



Dual-Target SARS-CoV-2 **STAR Complete**

Instructions for Use



L018180801096



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1. Intended Use

The LumiraDx Dual-Target SARS-CoV-2 STAR Complete is a rapid, non-isothermal nucleic acid amplification test (NAAT) utilizing qSTAR (quantitative Selective Temperature Amplification Reaction) technology for the detection and differentiation of nucleic acid from two distinct genes (nsp1 & orf8) from the SARS-CoV-2 genome in nasopharyngeal or anterior nasal swab samples collected dry or in transport medium from individuals suspected of COVID-19.

Results are for the identification of SARS-CoV-2 nucleic acid. SARS-CoV-2 nucleic acid is generally detectable in upper respiratory samples during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 nucleic acid; 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 definitive cause of disease. Negative results do not preclude infection with SARS-CoV-2 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 Dual-Target SARS-CoV-2 STAR Complete is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures.

Laboratories may be required to report all positive results to the appropriate Competent Health Authorities.

2. Summary and Explanation of the Test

Respiratory tract infections caused by respiratory viruses are a common cause of acute illness globally. Timely and accurate diagnosis of the cause of respiratory tract infections is important as disease severity can be increased in young, immunocompromised, or elderly individuals¹.

SARS-CoV-2

The World Health Organization (WHO) have named the disease caused by SARS-CoV-2 virus as coronavirus 2019 disease or COVID-19². 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 days³.

3. Principles of the Procedure

LumiraDx Dual-Target SARS-CoV-2 STAR Complete is a rapid, non-isothermal nucleic acid amplification test (NAAT) utilizing qSTAR (quantitative Selective Temperature Amplification Reaction) technology, which detects and differentiates nucleic acid from two distinct genes (nsp1 & orf8) from SARS-CoV-2 from dry swabs or swabs in transport medium directly from anterior nasal or nasopharyngeal swab samples collected dry or in transport medium within twenty-five minutes, without the need for up-front sample purification or extraction.

The LumiraDx Dual-Target SARS-CoV-2 STAR Complete Internal Control, Primer and Probe (IC/P) Mix is designed for the qualitative detection of nucleic acid from two distinct genes (nsp1 & orf8) from SARS-CoV-2 in nasopharyngeal swabs collected from individuals suspected of COVID-19 by their healthcare provider.

Target virions are lysed and amplified from respiratory samples in a single reaction. Lysis is achieved using detergents found in the LumiraDx Extraction Buffer. Nucleic acids present post-lysis are reverse transcribed and subsequently amplified by qSTAR using specific primers for each assay target. qSTAR amplification is achieved by cycling between two temperatures and using two distinct enzymes, a polymerase and a nicking enzyme. The polymerase is relatively favored at the higher temperature while the nicking enzyme is relatively favored at the lower temperature. Molecular beacons are used to specifically anneal and detect each target amplicon utilizing any of the following RT-PCR instruments: 96-Well: Applied Biosystems QuantStudio 5 (software version 1.5.1), Applied Biosystems QuantStudio 7 Flex (software version 1.3), Analytik Jena qTOWER³ (software version 4.1), or the Bio-Rad CFX Opus System (software version 2.2).

1 Couch, R.B. and Kasel, J.A. 1995. Influenza in Diagnostic Procedures for Viral, Rickettsial, and Chlamydial Infections. 7th Edition. 431-446.

2 World Health Organization: www.who.int.

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

4. Reagents and Materials

4.1. Materials Required (Provided)

Table 1. Dual-Target SARS-CoV-2 STAR Complete Kit Components and Volumes -

Component	96-Well Volume (L018180801096)
Dual-Target SARS-CoV-2 STAR Complete Positive Control Media (PCM)	250 µL
Dual-Target SARS-CoV-2 STAR Complete Negative Control Media (NCM)	1.5 mL
Dual-Target SARS-CoV-2 STAR Complete Salt Mix	1.0 mL
Dual-Target SARS-CoV-2 STAR Complete Extraction Buffer	500 µL
Dual-Target SARS-CoV-2 STAR Complete Internal Control & Primer Mix (IC/P Mix)	200 µL
Dual-Target SARS-CoV-2 STAR Complete Master Mix	2.0 mL

4.2. Materials Required (But Not Provided)

Table 2. Consumables Required (Not Provided)

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

Table 3. Reagents Required (Not Provided)

Reagents	Source	Catalog #
Sodium Hypochlorite Solution (Bleach)	ThermoFisher Scientific	SS290-1
70% Isopropanol (or 70% Ethanol)	General lab supplier	N/A
DNAZap™ (or equivalent)	ThermoFisher Scientific	AM9890
RNaseZap™ (or equivalent)	ThermoFisher Scientific	AM9782
Compatible Swabs and Transport Medium	General lab supplier	N/A
Saline, 0.85 or 0.90%	Hardy Diagnostics	U157 or U264
1x Phosphate Buffered Saline (PBS), pH 7.4	ThermoFisher Scientific	10010023
Transport Medium	Corning Transport Medium*	25-500-CM

*Or medium of similar formulation

Table 4. 96-Well Instruments and Consumables (Not Provided)

96-Well PCR Instruments & Consumables	Source	Catalog #
Applied Biosystems QuantStudio™ 5, 96-well block (software version 1.5.1)	ThermoFisher Scientific	A28574
Applied Biosystems MicroAmp Optical 96-Well Plate	ThermoFisher Scientific	4306737
Heat-Resistant Polypropylene Film for Raised-Rim Plates	VWR	89087-690
Applied Biosystems QuantStudio 7 Flex, 96-well block (software version 1.3)	ThermoFisher Scientific	4485698
Applied Biosystems MicroAmp Optical 96-Well Plate	ThermoFisher Scientific	4306737
Heat-Resistant Polypropylene Film for Raised-Rim Plates	VWR	89087-690
Bio-Rad CFX Opus 96 Real-Time PCR Instrument (software version 2.2)	Bio-Rad	12011319
Eppendorf twin.tec Real-Time PCR Plate 96-Well Skirted	Eppendorf	951022003
Heat-Resistant Polypropylene Film for Raised-Rim Plates	VWR	89087-690
qTOWER ³ or qTOWER ³ G (software version 4.1)	Analytik Jena	844-00553-4 or 844-00554-4
Eppendorf twin.tec Real-Time PCR Plate 96-Well Semi-Skirted	Eppendorf	951022043
Heat-Resistant Polypropylene Film for Raised-Rim Plates	VWR	89087-690

Table 5. Universal Consumables* (Not Provided)

Universal Consumables	Source	Catalog #
ThermalSeal A Sealing Film	Research Product International	202545
Heat-Resistant Polypropylene Film for Raised-Rim Plates	VWR	89087-690
Utility Sealing Film		
VWR Adhesive Film for Microplates	VWR	60941-070

Table 6. Equipment (Not Provided)

Equipment	Source	Catalog #
-80°C Laboratory Freezer	General lab supplier	N/A
-15°C to -25°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 Block(s)	General lab supplier	N/A
Dry Heat Block (Capable of heating PCR Plate to 65°C +/- 1°C for 5 minutes)	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

4.3. Reagent Storage, Handling, and Stability

- Upon receipt, store the LumiraDx Dual-Target SARS-CoV-2 STAR Complete kit between -15°C and -25°C.
- After initial use, freeze LumiraDx Dual-Target SARS-CoV-2 STAR Complete Reagents between -15°C and -25°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 Dual-Target SARS-CoV-2 STAR Complete Internal Control & Primer Mix (IC/P Mix).
- The Extraction Buffer, Internal Control/Primer Mix, and Master Mix must be thawed and kept on a cold block at all times during preparation and use.
- The external controls, PCM and NCM must be thawed and kept cold at all times during preparation and use.
- Repeated thawing and freezing of the reagents more than 3 times should be avoided, as this might affect the performance of the product.

