person has not used oral hygiene products within at least 30 minutes prior to collection.

6 Reagent Storage, Handling, and Stability

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

7 Sample Collection, Handling, and Storage

The following sample types are considered acceptable for use with LumiraDx SARS-CoV-2 RNA STAR Complete: nasal swabs, mid-turbinate swabs, nasopharyngeal swabs, oropharyngeal swabs, and saliva samples.

7.1 Sample Collection

- Swab samples 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 samples should be collected and placed into appropriate transport media, such as viral transport media (VTM), 0.85% saline solution, or phosphate buffer saline (PBS – calcium and magnesium free). Swabs provided in up to 3 mL of compatible transport media are acceptable, but to save on reagents and improve performance, one (1) mL of buffer is suggested.
- For dry swab collection - Respiratory samples 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 sample, add 1 mL of compatible transport media (VTM, 0.85% Saline, or PBS), soak the swab for 30 seconds then swirl the solution thoroughly by rotating the swab against the side of the tube 5 times (beware of cross-contamination from splashing). Discard the swab in biohazard waste.
- Saliva Sample Collection – Saliva samples must be self-collected in a healthcare setting under the supervision of a healthcare provider. Avoid coughing prior to harvesting of the sample. No eating, drinking, smoking, chewing of gum or brushing teeth for at least 30 minutes prior to sampling. No dental work or examination performed 24 hours prior to saliva collection. A minimum of one (1) mL volume should be transferred to any vial, such as a 5 mL tube, that does not contain preservatives.

7.2 Transporting Samples

- Samples 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 samples.
- If wet swab is expressed in a compatible buffer (i.e. viral transport media (VTM), 0.85% saline solution, or phosphate buffer saline (PBS – calcium and magnesium free)) store samples at 2 to 8 °C for up to 72 hours after collection. If a delay in testing or shipping is expected, store samples at -70°C or below and ship on dry ice.
- Dry swabs may be shipped in a dry tube without cold chain, with stability established up to 3 days for dry polyester or foam swabs. Additionally, if a delay in testing or shipping is expected, dry swabs can be expressed in saline (1 mL) and frozen for longer storage. Store frozen samples at -70°C or below and ship on dry ice.
- Saliva samples may be shipped without a cold chain requirement up to 48-hours post-collection.

7.3 Storing Samples

- Dry swab samples are stable either at room temperature for up to 48-hours, or refrigerated (2 to 8 °C) for up to 72 hours, before processing. Wet swab samples can be stored at room temperature or refrigerated (2 to 8 °C) for up to 72 hours before processing.
- Samples received cold can be stored refrigerated (2 to 8 °C) for up to 72 hours, from time of collection, before processing.
- If samples cannot be assayed within 72 hours of collection, both dry-swab and wet-swab samples should be frozen at ≤ -70 °C.
- Saliva samples are stable at room temperature for up to 5 days.

8 LumiraDx SARS-CoV-2 RNA STAR Complete Preparation

8.1 Sample Preparation

This assay is compatible with swabs stored in an empty (dry) tube, with swabs stored in compatible transport media (VTM, 0.85% Saline, or PBS) or with saliva. 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 independent samples and both must be included as external controls in every plate. See Section 16.1 for more information about the LumiraDx SARS-CoV-2 RNA STAR Controls.

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

1. Thaw LumiraDx SARS-CoV-2 RNA STAR Complete Positive Control Media (Pos. Ctrl. Med.) and LumiraDx SARS-CoV-2 RNA STAR Complete Negative Control Media (Neg. Ctrl. Med.) on a cold block, vortex the Pos. Ctrl. Med. and Neg. Ctrl. Med. for 5 seconds then centrifuge for 5 seconds to collect reagents at the bottom of the tube.
2. To assemble the 1x PCM (Positive Control Media), freshly dilute 20.0 μ L Pos. Ctrl. Med. with 60.0 μ L Neg. Ctrl. Med. in a pre-chilled microcentrifuge tube. To assemble the 1x NCM (Negative Control Media), always freshly pipette 80.0 μ L Neg. Ctrl. Med. into a pre-chilled microcentrifuge tube.
3. Swab samples (24 μ L) or saliva samples (22.8 μ L) will be added directly to the sample plate prepared in Section 8.3 or Section 8.4, respectively.
 - a. 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.
 - b. For saliva samples, at minimum one (1) mL saliva can be collected in any vial, such as a 5 mL tube, that does not contain preservatives. Centrifuge the saliva sample at 6000 rpm for 1 to 5 minutes then immediately transfer the supernatant¹ (22.8 μ L) to the sample plate prepared in Section 8.4. The remainder of the supernatant (~ 500 μ L) can be transferred to a microcentrifuge tube for storage or retesting and will not need future spinning.

8.2 qSTAR Guidelines

NOTE: Amplification technologies such as qSTAR, like PCR, are sensitive to accidental introduction of product from previous amplification reactions. Incorrect results could occur if either the sample 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 samples.
- 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 samples.
- 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 Saliva Reagent, 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.

¹ The pellet after spinning for 1 min may not be visible. Make sure to aspirate the supernatant from the top of the liquid level.

8.3 qSTAR Reagent Preparation – Swab Samples

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 thermocycler (i.e. Roche LC 480 II, ABI 7500 Fast Dx, ABI QS 5, ABI QS 7 Flex, ABI QS 7 Pro, Bio-Rad CFX96, Agilent AriaMx, or the Agilent Stratagene Mx3005P) prior to performing the instructions below to ensure the performance of this assay is maintained.

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

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

4. Assuming one reaction is needed, perform the following to make the Reaction Mix:
 - a. Combine 10.0 µL Salt Mix and 1.2 µL IC/P Mix in a pre-chilled microcentrifuge tube, mix by slowly pipetting up and down 4 times without introducing bubbles (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 4 to 6 times without introducing bubbles, centrifuge briefly to collect contents at bottom of tube, then place tube back on the cold block.
5. 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. 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 20 seconds to collect contents at bottom of plate.
6. Place the 96-well plate in a thermocycler and follow instrument specific protocols and analysis procedures detailed below.

8.4 qSTAR Reagent Preparation – Saliva Samples

It is necessary to make excess Saliva Extraction Mix and Reaction Mix to allow for pipetting error. Additionally, it is recommended to read through the following instructions before attempting to set up the Saliva Extraction Mix and 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 thermocycler (i.e. Roche LC 480 II, ABI 7500 Fast Dx, ABI QS 5, ABI QS 7 Flex, ABI QS 7 Pro, Bio-Rad CFX96, Agilent AriaMx, or the Agilent Stratagene Mx3005P) prior to performing the instructions below to ensure the performance of this assay is maintained.

1. Thaw the LumiraDx SARS-CoV-2 RNA STAR Complete kit components in a cold block equilibrated between 2 to 8 °C; i.e. LumiraDx SARS CoV-2 RNA STAR Complete Saliva Reagent, 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. Determine the number of reactions (N) to be prepared per assay:

Saliva Extraction Mix Setup	1 Rxn	100 Rxns	N Rxns
Saliva Reagent	1.2 µL	120 µL	N x 1.2 µL
Extraction Buffer	4.8 µL	480 µL	N x 4.8 µL
Total Volume	6.0 µL	600 µL	N x 6.0 µL

3. Assuming one reaction is needed, combine 1.2 µL Saliva Reagent and 4.8 µL Extraction Buffer to create the Saliva Extraction Mix in a pre-chilled microcentrifuge tube, mix by slowly pipetting up and down 4 to 6 times without introducing bubbles, then place tube back on the cold block.
4. Transfer 22.8 µL of saliva supernatant prepared in Step 3b of Section 8.1 and transfer 22.8 µL of external controls prepared in Step 2 of Section 8.1 into an appropriate, pre-chilled, 96-well plate. Add 6.0 µL Saliva Extraction Mix, per well of sample and external controls, and mix by slowly pipetting up and down 10 times without introducing bubbles. The addition and mixing of Saliva Extraction Mix 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.
5. Determine the number of reactions (N) to be prepared per assay:

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

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 (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 sample 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 thermocycler and follow instrument specific protocols and analysis procedures detailed below.

8.5 Stand-alone Instructions for Processing of a Single Sample

The following instructions provide an example for the processing of a single sample (can be a dry swab sample, a wet swab sample, or a saliva sample) 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 thermocycler (i.e. Roche LC 480 II, ABI 7500 Fast Dx, ABI QS 5, ABI QS 7 Flex, ABI QS 7 Pro, Bio-Rad CFX96, Agilent AriaMx, or the Agilent Stratagene Mx3005P) prior to performing the instructions below to ensure the performance of this assay is maintained.

1. Thaw the LumiraDx SARS-CoV-2 RNA STAR Complete kit components in a cold block equilibrated between 2 to 8 °C; i.e. LumiraDx SARS CoV-2 RNA STAR Complete Positive Control Media (Pos. Ctrl. Med.), LumiraDx SARS CoV-2 RNA STAR Complete Negative Control Media (Neg. Ctrl. Med.), LumiraDx SARS CoV-2 RNA STAR Complete Saliva Reagent, 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).
- 2a. **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 sample is provided wet, up to 3 mL of compatible transport media (VTM, 0.85% Saline, or PBS) is acceptable, but this higher volume may impact sensitivity.
- 2b. **For saliva sample**, at minimum one (1) mL saliva can be collected in any vial (e.g. 5 mL tube) that does not contain preservatives. Immediately before use, centrifuge the saliva sample at 6000 rpm for 1 to 5 minutes. If storage is needed, the supernatant (~ 500 µL) can be transferred to a microcentrifuge tube and will not need future spinning.
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 samples prepared in Step 2a or 22.8 µL of saliva supernatant prepared in Step 2b 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.
- 5a. **For swab sample**, add 4.8 µL of Extraction Buffer 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.
- 5b. **For saliva sample**, premix in a suitable pre-chilled tube (i.e. microcentrifuge tube or 5 mL tube) the Saliva Extraction Mix by combining N x 1.2 µL Saliva Reagent and N x 4.8 µL Extraction Buffer, then slowly pipette up and down 4 to 6 times without introducing bubbles and centrifuge. Transfer 6.0 µL Saliva Extraction Mix to the 96-well plate and mix by slowly pipetting up and down 10 times without introducing bubbles.
6. Determine the number of reactions (N) to be prepared per assay and prepare Reaction Mix in a suitable pre-chilled tube by following the order in the table below. Between each reagent, slowly mix by pipetting up and down 4 to 6 times without introducing bubbles and pulse centrifuge.

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

7. Transfer 31.2 µL of Reaction Mix to each well with sample and external controls. Mix by slowly pipetting up and down 10 times without introducing bubbles. Seal the 96-well plate using an appropriate optically clear adhesive and centrifuge the plate at 2000 rpm for 10 seconds to collect contents at bottom of plate.
8. Place the 96-well plate in a thermocycler and follow instrument specific protocols and analysis procedures detailed below.

8.6 Stand-alone Instructions for Sample Processing in a Deepwell Format

The following instructions provide an example for the processing of dry swab samples, using a deepwell plate, up to the final setup of the sample plate. The following language assumes 94 dry swab samples 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 thermocycler (i.e. Roche LC 480 II, ABI 7500 Fast Dx, ABI QS 5, ABI QS 7 Flex, ABI QS 7 Pro, Bio-Rad CFX96, Agilent AriaMx, or the Agilent Stratagene Mx3005P) prior to performing the instructions below to ensure the performance of this assay is maintained.

1. Thaw the LumiraDx SARS-CoV-2 RNA STAR Complete kit components in a cold block equilibrated between 2 to 8 °C; i.e. LumiraDx SARS CoV-2 RNA STAR Complete Positive Control Media (Pos. Ctrl. Med.), LumiraDx SARS CoV-2 RNA STAR Complete Negative Control Media (Neg. Ctrl. Med.), LumiraDx SARS CoV-2 RNA STAR Complete Salt Mix, LumiraDx SARS CoV-2 RNA STAR Complete Extraction Buffer, LumiraDx SARS CoV-2 RNA STAR Complete Internal Control & Primer Mix (IC/P Mix), and LumiraDx SARS CoV-2 RNA STAR Complete Master Mix. Invert each tube to mix then centrifuge for 5 seconds to collect reagents at the bottom of the tube (do not vortex samples).
2. Pour 100 mL of a compatible transport media (VTM, 0.85% Saline, or PBS) into a suitable reagent reservoir. Transfer one (1) mL to each deepwell using a multi-channel pipette. Leave two designated wells, A1 and A12, empty for the external controls assembled in Step 4.
3. Add a single dry swab sample 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 should be loaded with the NCM and position A12 should be loaded with the PCM.
5. Mix the sample 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 sample at the bottom of the well.
6. Assuming 96 reactions are needed, prepare Reaction Mix in a pre-chilled 5mL tube by following the order in the table below. Between each reagent, slowly mix by pipetting up and down 4 to 6 times without introducing bubbles.

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

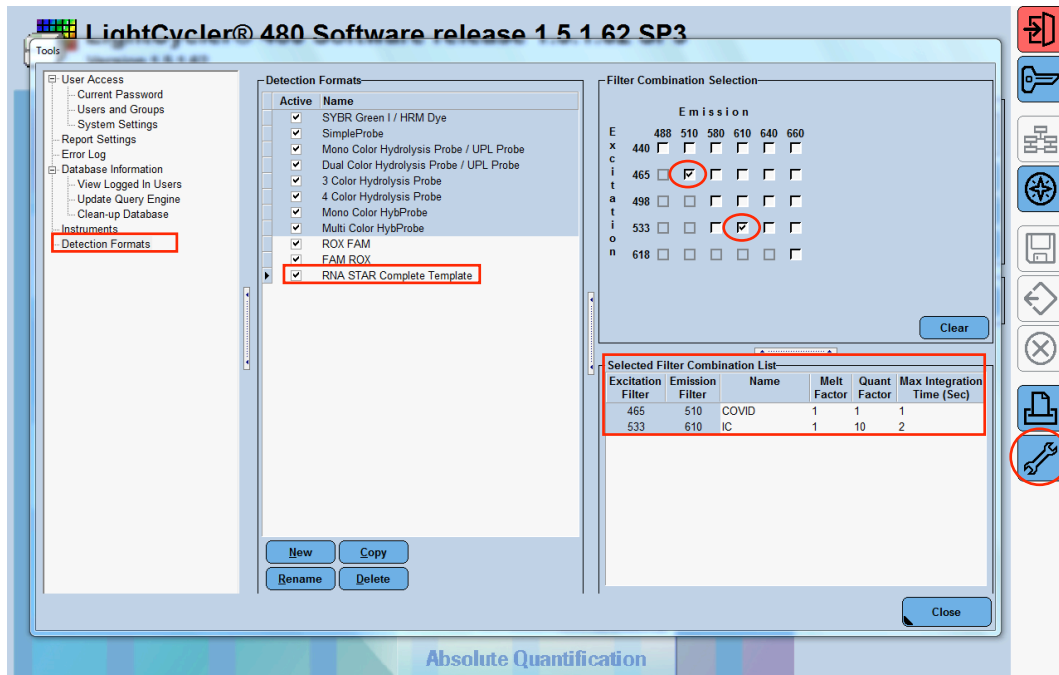
7. Transfer Reaction Mix to a pre-chilled reagent reservoir (for minimal dead volume) using a single channel pipette. Then, using a multi-channel pipette, transfer 31.2 µL of Reaction Mix to each well with sample and external controls. Mix by slowly pipetting up and down 10 times without introducing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

9 RNA STAR Complete Setup for Roche™ LightCycler 480 II

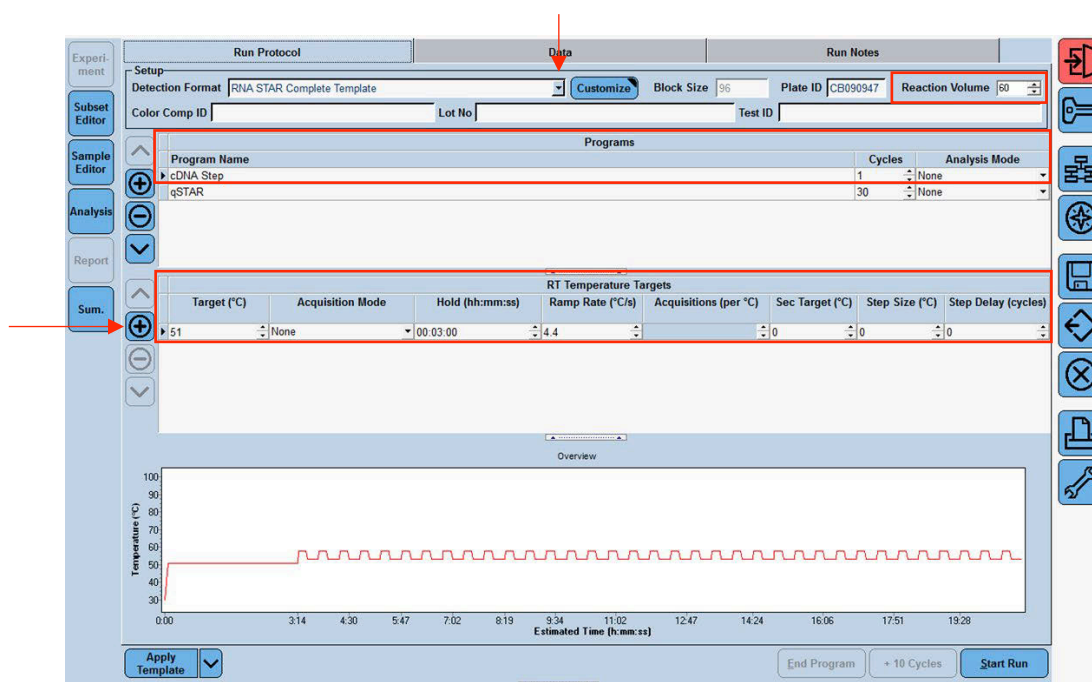
Refer to "User Manual Part Number 05152062001 0208" for additional information. The instrument programming instructions are intended for setup of an entire 96-well plate. If the entire plate is not used, please deselect wells as appropriate during analysis.

