



Instructions for Use

For Emergency Use Authorization (EUA) Only

Catalog #L018180030096
For *In Vitro* Diagnostic Use
Rx Use Only

ART-00014 Rev 7 · Rev Date 2023/04

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1 Intended Use

LumiraDx SARS-CoV-2 RNA STAR is a non-isothermal nucleic acid amplification qSTAR (Selective Temperature Amplification Reaction) method intended for the qualitative detection of nucleic acid from SARS-CoV-2 in upper respiratory specimens (such as nasal, mid-turbinate, nasopharyngeal and oropharyngeal swabs) and bronchoalveolar lavage specimens collected from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to laboratories - certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 USC, §263a that meet requirements to perform high complexity tests.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in upper respiratory and bronchoalveolar lavage specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

LumiraDx SARS-CoV-2 RNA STAR test is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures. LumiraDx SARS-CoV-2 RNA STAR test is only for use under the Food and Drug Administration's Emergency Use Authorization.

2 Summary and Explanation

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

LumiraDx SARS-CoV-2 RNA STAR has been designed to detect ORF1a genes from nucleic acid sequences from the genome of the SARS-CoV-2 RNA.

¹ World Health Organization: www.who.int.

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

3 Principles of the Procedure

LumiraDx SARS-CoV-2 RNA STAR is a rapid non-isothermal nucleic acid amplification technique utilizing qSTAR technology, which detects SARS-CoV-2 viral nucleic acid in 12 minutes following extraction. The SARS-CoV-2 Primer and probe set is designed for the qualitative detection of nucleic acid from SARS-CoV-2 in upper respiratory specimens (such as nasal, mid-turbinate, nasopharyngeal and oropharyngeal swabs) and bronchoalveolar lavage specimens collected from individuals suspected of COVID-19 by their healthcare provider.

Nucleic acids are isolated and purified from upper respiratory specimens (such as nasal, mid-turbinate, nasopharyngeal and oropharyngeal swabs and bronchoalveolar lavage specimens) using either an automated extraction protocol with the Qiagen DSP Virus/Pathogen Kit (catalog #937055; 400 µL input, 60 µL elution) on the Qiagen QIAasympyphony SP, or by manual extraction with either the MagMax Viral/Pathogen Nucleic Acid Isolation Kit (catalog #A42352; 400 µL input, 50 µL elution) or the QIAamp Viral RNA Mini Kit (catalog #52906; 280 µL input, 50 µL elution). The purified nucleic acid (10 µL) is reverse transcribed to form cDNA which is then subsequently amplified by qSTAR using primers to a target 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 has shown consistency and control of amplification, whilst maintaining the sensitivity of detection, to allow for reliable and accurate detection of infectious diseases 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), Agilent AriaMx (software version 1.71), or the Agilent Stratagene Mx3005P (software version 4.10) RT-PCR Instruments.

4 Materials Required (Provided)

Component	Description	Volume	Storage
LumiraDx SARS-CoV-2 RNA STAR Master Mix	COVID-19 Master Mix	1.0 mL	-25°C to -15°C
LumiraDx SARS-CoV-2 RNA STAR 50X Internal Control & Primer Mix (50X IC/P Mix)	COVID-19 Internal Control & Primer Mix	50 µL	-25°C to -15°C
LumiraDx SARS-CoV-2 RNA STAR 10X Salt Mix	COVID-19 Salt Mix	250 µL	-25°C to -15°C
LumiraDx SARS-CoV-2 RNA STAR Positive Extraction Control (Pos. Ext. Ctrl.)	COVID-19 Positive Control (ZeptoMetrix 50,000cp/mL)	500 µL	2°C to 8°C (-25°C to -15°C)
LumiraDx SARS-CoV-2 RNA STAR Negative Extraction Control (Neg. Ext. Ctrl.)	Molecular Grade Water	1.5 mL	-25°C to -15°C

5 Materials Required (But Not Provided)

Consumables	Source	Catalog #
Appropriate Personal Protective Equipment	General lab supplier	n/a
Aerosol Barrier Pipette Tips with Filters	General lab supplier	n/a
Microcentrifuge Tubes (DNase/RNase free), 0.6 to 5mL	General lab supplier	n/a
Powder-Free Nitrile Glove	General lab supplier	n/a
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
DNAZapTM (or equivalent)	ThermoFisher Scientific	AM9890
RNaseZapTM (or equivalent)	ThermoFisher Scientific	AM9782
Molecular Biology Grade Water	Corning	46-000-CM

Option for Extraction kits and Instruments – please see Section 9.1 below

Option for PCR Instruments & Recommended Consumables	Source	Catalog #
Roche LightCycler 480 II	Roche Life Science	5015278001
VWR Adhesive Film for Microplates	VWR	60941-070
LightCycler 480 Multiwell Plate 96, Clear	Roche Life Science	5102413001
Applied Biosystems 7500 Fast Dx Real-Time PCR Instrument	ThermoFisher Scientific	4406984
Applied Biosystems Optical Adhesive Covers	ThermoFisher Scientific	4360954
Applied Biosystems MicroAmp Fast Optical 96-Well Plate	ThermoFisher Scientific	4346906
Applied Biosystems QuantStudio 5	ThermoFisher Scientific	A28574
Applied Biosystems Optical Adhesive Covers	ThermoFisher Scientific	4360954
Applied Biosystems MicroAmp Optical 96-Well Plate	ThermoFisher Scientific	4306737
Agilent Aria Mx	Agilent Technologies	G8830A
AriaMx 96 Adhesive Seals	Agilent Technologies	401492
AriaMx 96 Well Plates, Skirted, LP	Agilent Technologies	401490
Agilent Stratagene Mx3005P	Agilent	401511
Applied Biosystems MicroAmp Clear Adhesive Film	ThermoFisher Scientific	4306311
Eppendorf twin.tec Real-Time PCR Plate 96-Well Semi-Skirted	Eppendorf	951022043

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

6 Warning and Precautions

- For *in vitro* Diagnostic Use (IVD).
- For prescription (Rx) use only.
- This LumiraDx SARS-CoV-2 RNA STAR Test has not been FDA cleared or approved; this Test has been authorized by FDA under an EUA for use by laboratories certified under CLIA meet requirements to perform high complexity tests.
- This LumiraDx SARS-CoV-2 RNA STAR Test has been authorized only for the detection of nucleic acid from SARS-CoV-2 virus and not for any other viruses or pathogens.
- This LumiraDx SARS-CoV-2 RNA STAR Test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of *in vitro* diagnostic tests for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food Drug and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.
- Follow standard precautions. All patient specimens 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 human specimens are handled.
- Handle all specimens as if infectious using safe laboratory procedures. Refer to Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with 2019-nCoV (<https://www.cdc.gov/coronavirus/2019-nCoV/lab-biosafety-guidelines.html>).
- Performance characteristics have been determined with human upper respiratory specimens from individuals with signs and symptoms of infection who are suspected of COVID-19.
- Use personal protective equipment such as (but not limited to) gloves and lab coats when handling kit reagents while performing this assay and handling materials including samples, reagents, pipettes, and other equipment and reagents.
- Dispose of unused kit reagents and human specimens according to local, state, and federal regulations.
- Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.
- Laboratories are required to report all positive results to the appropriate public health authorities.
- Lysis buffers used with the extraction methods described in section 9 on page 14 contain guanidinium thiocyanate or guanidine-containing materials. Highly reactive and/or toxic compounds may form if combined with sodium hypochlorite (bleach).

7 Reagent Storage, Handling, and Stability

- Upon receipt, store the LumiraDx SARS-CoV-2 RNA STAR test kit between -15 °C to -25 °C.
- After initial use, store LumiraDx SARS-CoV-2 RNA STAR Master Mix, LumiraDx SARS-CoV-2 RNA STAR 50X Internal Control & Primer Mix (50X IC/P Mix), LumiraDx SARS-CoV-2 RNA STAR 10X Salt Mix, LumiraDx SARS-CoV-2 RNA STAR Negative Extraction Control (Neg. Ext. Ctrl.) between -15 °C to -25 °C. Store LumiraDx SARS-CoV-2 RNA STAR Positive Extraction Control (Pos. Ext. Ctrl.) between 2 °C to 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 50X Internal Control & Primer Mix (50X IC/P Mix).
- The Internal Control/Primer Mix, Salts and Master Mix must be thawed and kept on a cold block or ice at all times during preparation and use.
- Controls must also be thawed and kept cold at all times during preparation and use.

8 Specimen Collection, Handling, and Storage

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

8.1 Collecting the Specimen

- Refer to *Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Persons for Coronavirus Disease 2019 (COVID-19)*. See <https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html>.
- Follow specimen collection device manufacturer instructions for proper collection methods.
- Swab specimens should be collected using only swabs with a synthetic tip, such as nylon or Dacron®, and an aluminum or plastic shaft. Calcium alginate swabs are unacceptable and cotton swabs with wooden shafts are not recommended.
- Respiratory specimens should be collected and placed into appropriate transport media, such as viral transport media (VTM) or DNA-RNA Shield, as described by CDC and WHO guidelines. See <https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html>.

8.2 Transporting Specimens

- Specimens must be packaged, shipped, and transported according to the current edition of the International Air Transport Association (IATA) Dangerous Goods Regulation. Follow shipping regulations for UN 3373 Biological Substance, Category B when sending potential SARS-CoV-2 specimens. Store specimens at 2-8 °C and ship overnight on ice pack. If a specimen is frozen ship at -70 °C or below, and ship overnight on dry ice.

8.3 Storing Specimens

- Specimens received cold should be stored refrigerated (2-8 °C) for up to 72 hours before processing.
- If a delay in specimen extraction is expected, store specimens at -70 °C or below.
- Specimens received frozen should be stored at -70 °C or below until processing.
- Store any residual specimens at -70 °C or below.
- Store purified nucleic acids at -70 °C or below.

9 Nucleic Acid Extraction

Performance of LumiraDx SARS-CoV-2 RNA STAR is dependent upon the amount and quality of template RNA purified from human specimens. The following commercially available RNA extraction kits and procedures have been qualified and validated for recovery and purity of RNA for use with LumiraDx SARS-CoV-2 RNA STAR:

1. Qiagen QIAasymphony DSP Virus/Pathogen Kit (software version 5.0.4). Refer to "User Manual Part Number 937055" for instructions.
2. Qiagen QIAamp Viral RNA Mini Kit. Refer to "User Manual Part Number HB-0354-006 QIAamp Viral RNA Mini Handbook" for instructions.
3. Applied Biosystems MagMax Viral/Pathogen Nucleic Acid Isolation Kit. Refer to "User Manual Part Number MAN0018072" for instructions.

Manufacturer's recommended procedures unless otherwise noted below are to be followed for all sample extractions.

9.1 Extraction Protocols Validated with the Test

Extraction Kit	Catalog Number	Test Sample ^b Volume	Protocol	Elution Volume
QIAasymphony DSP Virus/Pathogen Kit ^c	937055 (96 preps)	400µL ^a	QIAasymphony DSP Virus/Pathogen Kit Instructions for Use (Handbook) V1 (July 2015)	60µL
	937036 (192 preps)			
QIAamp Viral RNA Mini Kit	52904 (50 preps)	280µL	QIAamp Viral RNA Mini Handbook (July 2020) – Purification of Viral RNA (Spin Protocol)	50µL
	52906 (250 preps)			
MagMax Viral/Pathogen Nucleic Acid Isolation Kit	A42352 (100 preps)	400µL	Manual isolation of viral nucleic acid (RNA and DNA) from biofluids and transport media (6 December 2019)	50µL
	A42359 (1000 preps)			

^aSee the PRE-TREATMENT REQUIRED PRIOR TO EXTRACTION section if applicable.

^bUpper respiratory swab samples in 3 mL of appropriate Transport Media.

^cKit uses the QIAasymphony SP instrument.

CAUTION: Lysis buffers used with these extraction methods contain guanidinium thiocyanate or guanidine-containing materials. Highly reactive and/or toxic compounds may form if combined with sodium hypochlorite (bleach).

10 LumiraDx SARS-CoV-2 RNA STAR Preparation

10.1 Pre-Treatment Required Prior to Extraction

For extraction with the QIAasympphony DSP instrument, samples require off-board lysis pre-treatment. Follow the off-board lysis section in the QIAasympphony SP Protocol Sheet. This protocol requires Buffer ACL (Qiagen; catalog #939017) and ATL (Qiagen; catalog #939016) that are not provided with the QIAasympphony DSP Virus/Pathogen Kits.

10.2 Sample Extraction Protocol

The LumiraDx SARS-CoV-2 RNA STAR Negative Extraction Control (Neg. Ext. Ctrl.) and LumiraDx SARS-CoV-2 RNA STAR Positive Extraction Control (Pos. Ext. Ctrl.) should be treated as patient specimens and both must be processed in every extraction batch. See Section 16.1 on page 37 for more information about the LumiraDx SARS-CoV-2 RNA STAR Controls.

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

1. Thaw LumiraDx SARS-CoV-2 RNA STAR Positive Extraction Control (Pos. Ext. Ctrl.) and LumiraDx SARS-CoV-2 RNA STAR Negative Extraction Control (Neg. Ext. Ctrl.) on a cold block or ice.
2. Vortex the Pos. Ext. Ctrl. and Neg. Ext. Ctrl. for 5 seconds then centrifuge tubes at 280 xg for 5 seconds to collect reagents at the bottom of the tube.
3. To assemble the NEC (Negative Extraction Control) use the required volume for sample extraction (e.g. 400 μ L for the QIAasympphony DSP Virus/Pathogen Kit and MagMax Viral/Pathogen Nucleic Acid Isolation Kit; 280 μ L for the QIAamp Viral RNA Mini Kit).
4. To assemble the PEC (Positive Extraction Control) add 40 μ L Pos. Ext. Ctrl. and bring up to the required volume with Nuclease Free Water for sample extraction (e.g. 400 μ L for the QIAasympphony DSP Virus/Pathogen Kit and MagMax Viral/Pathogen Nucleic Acid Isolation Kit; 280 μ L for the QIAamp Viral RNA Mini Kit).
5. Extract upper respiratory samples in 3 mL of appropriate transport media, such as viral transport media (VTM) or DNA-RNA Shield. For BAL samples, collect 2 to 3 mL in a sterile container. Per the extraction protocol, use the appropriate volume for the test sample extraction (e.g. 400 μ L for the QIAasympphony DSP Virus/Pathogen Kit and MagMax Viral/Pathogen Nucleic Acid Isolation Kit; 280 μ L for the QIAamp Viral RNA Mini Kit).