5. Sample Collection, Handling, Storage, and Transport

Proper collection and handling of specimens is critical to laboratory diagnosis of infectious diseases. A specimen that is not collected correctly may lead to incorrect test results. Testing for respiratory viruses 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.

5.1. Sample Collection and Handling

- Follow the sample collection device’s instructions for proper collection and handling procedures.
- 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.
- **Wet swab:** specimens should be collected and placed into appropriate transport medium. Swabs provided in up to 3 mL of compatible transport medium are acceptable, however, for optimal performance, 1 mL of buffer is suggested.

- **Dry swab:** specimens should be collected and placed in a sterile, dry transport tube, such as a standard 15 mL Falcon tube. For elution of a dry swab specimen, add 1 mL of compatible transport medium and recap tube. Vortex the tube containing the swab for 30 seconds with intermittent pulsing. Incubate the swab at room temperature for at least 10 minutes. Remove (beware of cross-contamination from splashing) and discard the swab in the biohazard waste.
- For more information on the collection, storage and transport of samples suspected to contain SARS-CoV-2, refer to the World Health Organization Laboratory biosafety guidance related to coronavirus disease (COVID-19): interim guidance, 28 January 2021. <https://www.who.int/publications/i/item/WHO-WPE-GIH-2021.1>

5.2. Sample Storage

- Always follow the manufacturer's instructions for proper storage of samples.
- **Dry swab:** specimens should be stored as follows:
 - Room temperature (15-30°C) for up to 72 hours
 - Refrigerated (2-8°C) for up to 96 hours
- **Wet swab:** specimens should be stored as follows:
 - Room temperature (15-30°C) for up to 72 hours
 - Refrigerated (2-8°C) for up to 96 hours
 - Frozen ($\leq -20^{\circ}\text{C}$) for up to 1 week
- If a delay in testing is expected, store specimens at -20°C or below.
- If specimens cannot be tested within 72 hours of collection, specimens should be frozen at $\leq -20^{\circ}\text{C}$ may be frozen at $\leq -20^{\circ}\text{C}$ for up to 1 week until tested.
- Repeated thawing and freezing of the swabs should be avoided, as this might affect the performance of the product. Specimens may be frozen and thawed up to 3 times.

5.3. Sample Transport

- 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.
- Maintain sample storage conditions as described in the Sample Collection, Handling, Storage, and Transport section of this document.
- Samples must be shipped in accordance with applicable local, regional, national, and international transportation regulations.

6. Warnings and Precautions

- For *in vitro* Diagnostic Use (IVD).
- The Salt Mix and the Master Mix contains bovine serum albumin.
- LumiraDx Dual-Target SARS-CoV-2 STAR Complete is only for the detection of nucleic acid from SARS-CoV-2 and not for any other viruses or pathogens.
- Do not eat, drink, smoke, apply cosmetics or handle contact lenses in areas where reagents and human specimens are handled.
- Handle all specimens as if infectious using safe laboratory procedures.
- For more information on the collection, storage and transport of samples, refer to the World Health Organization Laboratory biosafety guidance related to coronavirus disease (COVID-19): interim guidance, 28 January 2021. <https://www.who.int/publications/i/item/WHO-WPE-GIH-2021.1>
- Performance characteristics have been determined with nasopharyngeal specimens from individuals with signs and symptoms of infection who are suspected of COVID-19.
- Use personal protective equipment such as, but not limited to, gloves and lab coats when handling kit reagents while performing this Test and handling materials including samples, reagents, pipettes, and other equipment and reagents.
- Dispose of unused kit reagents and human specimens according to local, regional, national, and international regulations.
- Negative results do not preclude infection with SARS-CoV-2 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.
- There is a risk of false positive values resulting from cross-contamination by target organisms, their nucleic acids or amplified product, or from non-specific signals in this Test.
- To avoid contamination of the environment and / or test reactions with SARS-CoV-2, do not open the reactions post-amplification.
- Report results to the appropriate public health authorities as required by local, regional, national, and international regulations.
- Reagents used with this test include guanidine-containing materials. Highly reactive and/or toxic compounds may form if combined with sodium hypochlorite (bleach).
- Only use listed components provided for LumiraDx Dual-Target SARS-CoV-2 STAR Complete; other LumiraDx products may not contain the same formulations as needed for this test.
- Deviations from the protocols, parameters, components, instruments, and instrument software versions described in this package insert may give erroneous results.

7. Real-Time PCR Instrument and Protocol Summary

The LumiraDx Dual-Target SARS-CoV-2 STAR Complete contains molecular beacons labeled with fluorophores for the detection and differentiation of nucleic acids from two distinct genes from SARS-CoV-2 as well as an internal control. A summary of the fluorophores and their excitation and emission wavelengths is found in **Table 7. Target/Fluorophores for LumiraDx Dual-Target SARS-CoV-2 STAR Complete**. Amplification temperature profiles for instruments are provided in **Figure 1.** and **Table 8a - Table 8d**. Optical and analysis settings for instruments are provided in **Table 9a and Table 9c**.

Detailed instructions for the setup of instruments are found in **Appendix A**.

NOTE: Heated lids should be used for all cycling reactions.