9.1 Programming the Run Template and Sample Template

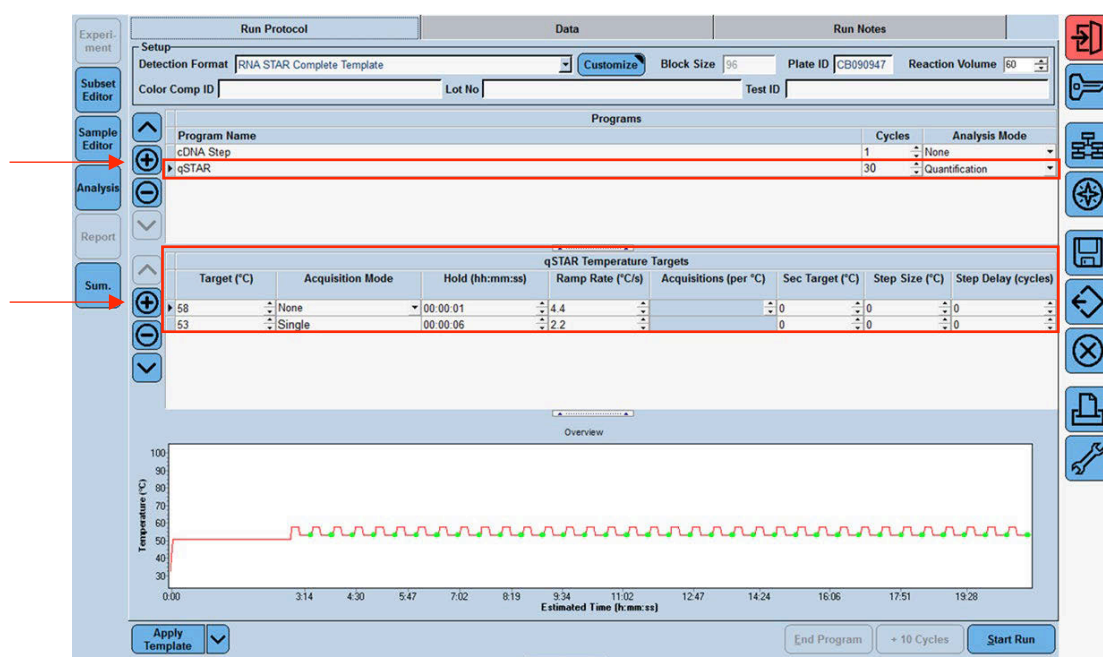
1. Launch the LightCycler (LC) 480 desktop software (version SW 1.5.1). If prompted, 'Log On' to the 'Traceable Database'.



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



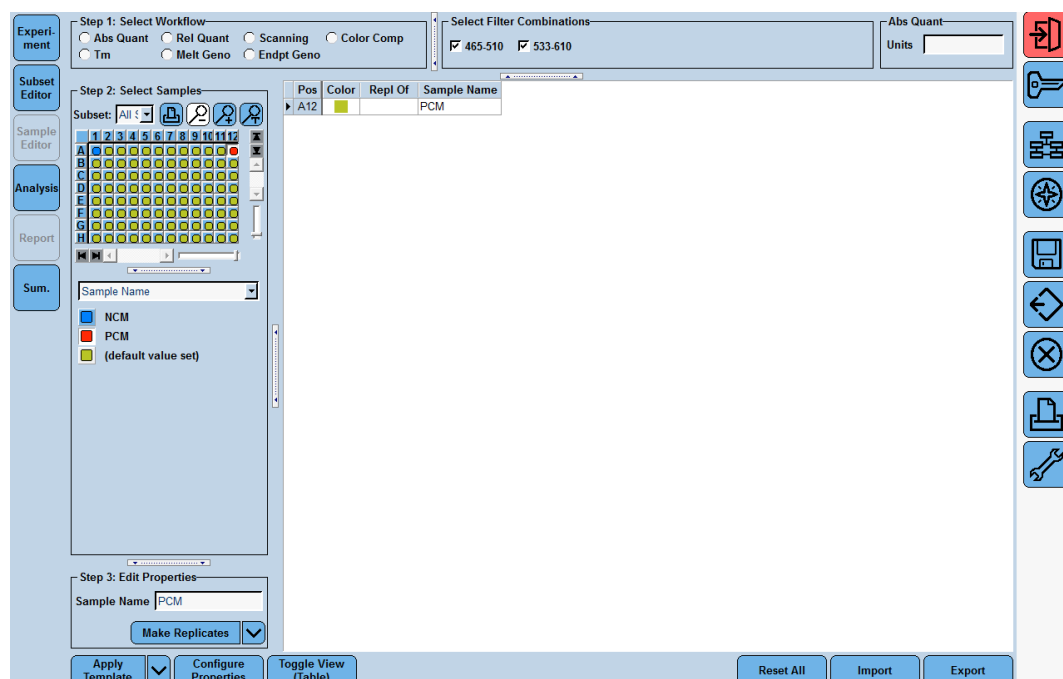
4. Select from the 'Detection Format' drop-down menu "RNA STAR Complete Template". Set 'Reaction Volume' to '60' in the upper right of the software. In the 'Program Name' section type "cDNA Step", set 'Cycles' to '1', and set 'Analysis Mode' to 'None'. Set cDNA Step 'RT Temperature Targets' as follows: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



5. Click the "+" button on the upper left of the software to add another 'Programs'. In the 'Program Name' section type "qSTAR", set 'Cycles' to '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 '0:00:01', 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 '0:00:06', set 'Ramp Rate (°C/s)' to '2.2', and set 'Sec Target (°C)', 'Step Size (°C)', and 'Step Delay (cycles)' to '0'.

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

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



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

9.2 Programming Initial Run and Creating Analysis Template

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

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

1. Launch the LightCycler (LC) 480 desktop software (version SW 1.5.1).
2. Click 'New Experiment from Template' and in the 'Run Templates' drop-down menu, select 'RNA STAR Complete Template' then click the 'Check' button to exit the 'Create Experiments from Template' window. Proceed to the 'Sample Editor' tab on the left of the software.
3. Select 'Apply Template' (do not select the drop-down menu next to the 'Apply Template' button) on the bottom left of the screen. Double-click the Templates folder then double-click the 'Sample Templates' folder and choose the 'RNA STAR Complete Sample Template'. Click the 'Check' button to exit the 'Apply Template' window. The NCM and PCM should be loaded into positions A1 and A12 of the plate. If not already selected, select all wells. Individual 'Sample Names' can be entered in the 'Step 3: Edit Properties' section if desired.
4. On the LightCycler 480 II instrument, push the Load Plate "↔" 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 is off' warning may appear. Click the 'Check' button to exit this warning. In the 'Save LightCycler 480 Experiment' window that opens, double-click the 'Experiments' folder and save under the 'Name' "RNA STAR Complete [YYMMDD_Plate#]" then click the 'Check' button to exit this window.
6. After the run has finished, proceed to the 'Analysis' tab on the left of the software. Choose 'Abs Quant/Fit Points' in the 'Create new analysis' section. Click the 'Check' button to exit the 'Create New Analysis' pop-up window.
7. Click the 'Background (2-6)' button on the software. Set 'Min Offset' to '0' and 'Max Offset' to '3' and click the 'Check' button to exit the 'Background Settings' window.
8. Confirm that 'Color Compensation' is '(Off)' for all analytes on the bottom of the software and confirm 'First Cycle' is set to '1' and 'Last Cycle' is set to '35'. Proceed to the 'Noise Band' tab at the upper middle of the software.
9. Select from the pull-down menu next to the 'Noiseband (Auto)' button at the bottom of the software select 'Noiseband (STD Mult)'. Click 'Filter Comb (456-510)' and select 'COVID'. Click the 'Check' button to exit the 'Filter Combination' window. Set 'STD Multiplier' to '22.0000'. Click 'Filter Comb (456-510)' and select 'IC'. Click the 'Check' button to exit the 'Filter Combination' window. Set 'STD Multiplier' to '22.0000'.
10. Save the new analysis protocol as a template for future use. In the lower left corner of the screen select the pull-down menu next to the 'Apply Template' button and click 'Save As Template'. Double-click the 'Templates' Folder. Select the 'Analysis Templates' Folder and 'Name' the file "RNA STAR Complete Analysis Template" and click the 'Check' button to exit the 'Save Template' window. Click 'Calculate' at the bottom of the software.
11. To create a report, select the 'Save' button on the right of the software. If prompted, define 'Enter the reason for the changes' as "Report" and click the 'Check' button to exit the window. Proceed to the 'Report' tab on the left of the software.
12. Select the box next to 'Results' in the 'General' tab under the 'Abs Quant/Fit Points' section. Click 'Generate' then click the 'Save' button on the right of the software. If prompted, define 'Enter the reason for the changes' as "Save" and click the 'Check' button to exit the window.
13. Remove the plate from the LightCycler 480 II instrument using the Load Plate "←→" button on the front of the instrument.
14. Remove the plate and discard the plate in a sealable waste bag or container.

9.3 Using the Run / Sample / Analysis Template

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

1. Launch the LightCycler (LC) 480 desktop software (version SW 1.5.1).
2. Click 'New Experiment from Template' and in the 'Run Templates' drop-down menu select 'RNA STAR Complete Template' then click the 'Check' button to exit the 'Create Experiments from Template' window. Proceed to the 'Sample Editor' tab on the left of the software.
3. Select 'Apply Template' (do not select the drop-down menu next to the 'Apply Template' button) on the bottom left of the screen. Double-click the Templates folder then double-click the 'Sample Templates' folder and choose the 'RNA STAR Complete Sample Template'. Click the 'Check' button to exit the 'Apply Template' window. The NCM and PCM should be loaded into positions A1 and A12 of the plate. If not already selected, select all wells. Individual 'Sample Names' can be entered in the 'Step 3: Edit Properties' section if desired.
4. On the LightCycler 480 II instrument, push the Load Plate "←→" 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 is off' warning may appear. Click the 'Check' button to exit this warning. In the 'Save LightCycler 480 Experiment' window that opens, double-click the 'Experiments' folder and save under the 'Name' "RNA STAR Complete [YYMMDD_Plate#]" then click the 'Check' button to exit this window.

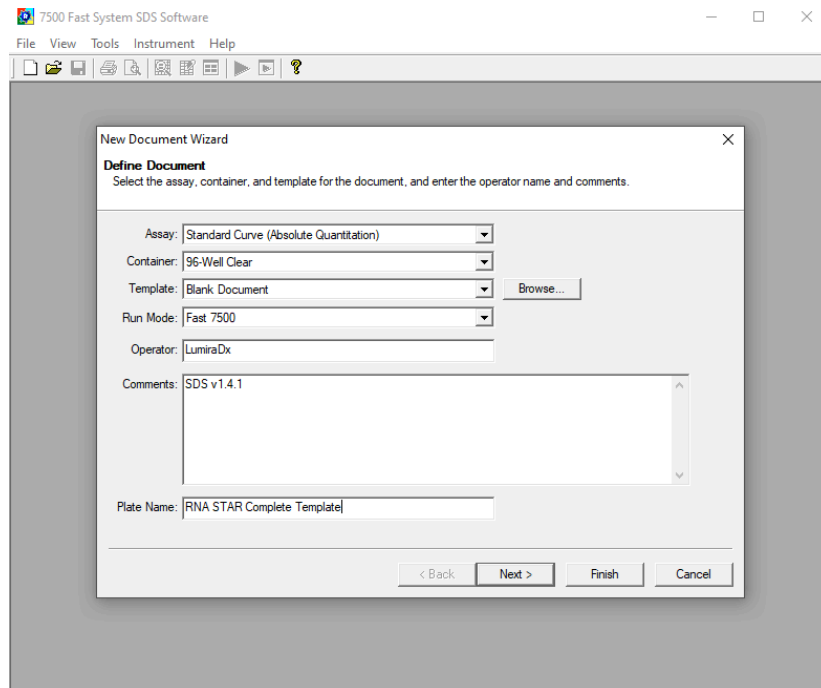
6. After the run has finished, proceed to the 'Analysis' tab on the left of the software. Choose 'Abs Quant/Fit Points' in the 'Create new analysis' section. Click the 'Check' button to exit the 'Create New Analysis' pop-up window.
7. In the lower left corner click 'Apply Template' (do not select the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

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

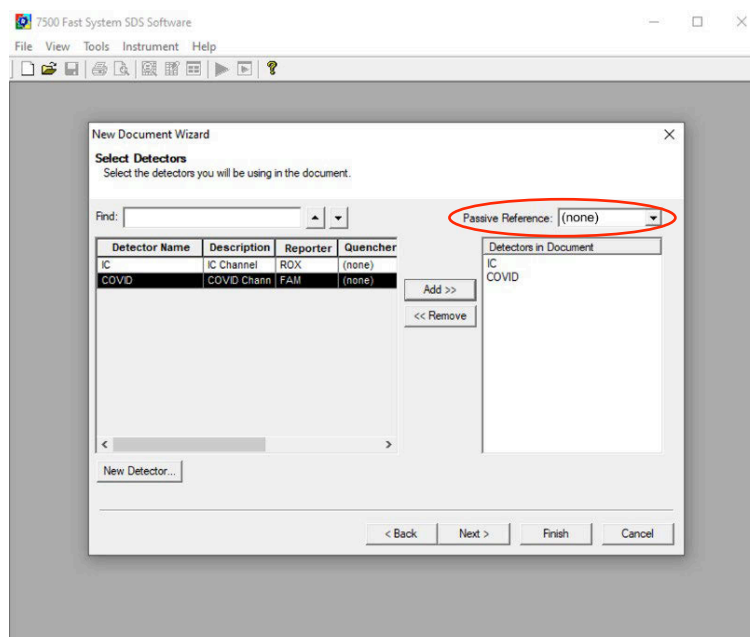
Refer to "User Manual Part Number 4406991" for additional information. The instrument programming instructions are intended for setup of an entire 96-well plate. If the entire plate is not used, please deselect wells as appropriate during analysis.

10.1 Programming the Run Template

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

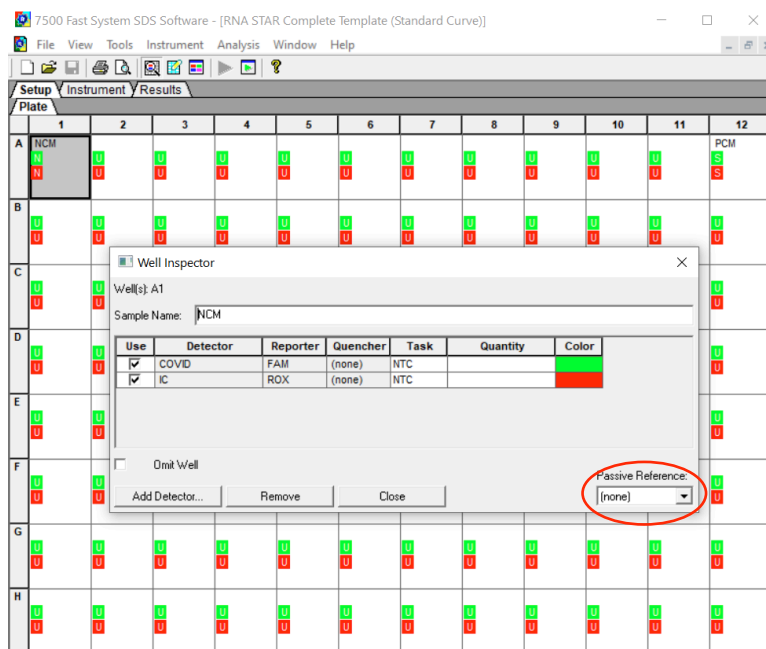


2. The 'Quick Startup document' window will open. Click 'Create New Document' to start the 'New Document Wizard'. In the 'Define Document' window verify the default settings: 'Assay' is set to 'Standard Curve (Absolute Quantification)', 'Container' is set to '96-Well Clear', 'Template' is set to 'Blank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



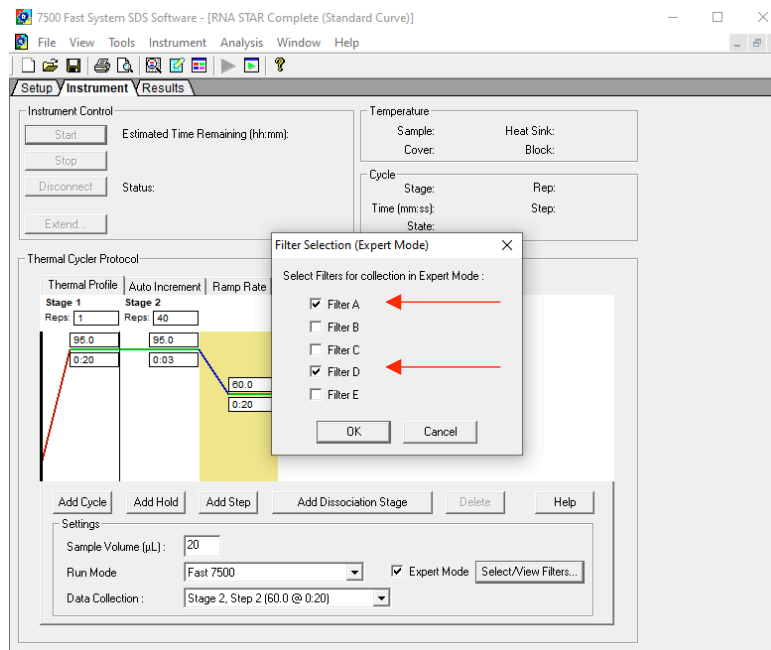
- In the 'Select Detectors' window, click 'New Detector' to open the 'New Detector' pop-up window and define these settings: define 'Name' as "COVID", define 'Description' to "COVID Channel", set 'Reporter' dye to 'FAM', set 'Quencher' to '(none)', and set 'Color' to 'Green'. Select 'OK'. Click 'New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