10.3 qSTAR Preparation

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

- Maintain separate areas for assay setup and handling of nucleic acids.
- 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 extracted nucleic acids.
- Wear a clean lab coat and powder-free disposable gloves (not previously worn) when setting up assays.
- Change gloves often and whenever contamination is suspected.
- Keep reagent and reaction tubes capped or covered as much as possible.
- LumiraDx SARS-CoV-2 RNA STAR Master Mix and LumiraDx SARS-CoV-2 RNA STAR 50X IC/P Mix must be thawed and maintained on a cold block or ice 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.

10.4 Reagent Preparation

All components should be kept on a cold block or ice to maintain the integrity of the reagents. It is necessary to make excess Reaction Mix to allow for pipetting error.

1. Thaw LumiraDx SARS-CoV-2 RNA STAR Master Mix, LumiraDx SARS-CoV-2 RNA STAR 50X IC/P Mix, and LumiraDx SARS-CoV-2 RNA STAR 10X Salt Mix on a cold block or ice.
2. Once thawed, invert each tube to mix and centrifuge at 280 xg for 5 seconds to collect reagents at the bottom of the tube (do not vortex samples).
3. Determine the number of reactions (N) to be prepared per assay:

Reaction Mix Setup	1 Rxn	100 Rxns	N Rxns
Water	2.0 µL	200 µL	N x 2.0 µL
LumiraDx SARS-CoV-2 RNA STAR 10X Salt Mix	2.5 µL	250 µL	N x 2.5 µL
LumiraDx SARS-CoV-2 RNA STAR 50X IC/P Mix	0.5 µL	50 µL	N x 0.5 µL
LumiraDx SARS-CoV-2 RNA STAR Master Mix	10 µL	1000 µL	N x 10 µL
Total Volume	15 µL	1500 µL	N x 15 µL

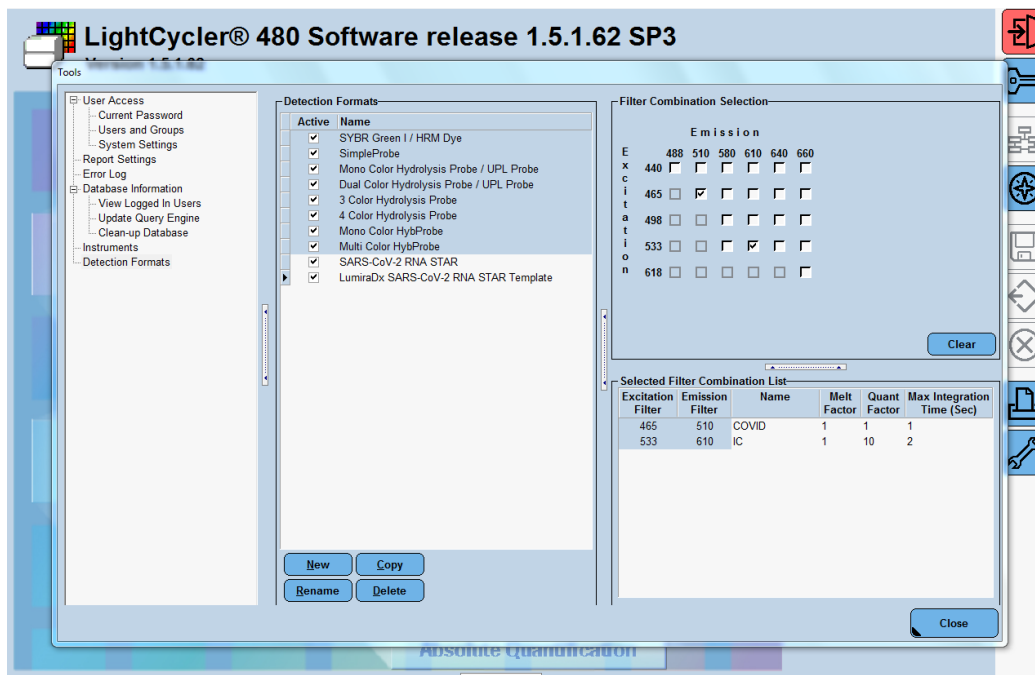
4. Assuming one reaction is needed, perform the following to make the Reaction Mix:
 - a. Combine 2 µL Water and 2.5 µL 10X Salt Mix in a pre-chilled microcentrifuge tube. Vortex for 5 seconds, centrifuge tubes at 280 xg for 5 seconds then place tube back on cold block or ice.
 - b. Add 0.5 µL 50X IC/P Mix to the Reaction Mix, mix by pipetting up and down 4 times, centrifuge briefly, then place tube back on cold block or ice.
 - c. Add 10 µL Master Mix to finalize the Reaction Mix, mix by slowly pipetting up and down 4 times without introducing air or bubbles (it is important to not vortex or spin down for an excessive amount of time once the Master Mix has been added), then place tube back on cold block or ice.
5. Transfer 15 µL of Reaction Mix into a pre-chilled 96-well plate. This plate should be kept cold until placing it onto the thermocycler.
6. Add 10 µL eluate from sample extraction (extraction controls or sample) into the same well.
7. Seal the 96-well plate using an optically clear adhesive.
8. Centrifuge the 96-well plate at 2000 rpm for 5 seconds to collect all reagents at the bottom of the plate.
9. Place the 96-well plate on a validated thermocycler and follow instrument specific protocols and analysis procedures detailed below.

11 RNA STAR Setup for Roche™ LightCycler 480 II

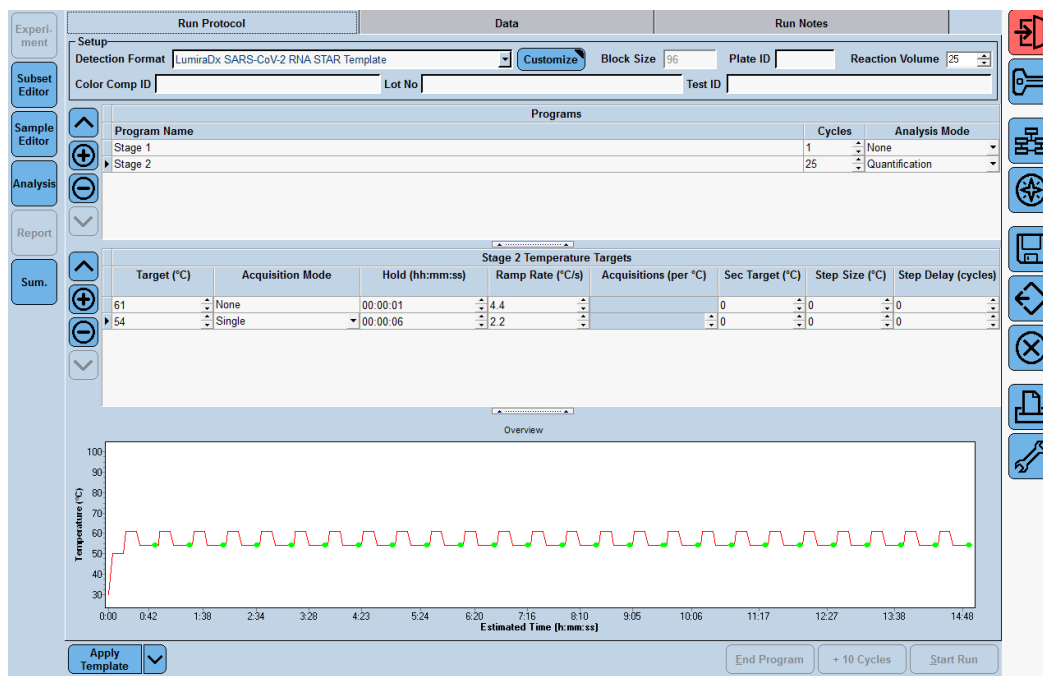
Refer to "User Manual Part Number 05152062001 0208" for additional information.

11.1 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'.



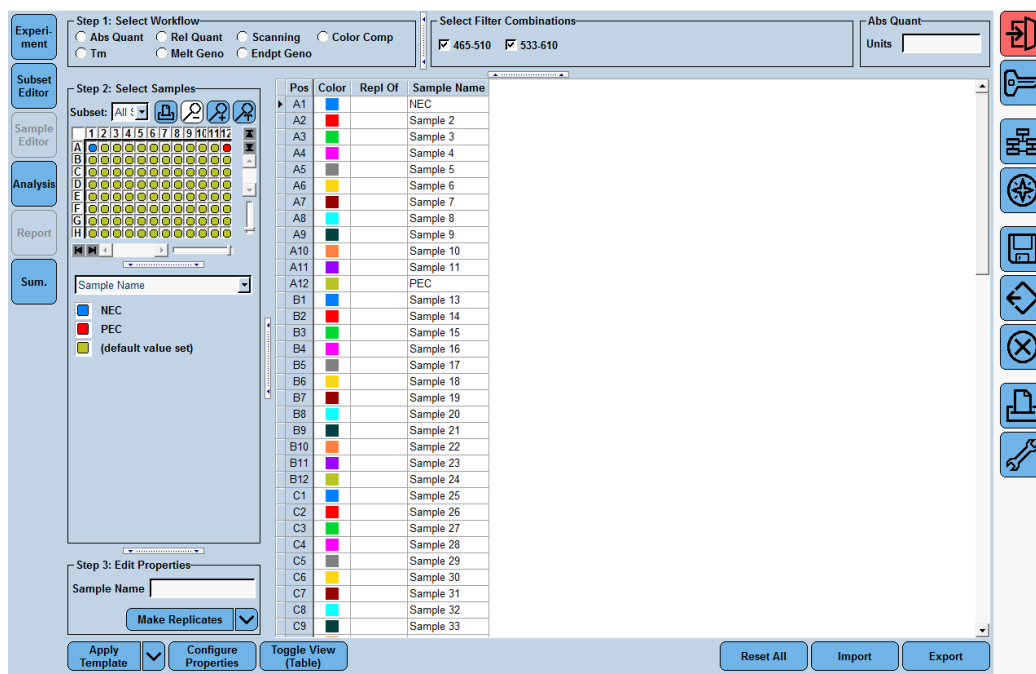
2. Click the 'Tools' button in the startup screen in the lower right of the software. Select 'Detection Formats' and click 'New' in the 'Tools' window that opens. Name the new 'Detection Formats' as "LumiraDx SARS-CoV-2 RNA STAR Template". In the 'Filter Combination Selection' section select '465-510'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



- Select from the 'Detection Format' drop-down menu "LumiraDx SARS-CoV-2 RNA STAR Template". Set 'Reaction Volume' to '25' in the upper right of the software. In the 'Program Name' section type 'Stage 1', set 'Cycles' to '1', and set 'Analysis Mode' to 'none'. Select the "+" button on the right of the software to add another program name. Name the next program 'Stage 2', set 'Cycles' to '25', and set 'Analysis Mode' to 'Quantification'. Select the 'Stage 1' line and change 'Stage 1 Temperature Targets' as follows: set 'Target (°C)' to '50', 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 'Stage 2' line and change 'Stage 2 Temperature Targets' as follows: set 'Target (°C)' to '61', set 'Acquisition Mode' to 'none', set 'Hold (hh:mm:ss)' to '00:00:01', set 'Ramp Rate (°C/s)' to '4.4', and set 'Sec Target (°C)', 'Step Size (°C)', and 'Step Delay (cycles)' to '0'. Select the "+" button to the right of 'Stage 2 Temperature Targets' to add another step. Set the second 'Stage 2 Temperature Targets' line as follows: set 'Target (°C)' to '54', set 'Acquisition Mode' to 'Single', set 'Hold (hh:mm:ss)' to '00:00:06', set 'Ramp Rate (°C/s)' to '2.2', and set 'Sec Target (°C)', 'Step Size (°C)', and 'Step Delay (cycles)' to '0'.

NOTE: The instrument will display a 14-minute run time, however the run time is less than 12 minutes, as the instrument does not accurately account for the 1 sec cycle at 61 °C.

- In the lower left corner of the click select the pull-down menu next to the 'Apply Template' button. Select 'Save As Template'. Double-click the 'Templates' folder. Click the 'Run Templates' folder and 'Name' the file "LumiraDx SARS-CoV-2 RNA STAR Template" and click the 'Check' button to exit the 'Save Template' window.



6. 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 'NEC' (Negative Extraction Control). Push the 'Enter' button on the keyboard. Click the 'A12' well and define the 'Sample Name' as 'PEC' (Positive Extraction Control). Push the 'Enter' button on the keyboard.
7. 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 "LumiraDx SARS-CoV-2 RNA STAR Sample Template" and click the 'Check' button to exit the 'Save Template' window.
8. Exit the software. If prompted, click 'No' to not save.

11.2 Programming Initial Run and Creating Analysis Template

It is recommended to setup the instrument up to step 4 before preparing the LumiraDx SARS-CoV-2 RNA STAR Master Mix, LumiraDx SARS-CoV-2 RNA STAR 10X Salt Mix and LumiraDx SARS-CoV-2 RNA STAR 50X IC/P Mix as defined in the qSTAR 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 or ice. Use 96-well plates and seals recommended by the instrument manufacturer (see Option for PCR Instruments & Recommended Consumables table above).

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

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 "LumiraDx SARS-CoV-2 RNA STAR 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 "LumiraDx SARS-CoV-2 RNA STAR Sample Template". Click the 'Check' button to exit the 'Apply Template' window. The NEC and PEC should be loaded into positions A1 and A12 of the plate. If not already selected, select all wells. Individual 'Sample Names' can be entered in the 'Step 3: Edit Properties' section if desired.

4. On the LightCycler 480 II instrument, push the Load Plate “↔” button on the front of the instrument. The instrument drawer will automatically come out. Insert the 96-well plate onto the instrument drawer. Align the notch of the 96-well plate with the notch on the instrument drawer. Push the Load Plate “↔” button to close the plate holder.
5. In the LightCycler (LC) 480 desktop software proceed to the ‘Experiment’ tab on the upper left of the software. Click ‘Start Run’ on the bottom right of the software. A ‘Plate Sensor if off’ warning may appear. Click the ‘Check’ button to exit this warning. In the ‘Save LightCycler 480 Experiment’ window that opens, double-click the ‘Experiments’ folder and save under the ‘Name’ “LumiraDx SARS-CoV-2 RNA STAR [YYMMDD_Plate#]” then click the ‘Check’ button to exit this window.
6. After the run has finished, proceed to the ‘Analysis’ tab on the left of the software. Choose ‘Abs Quant/Fit Points’ in the ‘Create new analysis’ section. Click the ‘Check’ button to exit the ‘Create New Analysis’ pop-up window.
7. Click the ‘Background (2-6)’ button on the software. Set ‘Min Offset’ to ‘0’ and ‘Max Offset’ to ‘5’ and click the ‘Check’ button to exit the ‘Background Settings’ window.
8. Confirm that ‘Color Compensation’ is ‘(Off)’ for all analytes on the bottom of the software and confirm ‘First Cycle’ is set to ‘1’ and ‘Last Cycle’ is set to ‘25’. Proceed to the ‘Noise Band’ tab at the upper middle of the software.
9. Select from the pull-down menu next to the ‘Noiseband (Auto)’ button at the bottom of the software select ‘Noiseband (STD Mult)’. Click ‘Filter Comb (456-510)’ and select ‘COVID’. Click the ‘Check’ button to exit the ‘Filter Combination’ window. Set ‘STD Multiplier’ to ‘10.0000’. Click ‘Filter Comb (456-510)’ and select ‘IC’. Click the ‘Check’ button to exit the ‘Filter Combination’ window. Set ‘STD Multiplier’ to ‘10.0000’.
10. Save the new analysis protocol as a template for future use. In the lower left corner of the screen select the pull-down menu next to the ‘Apply Template’ button and click ‘Save As Template’. Double-click the ‘Templates’ Folder. Select the ‘Analysis Templates’ Folder and ‘Name’ the file “LumiraDx SARS-CoV-2 RNA STAR 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.