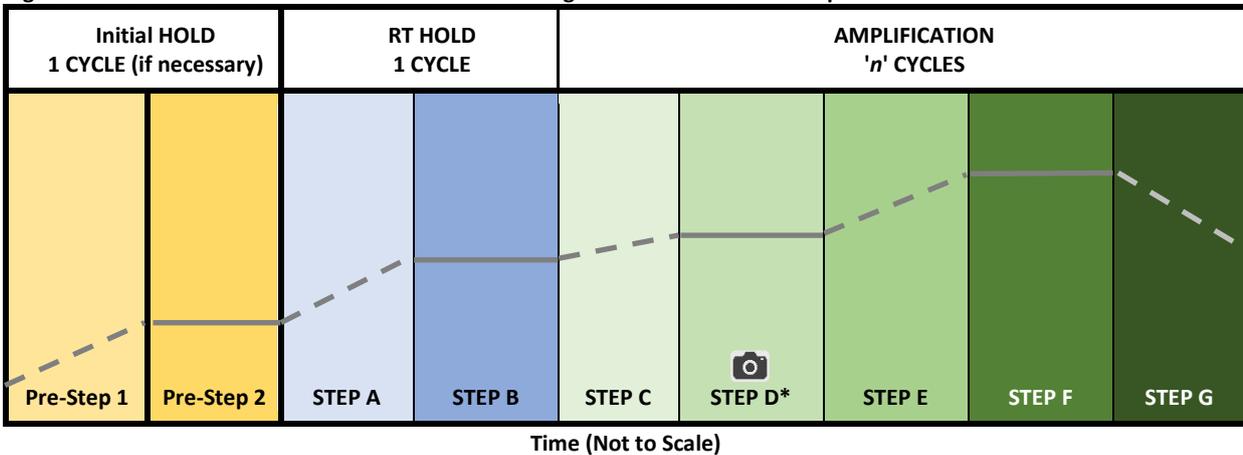
NOTE: No passive reference dyes should be selected in instrument settings.

NOTE: Thermocyclers should be programmed and queued-up prior to reagent preparation.

Table 7. Target/Fluorophores for LumiraDx Dual-Target SARS-CoV-2 STAR Complete

Target	Dye	Quencher	Excitation	Emission
SARS-CoV-2 Target 1	FAM	Dark Quencher	470	520
SARS-CoV-2 Target 2	Cy5/Mustang Purple™	Dark Quencher	648/640	668/682
Internal Control	ROX	Dark Quencher	580	623

Figure 1. General Thermal Profile for LumiraDx Dual-Target SARS-CoV-2 STAR Complete



* Image acquisition.

Table 8a. Thermal Profile for LumiraDx Dual-Target SARS-CoV-2 STAR Complete: Applied Biosystems QuantStudio 5 (96-Well)

Instrument		Applied Biosystems QS5	
Format		96-Wells	
Volume Setting		60 µL	
Amp Cycles		'20'	
Ramp Rate	Pre-Step 1 (Hold Stage 1)	2°C/sec	
Temperature/Time	Pre-Step 2 (Hold Stage 1)	30°C	1 min
Ramp Rate	STEP A (Hold Stage 2)	2°C/sec	
Temperature/Time	STEP B (Hold Stage 2)	51°C	3 min
Ramp Rate	STEP C	2°C/sec	
Temperature/Time	STEP D* 	51°C	25 sec
Ramp Rate	STEP E	2°C/sec	
Temperature/Time	STEP F	61°C	1 sec
Ramp Rate	STEP G	2°C/sec	

* Acquire image during **STEP D** of cycling (51°C).

NOTE: Heated lids should be used for all cycling reactions.
NOTE: No passive reference dyes should be selected in instrument settings.
NOTE: Thermocyclers should be programmed and queued-up prior to reagent preparation.

Table 8b. Thermal Profile for LumiraDx Dual-Target SARS-CoV-2 STAR Complete: Applied Biosystems QuantStudio 7 Flex (96-Well)

	Instrument	Applied Biosystems QS 7 Flex	
	Format	96-Wells	
	Volume Setting	60 µL	
	Amp Cycles	'20'	
Ramp Rate	Pre-Step 1 (Hold Stage 1)	1.946°C/sec	
Temperature/Time	Pre-Step 2 (Hold Stage 1)	30°C	1 min
Ramp Rate	STEP A (Hold Stage 2)	1.946°C/sec	
Temperature/Time	STEP B (Hold Stage 2)	51°C	3 min
Ramp Rate	STEP C	1.645°C/sec	
Temperature/Time	STEP D* 	52°C	25 sec
Ramp Rate	STEP E	1.946°C/sec	
Temperature/Time	STEP F	61°C	1 sec
Ramp Rate	STEP G	1.946°C/sec	

* Acquire image during **STEP D** of cycling (52°C).

NOTE: Heated lids should be used for all cycling reactions.
NOTE: No passive reference dyes should be selected in instrument settings.
NOTE: Thermocyclers should be programmed and queued-up prior to reagent preparation.

Table 8c. Thermal Profile for LumiraDx Dual-Target SARS-CoV-2 STAR Complete: Bio-Rad CFX OPUS (96-Well)

	Instrument	CFX OPUS	
	Format	96-Wells	
	Volume Setting	0 µL	
	Amp Cycles	'26'	
Temperature/Time	Pre-Step 1	Instrument default	
Temperature/Time	Pre-Step 2	45°C	00:00 (Forever)**
Ramp Rate	STEP A	Instrument default	
Temperature/Time	STEP B	49.5°C	3 min
Ramp Rate	STEP C	Instrument default	
Temperature/Time	STEP D* 	49°C	12 sec
Ramp Rate	STEP E	Instrument default	
Temperature/Time	STEP F	67.5°C	1 sec
Ramp Rate	STEP G	Instrument default	

* Acquire image during **STEP D** of cycling (49°C).

****PRE-STEP is to allow the lid to heat. Do not load RT-PCR Plate or continue to protocol STEP A until the lid has reached 70°C. Plate is inserted once the temperature is achieved. Once the plate has been loaded the HOLD step must be skipped to proceed with the rest of the protocol**

NOTE: No passive reference dyes should be selected in instrument settings.
NOTE: Thermocyclers should be programmed and queued-up prior to reagent preparation.

Table 8d. Thermal Profile for LumiraDx Dual-Target SARS-CoV-2 STAR Complete: Analytik Jena qTOWER³ (96-Well)

	Instrument	qTOWER³	
	Format	96-Well	
	Volume Setting	60 µL	
	Amp Cycles	'20'	
Ramp Rate	STEP A	2°C/sec	
Temperature/Time	STEP B	51°C	3 min
Ramp Rate	STEP C	2°C/sec	
Temperature/Time	STEP D*	49°C	10 sec
Ramp Rate	STEP E	2°C/sec	
Temperature/Time	STEP F	61°C	1 sec
Ramp Rate	STEP G	2°C/sec	

NOTE: Preheat Lid to 70°C

* Acquire image during **STEP D** of cycling (49°C).