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

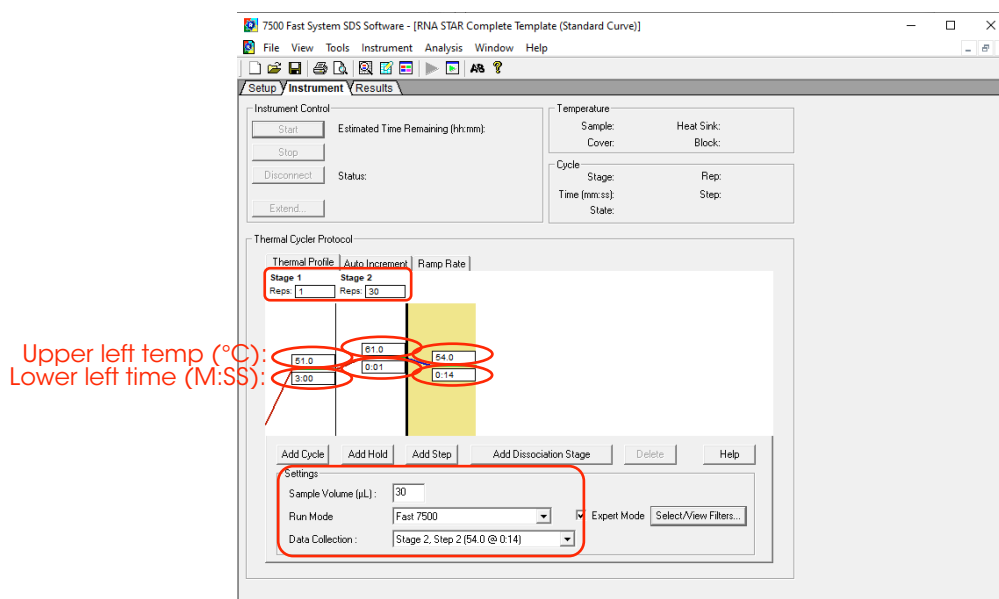


- In the 'Set Up Sample Plate' window click the gray box between "A" and "1" to select all wells then select checkboxes under the 'Use' section to add the COVID and IC Detector to all wells. Click 'Finish'. The wizard will close, and a screen will display the 'Setup' tab and 'Plate' tab. This will show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

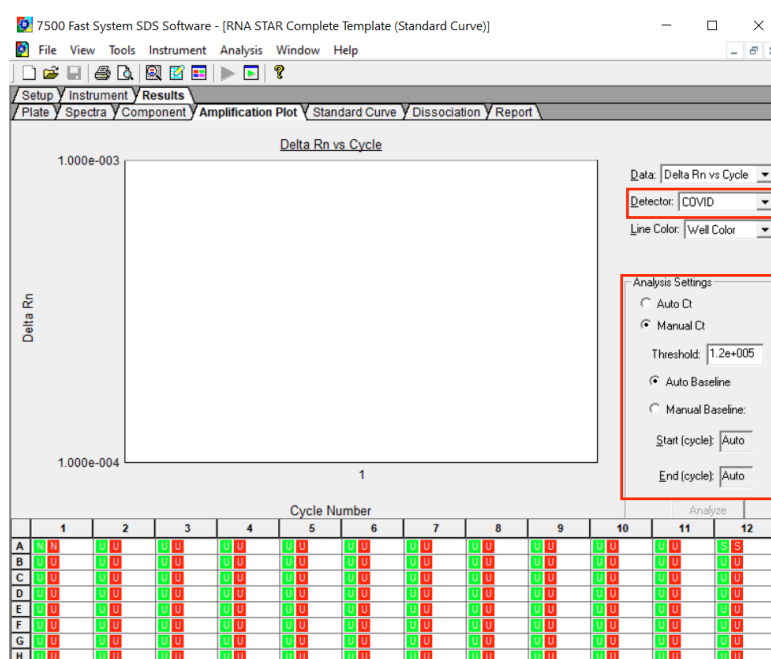
- Select location 'A12', right click and select 'Well Inspector'. In the 'Well Inspector' window, define the 'Sample Name' as "PCM" (Positive Control Media). Select the 'COVID' Detector line, under 'Task' select from the drop-down menu 'Standard'. Select the 'IC' Detector line, under 'Task' select from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



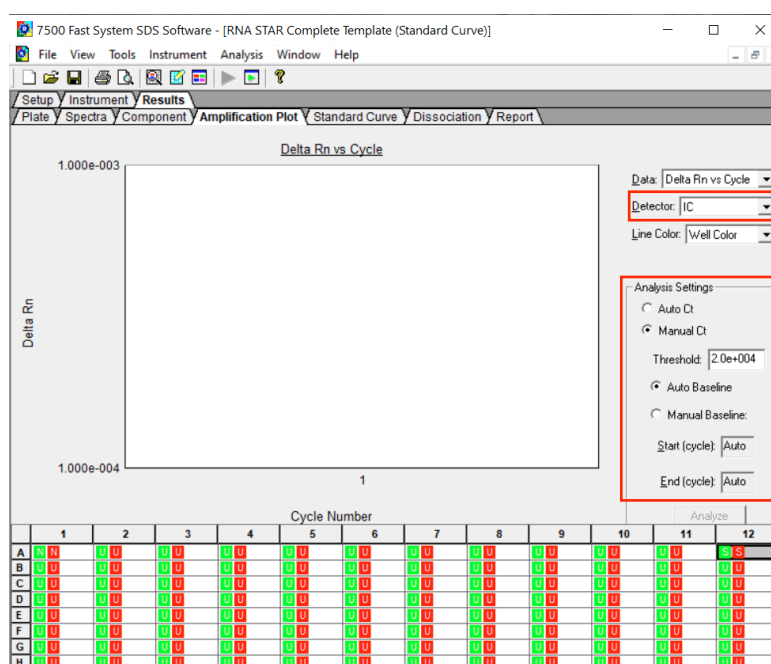
- Select the 'Instrument' tab (do not select 'Instrument' from the menu), check the box next to 'Expert Mode' then click 'Select/View Filters...' and check only the boxes for 'Filter A' and 'Filter D'. Click 'OK'.



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



8. Select the 'Amplification Plot' tab. From the 'Detector' drop-down menu in the top right corner, select 'COVID'. In the 'Analysis Settings' window, select the 'Manual Ct' circle and define 'Threshold' as "1.2e+005". Confirm that the 'Auto Baseline' circle is selected.



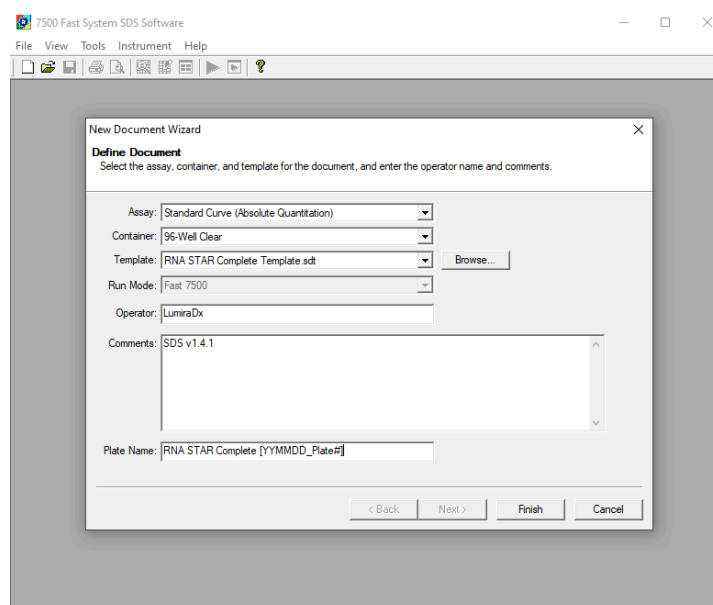
9. From the 'Detector' drop-down menu again, select 'IC' from the 'Detector'. In the 'Analysis Settings' section, select the 'Manual Ct' circle and define 'Threshold' as "2.0e+004". Confirm that the 'Auto Baseline' circle is selected.
10. Select 'File' from the menu then click 'Save As'. Save the template in file path "D:\Applied Biosystems\7500 Fast System\Template\" and hit the 'Enter' key to confirm file path. 'Name' the file as "RNA STAR Complete Template". Change 'File Type' to 'SDS Templates (*.sdt)'. Click 'Save'.
11. Exit the software. A prompt may appear to 'Save Changes to Plate 1'. Click 'No'.

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

10.2 Programming Runs Using the Run Template

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

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



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

11 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.

11.1 Programming Instructions for PC/Laptop-connected Instrument

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

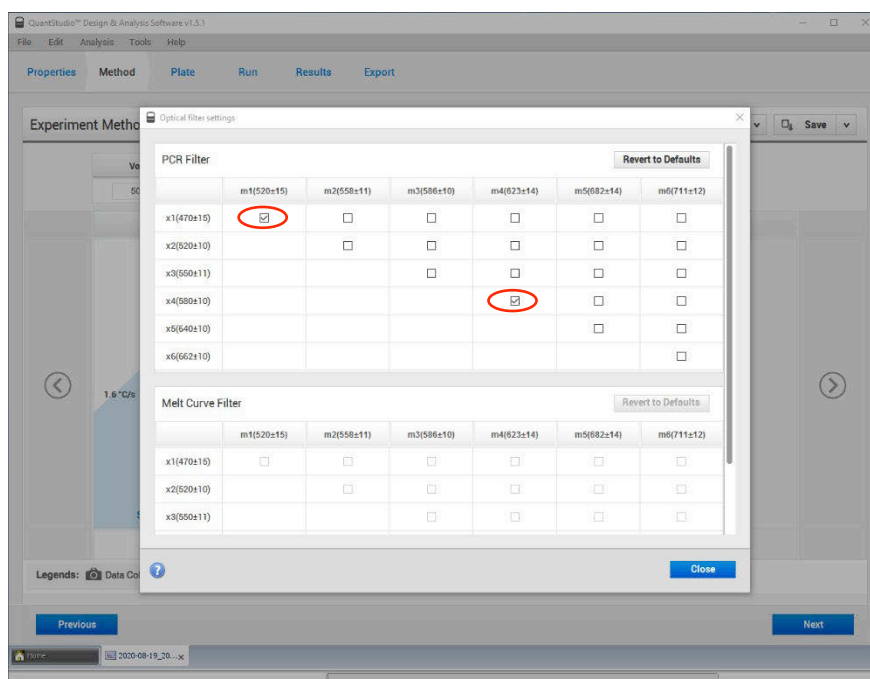
1. If the instrument touchscreen is in sleep mode (dark screen), touch the screen anywhere to activate the instrument. Sleep mode lowers the temperature of the heated cover. It is important that the heated cover idle at 105°C before loading a 96-well plate.

The screenshot shows the 'Experiment Properties' window in the QuantStudio™ Design & Analysis Software v1.5.1. The window has a menu bar (File, Edit, Analysis, Tools, Help) and a tab bar (Properties, Method, Plate, Run, Results, Export). The 'Properties' tab is selected. The 'Experiment Properties' section includes a 'Save' button and a 'Comments - optional' text area. The following fields are populated:

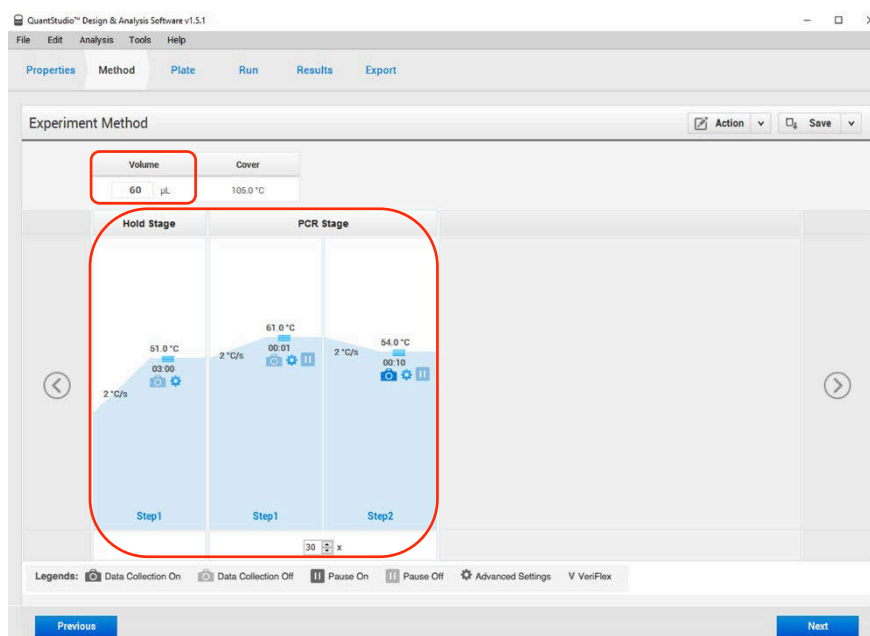
Field	Value
Name	RNA STAR Complete Template
Barcode	Barcode - optional
User name	User name - optional
Instrument type	QuantStudio™ 5 System
Block type	96-Well 0.2-mL Block
Experiment type	Standard Curve
Chemistry	Other
Run mode	Standard

A 'Next' button is located at the bottom right of the window.

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

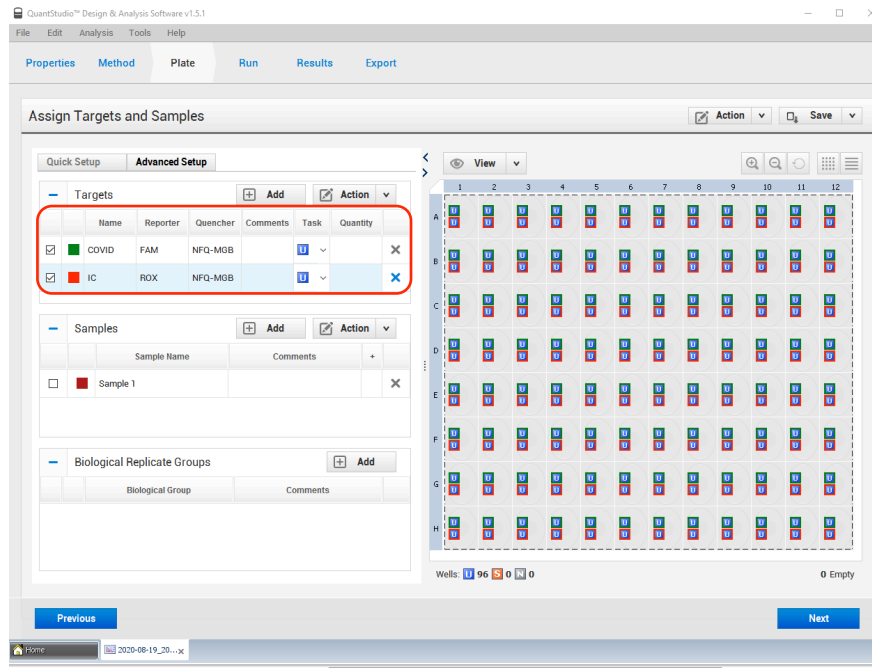


- Click 'Action' and select 'Optical filter settings' from the drop-down menu. In the 'PCR Filter' table, uncheck all filter combinations except the following: x1(470±15), m1(520±15), x4(580±10), m4(623±14)



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

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



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

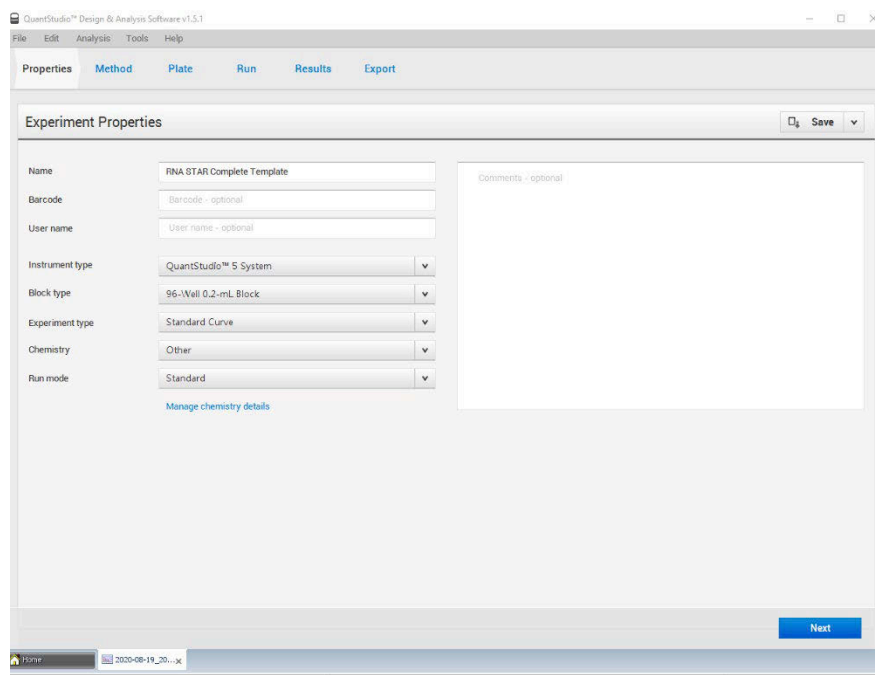
11.2 Analysis Instructions

1. Modify the analysis settings using the 'Results' tab in the 'QuantStudio Design & Analysis' Desktop Software (version v1.5.1). Select the desired wells for analysis in the Plate Layout then click on the Eye symbol above the 'Amplification Plot' to configure the plot as follows: set 'Plot Type' to ' ΔR_n vs Cycle', set 'Graph Type' to 'Log', and set 'Plot Color' to 'Target'. All other content remains unchanged. Click out of the window to accept changes.
2. Click the 'Gear' icon to the right of the 'Analyze' button in the top left of the software to open the 'Analysis Settings' window. In the 'Ct settings' tab, uncheck the 'Default settings' box under the 'Ct settings for COVID' section, uncheck the 'Automatic Threshold' box, and uncheck the 'Automatic Baseline' box. Enter "80,000" for the 'Threshold', set 'Baseline Start Cycle' to '1' and set 'End Cycle' to '4'. Select the 'IC' line then uncheck the 'Default settings' box, the 'Automatic Threshold' box, and the 'Automatic Baseline' box. Enter "15,000" for the 'Threshold', set 'Baseline Start Cycle' to '1' and set 'End Cycle' to '4'. Click 'Apply' to proceed the 'Export' tab.
3. Define the 'File Name' for the exported file ("RNA STAR Complete [YYMMDD_Plate#]"). Define the 'File name', choose the 'File Type' (default is QuantStudio as an .xls file), choose the 'Location' to export the file, and choose the 'Content' to be exported (the 'Results' box needs to be checked for Ct values). Click 'Customize' to proceed to what is to be exported within each Content item.
4. Select the 'All Fields' box to uncheck all content then check the boxes for the following content: 'Well', 'Well Position', 'Sample Name', 'Target Name', 'Task', 'Reporter', 'Quencher', and 'CT'. Click 'Close' to return to the 'Export' tab screen.
5. Click 'Save' to save the modified settings.
6. Click the 'Export' button to generate the export data file. The exported file will include a 'Results' section which contains the sample Ct values.
7. Close the software.