11.3 Programming Runs Using the Run, Sample, and Analysis Template

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 “LumiraDx SARS-CoV-2 RNA STAR 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 “LumiraDx SARS-CoV-2 RNA STAR Sample Template”. Click the ‘Check’ button to exit the ‘Apply Template’ window. The NEC and PEC should be loaded into positions A1 and A12 of the plate. If not already selected, select all wells. Individual ‘Sample Names’ can be entered in the ‘Step 3: Edit Properties’ section if desired.
4. On the LightCycler 480 II instrument, push the Load Plate “↔” button on the front of the instrument. The instrument drawer will automatically come out. Insert the 96-well plate onto the instrument drawer. Align the notch of the 96-well plate with the notch on the instrument drawer. Push the Load Plate “↔” button to close the plate holder.
5. In the LightCycler (LC) 480 desktop software proceed to the ‘Experiment’ tab on the upper left of the software. Click ‘Start Run’ on the bottom right of the software. A ‘Plate Sensor if off’ warning may appear. Click the ‘Check’ button to exit this warning. In the ‘Save LightCycler 480 Experiment’ window that opens, double-click the ‘Experiments’ folder and save under the ‘Name’ “LumiraDx SARS-CoV-2 RNA STAR [YYMMDD_Plate#]” then click the ‘Check’ button to exit this window.

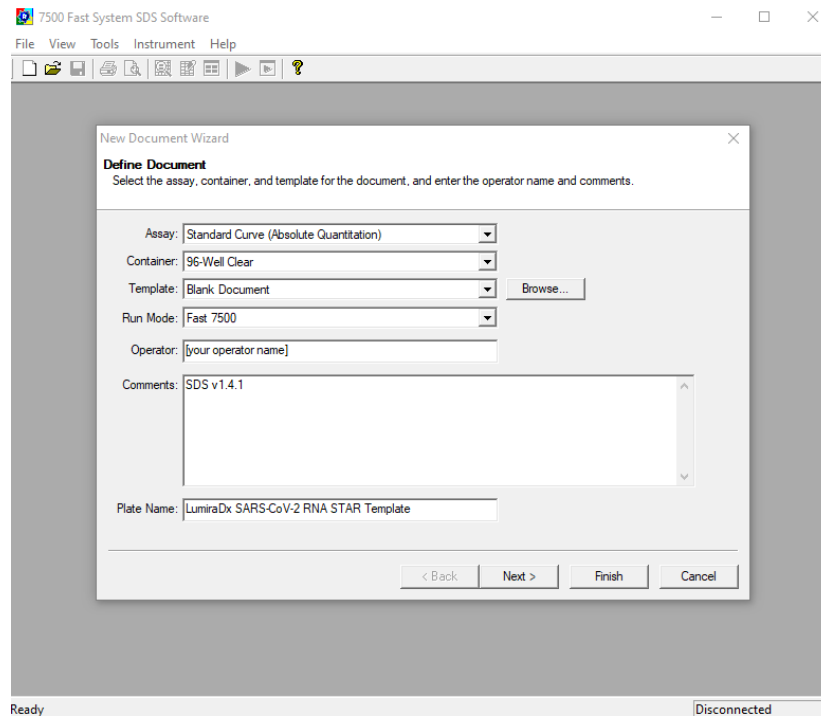
6. After the run has finished, proceed to the 'Analysis' tab on the left of the software. Choose 'Abs Quant/Fit Points' in the 'Create new analysis' section. Click the 'Check' button to exit the 'Create New Analysis' pop-up window.
7. In the lower left corner click 'Apply Template' (do not select the drop-down menu next to the 'Apply Template' button). Double-click the 'Templates' folder. Double click the 'Analysis Templates' folder and select the "LumiraDx SARS-CoV-2 RNA STAR 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 " \leftrightarrow " button on the front of the instrument.
11. Remove the plate and discard the plate in a sealable waste bag or container.

12 RNA STAR Setup for Applied Biosystems™ 7500 Fast Dx

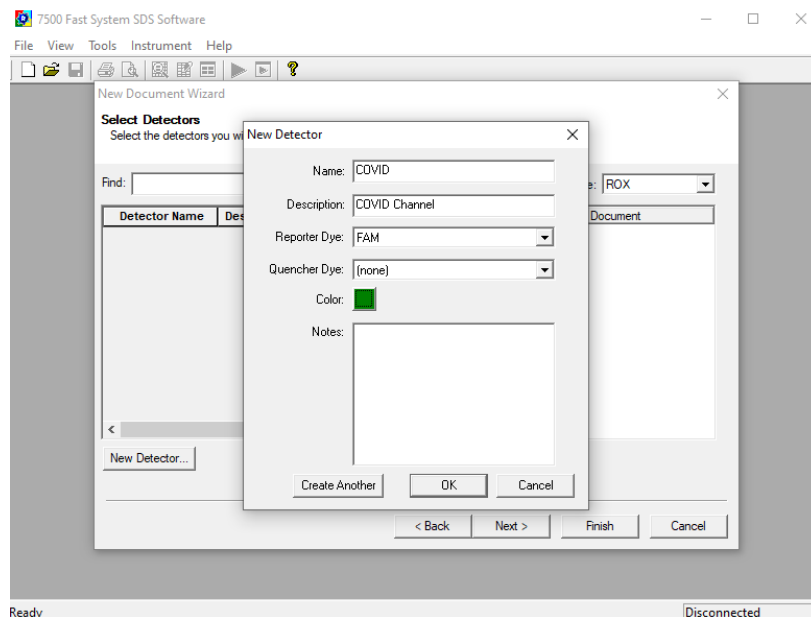
Refer to “User Manual Part Number 4406991” for additional information.

12.1 Programming the Run Template

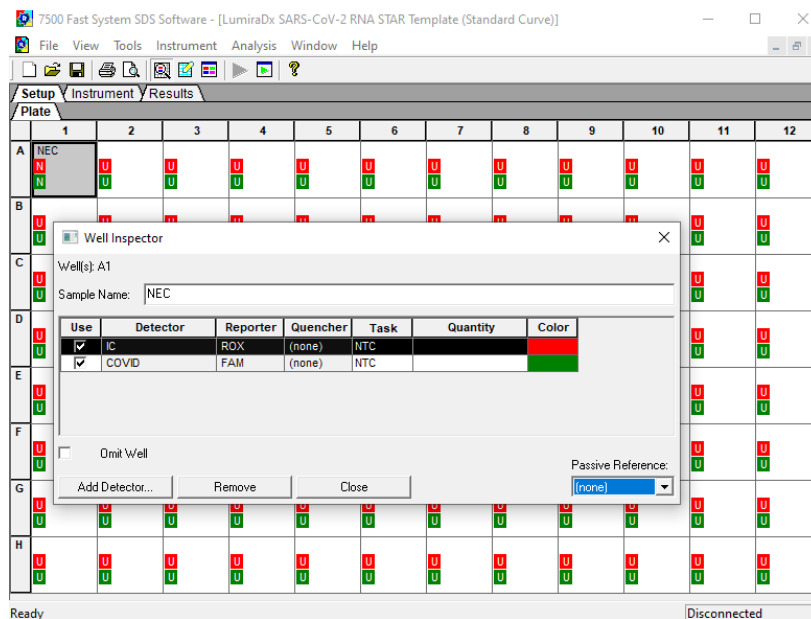
1. Launch the 7500 Fast System desktop software (version 1.4.1).



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



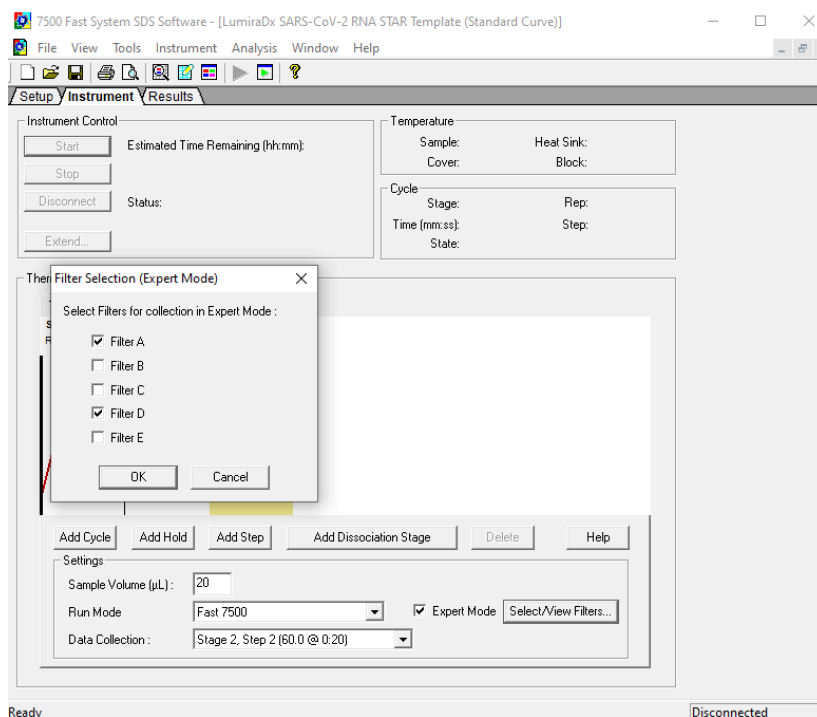
3. In the 'Select Detectors' window, click 'New Detector' to open the 'New Detector' pop-up window and define these settings: define 'Name' as "COVID", define 'Description' to "COVID Channel", set 'Reporter Dye' to 'FAM', set 'Quencher' to '(none)', and set 'Color' to 'Green'. Select 'OK'. Click 'New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



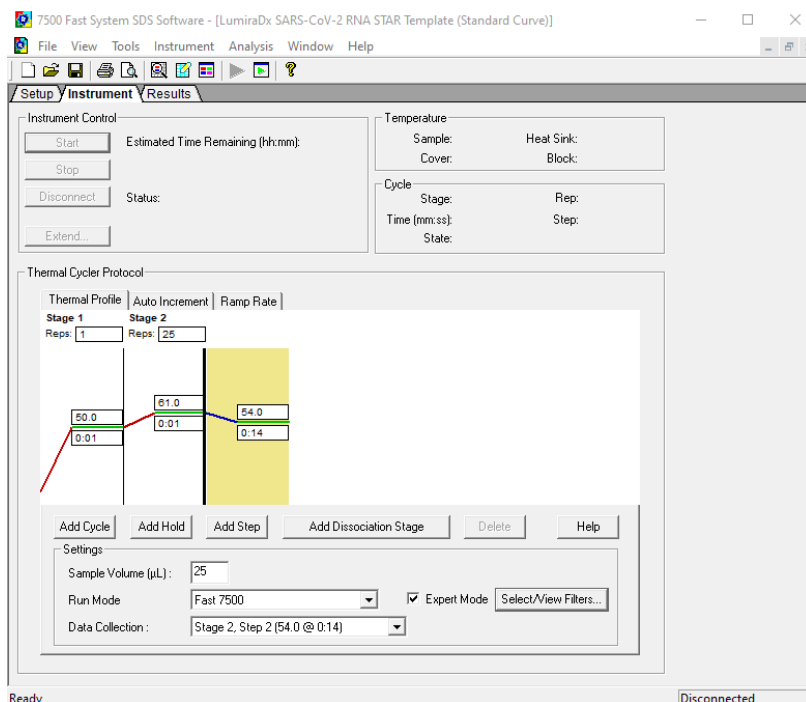
4. In the 'Set Up Sample Plate' window click the box between "A" and "1" to select all wells then select boxes under the 'Use' section to add the COVID and IC Detector to all wells. Click 'Finish'. The wizard will close, and a screen will display the 'Setup' tab and 'Plate' tab. This will show the sample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

5. Select location 'A12', right click and select 'Well Inspector'. In the 'Well Inspector' window, define the 'Sample Name' as "PEC" (Positive Extraction Control). 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'.

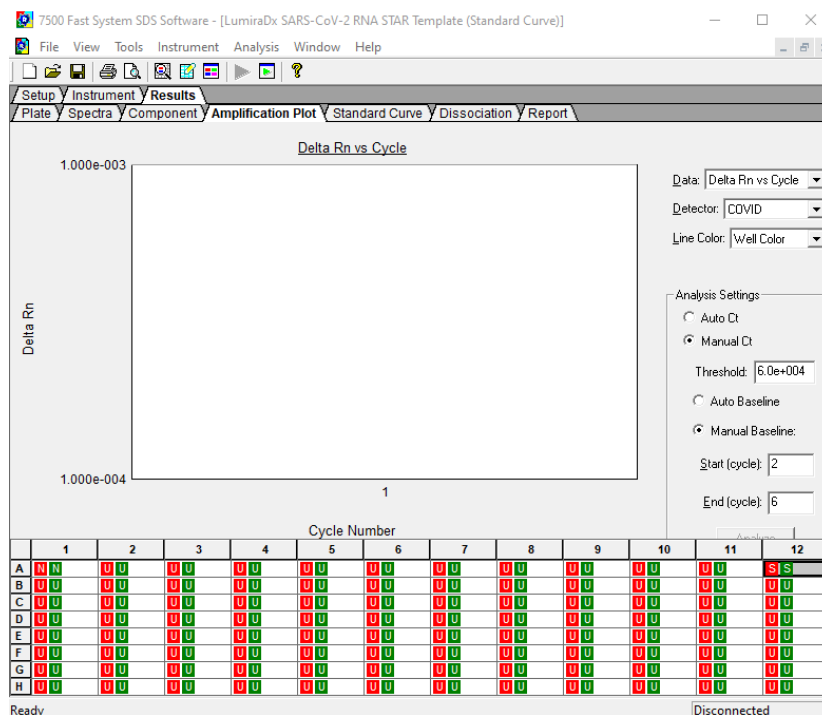
Note: If ROX is not removed as a Passive Reference dye the IC channel will be impacted and the run must be repeated.