Table 9a. Optical and Analysis Settings:

Applied Biosystems QuantStudio 5 and QuantStudio 7 Flex RT-PCR Instruments (96-Well)

Plate Format	System Target (Fluor)	Instrument Excitation/Detection Settings	Baseline Cycles	Threshold
QS5 96-Well	SARS-CoV-2 Target 1 (FAM)	470/520	1-2	100,000
	SARS-CoV-2 Target 2 (Cy5/Mustang Purple)	~640/~682	1-2	100,000
	Internal Control (ROX)	580/623	1-2	100,000
QS7 Flex 96-Well	SARS-CoV-2 Target 1 (FAM)	470/520	1-2	500,000
	SARS-CoV-2 Target 2 (Cy5/Mustang Purple)	~640/~682	1-2	250,000
	Internal Control (ROX)	580/623	1-2	500,000

Table 9b. Optical and Analysis Settings: Bio-Rad CFX OPUS RT-PCR Instrument

Plate Format	Target (Fluor)	Baseline Cycles	Threshold
96-Well	SARS-CoV-2 Target 1 (FAM)	1-2	5,000
	SARS-CoV-2 Target 2 (Cy5)	1-2	5,000
	Internal Control (ROX)	1-2	5,000

Table 9c. Optical and Analysis Settings: qTOWER³ RT-PCR Instrument (96-Well)

Plate Format	System Target (Fluor)	Baseline Cycles	Monitoring Threshold
96-Well	SARS-CoV-2 Target 1 (FAM)	1-6	1.5
	SARS-CoV-2 Target 2 (Cy5)	1-6	5
	Internal Control (ROX)	1-6	5

8. LumiraDx Dual-Target SARS-CoV-2 STAR Complete Workflow

8.1. Quality Control

Internal, positive, and negative controls are recommended to monitor reagent function and to demonstrate successful operation of the assay.

8.1.1. Internal Controls

- An Internal Control is present in each reaction of the LumiraDx Dual-Target SARS-CoV-2 STAR Complete assay for validation of enzyme, primer, and probe stability and performance.

8.1.2. Positive and Negative Controls

- Test all Positive and Negative Controls Media when running diagnostic samples and with each new lot of the LumiraDx Dual-Target SARS-CoV-2 STAR Complete kit to ensure all reagents and kit components are working properly.

8.2. Sample Preparation

LumiraDx Dual-Target SARS-CoV-2 STAR Complete removes sample purification and extraction by combining lysis and amplification in a single step. This assay is compatible with swabs stored in an empty (dry) tube or with swabs stored in transport medium.

NOTE: Please handle the PCM with care as it can cause false positives if accidentally spilled or handled carelessly. To reduce the risk of cross-contamination, handle the PCM in a separate area than where samples are processed and use separate pipette tips for all materials.

NOTE: Nucleic acid amplification technologies, including qSTAR, are extremely sensitive and accidental introduction of product from previous amplification reactions can result in false positive results. Incorrect results can occur if either the sample or the qSTAR reagents used in the amplification step become contaminated by accidental introduction of amplicon. Workflow in the laboratory should always proceed in a unidirectional manner to minimize the potential for such events.

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

8.2.1. Dry Swab (Standard Format)

- a. If swab is provided dry, transfer (1) mL of a compatible transport medium into the tube and recap tube.
- b. Vortex the tube containing the swab for 30 seconds with intermittent pulsing.
- c. Incubate the swab at room temperature for at least 10 minutes.
- d. Remove and discard the swab in biohazard waste.

NOTE: An aliquot of this sample will be directly loaded into the RT-PCR plate.

8.2.2. Dry Swab (Deep Well Format)

- a. Dispense 100mL of a compatible transport medium into a suitable reagent reservoir.
- b. Using a multi-channel pipette, transfer 1 mL of medium into each of 94 wells of the deep well reservoir, leaving A1 and A12 empty for external controls.
- c. Place and soak the swab(s) in the appropriate wells for at least 10 minutes then swirl thoroughly by rotating the swab up to 5 times.
- d. Express the liquid from the swab by rotating the swab against the side of the well while removing the swab from the well. (Beware of cross-contamination from splashing).
- a. Discard the swab in biohazard waste.

NOTE: An aliquot of this sample will be directly loaded into the RT-PCR plate.

8.2.3. Wet Swab

- a. No additional preparation is required, however, samples in greater than 3 mL of compatible transport medium may have reduced sensitivity due to lower concentration of virus in the medium. One (1) mL of transport medium is recommended.

NOTE: An aliquot of this sample will be directly loaded into the RT-PCR plate.

8.3. qSTAR Reagent Preparation and Plate Setup

Please read and thoroughly understand the following instructions before attempting to prepare the Reaction Mix and controls.

All components should be thawed and kept on a cold block equilibrated between 2 and 8°C to maintain the integrity of the reagents. It is recommended to queue the validated RT-PCR Instrument prior to performing the instructions below to ensure the performance of this assay is maintained.

8.3.1. 96-Well Format (60 µL Reaction Volume)

1. Control, Samples, and Extraction Buffer Preparation

- a. Thaw the LumiraDx Dual-Target SARS-CoV-2 STAR Complete PCM, NCM, and Extraction Buffer on a cold block equilibrated between 2 and 8°C.
- b. Vortex tubes for 5 seconds.
- c. Centrifuge the tubes for 5 seconds to collect reagents at the bottom of each tube.
- d. Directly transfer 23.0 µL of PCM, 23.0 µL of NCM, and 23.0 µL of sample(s) to the appropriate wells of the pre-chilled RT-PCR Plate.
- e. Add 5.0 µL of Extraction Buffer to each well containing controls and sample(s).

NOTE: The addition of Extraction Buffer can be simplified by using a multi-channel pipette.

- f. Thoroughly mix controls and sample(s) for at least 10 seconds. Avoid creating bubbles.
- g. Seal the RT-PCR plate with sealing film and spin down to collect the liquid at the bottom of the wells to ensure no reagent/sample is retained on the side walls of the wells.
- h. Place the plate at 65°C for 5 minutes then, immediately place the RT-PCR Plate back on the cold block.

NOTE: The heat from the 65°C plate could potentially increase the temperature of the cold block to above 8°C. It is recommended that the cold block be swapped with a fresh block (equilibrated to 2 to 8°C) for each setup.

NOTE: If condensation is present, the plate may be spun down to collect the liquid at the bottom of the wells.

2. Reaction Mix Preparation

- a. Thaw the LumiraDx Dual-Target SARS-CoV-2 STAR Complete Salt Mix, IC/P Mix, and Master Mix on a cold block equilibrated between 2 and 8°C.
- b. Obtain a sterile (RNase/DNase free) tube and place on a cold block.
- c. Determine the number of reactions (*n*) to be prepared per assay:

Table 10. Reaction Mix Volumes (60 µL Reactions)

Reaction Mix	1 Reaction	100 Reactions	<i>n</i> Reactions
Salt Mix	10.0 µL	1000.0 µL	<i>n</i> x 10.0 µL
IC/P Mix	2.0 µL	200.0 µL	<i>n</i> x 2.0 µL
Master Mix	20.0 µL	2000.0 µL	<i>n</i> x 20.0 µL
Total Volume	32.0 µL	3200.0 µL	<i>n</i> x 32.0 µL