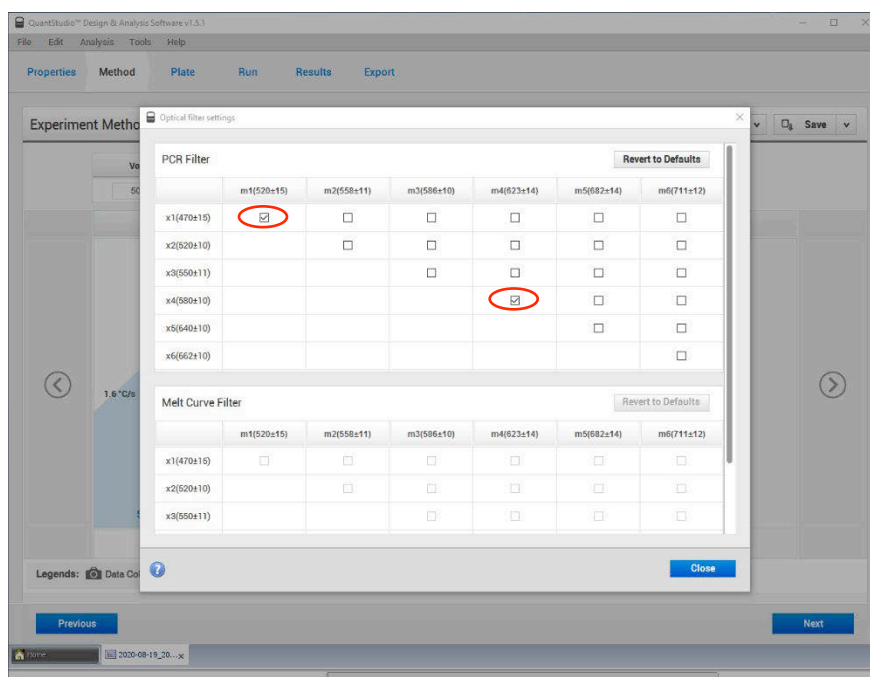
11.3 Programming Instructions for Stand-Alone Instrument

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

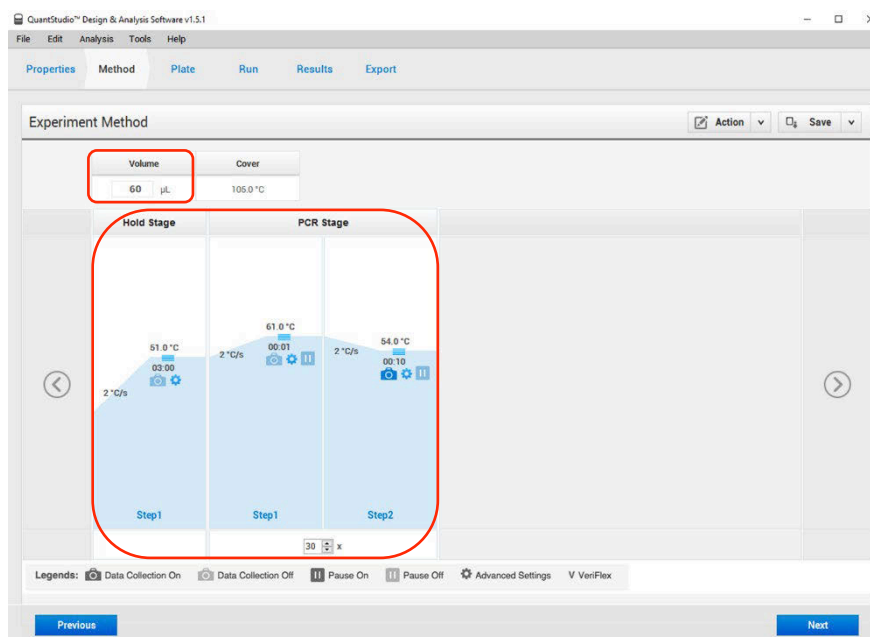
1. If the instrument touchscreen is in sleep mode (dark screen), touch the screen anywhere to activate the instrument. Sleep mode lowers the temperature of the heated cover. It is important that the heated cover idle at 105°C before loading a 96-well plate.



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



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



5. Click 'Close' to return to the 'Method' tab. Set the 'Volume' to '60 µL'. In the 'Hold Stage' section, hover over 'Step 2' and click the "-" button that appears just above the wording to delete this plateau. In the 'Hold Stage' section, change 'Step 1' settings as follows: set ramp rate to '2 °C/s', set temp to '51 °C', and set time to '03:00'. In the 'PCR Stage' section, change 'Step 1' settings as follows: set ramp rate to '2 °C/s', set temp to '61 °C', and set time to '00:01'. In the 'PCR Stage' section, change 'Step 2' settings as follows: set ramp rate to '2 °C/s', set temp to '54 °C', and set time to '00:10' (data collection step). At the bottom of the 'PCR Stage' section, set 'Cycles' to '30'. Then proceed to the 'Plate' tab.
6. Change 'Passive Reference' to 'None' in the 'Plate Attributes' window. Select all the wells from the plate layout then proceed to the 'Advanced Setup' tab in the 'Assign Targets and Samples' section.
7. Under the 'Targets' section, rename 'Target 1' to "COVID", change the default color to green, select 'FAM' as the 'Reporter' (the 'Quencher' is automatically entered as 'NFQ-MGB'), and checkmark the empty box to the left of the colored box to apply it to the wells. 'Add' a second target then rename to "IC", change the default color to red, select 'ROX' as the 'Reporter' (the 'Quencher' is automatically entered as 'NFQ-MGB'), and checkmark the empty box to the left of the colored box to apply it to the wells.
8. (Optional) Under the 'Samples' section, the 'Sample Name' can be added individually or pasted into the 96-well plate layout from an Excel file. Then proceed to the 'Run' tab.
9. Save the experiment as a template for subsequent runs by clicking the 'Save' down arrow and selecting the 'Save As' option. Name the template "RNA STAR Complete Template" and click 'Save'.
10. Copy the template (.edf) file to a USB drive.
11. Insert the USB drive into the designated slot in the front of the QuantStudio 5 instrument. On the instrument touchscreen, press 'Load Experiment'. Select 'USB' from the options provided, scroll to locate the saved template (.edf) file and select the file to import it.
12. Change the 'Run File Name' to "RNA STAR Complete [YYMMDD_Plate#]" then press 'Start Run'.
13. An alert will prompt the user to insert the sample plate. Press 'Open Drawer' to open instrument drawer, place the sample plate on the amplification block. Make sure to align A1 on the 96-well plate with the defined A1 position on the instrument drawer. Press 'Close Drawer' to close the instrument drawer and press 'Start Run'.
14. Following the run, the instrument touchscreen indicates when the run is 'Complete'. Press 'Transfer file' to export the file to the USB drive and remove the USB drive from the instrument.
15. Open the instrument drawer to remove the plate by pressing the arrow icon at the top right of the instrument touch screen. The instrument drawer will open.
16. Remove the plate and discard the plate in a sealable waste bag or container.

11.4 Analysis Instructions

1. Launch the QuantStudio Design & Analysis Desktop Software (version v1.5.1). Click 'Open' in the 'Open Existing Experiment' section then locate the experiment file (.eds) to be analyzed and click 'Open'.
2. Modify the analysis settings using the 'Results' tab in the QuantStudio Design & Analysis Desktop Software (version v1.5.1). Select the desired wells for analysis in the Plate Layout then click on the Eye symbol above the 'Amplification Plot' to configure the plot as follows: set 'Plot Type' to ' ΔR_n vs Cycle', set 'Graph Type' to 'Log', and set 'Plot Color' to 'Target'. All other content remains unchanged. Click out of the window to accept changes.
3. Click the 'Gear' icon to the right of the 'Analyze' button in the top left of the software to open the 'Analysis Settings' window. In the 'Ct settings' tab, uncheck the 'Default settings' box under the 'Ct settings for COVID' section, uncheck the 'Automatic Threshold' box, and uncheck the 'Automatic Baseline' box. Enter "80,000" for the 'Threshold', set 'Baseline Start Cycle' to '1' and set 'End Cycle' to '4'. Select the 'IC' line then uncheck the 'Default settings' box, the 'Automatic Threshold' box, and the 'Automatic Baseline' box. Enter "15,000" for the 'Threshold', set 'Baseline Start Cycle' to '1' and set 'End Cycle' to '4'. Click 'Apply' to proceed the 'Export' tab.
4. Define the 'File Name' for the exported file ("RNA STAR Complete [YYMMDD_Plate#]"). Define the 'File name', choose the 'File Type' (default is QuantStudio as an .xls file), choose the 'Location' to export the file, and choose the 'Content' to be exported (the 'Results' box needs to be checked for Ct values). Click 'Customize' to proceed to what is to be exported within each Content item.
5. Select the 'All Fields' box to uncheck all content then check the boxes for the following content: 'Well', 'Well Position', 'Sample Name', 'Target Name', 'Task', 'Reporter', 'Quencher', and 'CT'. Click 'Close' to return to the 'Export' tab screen.
6. Click 'Save' to save the modified settings. Click the 'Export' button to generate the export data file. The exported file will include a 'Results' section which contains the sample Ct values.
7. Close the software.

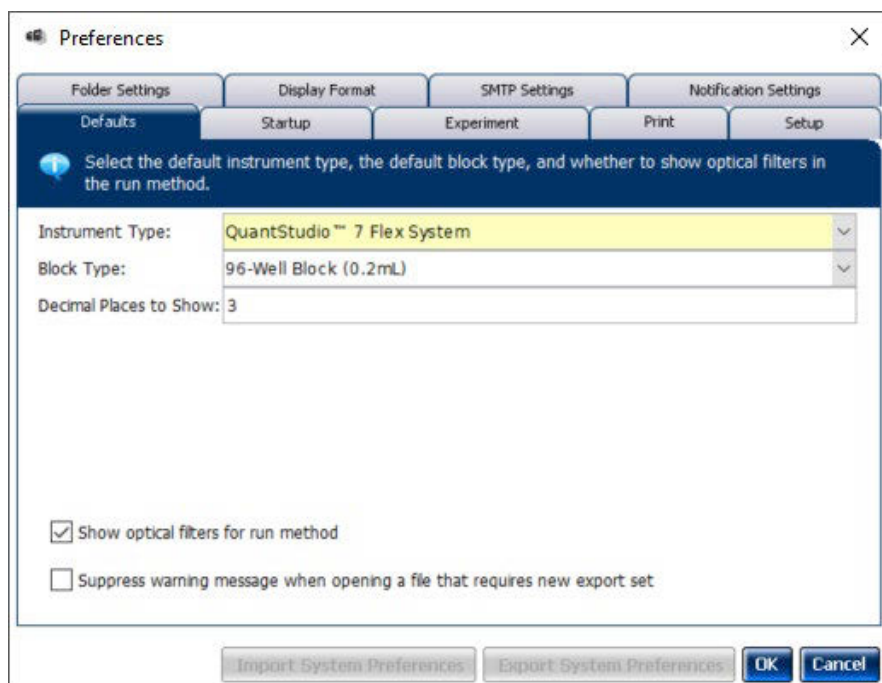
12 RNA STAR Complete Setup for Applied Biosystems™ QuantStudio 7 Flex

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.

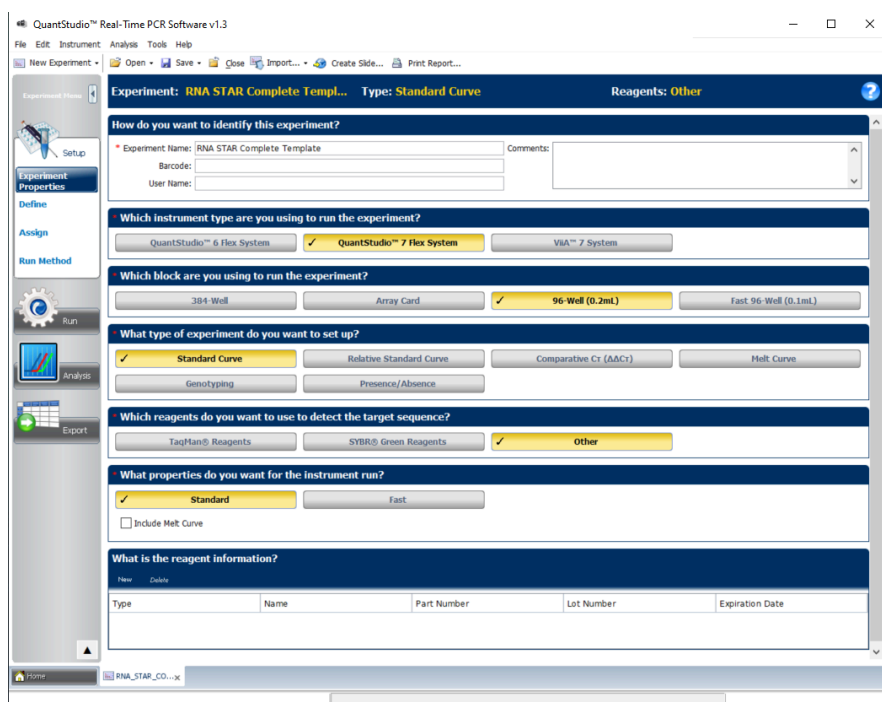
12.1 Programming Instructions for PC/Laptop-connected Instrument

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

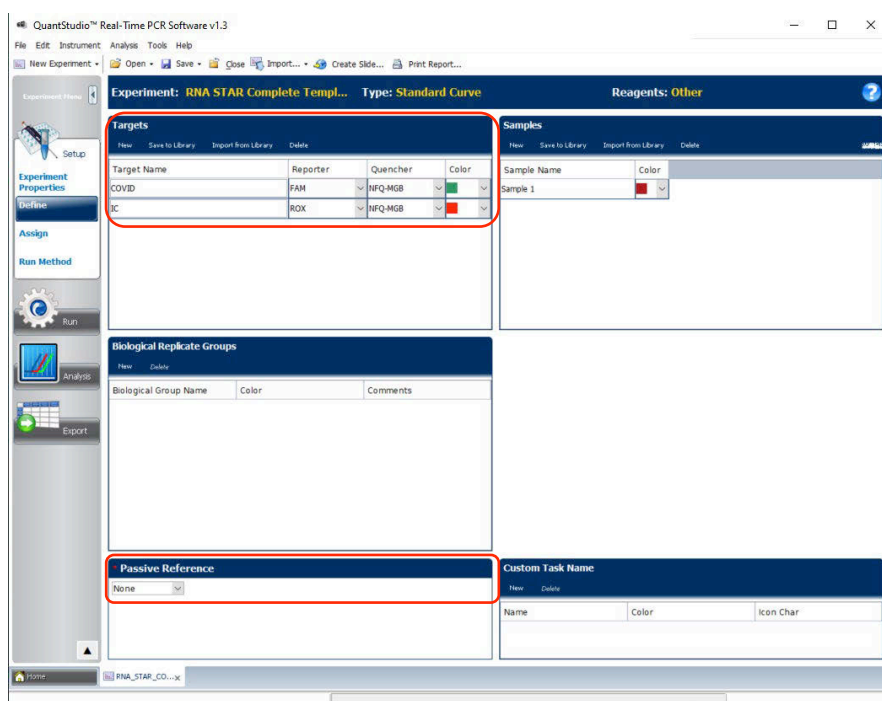
1. If the instrument touchscreen is in sleep mode (dark screen), touch the screen anywhere to activate the instrument. Sleep mode lowers the temperature of the heated cover. It is important that the heated cover idle at 105°C before loading a 96-well plate.
2. Launch the 'QuantStudio Real-Time PCR Software'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



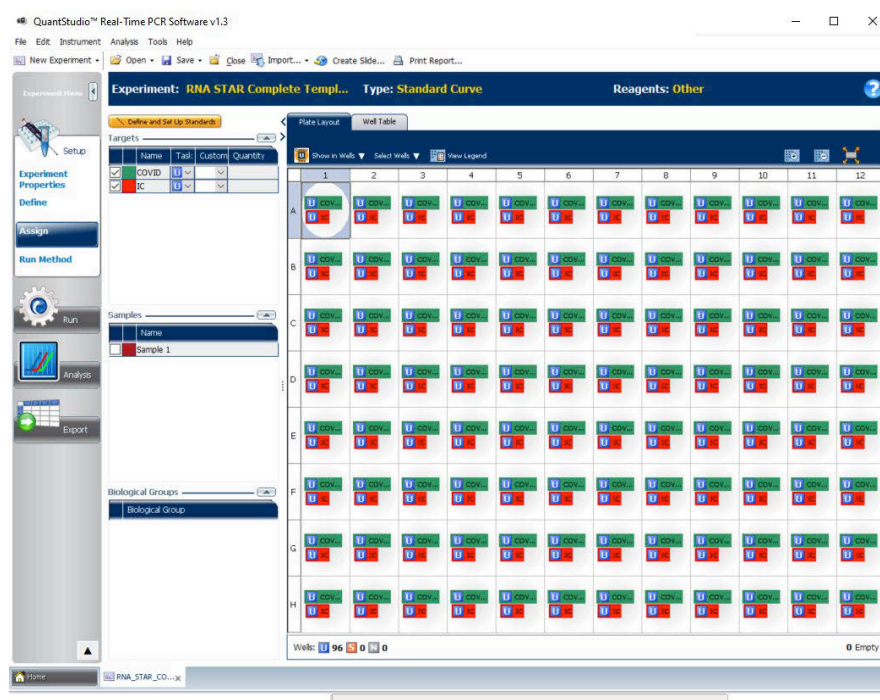
3. Confirm that the 'Instrument Type:' is set to 'QuantStudio™ 7 Flex System', the 'Block Type:' is set to '96-Well Block (0.2mL)', and that the 'Decimal Places to Show:' is set to '3'. Click the box next to 'Show optical filters for run method' to select this feature. Click on 'OK'.
4. Select 'Create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