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



7. Modify the default thermal profile for 'Stage 1' as follows: set 'Reps' to '1', set 'Temp' to '50', and set 'Time' to '0:01'. Modify the default thermo profile for 'Stage 2' as follows: set 'Reps' to '25', set 'Temp' (left) to '61', set 'Time' (left) to '0:01', set 'Temp' (right) to '54', and set 'Time' (right) to '0:14'. Under the 'Settings' section enter the following: set 'Sample Volume (μL)' to '25', set 'Run Mode' to '7500 Fast' (default), and set 'Data Collection' to 'Stage 2, Step 2(54.0 @ 0:14)'. Proceed to the 'Results' tab to set thresholds for each analyte.



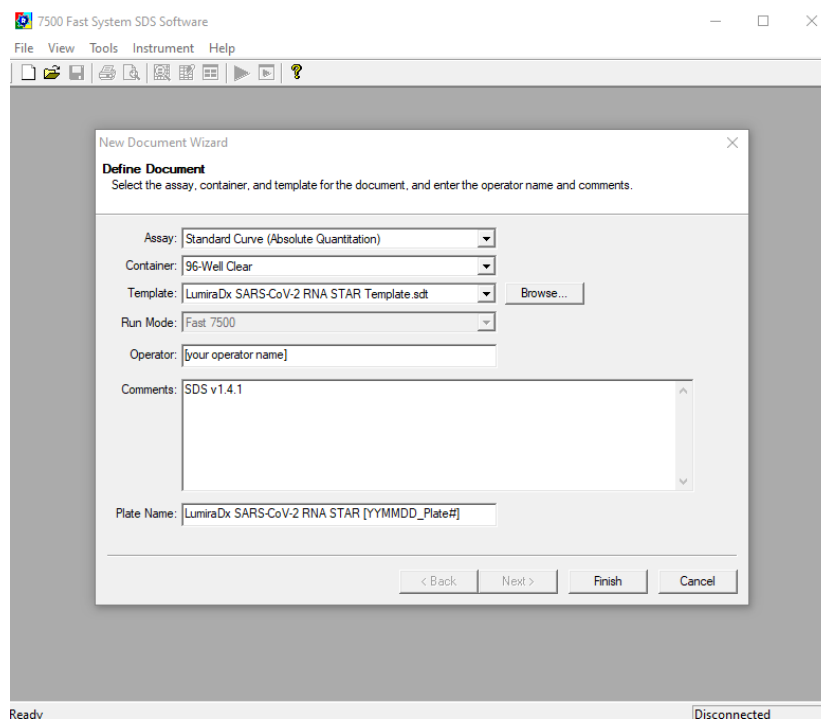
8. Select the 'Amplification Plot' tab then select 'COVID' from the 'Detector' drop-down menu in the top right corner and select the 'Manual Ct' box. In the 'Analysis Settings' window define 'Threshold' as "6.0e+004". In the 'Manual Baseline' window set the 'Start (cycle)' to '2' and set the 'End (cycle)' to '6'.

9. Select 'IC' from the 'Detector' drop-down menu in the top right corner and select the 'Manual Ct' box. In the 'Analysis Settings' section, define 'Threshold' as "1.2e+004". In the 'Manual Baseline' window set 'Start (cycle)' to '2' and set 'End (cycle)' to '6'.
10. Select 'File' from the menu then click 'Save As'. Save the template in file path "D:\Applied Biosystems\7500 Fast System\Template\" and hit the 'Enter' key to confirm file path. 'Name' the file as "LumiraDx SARS-CoV-2 RNA STAR 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'.

12.2 Programming Runs Using the Run Template

It is recommended to setup the instrument up to step 4 before preparing the LumiraDx SARS-CoV-2 RNA STAR Master Mix, LumiraDx SARS-CoV-2 RNA STAR 10X Salt Mix and LumiraDx SARS-CoV-2 RNA STAR 50X IC/P Mix as defined in the qSTAR 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 or ice. Use 96-well plates and seals recommended by the instrument manufacturer (see Option for PCR Instruments & Recommended Consumables table above).

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



2. The 'Quick Startup document' dialog window will open. Click 'Create New Document'. In the 'Define Document' window verify the default settings: 'Assay' is set to 'Standard Curve (Absolute Quantification)', 'Container' is set to '96-Well Clear', 'Template' is set to 'LumiraDx SARS-CoV-2 RNA STAR 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 "LumiraDx SARS-CoV-2 RNA STAR [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 "LumiraDx SARS-CoV-2 RNA STAR [YYMMDD_Plate#]". Confirm 'File Type' is set to 'SDS Document (*.sds)' and click 'OK'. A window will open asking for the "Reason for change of entry". Type "Run Setup" and select 'OK'. Proceed to the 'Instrument' tab.

5. Push circular button on the front of the Applied Biosystems 7500 Fast Dx instrument. Pull out the instrument drawer and load the plate. Make sure to align A1 on the 96-well plate with the defined A1 position in the instrument drawer. Close the instrument drawer by firmly pressing the circular button till drawer is closed.
6. In the 7500 Fast System desktop software (version 21 CFR Part II Software) click 'Start' to initiate the run in the 'Instrument Control' section.
7. Upon completion of the run, click 'OK' on the 'SDS Software' pop-up window. Go to the 'Results' tab then 'Amplification Plot' to then click 'Analyze'. Alternatively, type "Ctrl G" button on the keyboard.
8. 'Save' the file or type "Ctrl S" on the keyboard. A window will open asking for the "Reason for the change". Type "Data Analysis" and click 'OK'.
9. Click the 'Report' tab then select the whole plate and create report as desired. Remove the plate and discard the plate in a sealable waste bag or container.

13 RNA STAR Setup for Applied Biosystems™ QuantStudio 5

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

13.1 Programming Instructions for PC/Laptop-connected Instrument

It is recommended to setup the instrument up to step 11 before preparing the LumiraDx SARS-CoV-2 RNA STAR Master Mix, LumiraDx SARS-CoV-2 RNA STAR 10X Salt Mix and LumiraDx SARS-CoV-2 RNA STAR 50X IC/P Mix as defined in the qSTAR 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 or ice. 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.

QuantStudio™ Design & Analysis Software v1.5.1

File Edit Analysis Tools Help

Properties Method Plate Run Results Export

Experiment Properties Save

Name: LumiraDx SARS-CoV-2 RNA STAR 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

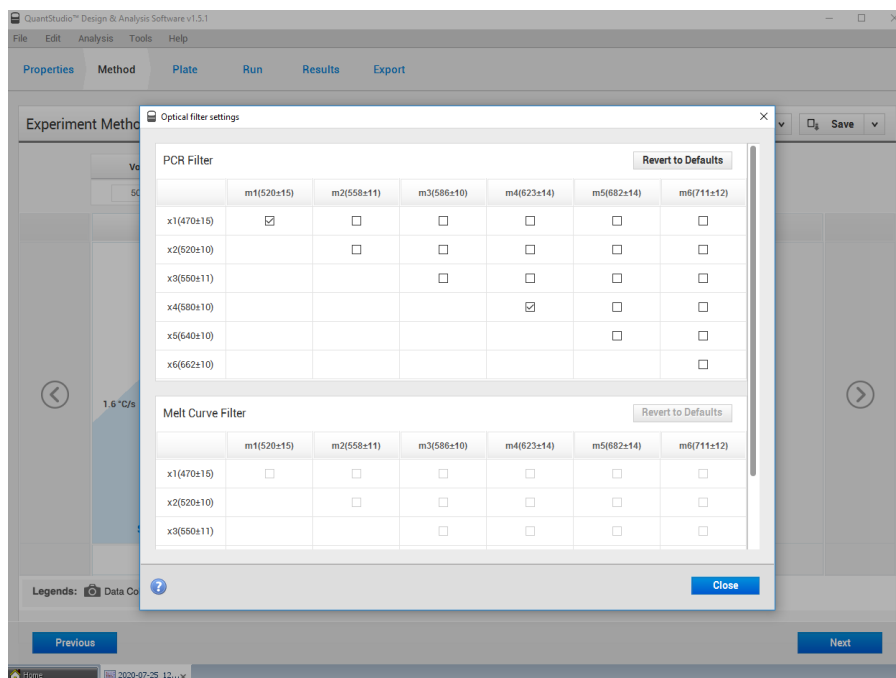
Run mode: Standard

[Manage chemistry details](#)

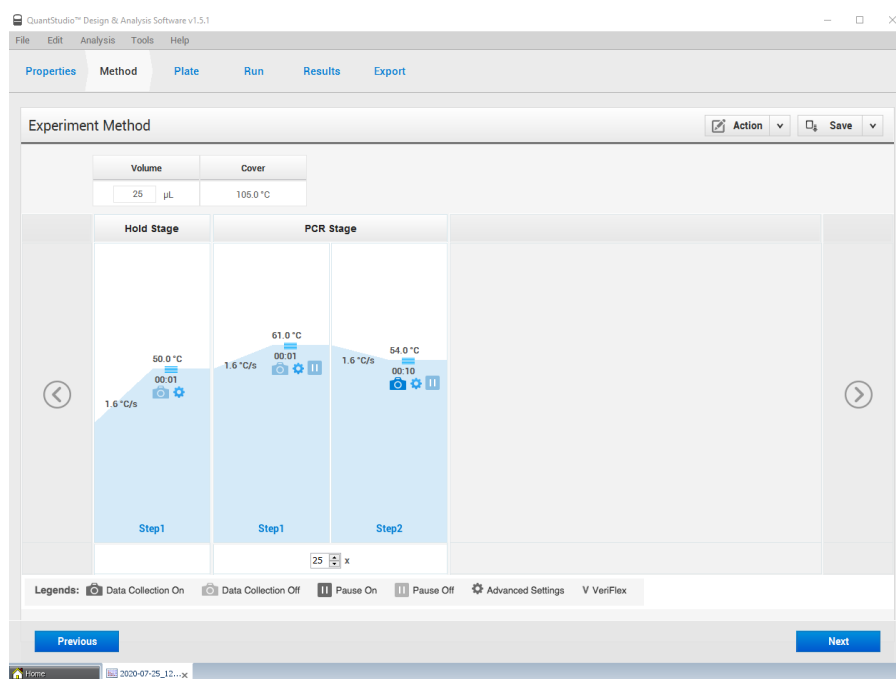
Comments - optional

Next

2. Launch the 'QuantStudio Design & Analysis' Desktop Software (version v1.5.1) and select 'Create New Experiment'. A window will open with the 'Properties' tab selected.
3. Enter the experiment properties as follows: name the experiment "LumiraDx SARS-CoV-2 RNA STAR 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 '25 µL'. Select the 'Hold Stage' and delete Step 2 by clicking the "-" button so only one segment remains. Change 'Step 1' settings as follows: set ramp rate to 1.6°C/s (default ramp rate), set temp to 50°C, and set time to 00:01.
- In the 'PCR Stage', change 'Step 1' settings as follows: set ramp rate to 1.6°C/s (default ramp rate), set temp to 61°C, and set time to 00:01. Change 'Step 2' settings as follows: set ramp rate to 1.6°C/s (default ramp rate), set temp to 54°C, and set time to 00:10 (data collection step).

7. At bottom of window, set 'Cycles' to '25'. Then proceed to the 'Plate' tab.
8. 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.
9. 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.
10. 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.
11. Save the experiment as a template for subsequent runs by clicking the 'Save' down arrow and selecting the 'Save As' option. Name the template "LumiraDx SARS-CoV-2 RNA STAR Template" and click 'Save'.
12. 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.
13. 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'.
14. 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.

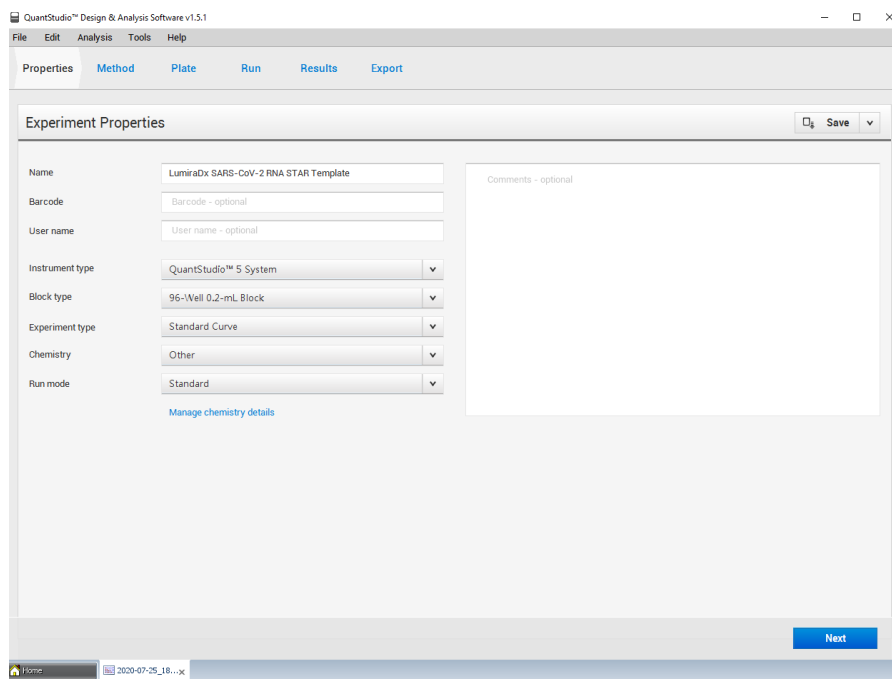
13.2 Analysis Instructions

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 "10,500" for the 'Threshold', set 'Baseline Start Cycle' to '1' and set 'End Cycle' to '6'. Select the 'IC' line then uncheck the 'Default settings' box, the 'Automatic Threshold' box, and the 'Automatic Baseline' box. Enter "20,000" for the 'Threshold', set 'Baseline Start Cycle' to '1' and set 'End Cycle' to '6'. Click 'Apply' to proceed the 'Export' tab.
3. Define the 'File Name' for the exported file ("LumiraDx SARS-CoV-2 RNA STAR [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.