- d. Vigorously vortex the Salt Mix only for 20 seconds, centrifuge for 5 seconds to collect reagent at the bottom of tube, and **IMMEDIATELY** add the appropriate volume to the pre-chilled tube.
- e. Invert the IC/P Mix to mix then centrifuge for 5 seconds to collect reagents at the bottom of the tube (do not vortex), and **IMMEDIATELY** add the appropriate volume to the Salt Mix.
- f. Mix thoroughly for at least 10 seconds (avoid creating bubbles). Centrifuge briefly (do not vortex and do not spin down for an excessive amount of time), then place tube back on the cold block.
- g. Invert the Master Mix to mix then centrifuge for 5 seconds to collect reagents at the bottom of the tube (do not vortex samples), and **IMMEDIATELY** add the appropriate volume to finalize the Reaction Mix.
- h. Mix thoroughly for at least 10 seconds (avoid creating bubbles). Centrifuge briefly, then place tube back on the cold block.
- i. Carefully remove the sealing film from the RT-PCR Plate and add 32.0 µL of Reaction Mix to each well with control or sample. Mix thoroughly for at least 10 seconds (avoid creating bubbles).
- j. Apply the appropriate optical adhesive plate film. Centrifuge the plate for at least 20 seconds at 2000 rpm to collect the reaction at the bottom of each well. After centrifugation confirm that no bubbles persist. If bubbles are observed repeat this step.
- k. Immediately place the 96-well plate in the pre-queued RT-PCR instrument.

8.4. Amplification

Once the RT-PCR Plate has been placed in a validated RT-PCR Instrument with the appropriate protocol selected based on **Section 7 Real-Time PCR Instrument and Protocol Summary** and start the run.

8.5. Interpretation and Reporting of Results

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

8.5.1. LumiraDx Dual-Target SARS-CoV-2 STAR Complete Controls

The LumiraDx Dual-Target SARS-CoV-2 STAR Complete Positive Control Media (PCM) is an external positive control needed to ensure test reagents are properly detecting both SARS-CoV-2 nucleic acid targets. It is comprised of a quantified SARS-CoV-2 template. The control is formulated in a proprietary matrix with purified, intact viral particles containing whole length genome, which have been rendered non-infectious.

The LumiraDx Dual-Target SARS-CoV-2 STAR Complete Negative Control Media (NCM) is an external, negative, control needed to monitor cross-contamination, or reagent contamination did not occur during sample processing or reaction setup and is comprised of 1x Phosphate Buffered Saline (PBS), pH 7.4.

An Internal Control (a component in the LumiraDx Dual-Target SARS-CoV-2 STAR Complete Internal Control and Primer Mix) consists of a synthetic RNA – that the assay primers can bind and amplify from – with a unique probe region for molecular beacon detection in the **ROX** channel. The Internal Control serves as a control for detection of inhibitors present in the specimen, assures that adequate amplification has taken place, and that the enzymes and primers were not inadvertently damaged during production, shipment, and storage.

The testing algorithm for all instruments is based on the standard practice of determining background fluorescence and calling a sample well positive if the change in fluorescent signal exceeds an established threshold. Background fluorescence levels and thresholds are calculated and applied to each run as shown in **Table 9a - Table 9c**. Positive and negative results are based on a sample well exceeding this threshold. No Cycle Threshold (Ct) cutoff is used for this testing algorithm, and it is expected that all reactions should occur between a Ct value of 3 to 25.

Failure of the PCM or NCM invalidates the qSTAR run and results should not be reported. The qSTAR assay should be repeated with fresh aliquots of external controls and sample. If the results continue to be invalid contact technical support. 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 11. Expected Results from External Controls on Applied Biosystems QuantStudio5, QuantStudio 7 Flex, Analytik Jena qTOWER³, Bio-Rad CFX Opus 96-Well Instruments

Control Type/Name	Used to Monitor	SARS-CoV-2 Target 1 (FAM) Expected Ct Value (Result)	SARS-CoV-2 Target 2 (Cy5/Mustang Purple) Expected Ct Value (Result)	IC (ROX) Expected Ct Value (Result)
PCM	Substantial reagent failure including primer and probe integrity	$3.0 \geq Ct \leq 25.0$ + (Positive)	$3.0 \geq Ct \leq 25.0$ + (Positive)*	$3.0 \geq Ct \leq 25.0$ + (Positive) OR - (Negative)*
NCM	Reagent and/or environmental contamination	Ct not detected - (Negative)	Ct not detected - (Negative)	$3.0 \geq Ct \leq 25.0$ + (Positive)

*The Internal Control or Target 2 is not required to amplify for the PCM to be deemed positive. See Table 15.

8.5.2. LumiraDx Dual-Target SARS-CoV-2 STAR Complete Sample Results

Assessment of a sample test result should be performed after the PCM and NCM have been examined and determined to be valid. If the Controls are not valid, the results cannot be interpreted.

Table 12. Ct Ranges for Positive Assay Results for the LumiraDx Dual-Target SARS-CoV-2 STAR Complete on Applied Biosystems QuantStudio5 and QuantStudio 7 Flex, Analytik Jena qTOWER³, Bio-Rad CFX Opus 96-Well Instruments

Sample or Control	Positive Ct Range*	IC (ROX) Ct Value	Interpretation of Results
SARS-CoV-2 Target 1 (FAM)	$3.0 \geq Ct \leq 25.0$	$3.0 \geq Ct \leq 25.0$ + (Positive) OR - (Negative)**	SARS-CoV-2 detected
SARS-CoV-2 Target 2 (Cy5/Mustang Purple)	$3.0 \geq Ct \leq 25.0$	$3.0 \geq Ct \leq 25.0$ + (Positive) OR - (Negative)**	SARS-CoV-2 detected

*For samples generating a Ct value greater than 0 and less than 3, perform 1:100 and/or 1:10 dilution using uninoculated compatible medium and process and test according to **Section 7**.

** The Internal Control is not required to amplify.

Table 13. Results Interpretation for the LumiraDx Dual-Target SARS-CoV-2 STAR Complete on Applied Biosystems QuantStudio5 and QuantStudio 7 Flex, Analytik Jena qTOWER³, Bio-Rad CFX Opus 96-Well Instruments

Sample			Interpretation and Reporting of Results
SARS-CoV-2 Target 1 (FAM)	SARS-CoV-2 Target 2 (Cy5/Mustang Purple)	IC (ROX)	
+ (Positive)	+ (Positive)	+ (Positive) OR - (Negative)*	SARS-CoV-2 detected. Report results.
+ (Positive)	- (Negative)	+ (Positive) OR - (Negative)*	SARS-CoV-2 detected. Report results.
- (Negative)	+ (Positive)	+ (Positive) OR - (Negative)*	SARS-CoV-2 detected. Report results.
- (Negative)	- (Negative)	+ (Positive)	SARS-CoV-2 not detected. Report results. Consider testing for other respiratory pathogens.
- (Negative)	- (Negative)	- (Negative)	Invalid. Do not report results. Retest the same processed sample. If the retest is also invalid obtain a new specimen and retest.

* The Internal Control is not required to amplify.