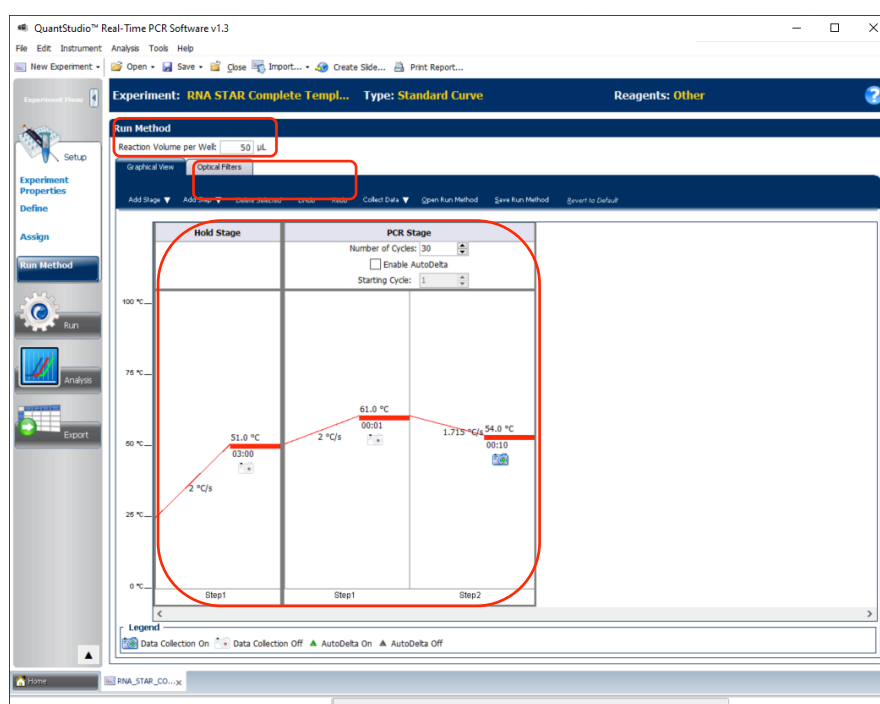
- On the left of the screen, the 'Experiment Properties' tab will be selected. Enter the 'Experiment Properties' as follows: define the 'Experiment Name' as "RNA STAR Complete Template", select the instrument type as 'QuantStudio™ 7 Flex System', select the block being used for the run as '96-Well (0.2mL)',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



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



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



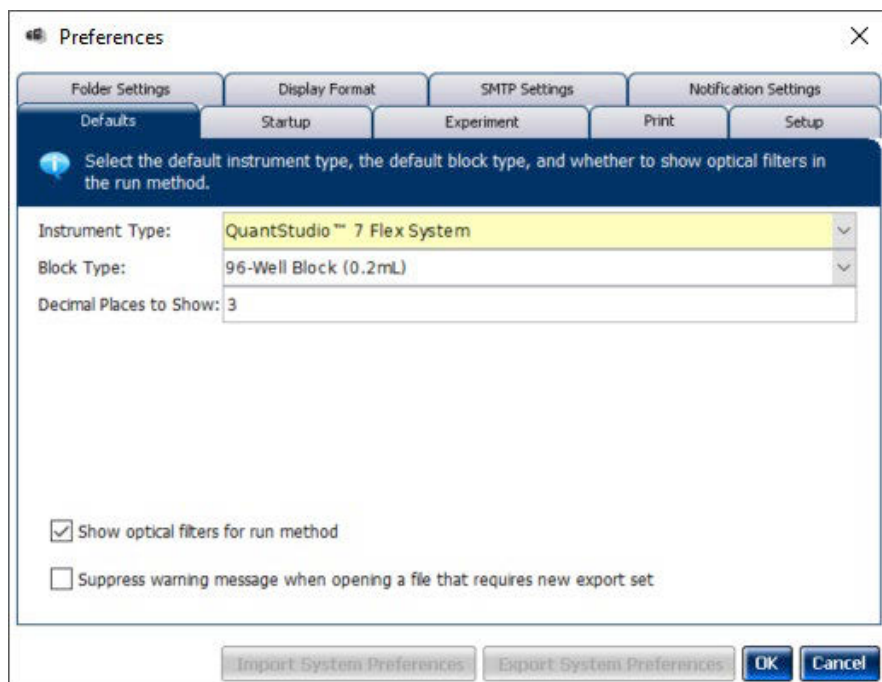
9. Under the 'Run Method' section, define the 'Reaction Volume per Well' to "50" (µL). In the 'Graphical View' section, select the 'Hold Stage' and delete one step by clicking the step and click 'Delete Selected' so one 'Hold Stage' segment remains. Change the 'Hold Stage' 'Step 1' settings as follows: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

5. Click 'Save' to save the modified settings.
6. Click the 'Export' button to generate the export data file. The exported file will include a 'Results' section which contains the sample Ct values.
7. Close the software.

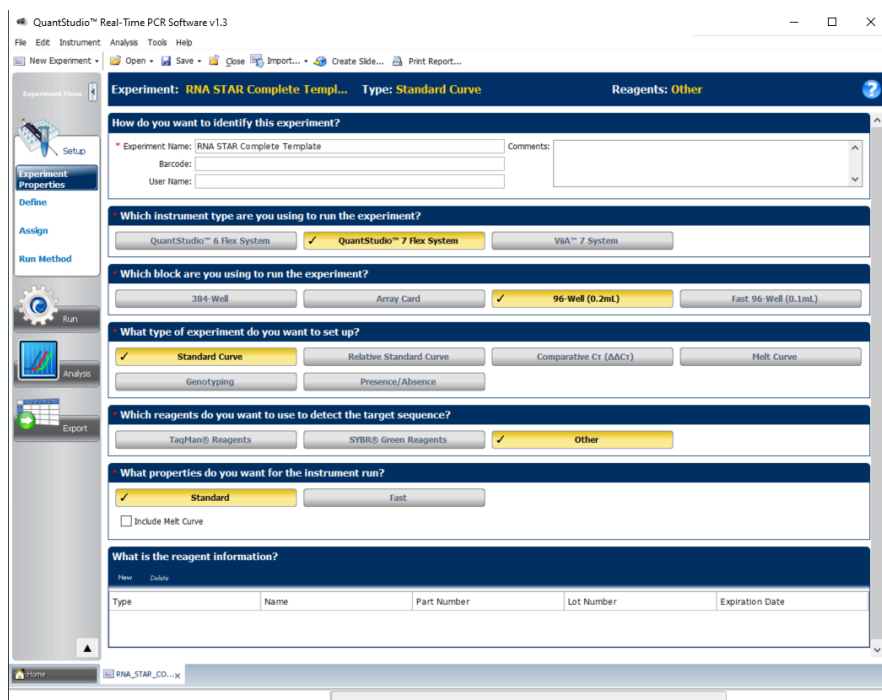
12.3 Programming Instructions for Stand-Alone Instrument

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

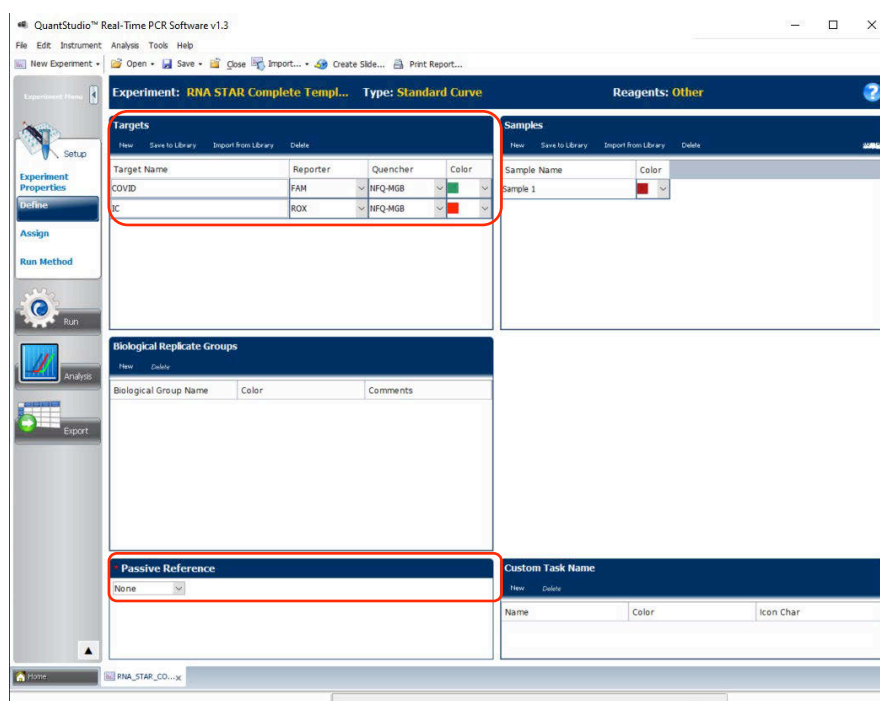
1. If the instrument touchscreen is in sleep mode (dark screen), touch the screen anywhere to activate the instrument. Sleep mode lowers the temperature of the heated cover. It is important that the heated cover idle at 105°C before loading a 96-well plate.
2. Launch the 'QuantStudio Real-Time PCR Software'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



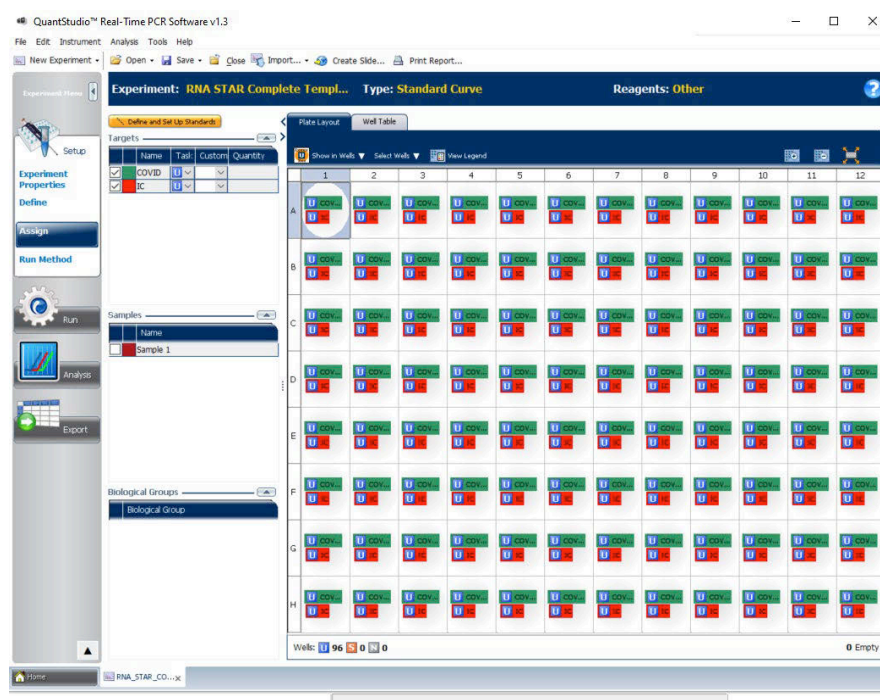
3. Confirm that the 'Instrument Type:' is set to 'QuantStudio™ 7 Flex System', the 'Block Type:' is set to '96-Well Block (0.2mL)', and that the 'Decimal Places to Show:' is set to '3'. Click the box next to 'Show optical filters for run method' to select this feature. Click 'OK'.
4. Select 'Create New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



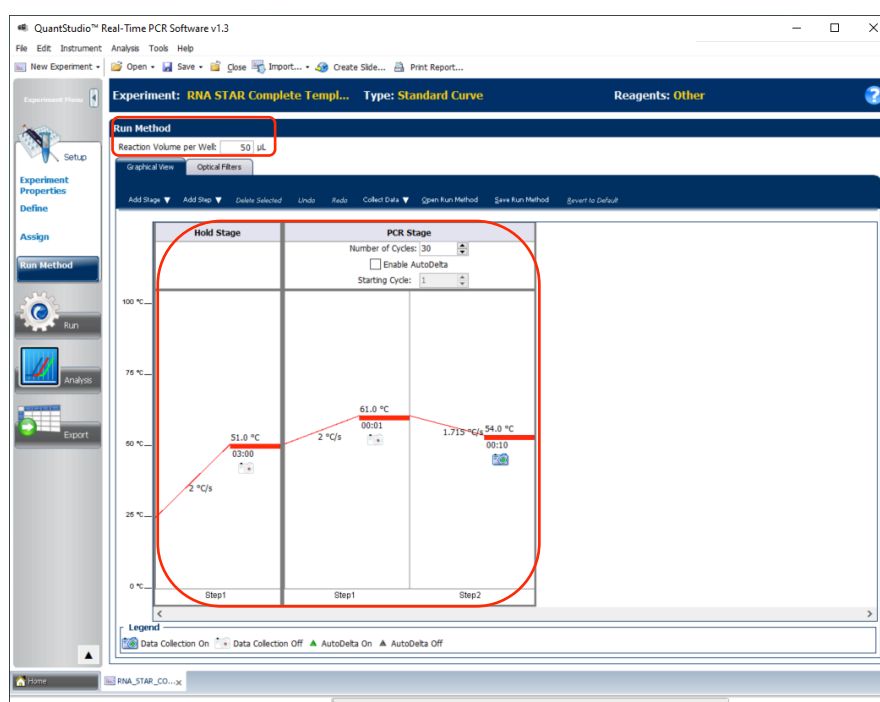
- On the left of the screen, the 'Experiment Properties' tab will be selected. Enter the 'Experiment Properties' as follows: define the 'Experiment Name' as "RNA STAR Complete Template", select the instrument type as 'QuantStudio™ 7 Flex System', select the block being used for the run as '96-Well (0.2mL)',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



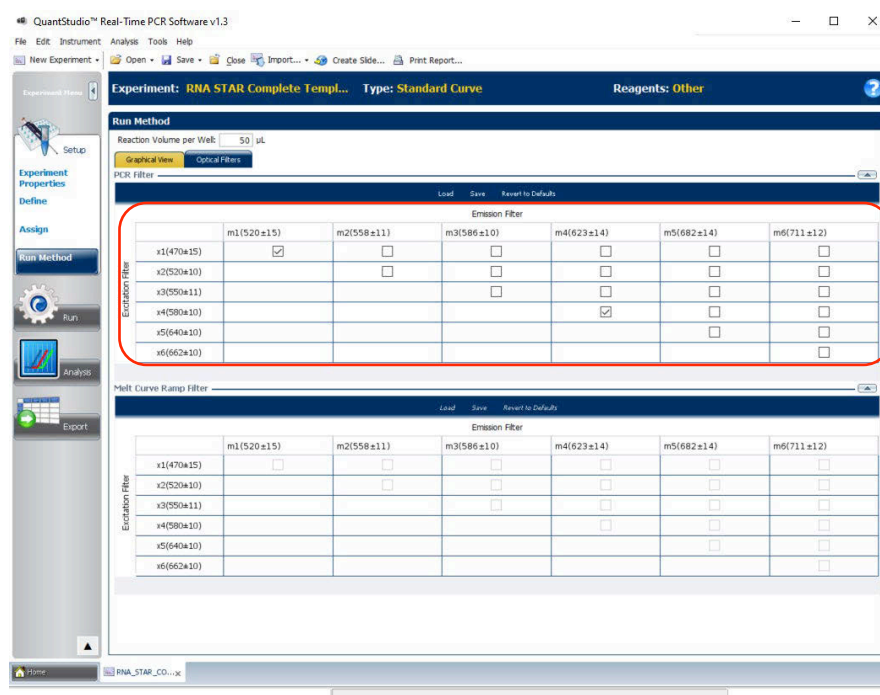
- Under the 'Targets' section: define the 'Target Name' for 'Target 1' as "COVID", select 'FAM' as the 'Reporter', change the 'Color' to green, and confirm the 'Quencher' is automatically entered as 'NFQ-MGB'. Add a second target by clicking 'New' (just above 'Target Name') then rename to 'IC', change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



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



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



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

12.4 Analysis Instructions

1. Launch the 'QuantStudio Real-Time PCR Software' Desktop Software (version v1.3). Click 'Analyze Experiment' in the 'Analyze' section then locate the experiment file (.eds) to be analyzed and click 'Open'.
2. Modify the analysis settings using the 'Results' tab in the 'QuantStudio Real-Time PCR Software' Desktop Software (version v1.3). Select the desired wells for analysis in the Plate Layout then configure the 'Amplification Plot' as follows: set 'Plot Type' to 'ΔRn vs Cycle', set 'Graph Type' to 'Log', and set 'Plot Color' to 'Target'.
3. Click the 'Analysis Settings' to the right of the 'Analyze' button in the top left of the software to open the 'Analysis Settings' window. In the 'Ct settings' tab, uncheck the 'Default settings' box under the 'Ct settings for COVID' section, uncheck the 'Automatic Threshold' box, and uncheck the 'Automatic Baseline' box. Enter "80,000" for the 'Threshold', set 'Baseline Start Cycle' to '1' and set 'End Cycle' to '3'. Select the 'IC' line then uncheck the 'Default settings' box, the 'Automatic Threshold' box, and the 'Automatic Baseline' box. Enter "15,000" for the 'Threshold', set 'Baseline Start Cycle' to '1' and set 'End Cycle' to '3'. Click 'Apply' to proceed to the 'Export' tab.
4. Define the 'File Name' for the exported file ("RNA STAR Complete [YYMMDD_Plate#]"). Define the 'File name', choose the 'File Type' (default is QuantStudio as an .xls file), choose the 'Location' to export the file, and choose the 'Content' to be exported (the 'Results' box needs to be checked for Ct values). Click 'Customize' to proceed to what is to be exported within each Content item.

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

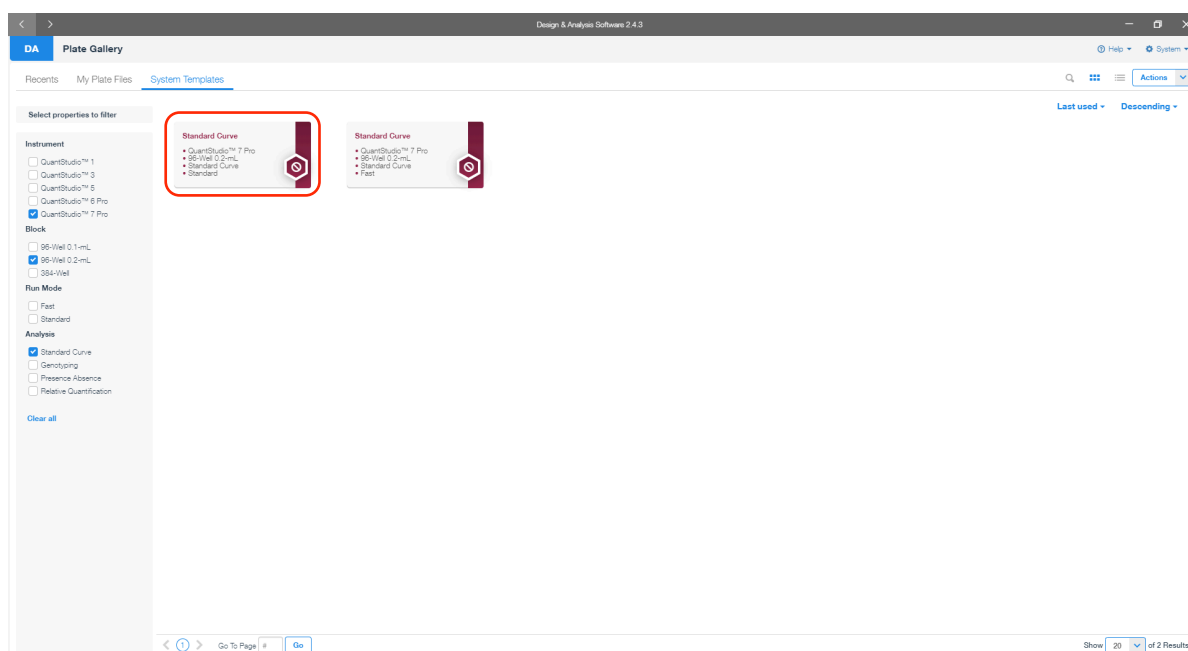
13 RNA STAR Complete Setup for Applied Biosystems™ QuantStudio 7 Pro

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.