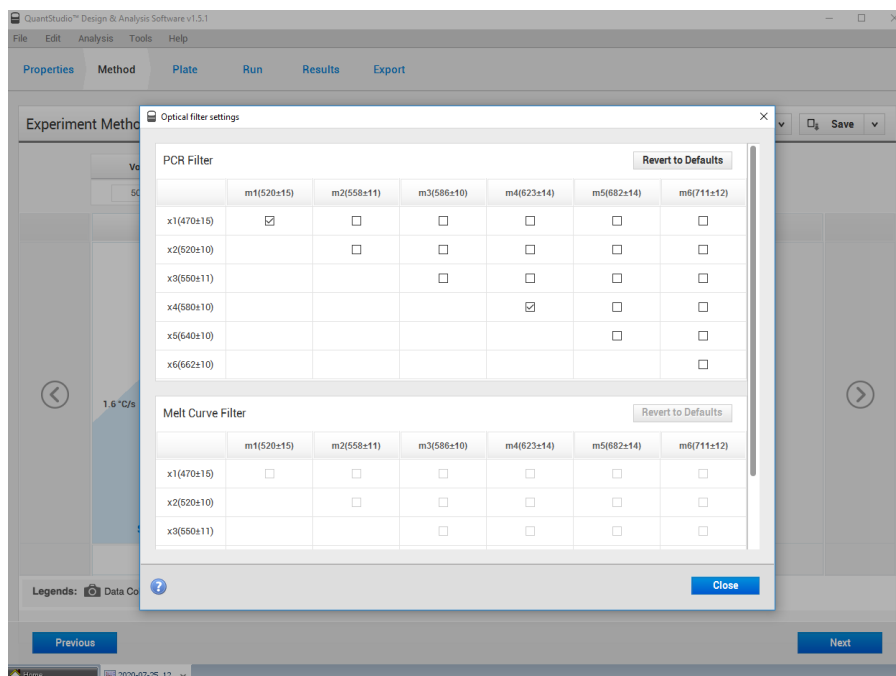
13.3 Programming Instructions for Stand-Alone Instrument

It is recommended to setup the instrument up to step 14 before preparing the LumiraDx SARS-CoV-2 RNA STAR Master Mix, LumiraDx SARS-CoV-2 RNA STAR 10X Salt Mix and LumiraDx SARS-CoV-2 RNA STAR 50X IC/P Mix as defined in the qSTAR 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 or ice. Use 96-well plates and seals recommended by the instrument manufacturer (see Option for PCR Instruments & Recommended Consumables table above).

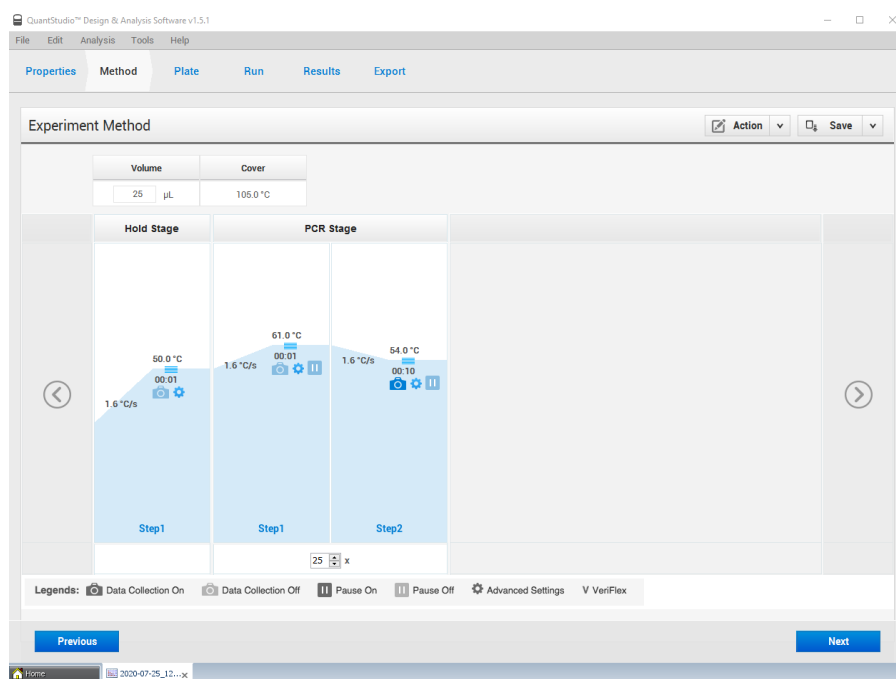
1. If the instrument touchscreen is in sleep mode (dark screen), touch the screen anywhere to activate the instrument. Sleep mode lowers the temperature of the heated cover. It is important that the heated cover idle at 105°C before loading a 96-well plate.



2. An experiment template is required for the QuantStudio 5 instrument when using the touch screen. Create the template by launching the 'QuantStudio Design & Analysis' Desktop Software (version v1.5.1). Select 'Create New Experiment'. A window will open with the 'Properties' tab selected.
3. Enter the experiment properties as follows: name the experiment "LumiraDx SARS-CoV-2 RNA STAR 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 '25 µL'. In the 'Hold Stage', delete Step 2 by clicking the "-" button so only one segment remains. Change 'Step 1' settings as follows: set ramp rate to 1.6°C/s (default ramp rate), set temp to 50°C, and set time to 00:01.
6. In the 'PCR Stage', change 'Step 1' settings as follows: set ramp rate to 1.6°C/s (default ramp rate), set temp to 61°C, and set time to 00:01. Change 'Step 2' settings as follows: set ramp rate to 1.6°C/s (default ramp rate), set temp to 54°C, and set time to 00:10 (data collection step).

7. At bottom of window, set 'Cycles' to '25'. Then proceed to the 'Plate' tab.
8. 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.
9. 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.
10. 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.
11. Save the experiment as a template for subsequent runs by clicking the 'Save' down arrow and selecting the 'Save As' option. Name the template "LumiraDx SARS-CoV-2 RNA STAR Template" and click 'Save'.
12. Copy the template (.edt) file to a USB drive.
13. Insert the USB drive into the designated slot in the front of the QuantStudio 5 instrument. On the instrument touchscreen, press 'Load Experiment'. Select 'USB' from the options provided, scroll to locate the saved template (.edt) file and select the file to import it.
14. Change the 'Run File Name' to "LumiraDx SARS-CoV-2 RNA STAR [YYMMDD_Plate#]" then press 'Start Run'.
15. 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'.
16. 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.
17. 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.
18. Remove the plate and discard the plate in a sealable waste bag or container.

13.4 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 "10,500" for the 'Threshold', set 'Baseline Start Cycle' to '1' and set 'End Cycle' to '6'. Select the 'IC' line then uncheck the 'Default settings' box, the 'Automatic Threshold' box, and the 'Automatic Baseline' box. Enter "20,000" for the 'Threshold', set 'Baseline Start Cycle' to '1' and set 'End Cycle' to '6'. Click 'Apply' to proceed the 'Export' tab.
4. Define the 'File Name' for the exported file ("LumiraDx SARS-CoV-2 RNA STAR [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.

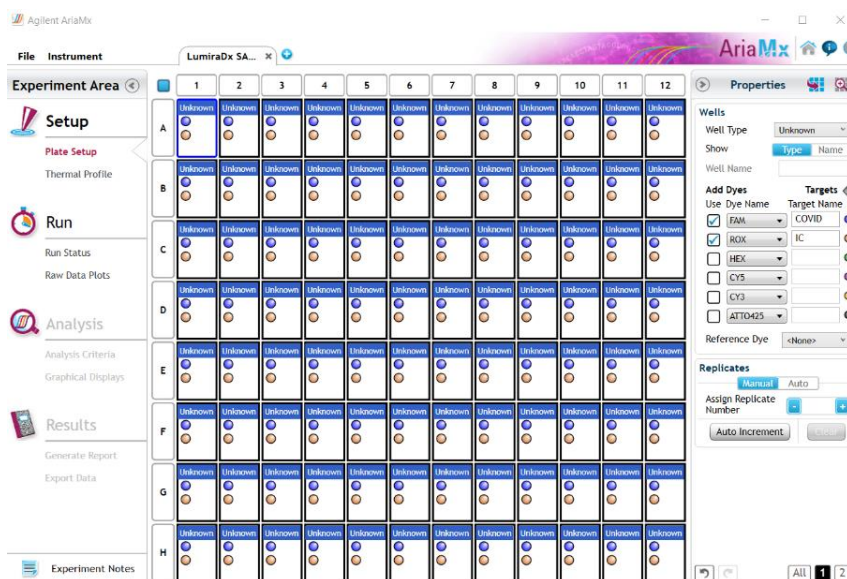
14 RNA STAR Setup for Agilent™ AriaMx

Refer to “User Manual Part Number G8830A” for additional information.

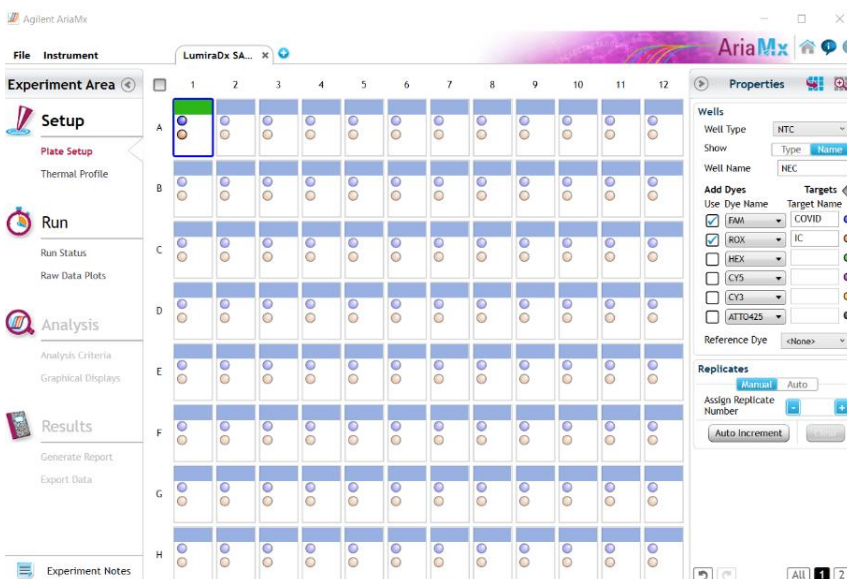
14.1 Programming Instructions for PC/Laptop-connected Instrument

It is recommended to setup the instrument up to step 6 before preparing the LumiraDx SARS-CoV-2 RNA STAR Master Mix, LumiraDx SARS-CoV-2 RNA STAR 10X Salt Mix and LumiraDx SARS-CoV-2 RNA STAR 50X IC/P Mix as defined in the qSTAR 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 or ice. Use 96-well plates and seals recommended by the instrument manufacturer (see Option for PCR Instruments & Recommended Consumables table above).

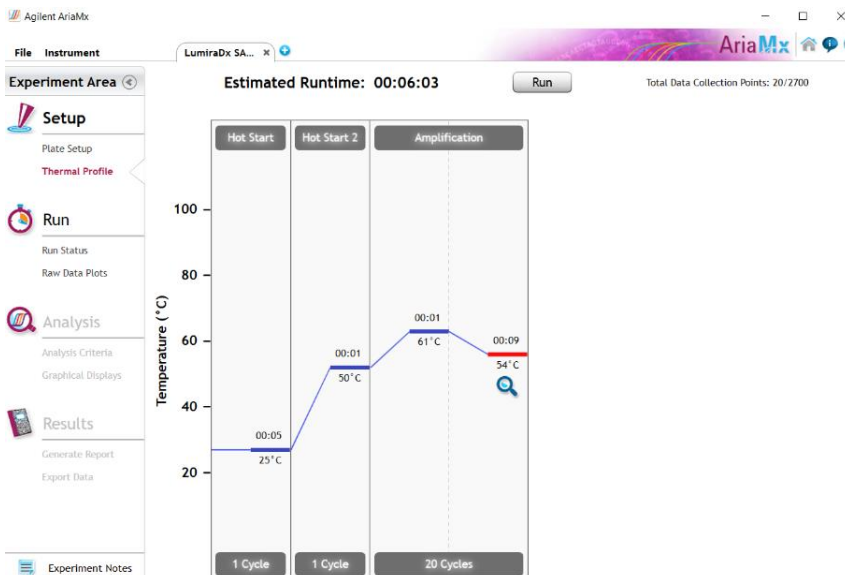
1. Launch the Agilent AriaMx desktop software (version 1.71). Select ‘Quantitative PCR Fluorescence Probe’ in the ‘Experiment Types’ section (do not double click). Define the ‘Experiment Name’ as “LumiraDx SARS-CoV-2 RNA STAR Template” and click ‘Create’.
2. Select all the wells and in the ‘Properties’ section on the right of the screen set ‘Well Type’ to ‘Unknown’, select the ‘FAM’ and ‘ROX’ boxes, and toggle the ‘Target’ arrow (across from ‘Add Dyes’). Set the ‘Target Name’ for ‘FAM’ to “COVID” and ‘ROX’ to “IC”.



3. Select just well 'A1' and re-set 'Well Type' to 'NTC' (No Template Control) from the drop-down menu. Click the 'Name' button next to and define the 'Well Name' as "NEC" (Negative Extraction Control). 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 "PEC" (Positive Extraction Control). The other wells will remain 'Unknown' unless defined as Samples Names (optional).



- Proceed to the 'Thermal Profile' tab on the left of the software and add another 'Hot Start' segment to the left of the default 'Hot Start' segment by click on the "+" (Add Segment) icon on the left edge of the existing segment. Choose 'Hot Start' under the 'New Segment' window and modify the default thermal profile for 'Hot Start' as follows: set 'Temp' to '25°C', set 'Time' to '00:05' and confirm 'Cycle' is automatically set to '1'. Modify the default thermal profile for 'Hot Start 2' as follows: set 'Temp' to '50°C', set 'Time' to '00:01' and confirm 'Cycle' is automatically set to '1'. Modify the default thermal profile for 'Amplification' as follows: set 'Temp' (left) to '61°C', set 'Time' (left) to '00:01', set 'Temp' (right) to '54°C', set 'Time' (right) to '00:09' (Data collection step), and set 'Cycle' to '20'.



- Proceed to save the file by clicking 'File' from the menu and choose 'Save As Template' from the drop-down menu. Name the file "LumiraDx SARS-CoV-2 RNA STAR Template" and click 'Save'.
- Click 'Run' and, once instrument connection is made, click 'Send Config'. The software will prompt you to save the experiment again. Click 'Save' and define 'File Name' as "LumiraDx SARS-CoV-2 RNA STAR [YYMMDD_Plate#]" and click 'Save'.
- On the Agilent AriaMx instrument touchscreen, locate and press the 'Monitor' icon (🔍) at the bottom of the touch screen. Press 'Open Primed Experiment' from pop-up menu. Press the 'Thermal Profile' tab on the left side of the touch screen then press 'Run Experiment' to initiate the run. The instrument lid will begin its Warm-Up procedure (~2 minutes). Press the 'Pause' button when it is activated (green arrow). The 'Pause' button is active once the lid reaches 100 °C. Lift up the instrument door, pull the block lid out to unlock then lift the block lid and load the plate.