9. Limitations

- For export only.
- Do not use reagents past their expiration date.
- This test is qualitative and does not provide quantitative value(s) for target organism(s) in the sample.
- Detection of nucleic acids is dependent upon proper sample collection, handling, transportation, storage, and preparation. Improper collection, transport, or storage of samples may hinder the ability of this test to detect the target sequences.
- This test cannot rule out diseases caused by other bacterial or viral pathogens.
- Negative results do not preclude infection with a target organism and should not be the sole basis for treatment of patient management decisions.
- The performance of LumiraDx Dual-Target SARS-CoV-2 STAR Complete was assessed using nasopharyngeal swab specimens only.
- There is a risk of false negative results due to the presence of sequence variants in the viral targets of this test.
- Inhibitors present in the specimen and/or errors in following the Test 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.
- This test performance was not established in immunocompromised patients.
- The clinical performance of this test has not been established in all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of target viruses and their prevalence, which change over time.

10. Performance Characteristics

10.1. Analytical Studies

10.1.1. Analytical Sensitivity: (Spiked Sample Matrix)

The analytic sensitivity, or limit of detection (LoD) of the LumiraDx Dual-Target SARS-CoV-2 STAR Complete was determined using virus spiked directly into negative sample matrix consisting of pooled negative nasopharyngeal swab medium and tested on a validated RT-PCR instrument.

To determine the LoD serial dilutions were of virus were and spiked into negative sample matrix. A range-finding study was performed with 5 replicates at each concentration to determine the point at which at least 1 of the 5 replicates was not detected. 96 replicates per concentration were then performed based on the range-finding study. The lowest concentration at which at least 95% of replicates were positive was determined to be the LoD. The confirmed LoD for virus spiked into negative sample matrix is shown in **Table 14**.

Table 14. LumiraDx Dual-Target SARS-CoV-2 STAR Complete LOD Determination (Spiked Sample Matrix)

Organism	Strain	LoD Concentration
SARS-CoV-2	USA-WA1/2020	1.54 x 10 ¹ TCID ₅₀ /mL

10.1.2. Analytical Sensitivity: LoD (Spiked Swab)

The analytical sensitivity, or limit of detection (LoD) of the LumiraDx Dual-Target SARS-CoV-2 STAR Complete was determined using virus diluted in negative sample matrix and spiked onto swabs which were then eluted into 1x PBS, pH 7.4 and tested in a validated RT-PCR instrument.

Based on the established LoD, 96 replicates per concentration were performed. The lowest concentration at which at least 95% of replicates were positive was determined to be the LoD. The confirmed LoD for swabs spiked with virus and eluted into negative sample matrix is shown in **Table 15**.

Table 15. LumiraDx Dual-Target SARS-CoV-2 STAR Complete LOD Determination (Spiked Swab)

Organism	Strain	LoD Concentration
SARS-CoV-2	USA-WA1/2020	3.07 x 10 ¹ TCID ₅₀ /mL

10.1.3. Analytical Sensitivity: LoD (Dry Swab)

The analytic sensitivity, or limit of detection (LoD) of the LumiraDx Dual-Target SARS-CoV-2 STAR Complete was determined using swabs spiked with virus, **dried**, and eluted into negative sample matrix and tested on a validated RT-PCR instrument.

Based on the established LoD, 96 replicates per concentration were performed. The lowest concentration at which at least 95% of replicates were positive was determined to be the LoD. The confirmed LoD for swabs spiked with virus, dried, and eluted into negative sample matrix is shown in **Table 16**.

Table 16. LumiraDx Dual-Target SARS-CoV-2 STAR Complete LOD Determination (Dry Swab)

Organism	Strain	LoD Concentration
SARS-CoV-2	USA-WA1/2020	3.07 x 10 ¹ TCID ₅₀ /mL

10.1.4. Inclusivity

Inclusivity of the LumiraDx SARS-CoV-2 STAR Complete was assessed by testing low concentrations (~3x LoD) of virus (rendered non-infectious) using 3 replicates each for several strains of SARS-CoV-2, including important variants tested on a validated RT-PCR instrument. A summary of the results from inclusivity testing is shown in **Table 17**. The LumiraDx Dual-Target SARS-CoV-2 STAR Complete successfully detected all inclusivity strains at the concentrations tested.

Table 17. LumiraDx Dual-Target SARS-CoV-2 STAR Complete Inclusivity

Organism	Strain	Testing Concentration	Percent Detection (Pos/Tot)
SARS-CoV-2	Variant B.1.1.7 (Alpha)	3x LoD	100%
	Variant B.1.351 (Beta)	3x LoD	100%
	Variant B.1.617.2 (Delta)	3x LoD	100%
	Variant B.1.1.529 (Omicron)	3x LoD	100%

10.1.5. Inclusivity (In Silico)

A sampling of approximately 1,000,000 sequences from the SARS-CoV-19 GISAID database were pulled randomly and processed for quality and completeness in May of 2022. High-coverage and full-length sequences were used in the analysis. A summary of the results of the in silico inclusivity analysis by target and by variant are shown in **Table 18** and **Table 19**. These data indicate that the LumiraDx Dual-Target SARS-CoV-2 STAR Complete is predicted to detect ~99.82% of all sequences assessed at the time of analysis.

Table 18. LumiraDx Dual-Target SARS-CoV-2 STAR Complete In Silico Inclusivity by Target

Assay Target	Homology to Analyzed Sequences
Target 1	>99%
Target 2	>99%*
Target 1, Target 2 Combined	99.8%

*With one or fewer mismatches

Table 19. LumiraDx Dual-Target SARS-CoV-2 STAR Complete In Silico Inclusivity by Variant

Variant	Oligonucleotide Homology of Target 1 to Analyzed Sequences	Oligonucleotide Homology of Target 2 to Analyzed Sequences
Omicron (BA.1, BA.2, BA.3, BA.4, BA.5, XE)	>99%*	>99%
Delta (B.1.617.2)	>99%*	>99%

*With one or fewer mismatches

10.1.6. Analytical Specificity: Exclusivity and Cross-Reactivity

Exclusivity and cross-reactivity for the LumiraDx Dual-Target SARS-CoV-2 STAR Complete were assessed by testing a panel of 43 microorganisms commonly found in the upper respiratory tract or which are genetically similar to SARS-CoV-2 at high-titers, as noted in **Table 20**. Each organism was diluted in negative sample matrix and tested in triplicate. Testing was performed for each of the off-panel organisms alone and in the presence of each of the on-panel organisms at 3x LOD on a

validated RT-PCR instrument. The results of this study are shown in **Table 20**. None of the organisms produced false positive results at the concentrations tested and all replicates of each SARS-CoV-2 target were detected at 3x LOD in the presence of high-levels of off-panel organism.

NOTE: Concentrations are reported in TCID₅₀ (Median Tissue Culture Infectious Dose), CFU (Colony Forming Units), CCU (Colony Changing Units), Copies (genomic copies or genomic equivalents), and IFU (Inclusion Forming Units).