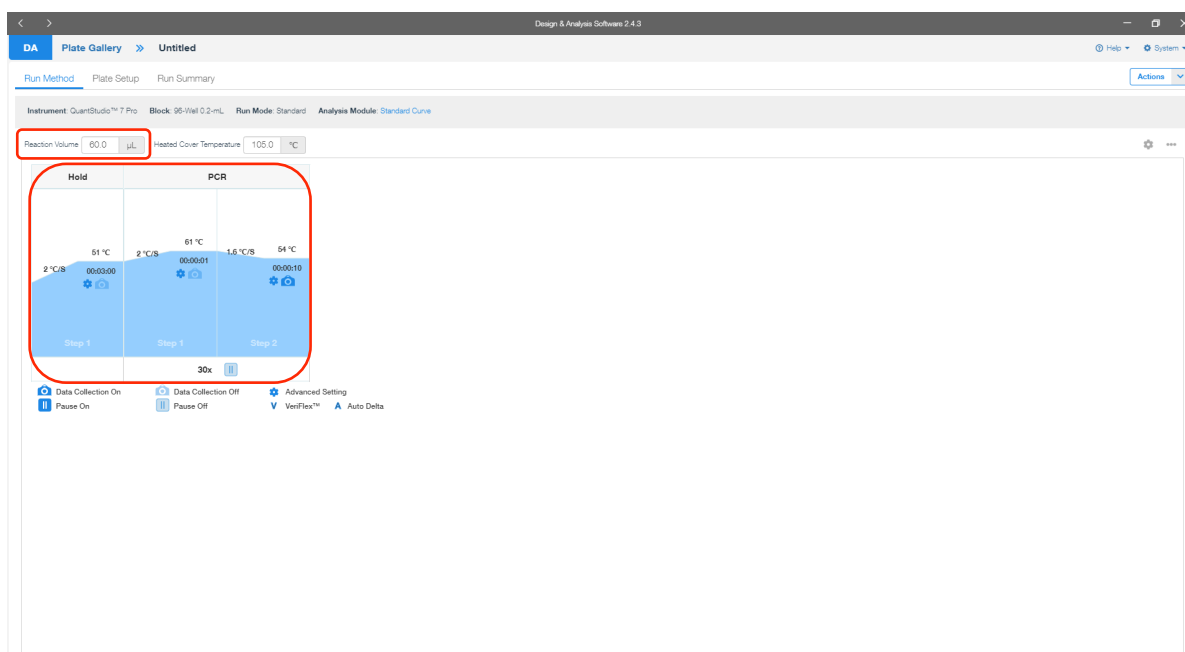
13.1 Programming Instructions for PC/Laptop-connected Instrument

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 recommended by the instrument manufacturer (see Option for PCR Instruments & Recommended Consumables table above).

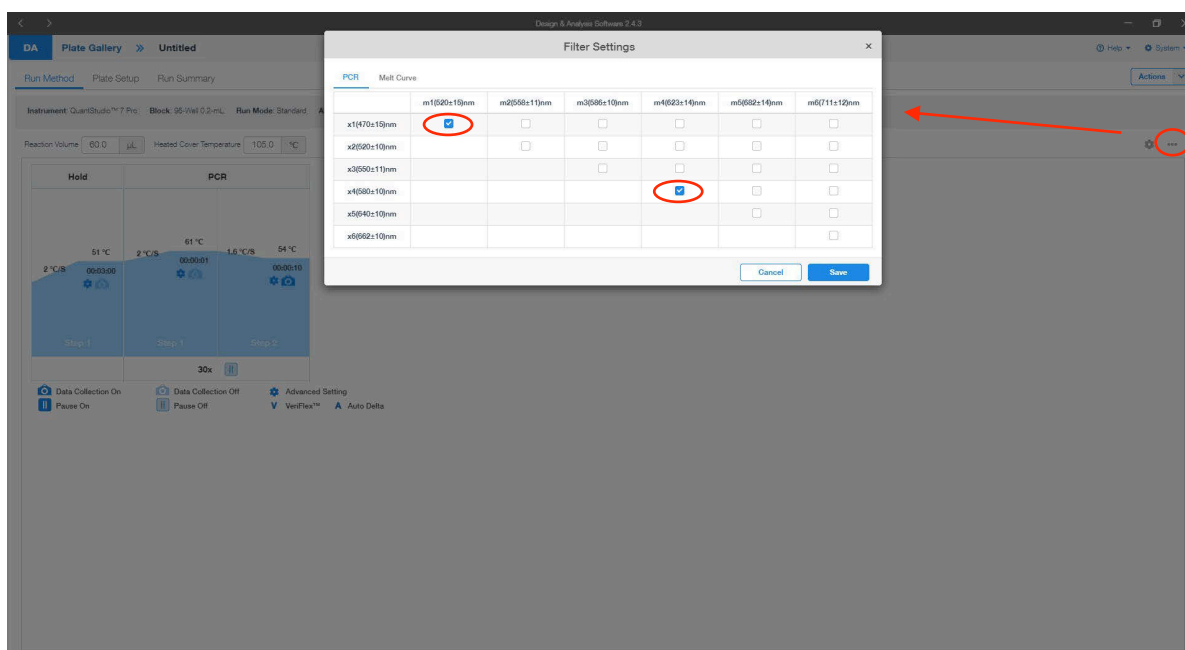
1. If the instrument touchscreen is in sleep mode (dark screen), touch the screen anywhere to activate the instrument. Sleep mode lowers the temperature of the heated cover. It is important that the heated cover idle at 105°C before loading a 96-well plate.



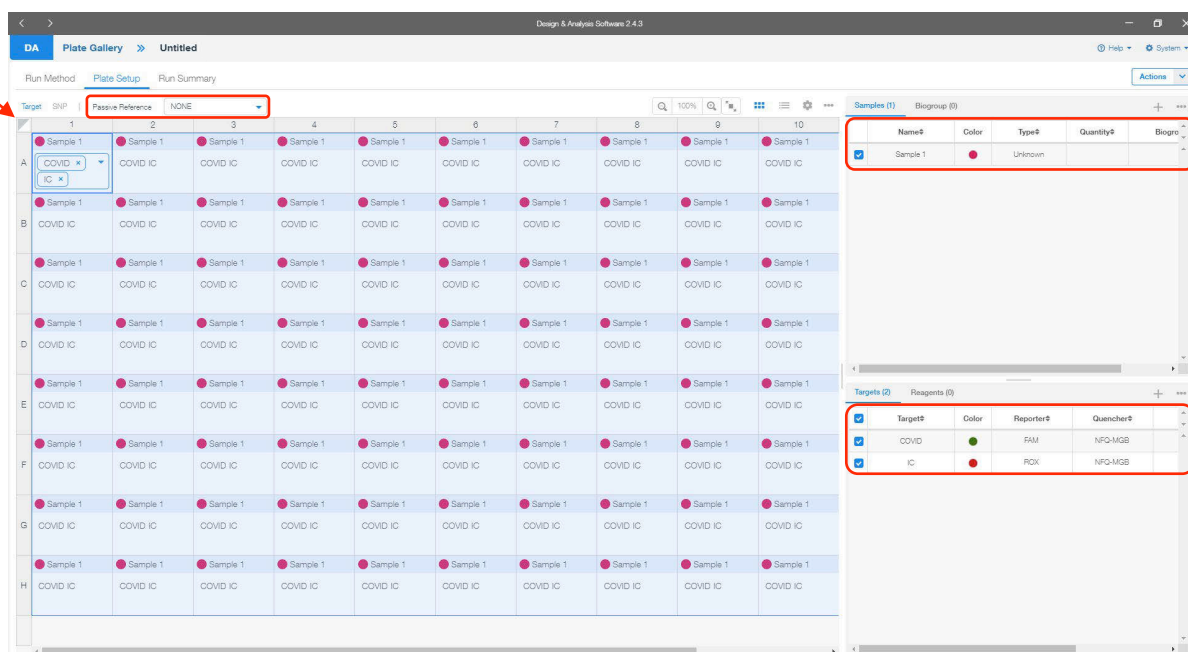
2. Launch the 'Design & Analysis' Desktop Software (version v2.4.3) and click 'Set Up Plate'. In the new screen, click on 'Standard Curve, QuantStudio™ 7 Pro, 96-Well 0.2mL, Standard Curve, Standard' to begin setup.



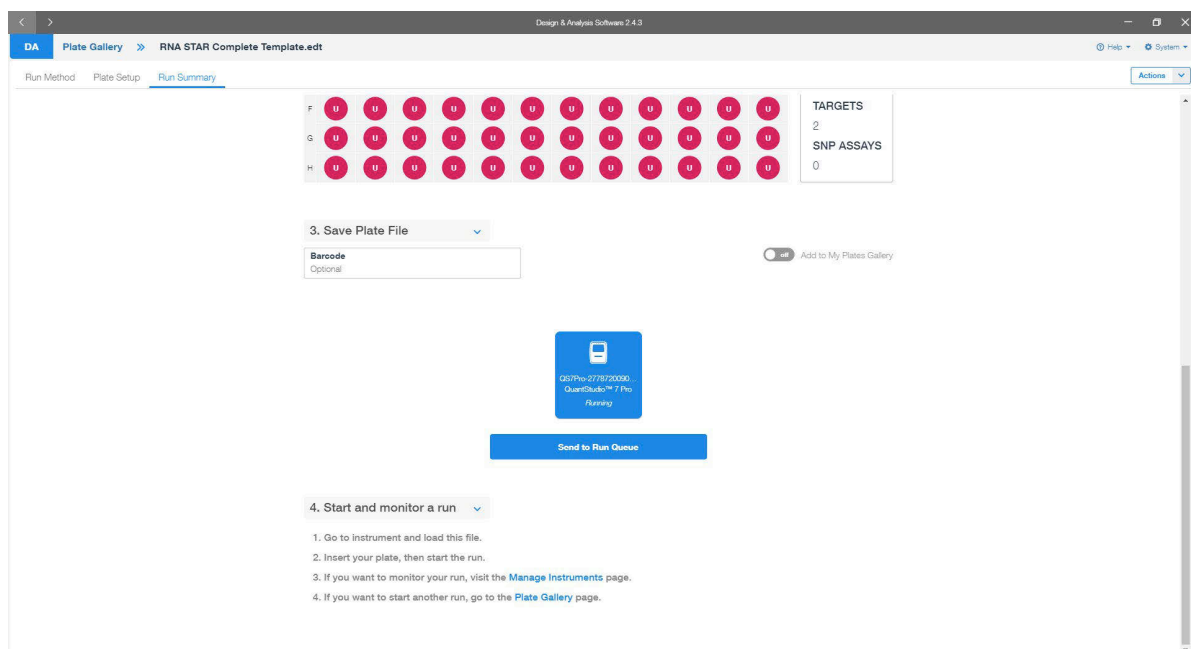
3. A new window will open with the 'Run Method' tab selected. Set the 'Reaction Volume' to '60.0 µL'. In the 'Hold Stage' section, hover over 'Step 2' and click the "-" button that appears just above the wording to delete this plateau. In the 'Hold Stage' section, change 'Step 1' settings as follows: 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'.




4. 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: '4x1(70±15)nm', 'm1(520±15)nm'; and 'x4(580±10)nm', 'm4(623±14)nm'. Click 'Save' to dismiss the window. Then proceed to the 'Plate Setup' tab.



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

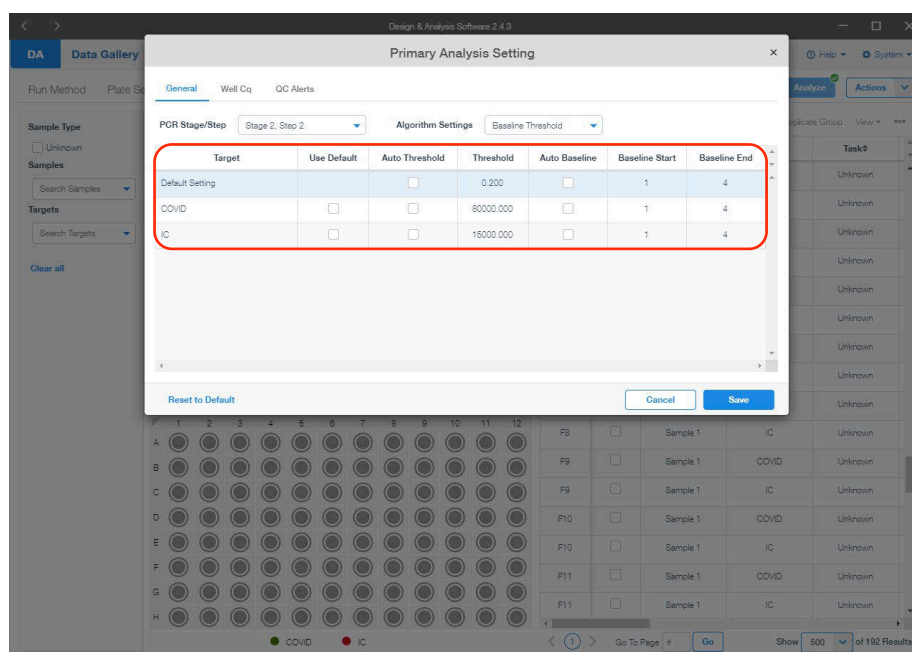


- Scroll down to the '3. Save Plate File' section and click 'QuantStudio™ 7 Pro' instrument icon. In the top right corner click 'Actions' and using the drop-down menu click 'Save As...'. Name the 'File Name' as 'RNA STAR Complete Template.edt' ('.edt' identifies this file as a template). Chose a 'Folder'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

- On the home instrument touch screen, press 'Load Plate File'. In new screen in the 'Run Queue' folder, press 'RNA STAR Complete Template' in the 'Plate file' section. In the 'Data file properties' screen, re-name the 'Data file name' as "RNA STAR Complete [YYMMDD_Plate#]". Press 'Enter' to confirm 'Data file name'.
- At the top right of the screen, press the tray button, , to open instrument drawer. Place the sample plate on the amplification block. Make sure to align A1 on the 96-well plate with the defined A1 position on the instrument drawer. Press the same tray button to close the instrument drawer.
- At the bottom of the screen, press 'Start run' to start the run. A 'Did you remember to insert your plate?' screen will occur and press 'Start run' again to start the run.
- Following the run, the instrument touchscreen indicates 'Run Completed'. Press 'Transfer file', insert a USB drive, press 'USB drive', and press 'Done' to export the file to the USB drive. Remove the USB drive from the instrument and press 'Close'.
- Open the instrument drawer to remove the plate by pressing the arrow icon at the top right of the instrument touch screen. The instrument drawer will open.
- Remove the plate and discard the plate in a sealable waste bag or container.

13.2 Analysis Instructions

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



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

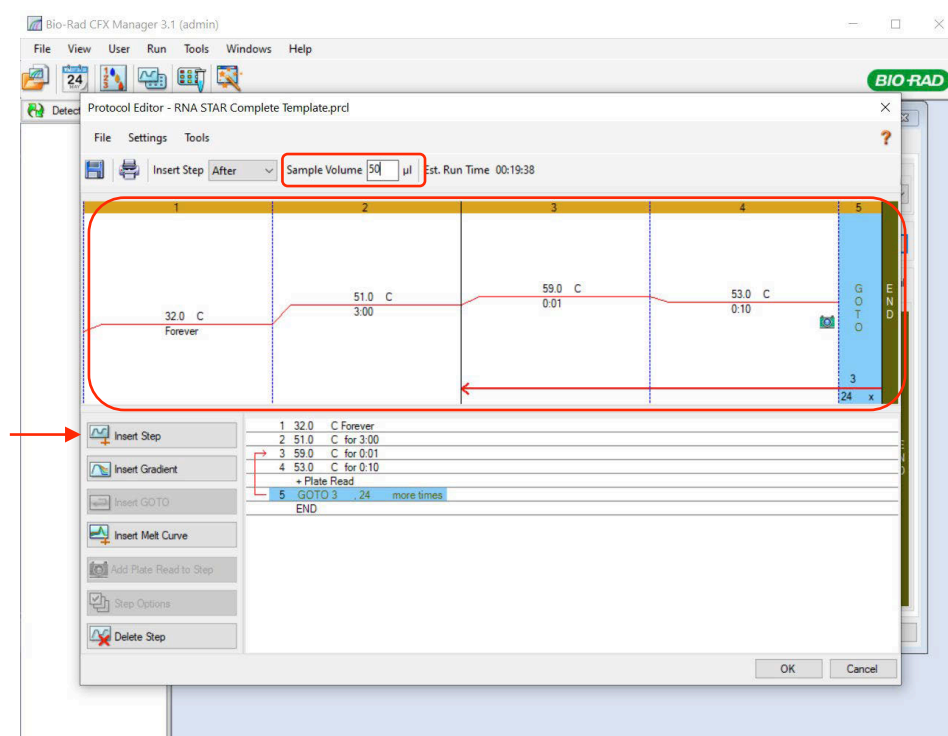
14 RNA STAR Complete Setup for Bio-Rad CFX96 Touch System

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.