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

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

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

14.2 Analysis Instructions

- In the Agilent AriaMx desktop software (version 1.71), select 'Analysis Criteria' from the 'Experiment Area' panel on the left side of the software then select the appropriate wells for analysis using the plate map.
- Select 'Graphical Displays' from the 'Experiment Area' panel on the left side of the screen, then confirm in the 'Amplification Plots' panel on the right side of the software that 'Fluorescence Term' is set to 'ΔR' and 'Smoothing' is 'On'. Expand the section under 'Amplification Plots' by toggling the 'Arrow' icon (if needed) then click 'Adjust'. In the 'Baseline Correction' window, click 'Select All' to highlight all the wells to be analyzed. Set 'Start Cycle' to '1' and set 'End Cycle' to '5' then click 'Apply'. To exit the window, click 'OK'.

3. In the 'Amplification Plots' panel, change 'Graph Type' to 'Log'. Change automatically generated 'Threshold Fluorescence' values by defining 'COVID' as "120" then click the 'Unlock' icon immediately next to the newly defined threshold to lock the setting, additionally, define 'IC' as "100" then click the 'Unlock' icon immediately next to the newly defined threshold to lock the setting. Set 'Background Based Threshold' for 'Cycle Range' as '1' thru '1' and set 'Sigma Multiplier' to '10'.
4. 'Threshold Fluorescence' values are automatically generated for 'COVID' and 'IC'. Set 'Background Based Threshold' for 'Cycle Range' as '1' thru '1' and set 'Sigma Multiplier' to '10'.
5. Select 'Generate Report' from the 'Experiment Area' panel on the left side of the screen, then confirm in the 'Report Configuration' panel that 'Definition' is set to 'Default'. The 'Report' type can be set to 'PDF' or 'PowerPoint'. In the 'Items' section, select the Pencil button next to the 'Tabular Results' line and set 'Include Target Information' to 'Yes'. Check mark the following results: 'Well', 'Well Type', 'Well Name', 'Dye', 'Target', 'Cq (ΔR)', and 'Final Call (ΔR)'. All other headings should be unchecked. Click 'OK' to exit the 'Tabular Results Properties' window. All other report features *can* remain unchanged. Click 'Generate Report', define 'File Name' and click 'Save'.
6. Select 'Export Data' from the 'Experiment Area' panel on the left side of the screen, then confirm in the 'Export Configuration' panel that 'Definition' is set to 'Default'. The file type can be set to 'Excel', 'Text', 'LIMS', 'RDML'. In the 'Items' section, select the Pencil button next to the 'Tabular Results' line and set 'Include Target Information' to 'Yes'. Check mark the following results: 'Well', 'Well Type', 'Well Name', 'Dye', 'Target', 'Cq (ΔR)', and 'Final Call (ΔR)'. All other headings should be unchecked. Click 'OK' to exit the 'Column Options' window then click 'Export Data' to generate the report.
7. To save the run, click 'File' from the menu and choose 'Save' from the drop-down menu.
8. Close the software.

14.3 Programming Instructions Using Instrument Touch Screen

It is recommended to setup the instrument up to step 4 before preparing the LumiraDx SARS-CoV-2 RNA STAR Master Mix, LumiraDx SARS-CoV-2 RNA STAR 10X Salt Mix and LumiraDx SARS-CoV-2 RNA STAR 50X IC/P Mix as defined in the qSTAR 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 or ice. Use 96-well plates and seals recommended by the instrument manufacturer (see Option for PCR Instruments & Recommended Consumables table above).

1. On the Agilent AriaMx instrument touchscreen, press the 'Home' button then press 'New Experiment' and press 'Quantitative PCR Fluorescence Probe'.
2. On the 'Plate Setup' screen, select all the wells then press 'Well Types' and press 'Unknown' from the drop-down list. Check the boxes next to 'ROX' and 'FAM' and proceed to the 'Thermal Profile' tab.
3. Select the 'Hot Start' window and add another 'Hot Start' segment to the left of the default 'Hot Start' segment by pressing the "+" button. Select 'Hot Start' as the 'New' segment. Modify the default thermal profile for 'Hot Start' as follows: set 'Temp' to '25 °C', set 'Time' to '00:05', and set 'Cycle' to '1'. Modify the default thermal profile for 'Hot Start 2' as follows: set 'Temp' to '50 °C', set 'Time' to '00:01', and set 'Cycle' to '1'. Modify the default thermal profile for 'Amplification' as follows: set 'Temp' (left) to '61 °C', set 'Time' (left) to '00:01', set 'Temp' (right) to '54 °C', set 'Time' (right) to '00:09' (data collection step), and set 'Cycle' to '20'. 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 "LumiraDx SARS-CoV-2 RNA STAR [YYMMDD_Plate#]" and press 'Save'.
5. The instrument lid will begin its Warm-Up procedure (~2 minutes). Press the 'Pause' button when it is activated (green arrow). The 'Pause' button is active once the lid reaches 100 °C. Lift up the instrument door, pull the block lid out to unlock then lift the block lid and load the plate.

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

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

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

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

14.4 Analysis Instructions

9. 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".
10. 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 "NEC" (Negative Extraction Control). 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 "PEC" (Positive Extraction Control). The other wells will remain 'Unknown' unless defined as Samples Names (optional).
11. 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.
12. 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 '5' then click 'Apply'. To exit the window, click 'OK'.
13. In the 'Amplification Plots' panel, change 'Graph Type' to 'Log'. Change automatically generated 'Threshold Fluorescence' values by defining 'COVID' as "120" then click the 'Unlock' icon immediately next to the newly defined threshold to lock the setting, additionally, define 'IC' as "100" then click the 'Unlock' icon immediately next to the newly defined threshold to lock the setting.
14. 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'.
15. 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.
16. To save the run, click 'File' from the menu and choose 'Save' from the drop-down menu.
17. Close the software.

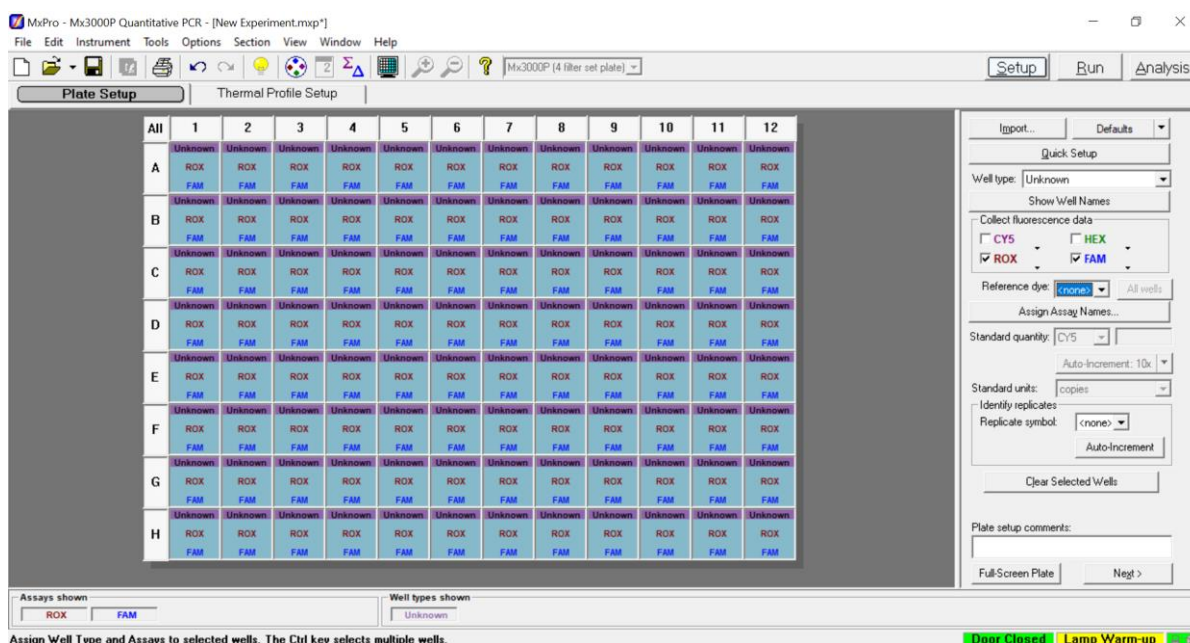
15 RNA STAR Setup for Agilent™ Stratagene Mx3005P

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

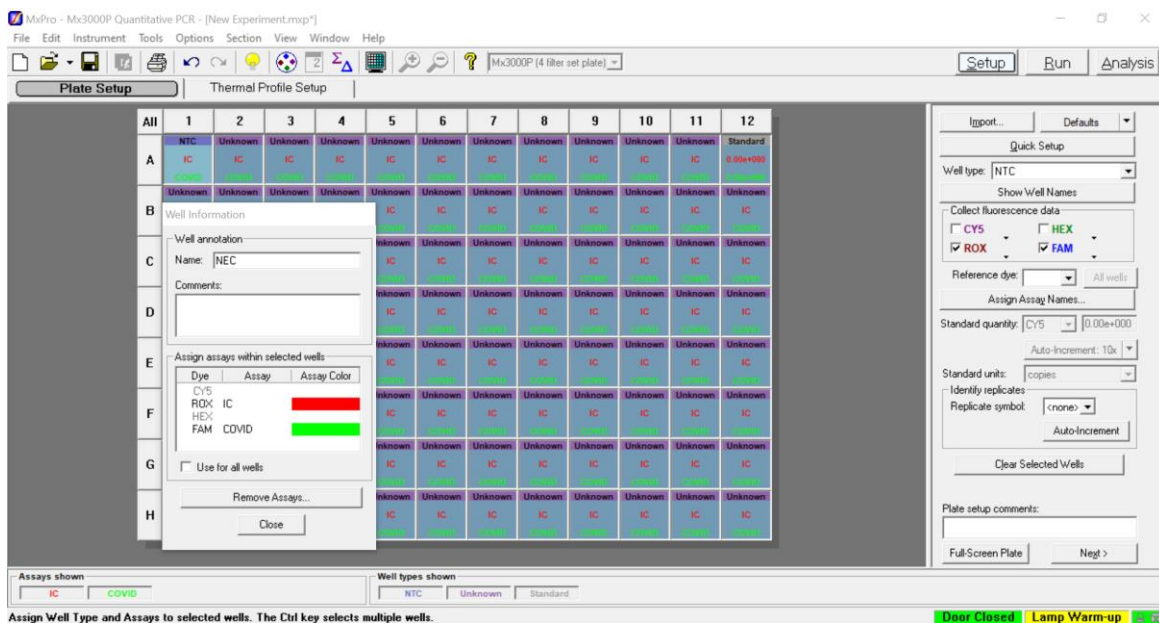
15.1 Programming Instructions for PC/Laptop-connected Instrument

It is recommended to setup the instrument up to step 6 before preparing the LumiraDx SARS-CoV-2 RNA STAR Master Mix, LumiraDx SARS-CoV-2 RNA STAR 10X Salt Mix and LumiraDx SARS-CoV-2 RNA STAR 50X IC/P Mix as defined in the qSTAR 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 or ice. Use 96-well plates and seals recommended by the instrument manufacturer (see Option for PCR Instruments & Recommended Consumables table above).

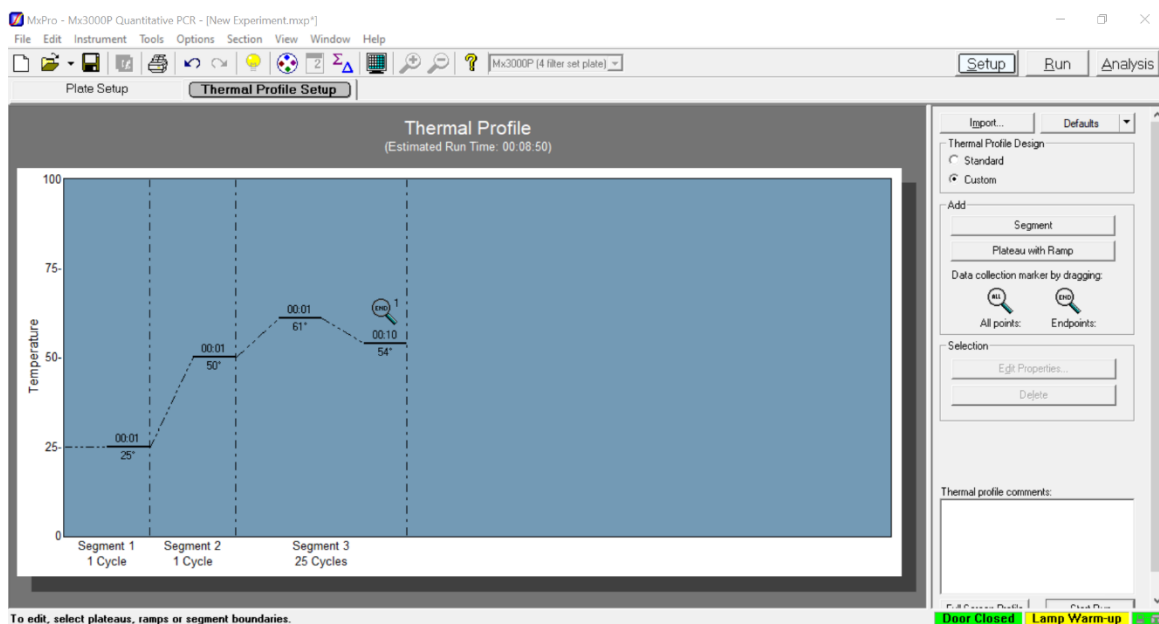
1. Launch the MxPro desktop software (version 4.10). Select ‘Quantitative PCR (Multiple Standards)’ in the ‘New Options’ window, check the box next to ‘Turn lamp on for warm-up?’, and click the ‘OK’ button. The lamp requires 20 minutes to properly warm-up.