Table 20. LumiraDx Dual-Target SARS-CoV-2 STAR Complete Exclusivity and Cross-Reactivity

Off-Panel Organism	Strain	Off-Panel Organism Concentration Tested	Off-Panel Organism Testing Result	SARS-CoV-2 Testing Result
Adenovirus	Type 1	1.00 x 10 ⁵ TCID ₅₀ /mL	Not Detected	Detected
Adenovirus	Type 5	1.00 x 10 ⁵ TCID ₅₀ /mL	Not Detected	Detected
Adenovirus	Type 7	1.00 x 10 ⁵ TCID ₅₀ /mL	Not Detected	Detected
Cytomegalovirus		2.00 x 10 ⁴ TCID ₅₀ /mL	Not Detected	Detected
Enterovirus	Type D68	6.20 x 10 ⁴ TCID ₅₀ /mL	Not Detected	Detected
Epstein-Barr Virus		1.00 x 10 ⁵ copies/mL	Not Detected	Detected
Human coronavirus	229 E	1.70 x 10 ⁴ TCID ₅₀ /mL	Not Detected	Detected
Human coronavirus	HKU1	2.70 x 10 ⁴ Copies/mL	Not Detected	Detected
Human coronavirus	NL63	5.00 x 10 ³ TCID ₅₀ /mL	Not Detected	Detected
Human coronavirus	OC43	1.00 x 10 ⁵ TCID ₅₀ /mL	Not Detected	Detected
Human metapneumovirus (hMPV)		1.00 x 10 ⁵ TCID ₅₀ /mL	Not Detected	Detected
Influenza A	Denver/1/57	1.00 x 10 ⁵ TCID ₅₀ /mL	Not Detected	Detected
Influenza B	GL/1739/54	1.00 x 10 ⁵ TCID ₅₀ /mL	Not Detected	Detected
Measles		2.00 x 10 ⁴ TCID ₅₀ /mL	Not Detected	Detected
MERS coronavirus		2.00 x 10 ⁴ TCID ₅₀ /mL	Not Detected	Detected
Mumps		1.00 x 10 ⁵ TCID ₅₀ /mL	Not Detected	Detected
Parainfluenza virus	Type 1	6.20 x 10 ⁴ TCID ₅₀ /mL	Not Detected	Detected
Parainfluenza virus	Type 2	7.50 x 10 ⁴ TCID ₅₀ /mL	Not Detected	Detected
Parainfluenza virus	Type 3	1.00 x 10 ⁵ TCID ₅₀ /mL	Not Detected	Detected
Parainfluenza virus	Type 4a	2.70 x 10 ³ TCID ₅₀ /mL	Not Detected	Detected
Respiratory syncytial virus (RSV)		1.70 x 10 ⁴ TCID ₅₀ /mL	Not Detected	Detected
Rhinovirus	Type 1A	8.00 x 10 ³ TCID ₅₀ /mL	Not Detected	Detected
SARS coronavirus		2.60 x 10 ⁴ TCID ₅₀ /mL	Not Detected	Detected
<i>Bordetella pertussis</i>		1.00 x 10 ⁶ CFU/mL	Not Detected	Detected
<i>Chlamydia pneumoniae</i>		1.00 x 10 ⁶ IFU/mL	Not Detected	Detected
<i>Corynebacterium diphtheriae</i>		1.00 x 10 ⁶ CFU/mL	Not Detected	Detected
<i>Escherichia coli</i>		1.00 x 10 ⁶ CFU/mL	Not Detected	Detected
<i>Haemophilus influenzae</i>		1.00 x 10 ⁶ CFU/mL	Not Detected	Detected
<i>Lactobacillus salivarius</i>		1.00 x 10 ⁶ CFU/mL	Not Detected	Detected
<i>Legionella pneumophila</i>		1.00 x 10 ⁶ CFU/mL	Not Detected	Detected
<i>Moraxella catarrhalis</i>		1.00 x 10 ⁶ CFU/mL	Not Detected	Detected
<i>Mycobacterium tuberculosis</i>		1.00 x 10 ⁶ CFU/mL	Not Detected	Detected
<i>Mycoplasma pneumoniae</i>		1.00 x 10 ⁶ CCU/mL	Not Detected	Detected
<i>Neisseria gonorrhoeae</i>		1.00 x 10 ⁶ CFU/mL	Not Detected	Detected
<i>Neisseria meningitidis</i>		1.00 x 10 ⁶ CFU/mL	Not Detected	Detected
<i>Pneumocystis jirovecii</i>		1.00 x 10 ⁶ CFU/mL	Not Detected	Detected
<i>Pseudomonas aeruginosa</i>		1.00 x 10 ⁶ CFU/mL	Not Detected	Detected
<i>Staphylococcus aureus</i>		1.00 x 10 ⁶ CFU/mL	Not Detected	Detected
<i>Staphylococcus epidermidis</i>		1.00 x 10 ⁶ CFU/mL	Not Detected	Detected
<i>Streptococcus pneumoniae</i>		1.00 x 10 ⁶ CFU/mL	Not Detected	Detected
<i>Streptococcus pyogenes</i>		1.00 x 10 ⁶ CFU/mL	Not Detected	Detected
<i>Streptococcus salivarius</i>		1.00 x 10 ⁶ CFU/mL	Not Detected	Detected
<i>Candida albicans</i>		1.00 x 10 ⁶ CFU/mL	Not Detected	Detected

10.1.7. Exclusivity (In Silico)

Exclusivity for the LumiraDx Dual-Target SARS-CoV-2 STAR Complete was assessed in silico by evaluating sequences of pathogens potentially present in respiratory specimens and/or with genetic similarities to target organisms. A summary of the organisms assessed for exclusivity is shown in

Table 21. No cross-reactivity is predicted based on the in silico analysis.

Table 21. LumiraDx Dual-Target SARS-CoV-2 STAR Complete In Silico Exclusivity Organisms

Viruses	Bacteria	Eukaryotes
Adenovirus (Type 1)	<i>Bordetella pertussis</i>	<i>Candida albicans</i>
Adenovirus (Type 5)	<i>Chlamydia pneumoniae</i>	Human genome
Adenovirus (Type 7)	<i>Corynebacterium diphtheriae</i>	
Cytomegalovirus	<i>Escherichia coli</i>	
Enterovirus D68	<i>Haemophilus influenzae</i>	
Epstein-Barr Virus	<i>Lactobacillus salivarius</i>	
Human coronavirus (229E)	<i>Legionella pneumophila</i>	
Human coronavirus (HKU1)	<i>Moraxella catarrhalis</i>	
Human coronavirus (NL63)	<i>Mycobacterium tuberculosis</i>	
Human coronavirus (OC43)	<i>Mycoplasma pneumoniae</i>	
Human metapneumovirus (hMPV)	<i>Neisseria gonorrhoeae</i>	
Influenza A (H1N1, H3N2)	<i>Neisseria meningitidis</i>	
Influenza B (Victoria, Yamagata)	<i>Pneumocystis jirovecii</i>	
Measles	<i>Pseudomonas aeruginosa</i>	
MERS coronavirus	<i>Staphylococcus aureus</i>	
Mumps	<i>Staphylococcus epidermidis</i>	
Parainfluenza virus (Type 1)	<i>Streptococcus pneumoniae</i>	
Parainfluenza virus (Type 2)	<i>Streptococcus pyogenes</i>	
Parainfluenza virus (Type 3)	<i>Streptococcus salivarius</i>	
Parainfluenza virus (Type 4a)		
Respiratory syncytial virus (RSV)		
Rhinovirus (Type 1A)		
SARS Coronavirus		