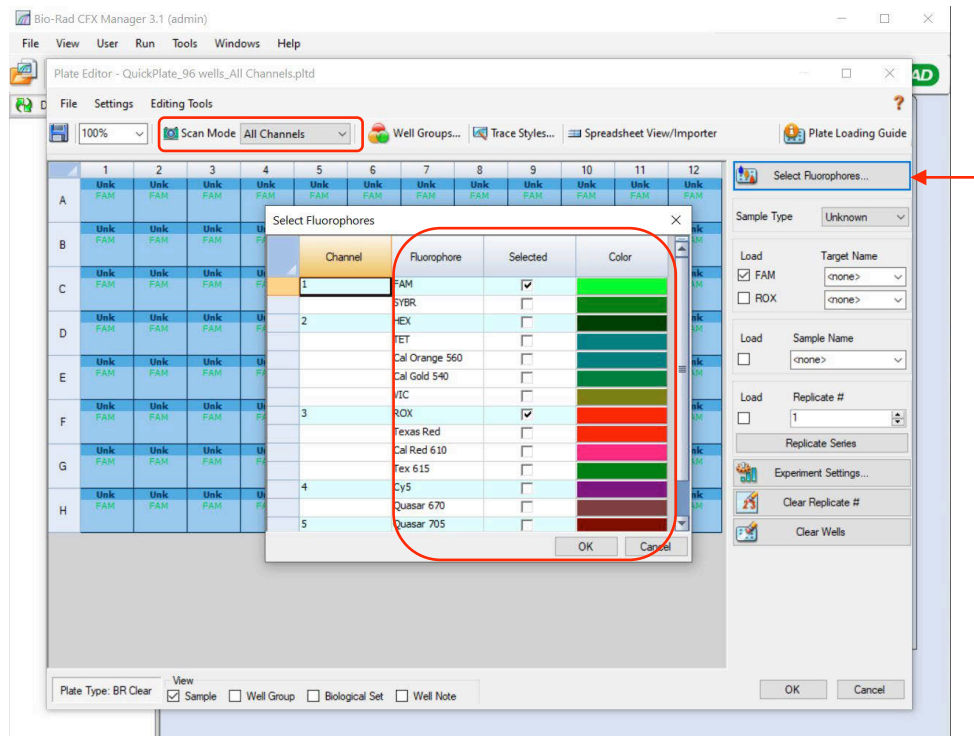
14.1 Programming Instructions for PC/Laptop-connected instrument

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

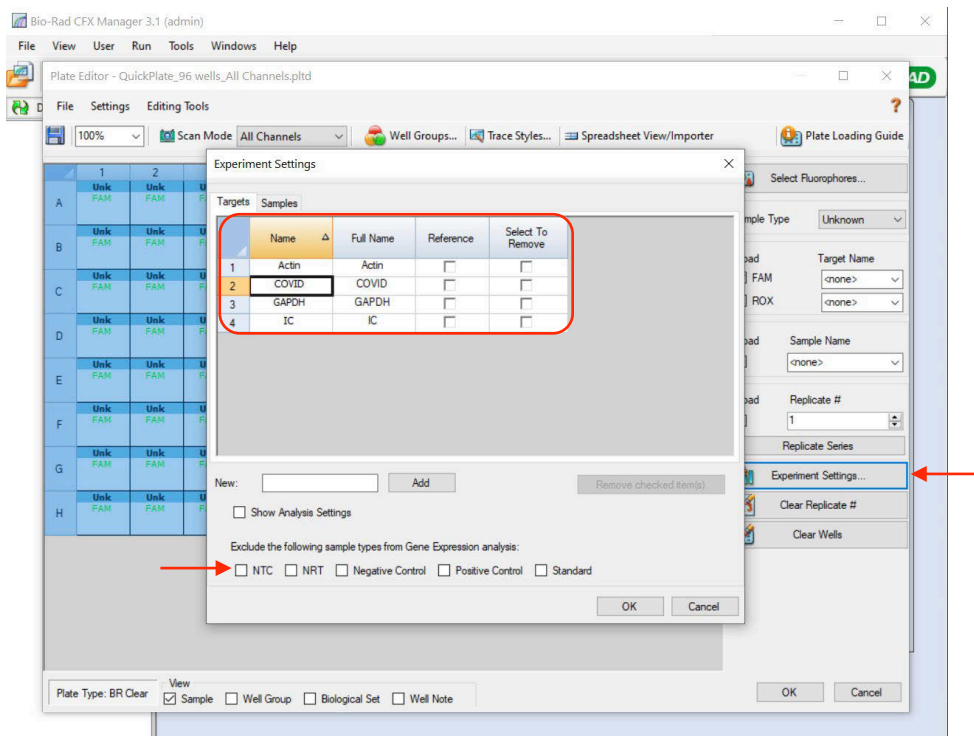
1. Launch the 'Bio-Rad CFX Manager' Desktop Software (version v3.1). In the 'Startup Wizard' pop-up window, select 'User-defined' as the run type then, in the 'Run Setup' pop-up window, select 'Edit Selected...' on the right of the window under the 'Protocol' tab to begin setup.



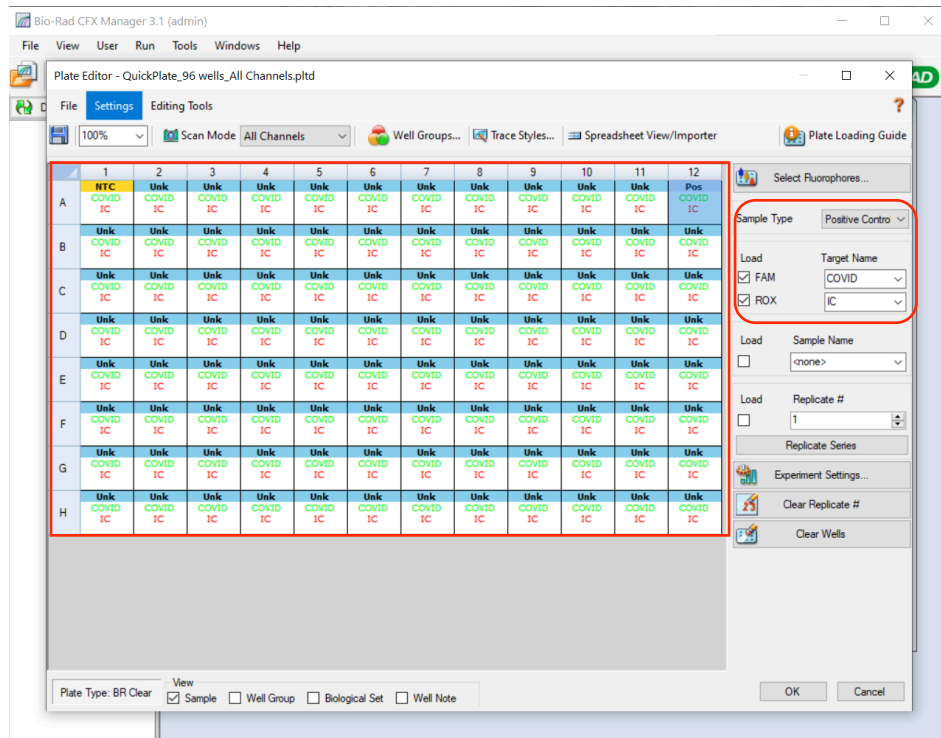
2. The 'Protocol Editor' pop-up window will open. Set the 'Sample Volume' to "50" µL (the sample volume should not be set higher than this). Click 'Insert Step' to add a total of five steps to the protocol. Edit steps '1' through '5' as follows: set step '1' temp to '32.0' C, set '1' time to '0:00' (this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



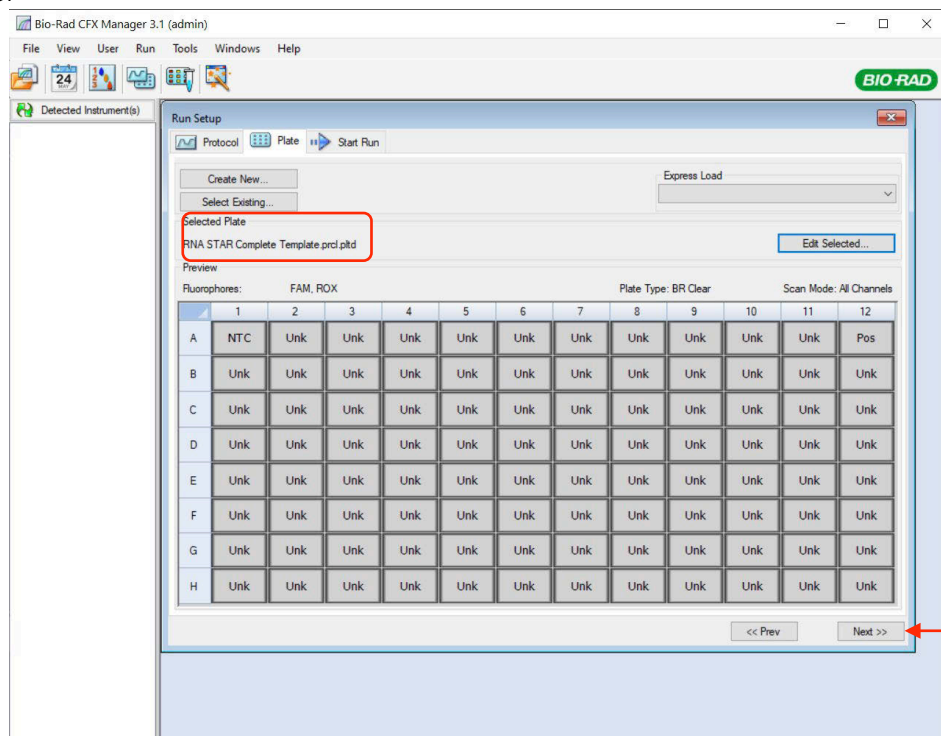
- Proceed to the 'Plate' tab by selecting the 'Next' button on the bottom right of the 'Protocol Editor' window. Select 'Edit Selected...' on the right of the window then select the entire plate by selecting the gray box between position 'A' and 'I' on the plate. Ensure that 'Scan Mode' is set to 'All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



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



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



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

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

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

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

14.2 Analysis Instructions

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

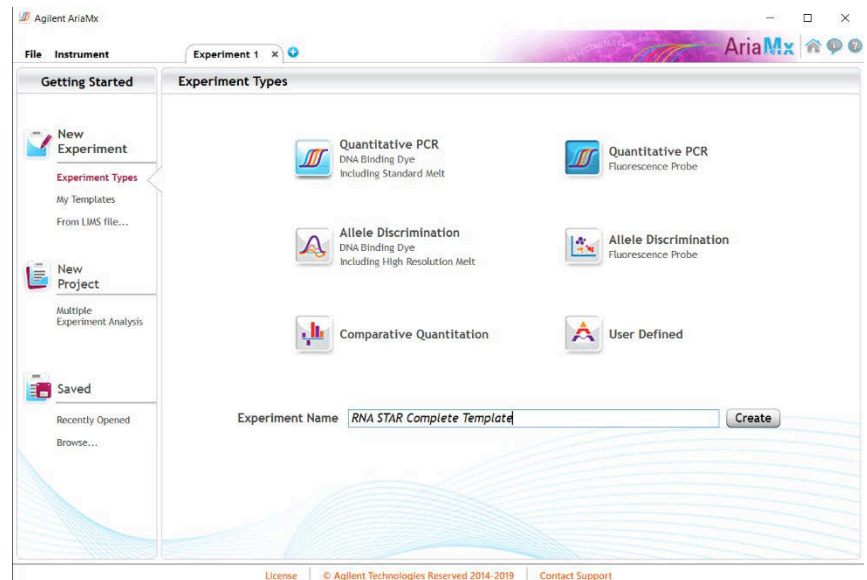
15 RNA STAR Complete Setup for Agilent™ AriaMx

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.

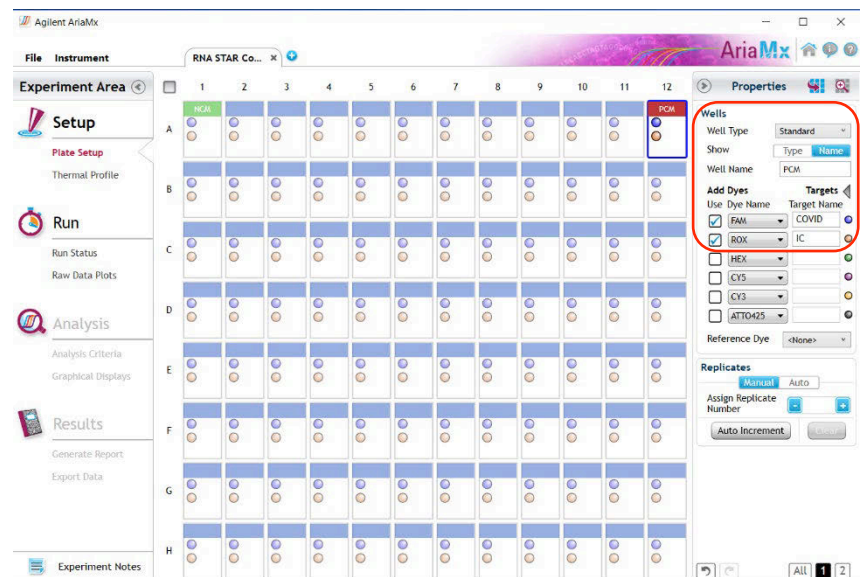
15.1 Programming Instructions for PC/Laptop-connected Instrument


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

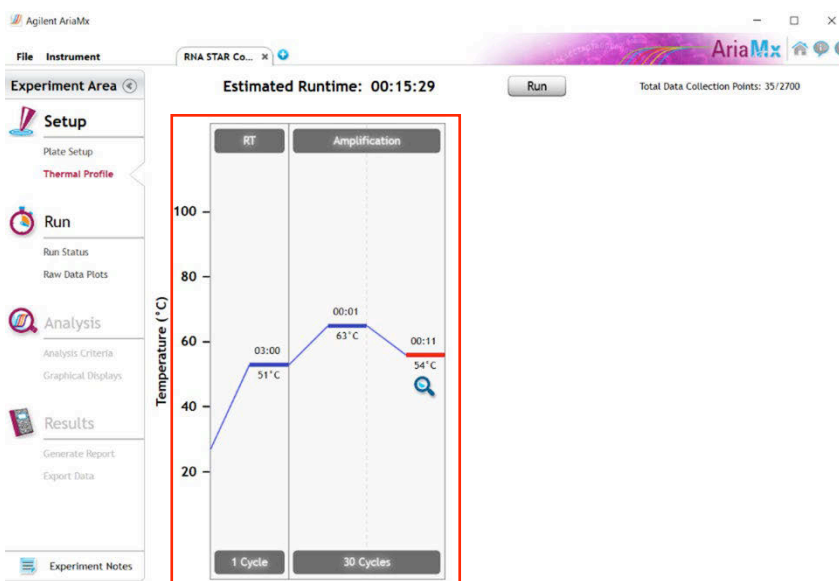
1. Launch the Agilent AriaMx desktop software (version 1.71). Select 'Quantitative PCR Fluorescence Probe' in the 'Experiment Types' section (do not double click). Define the 'Experiment Name' as "RNA STAR Complete Template" and click 'Create'.



2. Select all the wells and in the 'Properties' section on the right of the screen set 'Well Type' to 'Unknown' using the drop-down menu, select the 'FAM' and 'ROX' boxes, and toggle the 'Target' arrow (across from 'Add Dyes'). Set the 'Target Name' for 'FAM' to "COVID" and 'ROX' to "IC".
3. Select just well 'A1' and re-set 'Well Type' to 'NTC' using the drop-down menu. Click the 'Name' button just below and define the 'Well Name' as "NCM" (Negative Control Media). Select just well 'A12' and re-set 'Well Type' to 'Standard' using the drop-down menu. Click the 'Name' button just below and define the 'Well Name' as "PCM" (Positive Control Media). The other wells will remain 'Unknown' unless defined as Samples Names (optional).



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. Lift up the instrument door, pull the block lid out to unlock then lift the block lid and load the plate.



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

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

8. Upon completion of the run, close the 'Run Data Plots' screen by selecting the "X". Remove the plate and discard the plate in a sealable waste bag or container.

15.2 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 'Apply'. To exit the window, click 'OK'.
3. In the 'Amplification Plots' panel, change 'Graph Type' to 'Log'. Change automatically generated 'Threshold Fluorescence' values by defining 'COVID' as "350" then click the 'Unlock' icon immediately next to the newly defined threshold to lock the setting, additionally, define 'IC' as "120" then click the 'Unlock' icon immediately next to the newly defined threshold to lock the setting. Set 'Background Based Threshold' for 'Cycle Range' as '1' thru '1' and set 'Sigma Multiplier' to '10'.

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

15.3 Programming Instructions Using Instrument Touch Screen

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

1. On the Agilent AriaMx instrument touchscreen, press the 'Home' button then press 'New Experiment' and press 'Quantitative PCR Fluorescence Probe'.
2. On the 'Plate Setup' screen, select all the wells then press 'Well Types' and press 'Unknown' using the drop-down list. Check the boxes next to 'ROX' and 'FAM' and proceed to the 'Thermal Profile' tab.
3. Press 'Hold Start' and press the 'X' that appears next to this to delete this section. In the 'Amplification' section, press the left "+" icon near the bottom of the section. In the 'New' section that appears click 'RT'. Modify the default thermal profile for 'RT' as follows: set temp (lower left) to '51 °C', set time to (upper left) '03:00' and confirm 'Cycle' is automatically set to '1'. Modify the default thermal profile for 'Amplification' as follows: set temp (lower middle) to '63 °C', set time (upper middle) to '00:01', set temp (lower right) to '54 °C', set time (upper right) to '00:11' (this is the data collection step), and set 'Cycles' to '30'. Proceed to initiate the run by pressing the 'Run Experiment' button at the top of the screen.
4. A message box opens asking you to save the experiment. Press 'OK'. Name 'New File Name' as "RNA STAR Complete [YYMMDD_Plate#]" and press 'Save'.
5. The instrument lid will begin its Warm-Up procedure (~2 minutes). Press the 'Pause' button when it is activated (green arrow). The 'Pause' button is active once the lid reaches 100 °C. Lift up the instrument door, pull the block lid out to unlock then lift the block lid and load the plate.