2. On the 'Plate Setup' screen, select all the wells and on the right of the software 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 re-set 'Well Type' to 'NTC' (No Template Control). Select just well 'A12' and re-set 'Well Type' to 'Standard'. Double-click well 'A1' and define the 'Name' as "NEC" (Negative Extraction Control). Click 'Close'. Double-click well 'A12' and define the 'Name' as "PEC" (Positive Extraction Control). 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. Click 'Segment' on the right of the screen in the 'Add' section to add a segment. Select the 'Segment 3' window and click 'Delete' on the right of the screen in the 'Selection' section. Select the 'Segment 2' window then click 'Segment'. Select the new 'Segment 3' window then select 'Plateau with Ramp' on the right of the screen in the 'Add' section. Modify the default thermal profile for 'Segment 1' as follows: set 'Temp' to '25°', set 'Time' to '00:01', and set 'Cycle' to '1'. Modify the default thermal profile for 'Segment 2' as follows: set 'Temp' to '50°', set 'Time' to '00:01', and set 'Cycle' to '1'. Modify the default thermal profile for 'Segment 3' as follows: set 'Temp' (left) to '61°', set 'Time' (left) to '00:01', set 'Temp' (right) to '54°', set 'Time' (right) to '00:10', set 'Cycle' to '25', and drag the 'Endpoints:' symbol from the right of screen in the 'Add' section to the right 'Temp' to add a data collection step.

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

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

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

10. 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 "LumiraDx SARS-CoV-2 RNA STAR [YYMMDD_Plate#]" and click 'Save'. The run is started.
11. 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.
12. Remove the plate and discard the plate in a sealable waste bag or container.

15.2 Analysis Instructions

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

16 Interpretation of Results and Reporting

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

16.1 LumiraDx SARS-CoV-2 RNA STAR Controls

1. The LumiraDx SARS-CoV-2 RNA STAR Negative Extraction Control (Neg. Ext. Ctrl.) consists of Nuclease-Free Water to control for cross-contamination, or reagent contamination from the extraction process. The Neg. Ext. Ctrl. should be treated as a patient specimen and processed with every extraction batch.
2. The LumiraDx SARS-CoV-2 RNA STAR Positive Extraction Control (Pos. Ext. Ctrl.) is needed to ensure test reagents are properly detecting SARS-CoV-2 and is comprised of a quantified NATtrol™ SARS-Related Coronavirus 2 (SARS-CoV-2) External Run Control (ZeptoMetrix Corporation; 50,000 copies / mL). The working concentration of the Pos. Ext. Ctrl. is 5,000 copies / mL and this should be processed with every extraction batch. 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.
3. An Internal Control consists of a 40-bp synthetic RNA that the SARS-CoV-2 assay primers can bind and amplify from, with a unique probe region for molecular beacon detection in the ROX channel. The Internal Control is included in the LumiraDx SARS-CoV-2 RNA STAR 50X Internal Control & Primer Mix (50X IC/P Mix). The Internal Control serves as a control for detection of inhibitors present in the specimen, assures that adequate amplification has taken place, and that the enzymes and primers were not inadvertently damaged during production, shipment, and storage.

The testing algorithm for all instruments is based on the standard practice of determining background fluorescence and calling a sample well positive if the change in fluorescent signal exceeds an established threshold. Background fluorescence levels are calculated from cycles 1-6 for the Roche LightCycler 480 II and the Applied Biosystems QuantStudio 5. The Agilent Aria Mx uses cycles 1-4, the Applied Biosystems 7500 Fast Dx uses cycles 2-6 and the Agilent Stratagene Mx3005p uses the Adaptive Baseline settings. A threshold (further described starting in section 11, for each instrument) is applied to the run. Positive and negative results are based on a sample well exceeding this threshold. No Ct cutoff is used for the testing algorithm, and it is expected that all reactions should occur between a Ct value of 3 to 25 (3 to 20 for the Agilent Aria Mx).

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

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

Control Type/Name	Used to Monitor	SARS-CoV-2 (FAM)	Expected Ct Values	Internal Control (ROX)	Expected Ct Values
Pos. Ext. Ctrl.	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. Ext. Ctrl.	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. Ext. Ctrl. to be deemed positive.

Table 2. Expected Results from Controls (on Agilent AriaMx)

Control Type/Name	Used to Monitor	SARS-CoV-2 (FAM)	Expected Ct Values	Internal Control (ROX)	Expected Ct Values
Pos. Ext. Ctrl.	Substantial reagent failure including primer and probe integrity	+	$3.0 \leq Ct \leq 20.0$	+ or - (Can be positive or negative)	$3.0 \leq Ct \leq 20.0^*$
Neg. Ext. Ctrl.	Reagent and/or environmental contamination	-	None detected	+	$3.0 \leq Ct \leq 20.0$

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

16.2 Interpretation of Patient Specimen Results

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

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

Assay Results	SARS-CoV-2 (FAM)	Internal Control (ROX)	Interpretation of Results	Notes
SARS-CoV-2 Positive ("+")	$3.0 \leq Ct \leq 25.0$	$3.0 \leq Ct \leq 25.0^*$	SARS-CoV-2 Viral RNA detected; IC possibly detected	Report results to healthcare provider and CDC.
Negative ("-")	No Ct detected	$3.0 \leq Ct \leq 25.0$	No SARS-CoV-2 Viral RNA detected; IC detected	Report results to healthcare provider. Consider testing for other respiratory pathogens.
Invalid	No Ct detected	No Ct detected	No SARS-CoV-2 Viral RNA detected; No IC detected	Invalid test. Retest the same processed sample. If the second test is also invalid obtain a new specimen and retest.

*The Internal Control is not required to amplify.

Table 4. Interpretation of SARS-CoV-2 RNA STAR (on Agilent AriaMx)

Assay Results	SARS-CoV-2 (FAM)	Internal Control (ROX)	Interpretation of Results	Notes
SARS-CoV-2 Positive ("+")	$3.0 \leq Ct \leq 20.0$	$3.0 \leq Ct \leq 20.0^*$	SARS-CoV-2 Viral RNA detected; IC possibly detected	Report results to healthcare provider and CDC.
Negative ("-")	No Ct detected	$3.0 \leq Ct \leq 20.0$	No SARS-CoV-2 Viral RNA detected; IC detected	Report results to healthcare provider. Consider testing for other respiratory pathogens.
Invalid	No Ct detected	No Ct detected	No SARS-CoV-2 Viral RNA detected; No IC detected	Invalid test. Retest the same processed sample. If the second test is also invalid obtain a new specimen and retest.

*The Internal Control is not required to amplify.

17 Quality Control

- Quality control requirements must be performed in conformance with local, state, and federal regulations or accreditation requirements and the user's laboratory standard quality control procedures. For further guidance on appropriate quality control practices, refer to 42 CFR 493.1200.
- Quality control procedures are intended to monitor reagent and assay performance.
- Test all Positive and Negative Extraction Controls when running diagnostic samples and with each new Test lot to ensure all reagents and Test components are working properly.
- Good laboratory practice (cGLP) recommends running a Positive Extraction Control and Negative Extraction Control in each nucleic acid isolation batch.
- All samples include an Internal Control for validation of enzyme, primer, and probe stability.

18 Limitations

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

19 Conditions of Authorization for the Laboratory

The “LumiraDx SARS-CoV-2 RNA STAR Letter of Authorization”, along with the authorized “Fact Sheet for Healthcare Providers”, the authorized “Fact Sheet for Patients”, and other authorized labeling are available on the FDA website: <https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/vitro-diagnostics-euas>.

However, to assist clinical laboratories using LumiraDx SARS-CoV-2 RNA STAR, the relevant Conditions of Authorization are listed below:

1. Authorized laboratories³ using LumiraDx SARS-CoV-2 RNA STAR will include with test result reports, all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.
2. Authorized laboratories using LumiraDx SARS-CoV-2 RNA STAR will use the product as outlined in the authorized labeling. Deviations from the authorized procedures, including the authorized instruments, authorized extraction methods, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to use this product are not authorized under this EUA.
3. Authorized laboratories that receive the LumiraDx SARS-CoV-2 RNA STAR test will notify the relevant public health authorities of their intent to run this product prior to initiating testing.
4. Authorized laboratories using LumiraDx SARS-CoV-2 RNA STAR will have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.
5. Authorized laboratories will collect information on the performance of LumiraDx SARS-CoV-2 RNA STAR and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and LumiraDx (via email: customerservices.US@lumiradx.com) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of your product of which they become aware.
6. All laboratory personnel using LumiraDx SARS-CoV-2 RNA STAR must be appropriately trained in nucleic acid amplification techniques and use appropriate laboratory and personal protective equipment when handling this Test and use this product in accordance with the authorized labeling.
7. LumiraDx, authorized distributors, and authorized laboratories using LumiraDx SARS-CoV-2 RNA STAR will ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records will be made available to FDA for inspection upon request.

³ The letter of authorization refers to, “Laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform high complexity tests” as “authorized laboratories.”

20 Performance Characteristics

20.1 Limit of Detection (LoD)

20.1.1 Purpose

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

20.1.2 Procedure

Sample matrix pools were prepared from nasopharyngeal (NP) swab samples and spiked with quantified genomic RNA from SARS Related Coronavirus 2, Isolate USAWA1/2020, NR52285 (ATCC® VR1986D™). The RNA was spiked into the nasopharyngeal (NP) swab sample matrix pools at 0, 250, 500, and 750 copies/mL. Automated extraction with the Qiagen DSP Virus/Pathogen Kit (catalog #937055; 400 µL input, 60 µL elution) was performed on the Qiagen QIAAsymphony SP. The LumiraDx SARS-CoV-2 RNA STAR was conducted per the standard assay protocol on the Agilent Stratagene Mx3005P (software version 4.10) RT-PCR Instrument.

20.1.3 Results and Conclusions

The results are shown in Table 5 for the preliminary LoD studies. The preliminary LoD was expected to be 500 copies/mL.

Table 5. Preliminary LoD study.

Concentration (Copies/mL)	Observed Properties		
	Method	Replicates Detected	Mean Ct Value
0	Qiagen DSP Viral (Automated)	0/3	n/a
250	Qiagen DSP Viral (Automated)	2/3	16.5
500	Qiagen DSP Viral (Automated)	3/3	13.3
750	Qiagen DSP Viral (Automated)	3/3	13.3

The LoD was confirmed by processing and testing 20 replicates at 0, 250, 500 and 750 copies / mL using automated extraction with the Qiagen DSP Virus/Pathogen Kit (catalog #937055; 400 µL input, 60 µL elution) on the Qiagen QIAAsymphony SP then running the purified nucleic acid in LumiraDx SARS-CoV-2 RNA STAR on the Agilent Stratagene Mx3005P (software version 4.10) RT-PCR Instrument. Based on the automated process study data, the LoD is 500 copies / mL, as shown in Table 6.

Table 6. LoD confirmation study.

Concentration (Copies/mL)	Observed Properties		
	Method	Replicates Detected	Mean Ct Value
0	Qiagen DSP Viral (Automated)	0/3	n/a
250	Qiagen DSP Viral (Automated)	15/21	14.7
500	Qiagen DSP Viral (Automated)	19/20	14.8
750	Qiagen DSP Viral (Automated)	21/21	13.2

Additional extraction methods (MagMax Viral/Pathogen Nucleic Acid Isolation Kit (Catalog Number: A42352; 400 µL input, 50 µL elution) and the QIAamp Viral RNA Mini Kit (Catalog Number: 52906; 280 µL input, 50 µL elution) were evaluated during the LoD study. LumiraDx SARS-CoV-2 RNA STAR showed >95% detection at 750 copies/mL and 250 copies/mL with the QIAamp Viral Mini and the MagMax Viral/Pathogen Kit. Results demonstrated that the alternative extraction methods generated comparable LoD results when compared to the automated Qiagen DSP Viral method.

20.2 RT-PCR Instrument Validation

20.2.1 Purpose

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

20.2.2 Procedure

Quantitated genomic RNA from SARS Related Coronavirus 2, Isolate USAWA1/2020, NR52285 (ATCC® VR1986D™) was used for this testing. Respiratory matrix prepared from negative nasopharyngeal swabs spiked with 250 copies/mL (0.5x LoD) and 500 copies/mL (1x LoD) was extracted with the Qiagen DSP Virus/Pathogen Kit (catalog #937055; 400 µL input, 60 µL elution) on the Qiagen QIAasympyony SP.

20.2.3 Results and Conclusions

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

Table 7. LumiraDx SARS-CoV-2 RNA STAR on different RT-PCR Instruments.

Concentration (Copies/mL)		Roche LightCycler 480 II	Applied Biosystems 7500 Fast Dx	Applied Biosystems QuantStudio 5	Agilent AriaMx	Agilent Stratagene Mx3005P
0	FAM (COVID)	0/5	0/5	0/5	0/5	0/5
	ROX (IC)	5/5	5/5	5/5	5/5	5/5
250	FAM (COVID)	3/5	4/5	3/5	4/5	3/5
	ROX (IC)	5/5	5/5	5/5	5/5	5/5
500	FAM (COVID)	5/5	5/5	5/5	5/5	5/5
	ROX (IC)	5/5	5/5	5/5	5/5	5/5

20.3 Inclusivity (Analytical Sensitivity)

20.3.1 Purpose

To demonstrate inclusivity for all SARS-CoV-2 sequences in the GISAID database⁴ through *in silico* analysis of the amplification region.

20.3.2 Procedure

LumiraDx SARS-CoV-2 RNA STAR detects an amplicon from ORF1a of SARS-CoV-2. To ensure the performance of this Test design, an *in silico* analysis was performed to confirm Test inclusivity. A multisequence alignment was generated with complete high coverage SARS-CoV-2 sequences maintained in the GISAID database submitted between the dates of December 24, 2019, and June 14, 2020 (n = 46,031). The amplicon region of interest was then compared for identity to the respective regions in the alignment.

20.3.3 Results & Conclusions

An *in silico* analysis of 46,031 publicly available SARS-CoV-2 nucleic acid sequences in GISAID as of June 14, 2020 with amplicon target sequence that only include the amplicon region for LumiraDx SARS-CoV-2 RNA STAR. Over 99% homology was demonstrated between the target sequence and database genomes. The 1% of sequences that did not match fell into two categories. Approximately 0.58% of the sequences that did not match had a single base mismatch within the amplicon region. Of the remaining unmatched sequences, approximately 0.42%, had full homology for the bases that had been sequenced, however the remaining bases in the genomes were ambiguous and were represented by an "N". Based on the high percentage of analyzed sequences having no mismatches, and of the very few sequences with mismatches (only had a single mismatched base), the likelihood of a false negative is very low.

20.4 Cross Reactivity: *In silico* Analysis

20.4.1 Purpose

To demonstrate, through *in silico* analysis, the specificity of the LumiraDx SARS-CoV-2 RNA STAR to exclude amplification of RNA or DNA originating from organisms other than SARS-CoV-2.

20.4.2 Procedure

To ensure the performance of this Test, *in silico* analysis was performed to confirm the LumiraDx SARS-CoV-2 RNA STAR exclusivity. Amplicon sequences were aligned with genomic sequences from organisms listed in Table 8.