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

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

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

15.4 Analysis Instructions

1. From USB drive, load the saved experiment by double-clicking the experiment file. The Agilent AriaMx desktop software (version 1.71) launches and the 'Plate Setup' screen opens. Select all the wells and in the 'Properties' section on the right of the screen set 'Well Type' to 'Unknown', select the 'FAM' and 'ROX' boxes, and toggle the 'Target' arrow (across from 'Add Dyes'). Set the 'Target Name' for 'FAM' to "COVID" and 'ROX' to "IC".
2. Select just well 'A1' and re-set 'Well Type' to 'NTC' (No Template Control) from the drop-down menu. Click the 'Name' button next to and define the 'Well Name' as "NCM" (Negative Control Media). Select just well 'A12' and re-set 'Well Type' to 'Standard' from the drop-down menu. Click the 'Name' button next to and define the 'Well Name' as "PCM" (Positive Control Media). The other wells will remain 'Unknown' unless defined as Samples Names (optional).
3. Select 'Analysis Criteria' from the 'Experiment Area' panel on the left side of the software then select the appropriate wells for analysis using the plate map.
4. Select 'Graphical Displays' from the 'Experiment Area' panel on the left side of the screen, then confirm in the 'Amplification Plots' panel on the right side of the software that 'Fluorescence Term' is set to ' ΔR ' and 'Smoothing' is 'On'. Expand the section under 'Amplification Plots' by toggling the 'Arrow' icon (if needed) then click 'Adjust'. In the 'Baseline Correction' window, click 'Select All' to highlight all the wells to be analyzed. Set 'Start Cycle' to '1' and set 'End Cycle' to '4' then click 'Apply'. To exit the window, click 'OK'.
5. In the 'Amplification Plots' panel, change 'Graph Type' to 'Log'. Change automatically generated 'Threshold Fluorescence' values by defining 'COVID' as "350" then click the 'Unlock' icon immediately next to the newly defined threshold to lock the setting, additionally, define 'IC' as "120" then click the 'Unlock' icon immediately next to the newly defined threshold to lock the setting.
6. Select 'Generate Report' from the 'Experiment Area' panel on the left side of the screen, then confirm in the 'Report Configuration' panel that 'Definition' is set to 'Default'. The 'Report' type can be set to 'PDF' or 'PowerPoint'. In the 'Items' section, select the Pencil button next to the 'Tabular Results' line and set 'Include Target Information' to 'Yes'. Check mark the following results: 'Well', 'Well Type', 'Well Name', 'Dye', 'Target', 'Cq (ΔR)', and 'Final Call (ΔR)'. All other headings should be unchecked. Click 'OK' to exit the 'Tabular Results Properties' window. All other report features *can* remain unchanged. Click 'Generate Report', define 'File Name' and click 'Save'.
7. Select 'Export Data' from the 'Experiment Area' panel on the left side of the screen, then confirm in the 'Export Configuration' panel that 'Definition' is set to 'Default'. The file type can be set to 'Excel', 'Text', 'LIMS', 'RDML'. In the 'Items' section, select the Pencil button next to the 'Tabular Results' line and set 'Include Target Information' to 'Yes'. Check mark the following results: 'Well', 'Well Type', 'Well Name', 'Dye', 'Target', 'Cq (ΔR)', and 'Final Call (ΔR)'. All other headings should be unchecked. Click 'OK' to exit the 'Column Options' window then click 'Export Data' to generate the report.
8. To save the run, click 'File' from the menu and choose 'Save' from the drop-down menu.
9. Close the software.

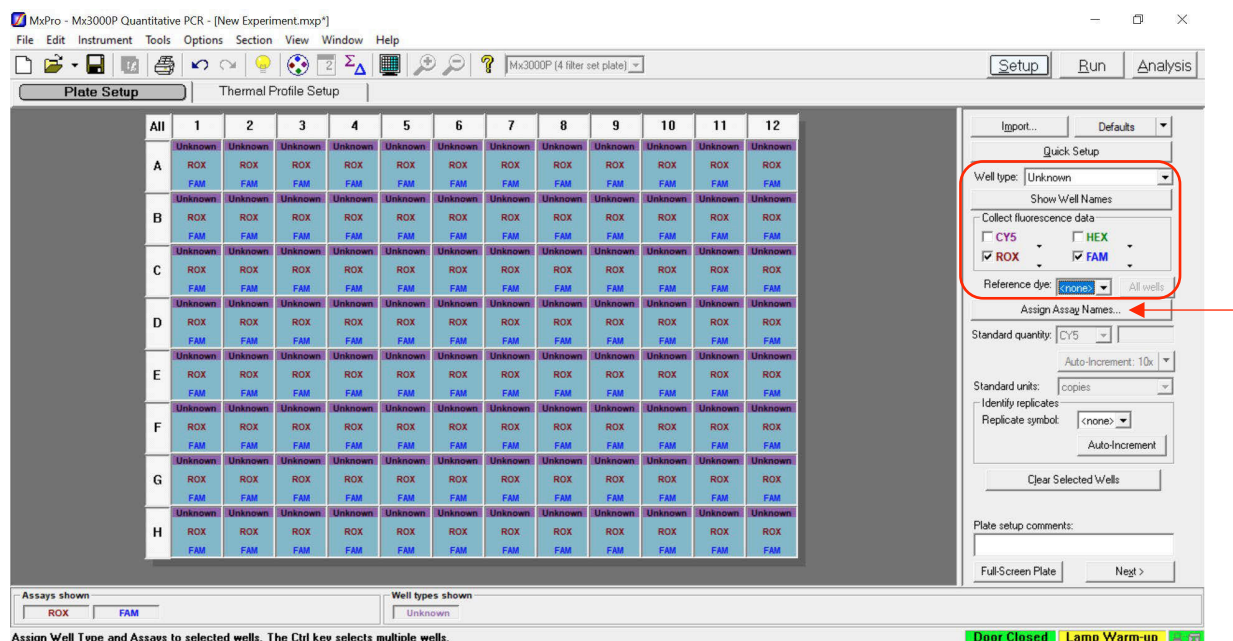
16 RNA STAR Complete Setup for Agilent™ Stratagene Mx3005P

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.

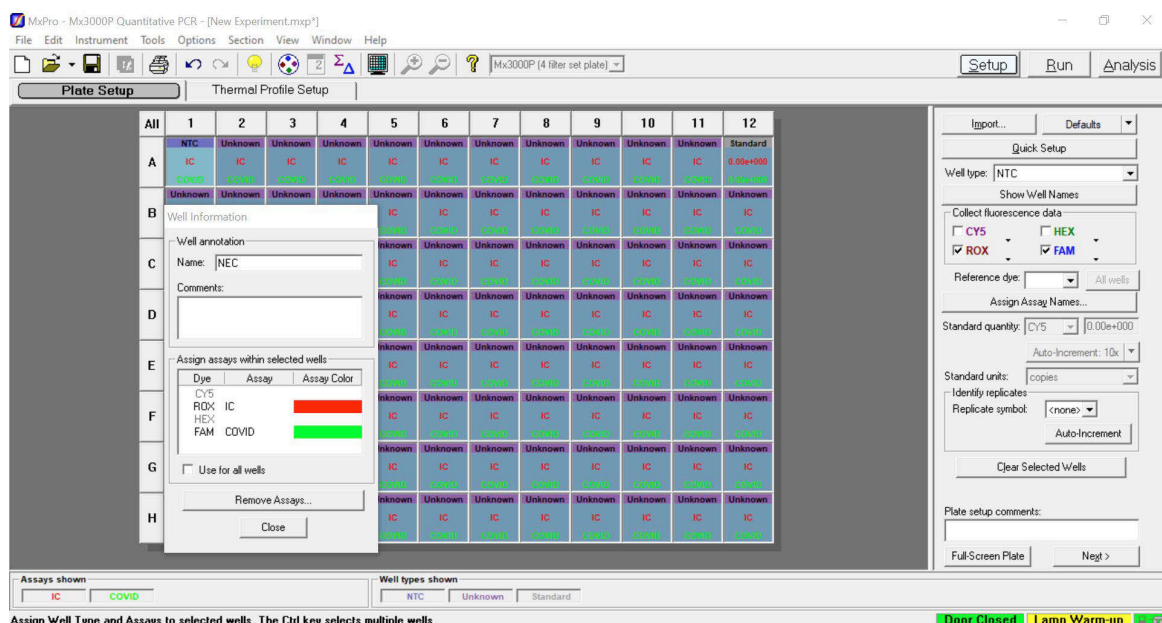
16.1 Programming Instructions for PC/Laptop-connected Instrument

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

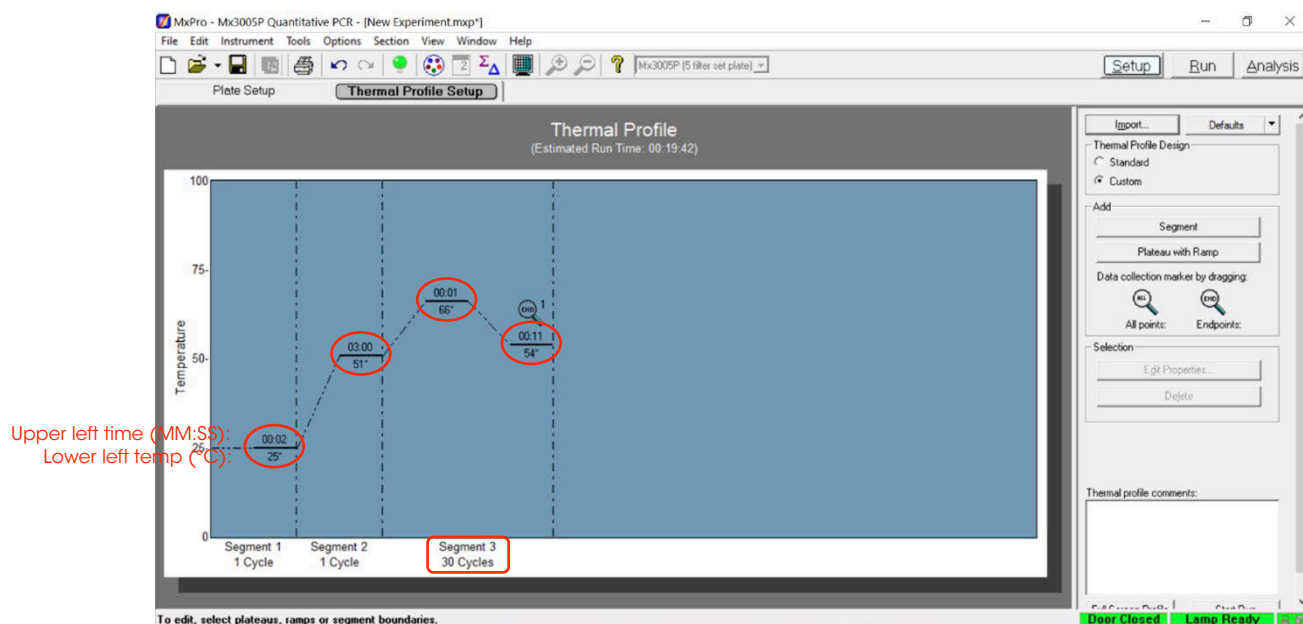
1. Launch the MxPro desktop software (version 4.10). Select 'Quantitative PCR (Multiple Standards)' in the 'New Options' window, check the box next to 'Turn lamp on for warm-up?', and click the 'OK' button. The lamp requires 20 minutes to properly warm-up.



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



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



5. On the right of the screen in the 'Add' section, click 'Segment' to add a segment. A new 'Segment 1' should appear in the 'Thermal Profile'. In the 'Segment 3' window, select the third plateau line then, on the right of the screen in the 'Selection' section, click 'Delete' to delete the third plateau. Modify the default thermal profile for 'Segment 1' as follows: set 'Temp' (lower left) to '25°', set 'Time' (upper left) to '00:02', and confirm 'Cycle' is set to '1'. Modify the default thermal profile for 'Segment 2' as follows: set 'Temp' (lower left) to '51°', set 'Time' (upper left) to '03:00', and confirm 'Cycle' is set to '1'. Modify the default thermal profile for 'Segment 3' as follows: set 'Temp' (lower left) to '66°', set 'Time' (upper middle) to '00:01', set 'Temp' (lower right) to '54°', set 'Time' (upper right) to '00:11', set 'Cycles' to '30'. Click 'Instrument' from the menu and choose 'Filter Set Gain Settings'. Verify the settings for 'ROX' and 'FAM' are set to 'x1' and 'x1', respectively. Click 'OK' to apply the changes and close the menu.

NOTE: If using different 96-well plates and seals than compatible for this instrument, please contact customer service.

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

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

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

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

16.2 Analysis Instructions

1. Click 'Analysis Term Settings' in the 'Select data collection ramp/plateau' section on the right of the software in the 'Analysis Selection/Setup' tab. In the 'Active Settings' section select 'Non-adaptive Baseline' and set the 'Start cycle' window to '1' and the 'through' window to '4'. Click on the 'Smoothing' tab and under 'Active settings' uncheck the 'Moving average' box. Click 'OK' to exit the 'Analysis Term Settings' window. Proceed to 'Results' tab near the top of the software.
2. Verify 'Fluorescence' is set to 'dR' using the drop-down menu on the right of the software. In the 'Threshold Fluorescence' section, change the values for 'IC' and "1000" then click the 'Unlock' icon immediately next to the newly defined threshold to lock the setting, and then change the value for 'COVID' to "1475" then click the 'Unlock' icon immediately next to the newly defined threshold to lock the setting. Right-click the Y-axis plot in the 'Amplification Plots' section and change 'Scale Y Axis' to 'Log'. In the 'Area to Analyze' section on the right of the software, select the 'Text Report'. Under the 'Column' section, select the boxes next to the following items to include in the final export file: 'Well', 'Well Name', 'Dye', 'Assay', 'Well Type', 'Ct (dR)', and 'Final Call (dR)'. Deselect all other column headings.
3. Export the results by right-clicking anywhere in the tabular text report. Choose 'Export Text Report' then click 'Export Text Report to Excel'.
4. From 'File' in the menu, choose 'Save' from the drop-down menu.
5. Exit the software

17 Analysis of Results

All controls should be examined prior to interpretation of samples.

17.1 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 kit reagents are properly detecting SARS-CoV-2 nucleic acids. It is comprised of a quantified NATrol™ 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 for swab samples and 285 copies for saliva samples². The control is formulated in a proprietary matrix with purified, intact viral particles containing whole length genome. The virus particles have been chemically modified to render them non-infectious and refrigerator stable.
2. The LumiraDx SARS-CoV-2 RNA STAR Complete Negative Control Media (Neg. Ctrl. Med.) is an external, negative, control needed to ensure cross-contamination, or reagent contamination from the upper respiratory swab sample 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 sample, assures that adequate amplification has taken place, and that the enzymes and primers were not inadvertently damaged during production, shipment, and storage.

The assay algorithm for all instruments is based on the standard practice of determining background fluorescence and calling a sample well positive if the change in fluorescent signal exceeds an established threshold. Background fluorescence levels are calculated from cycles 1 to 4 for the Roche LightCycler 480 II, Agilent Aria Mx, Applied Biosystems QuantStudio 5, Applied Biosystems QuantStudio 7 Pro, and the Agilent Stratagene Mx3005p. The Applied Biosystems 7500 Fast Dx uses auto baseline for background, Bio-Rad CFX96 uses cycles 1-2, and the Applied Biosystems QuantStudio 7 Flex uses cycles 1-3. A threshold (further described starting in section 9, for each instrument) is applied to the run. Positive and negative results are based on a sample well exceeding this threshold. No Cycle Threshold (Ct) cutoff is used for this assay algorithm, and it is expected that all reactions should occur between a Ct value of 3 to 30.

Failure of either the Positive (Pos. Ctrl. Med.) or Negative (Neg. Ctrl. Med.) Controls Media invalidates the qSTAR run. The qSTAR assay should be repeated with the aliquot of external controls and samples first. If the results continue to be invalid, assay another aliquot of the external controls and sample or obtain a new sample. If the Internal Control (IC) fails to amplify (in the absence of a positive signal), the qSTAR assay should be repeated as described above.

Table 1. Expected Results from External Controls (on Roche LightCycler 480 II, Applied Biosystems 7500 Fast Dx, Applied Biosystems QuantStudio 5, Applied Biosystems QuantStudio 7 Flex, Applied Biosystems QuantStudio 7 Pro, Agilent Aria Mx, and Agilent Stratagene Mx3005P).

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

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

² A four-fold dilution of the stock is made using Molecular Biology Grade Water (Neg. Ctrl. Med.) and 24 µL or 22.8 µL is added directly to the reaction for swab or saliva samples, respectively.

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

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

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

17.2 Analysis of Experimental Sample Results

Assessment of a sample 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 sample results cannot be interpreted.

Table 3. Interpretation of LumiraDx SARS-CoV-2 RNA STAR Complete (on Roche LightCycler 480 II, Applied Biosystems 7500 Fast Dx, Applied Biosystems QuantStudio 5, Applied Biosystems QuantStudio 7 Flex, Applied Biosystems QuantStudio 7 Pro, Agilent AriaMx, and the Agilent Stratagene Mx3005P).

Assay Results	SARS-CoV-2 (FAM)	Internal Control (ROX)	Interpretation of Results	Notes
SARS-CoV-2 Positive ("+")	$3.0 \leq Ct \leq 30.0$	$3.0 \leq Ct \leq 30.0^*$	SARS-CoV-2 Viral RNA detected; IC possibly detected	
Negative ("-")	No Ct detected	$3.0 \leq Ct \leq 30.0$	No SARS-CoV-2 Viral RNA detected; IC detected	
Invalid	No Ct detected	No Ct detected	No SARS-CoV-2 Viral RNA detected; No IC detected	Invalid result. Retest the sample or obtain a new sample for testing.

*The Internal Control is not required to amplify.

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

Assay Results	SARS-CoV-2 (FAM)	Internal Control (ROX)	Interpretation of Results	Notes
SARS-CoV-2 Positive ("+")	$3.0 \leq Ct \leq 25.0$	$3.0 \leq Ct \leq 25.0^*$	SARS-CoV-2 Viral RNA detected; IC possibly detected	
Negative ("-")	No Ct detected	$3.0 \leq Ct \leq 25.0$	No SARS-CoV-2 Viral RNA detected; IC detected	
Invalid	No Ct detected	No Ct detected	No SARS-CoV-2 Viral RNA detected; No IC detected	Invalid result. Retest the sample or obtain a new sample for testing.

*The Internal Control is not required to amplify.

18 Quality Control

- Quality control procedures are intended to monitor reagent and assay performance.
- Assay all Positive and Negative Controls Media when running experimental samples and with each new lot of the LumiraDx SARS-CoV-2 RNA STAR Complete assay 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 confirming enzyme, primer, and probe stability.
- Do not use reagents past their expiration date.
- Inhibitors present in the sample, cross-contamination, and/or errors in following the assay procedure may impact results.

19 Contact Information, Ordering, and Product Support

For ordering, contact LumiraDx at:

Website: www.lumiradx.com.

Email: customerservices@lumiradx.com.

For product information, contact LumiraDx at:

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

For technical support, contact LumiraDx at:

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

For return policy, contact LumiraDx at:

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

Intellectual property

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

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LumiraDx US Office

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