⁴ *GISAID (Global Initiative on Sharing All Influenza Data). Initiative. GISAID. <https://www.epicov.org/epi3/frontend#5c74af>. Accessed June 14, 2020.

20.4.3 Results and Conclusions

In silico analysis confirmed organisms listed in Table 8 maintain less than 80% homology with the SARS-CoV-2 qSTAR primers and probes.

Table 8. *In silico* homology for potential cross reactivity with LumiraDx SARS-CoV-2 RNA STAR.

Pathogen	Strain	GenBank Accession #	% Homology
Human coronavirus 229E	Seattle/USNSC9724/2018	MN369046.1	59.5 %
Human coronavirus OC43	MDS12	MK303623.1	57.1 %
Human coronavirus HKU1	SI17244	MH940245.1	54.8 %
Human coronavirus NL63	Seattle/USNSC0768/2019	MN306040.1	59.5 %
SARS CoV-1	Urbani isolate icSARS-C7-MA	MK062184.1	59.5 %
MERS CoV	Hu/Riyadh-KSA1801382/2018	MN723544.1	57.1 %
Adenovirus C1	SG09/HAdVC1/2016	MN513345.1	57.1 %
Human Metapneumovirus (hMPV)	HMPV/Seattle/USA/SC0380/2019	KC562241.1	59.5 %
Parainfluenza virus 1	HPIV1/USA/38078 A/2011	KF530203.1	59.5 %
Parainfluenza virus 2	HPIV2/Los Angeles/USA/C HLA18/2016	MK167027.1	59.5 %
Parainfluenza virus 3	HPIV3/Seattle/USA/SC9406/2019	MN306052.1	57.1 %
Parainfluenza virus 4	HPIV4b/Seattle/USA/SC9597/2019	MN306058.1	59.5 %
Influenza A	H3N2 A/Kitakyushu/ 159/93 PB1	AF037418.1	61.9 %
Influenza B	B/Iowa/14/2017	CY236691.1	57.1 %
Enterovirus EV68	USA/CA/2014-RGDS-1025	MK681490.1	59.5 %
Respiratory syncytial virus	RSV-A/US/BID-V8392/2003	MG027862.1	59.5 %
Rhinovirus	Rhinovirus 59 strain 16-J2	KY629935.1	57.1 %
<i>Chlamydia pneumoniae</i>	AR39	AE002161.1	64.3 %
<i>Haemophilus influenzae</i>	10P129H1	CP029620.1	61.9 %
<i>Legionella pneumophila</i>	Albuquerque 1 (D-7474)	CP021286.1	64.3 %
<i>Mycobacterium tuberculosis</i>	TCDC11	CP046728	66.7 %
<i>Mycobacterium tuberculosis</i>	H37Rv	NC_000962	66.7 %
<i>Streptococcus pneumoniae</i>	R6CIB17	CP038808.1	64.3 %
<i>Streptococcus pyogenes</i>	1085	CP047120.1	66.7 %
<i>Bordetella pertussis</i>	A340	CP033420.1	64.3 %
<i>Mycoplasma pneumoniae</i>	M129	U00089.2	64.3 %
<i>Candida albicans</i>	TIMM1768	CP032012.1	66.7 %
<i>Pseudomonas aeruginosa</i>	VIT PC9	CP048791.1	66.7 %
<i>Staphylococcus epidermidis</i>	1457	CP020463.1	66.7 %
<i>Streptococcus salivarius</i>	57.1	CP002888.1	66.7 %

20.5 Cross Reactivity: Wet Testing

20.5.1 Purpose

To demonstrate, through wet testing, the specificity of the LumiraDx SARS-CoV-2 RNA STAR using pooled samples of the High Priority organisms and priority pathogens (NATrol Pneumonia Panel – Quantifiable Bacteria; ZeptoMetrix; Catalog #NATPPA-Bio) shown in Table 9 tested in the absence or presence of SARS-CoV-2.

Table 9. List of Organisms analyzed by wet testing for potential cross reactivity with the LumiraDx SARS-CoV-2 RNA STAR grouped into pools.

	Organism	Strain	Panel
Group 1	Negative	n/a	n/a
	<i>Acinetobacter baumannii</i>	307-0294	Pneumonia Panel
	Adenovirus Type 3	n/a	Atypical Panel
Group 2	Adenovirus Type 31	n/a	Atypical Panel
	<i>Enterobacter cloacae</i>	Z101	Pneumonia Panel
	<i>Escherichia coli</i>	Z297	Pneumonia Panel
	<i>Haemophilus influenzae</i>	MinnA	Pneumonia Panel
	<i>Moraxella catarrhalis</i>	Ne11	Pneumonia Panel
	<i>Klebsiella aerogenes</i>	Z052	Pneumonia Panel
Group 3	<i>Klebsiella oxytoca</i>	Z115	Pneumonia Panel
	<i>Klebsiella pneumoniae</i>	KPC2	Pneumonia Panel
	<i>Klebsiella pneumoniae</i>	Z460; NDM-1	Pneumonia Panel
	<i>Klebsiella pneumoniae</i>	Z138; OXA-48	Pneumonia Panel
	<i>Chlamydia pneumoniae</i>	CWL-029	Atypical Panel
Group 4	Metapneumovirus 8	n/a	Resp Panel
	Influenza A H1N1	A/Singapore/63/04	Resp Panel
	Influenza A H1N1 (2009)	A/NY/02/09	Resp Panel
	Influenza A H3	A/Brisbane/10/07	Resp Panel
	Influenza B	B/Florida/02/06	Resp Panel
Group 5	Coronavirus 229E	n/a	Resp Panel
	Coronavirus NL63	n/a	Atypical Panel
	Coronavirus OC43	n/a	Resp Panel
	Coronavirus SARS	n/a	Resp Panel
	Enterovirus	n/a	Resp Panel
	<i>Legionella pneumophila</i>	Philadelphia	Atypical Panel
	Metapneumovirus 8	Peru6-2003	Atypical Panel
Group 6	<i>Mycoplasma pneumoniae</i>	M129	Atypical Panel
	Parainfluenza virus Type 1	n/a	Resp Panel
	Parainfluenza virus Type 1	n/a	Resp Panel
	Parainfluenza virus Type 2	n/a	Resp Panel
	Parainfluenza virus Type 3	n/a	Resp Panel
	Respiratory Syncytial Virus A	n/a	Resp Panel
	Respiratory Syncytial Virus B	CH93(18)-(18)	Resp Panel
	Rhinovirus	n/a	Resp Panel
	Rhinovirus 1A	n/a	Atypical Panel
Group 7	RSV A2	n/a	Atypical Panel
	<i>Proteus mirabilis</i>	Z050	Pneumonia Panel
	<i>Serratia marcescens</i>	Z053	Pneumonia Panel
	<i>Staphylococcus aureus</i>	MRSA, COL	Pneumonia Panel
	<i>Staphylococcus agalactiae</i>	Z019	Pneumonia Panel
	<i>Staphylococcus pneumoniae</i>	Z022	Pneumonia Panel
	<i>Staphylococcus pyogenes</i>	Z018	Pneumonia Panel
	<i>Mycobacterium tuberculosis</i>	2.30E+05	n/a
	<i>Candida albicans</i>	4.40E+05	n/a

20.5.2 Procedure

To ensure the performance of the LumiraDx SARS-CoV-2 RNA STAR design, wet testing was performed to confirm potential cross-reactivity.

20.5.3 Results and Conclusions

The pooled samples confirmed that the organisms listed in Table 9 did not interact with the LumiraDx SARS-CoV-2 RNA STAR run on the Agilent Stratagene Mx3005P (version 4.10) RT-PCR Instrument or interfere with detection of SARS-CoV-2 as seen in Table 10.

Table 10. Wet testing analysis for potential cross-reactivity with the LumiraDx SARS-CoV-2 RNA STAR.

SARS-CoV-2 Concentration	Observed Properties			
	Method	Cross Reactivity Group	Replicates Detected	Mean Ct Value
0 copies/ml	Qiagen DSP Viral (Automated)	n/a	0/2	n/a
		Group 1	0/2	n/a
		Group 2	0/2	n/a
		Group 3	0/2	n/a
		Group 4	0/2	n/a
		Group 5	0/2	n/a
		Group 6	0/2	n/a
		Group 7	0/2	n/a
1 x LoD	Qiagen DSP Viral (Automated)	n/a	2/2	13.5
		Group 1	2/2	14.0
		Group 2	2/2	13.0
		Group 3	2/2	14.0
		Group 4	2/2	13.5
		Group 5	2/2	14.5
		Group 6	2/2	14.5
		Group 7	2/2	13.5

20.6 Clinical Evaluation

20.6.1 Purpose

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

20.6.2 Procedure

A panel of 31 negative and 34 positive nasopharyngeal samples were prepared and tested in a randomized blind study. Each sample was processed by manual extraction with the MagMax Viral/Pathogen Nucleic Acid Isolation Kit (catalog #A42352) and tested with the LumiraDx SARS-CoV-2 RNA STAR test and the FDA EUA RT-PCR test. The LumiraDx SARS-CoV-2 RNA STAR was conducted per the standard assay protocol on the Applied Biosystems 7500 Fast Dx PCR Instrument.

20.6.3 Results and Conclusions

Positive and negative percent agreements between the LumiraDx SARS-CoV-2 RNA STAR test when compared to an approved FDA EUA RT-PCR test is shown below in Table 11. Confidence limits at the 95% level are also included. LumiraDx SARS-CoV-2 RNA STAR performed with 97.06% Positive Agreement and 96.77% Negative Agreement for 34 positive and 31 negative samples when compared to the FDA EUA RT-PCR test.

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

		FDA EUA RT-PCR Test (Comparator)		
		Positive	Negative	Total
LumiraDx SARS-CoV-2 RNA STAR	Positive	33	1	34
	Negative	1 *	30	31
	Total	34	31	65
Percent Agreement		97.06% (Pos.)	96.77% (Neg.)	
95% Confidence Interval		85.08 – 99.50%	83.80 – 99.40%	

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

21 FDA SARS-CoV-2 Reference Panel Testing

The evaluation of sensitivity and MERS-CoV cross-reactivity was performed using reference material (T1), blinded samples and a standard protocol provided by the FDA. The study included a range finding study and a confirmatory study for LoD. Blinded sample testing was used to establish specificity and to confirm the LoD. The extraction method was completed using automated extraction with the Qiagen DSP Virus/Pathogen Kit (Catalog #937055; 400 μ L input, 60 μ L elution) performed on the Qiagen QIA Symphony SP. The purified sample (10 μ L) was analyzed by the LumiraDx SARS-CoV-2 RNA STAR test per the standard assay protocol (LumiraDx SARS-CoV-2 RNA STAR Information for Use) on the Agilent Stratagene Mx3005P (software version 4.10) Instrument. The results are summarized in Table 12.

Table 12. Summary of LoD Confirmation Result Using the FDA SARS-CoV-2 Reference Panel

Reference Materials Provided by FDA	Specimen Type	Product LoD	Cross- Reactivity
SARS-CoV-2	Nasopharyngeal	5.4x10 ³ NDU/mL	N/A
MERS-CoV	(NP) swab	N/A	ND

NDU/mL = RNA NAAT detectable units/mL

N/A: Not applicable

ND: Not detected

22 Contact Information, Ordering, and Product Support

For ordering, contact LumiraDx at:

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

For product information, contact LumiraDx at:

Email (US): customerservices.US@lumiradx.com. Include "LumiraDx SARS-CoV-2 RNA STAR" 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" in the subject line.

For return policy, contact LumiraDx at:

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

Intellectual property

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

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Manufacturer information

LumiraDx UK Ltd, Building 115, Bedford Technology Park, Thurleigh, Bedford MK44 2YA, UK. Company Number 09206123.

LumiraDx US Office

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



LumiraDx SARS-CoV-2 RNA STAR

Product Insert

SPEC-30077 Rev1

ART-00003 Rev5

For *in vitro* diagnostic use

Emergency Use Authorization (EUA) only

Rx Only

IVD

Intended use

LumiraDx SARS-CoV-2 RNA STAR is a non-isothermal nucleic acid amplification qSTAR (Selective Temperature Amplification Reaction) method intended for the qualitative detection of nucleic acid from SARS-CoV-2 in upper respiratory specimens (such as nasal, mid-turbinate, nasopharyngeal and oropharyngeal swabs) and bronchoalveolar lavage specimens collected from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to laboratories - certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 USC. §263a that meet requirements to perform high complexity tests.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in respiratory specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

LumiraDx SARS-CoV-2 RNA STAR is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures. LumiraDx SARS-CoV-2 RNA STAR is only for use under the Food and Drug Administration's Emergency Use Authorization.

Procedure/interpretation/limitations

Users should refer to the LumiraDx SARS-CoV-2 RNA STAR Instructions for Use posted on the FDA website for all IVD products used under Emergency Use Authorization, <https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19/emergency-use-authorizations-medical-devices/in-vitro-diagnostics-euas>.

Package contents

Component	Description	Amount	Storage
LumiraDx SARS-CoV-2 RNA STAR Master Mix	COVID-19 Master Mix	1.0mL	-25°C to -15°C
LumiraDx SARS-CoV-2 RNA STAR 50X Internal Control & Primer Mix (50X IC/P Mix)	COVID-19 Internal Control & Primer Mix	50µL	-25°C to -15°C
LumiraDx SARS-CoV-2 RNA STAR 10X Salt Mix	COVID-19 Salt Mix	250µL	-25°C to -15°C
LumiraDx SARS-CoV-2 RNA STAR Positive Extraction Control (Pos. Ext. Ctrl.)	COVID-19 Positive Control (ZeptoMetrix 50,000cp/mL)	0.5mL	2°C to 8°C (-25°C to -15°C)
LumiraDx SARS-CoV-2 RNA STAR Negative Extraction Control (Neg. Ext. Ctrl.)	Molecular Grade Water	1.5mL	-25°C to -15°C

Storage instructions

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

Precautions

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

This Test has not been FDA cleared or approved; this Test has been authorized by FDA under an EUA for use by laboratories certified under CLIA, that meet requirements to perform high complexity tests.

This Test has been authorized only for the qualitative detection of nucleic acids from SARS-CoV-2 and not for any other viruses or pathogens.

This Test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of *in vitro* diagnostic tests for

detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.

Reagent complaints/questions

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

For return policy

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

Limited warranty

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

to the Limited Warranty shall be made in writing within the applicable Limited Warranty period.

Intellectual property

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

Legal notices

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Manufacturer information

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Company Number 09206123.









LumiraDx US Office

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

Symbols Glossary

References

- 1. World Health Organization www.who.int.
- 2. Centers for Disease Control and Prevention www.cdc.org.

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