10.1.8. Potentially Interfering Substance

Substances commonly found in respiratory samples, substances that could be introduced during specimen collection, and medications commonly used to treat congestion, allergies, or asthma symptoms which could potentially interfere with the LumiraDx Dual-Target SARS-CoV-2 STAR Complete were individually evaluated at clinically relevant concentrations against low titer (3x LoD) SARS-CoV-2 in negative clinical matrix on a validated RT-PCR instrument. A summary of substances tested is shown in **Table 22**. No inhibition was observed from any of the substances at the concentrations tested.

Table 22. LumiraDx Dual-Target SARS-CoV-2 STAR Complete Potentially Interfering Substances

Potentially Interfering Substance	Active Ingredient	Testing Concentration
Beclomethasone	Beclomethasone	0.1 mg/mL
Budesonide	Budesonide	0.1 mg/mL
Chloraseptic® Max	Phenol (1.5%), Glycerin (33%)	5% (v/v), or 0.075% Phenol, 1.65% Glycerin
Cold & Flu Relief Cough Syrup	Acetaminophen (325 mg), Dextromethorphan HBr (10 mg), Guaifenesin (200 mg), Phenylephrine (5 mg) per 15mL	5% (v/v), or Acetaminophen 1.08 mg/mL, Dextromethorphan 0.033 mg/mL, Guaifenesin 0.667 mg/mL, Phenylephrine 0.0167 mg/mL
Dexamethasone	Dexamethasone	0.1 mg/mL
Flonase	Fluticasone Propionate (50 mcg)	5% (v/v)
Flunisolide	Flunisolide (0.025%)	0.1 mg/mL
Mometasone Furoate	Mometasone Furoate	0.1 mg/mL
Mucin, Type I-S (bovine)	Purified mucin protein	0.25 mg/mL
Mupirocin	Mupirocin	0.1 mg/mL
Nasacort	Triamcinolone acetonide (55 mcg)	5% (v/v)
Saline Nasal Spray	Sodium chloride (0.65%)	5% (v/v) or 0.0325% NaCl
Oxymetazoline Nasal Spray	Oxymetazoline HCl (0.05%)	5% (v/v) or 0.0025% Oxymetazoline HCl
Phenylephrine Nasal Spray	Phenylephrine HCl (1%)	5% (v/v) or 0.05% Phenylephrine HCl
Tobramycin	Tobramycin	0.033 mg/mL
Vicks® VapoCOOL™ (throat lozenge)	Benzocaine, Menthol	15 mg/mL, 20 mg/mL
Zanamivir	Zanamivir	0.75 mg/mL
Zicam® (Nasal gel, homeopathic allergy relief)	Galphimia glauca, Luffa operculata, Sabadilla	5% (v/v)
Whole Blood (Human)	N/A	2.5% (v/v)

10.1.9. Repeatability

To assess the repeatability of the LumiraDx Dual-Target SARS-CoV-2 STAR Complete, SARS-CoV-2 virus (rendered non-infectious) was spiked into negative clinical matrix at low and moderate positive concentrations (2x and 5x LoD) and tested over 6 days by two operators with two distinct reagent lots with four replicates per condition along with negative controls on a validated RT-PCR instrument. 100% of replicates were in agreement with the expected result demonstrating the repeatability of the LumiraDx Dual-Target SARS-CoV-2 STAR Complete. A summary of repeatability testing conditions is shown in **Table 23**.

Table 23. LumiraDx Dual-Target SARS-CoV-2 STAR Complete Repeatability Conditions

Condition	Negative Replicates	2x LoD Replicates	5x LoD Replicates
Days	6	6	6
Reagent Lots	2	2	2
Operators	2	2	2
Replicates/Condition	4	4	4
Totals	96	96	96

10.1.10. Reproducibility

To assess the reproducibility of the LumiraDx Dual-Target SARS-CoV-2 STAR Complete, SARS-CoV-2 virus (rendered non-infectious) was spiked into negative clinical matrix at low and moderate positive concentrations (2x and 5x LoD) and tested over 6 days by two operators at three distinct sites with three replicates per condition along with negative controls using a validated RT-PCR instrument. 100% of replicates were in agreement with the expected result demonstrating the reproducibility of the LumiraDx Dual-Target SARS-CoV-2 STAR Complete. A summary of reproducibility testing conditions is shown in **Table 24**.

Table 24. LumiraDx Dual-Target SARS-CoV-2 STAR Complete Reproducibility Conditions

Condition	Negative Replicates	2x LoD Replicates	5x LoD Replicates
Number of Sites	3	3	3
Days	6	6	6
Operators	2	2	2
Replicates/Condition	3	3	3
Totals	108	108	108

10.2 Clinical Study

To demonstrate positive and negative percent agreement of the LumiraDx Dual-Target SARS-CoV-2 STAR Complete as compared to a highly sensitive CE-Marked RT-PCR (comparator) test, 378 nasopharyngeal swab specimens were prepared and tested in a blind study for both assays. Positive and negative percent agreement between the LumiraDx Dual-Target SARS-CoV-2 STAR Complete and the comparator test are summarized in **Table 25**.

Table 25. LumiraDx Dual-Target SARS-CoV-2 STAR Complete Positive and Negative Percent Agreement

		Comparator		
		Positive	Negative	Total
LumiraDx Dual-Target SARS-CoV-2 STAR Complete	Positive	167	0	167
	Negative	4*	207	211
	Total	171	207	378
Percent Agreement		97.7% (Pos)	100% (Neg)	
95% Confidence Interval		94.1-99.4%	98.2-100%	

*Of the four false negative samples, all were tested on additional CE-Marked RT-PCR test, one was negative and three were positive for SARS-CoV-2.

10.3 Equivalency Studies

10.3.1 Transport Medium Equivalency

A sample matrix equivalency study was performed to demonstrate the equivalency between four transport media. Several transport media were spiked with heat-inactivated virus at 1x LoD (a minimum of 20 replicates) and run on a validated RT-PCR instrument. Detection of $\geq 95\%$ of positive samples at 1x LoD and 100% detection of IC in negative samples with no false positive results were necessary to determine equivalency for each of the media tested. A summary of media tested and determined to be equivalent is shown in **Table 26**.

Table 26. LumiraDx Dual-Target SARS-CoV-2 STAR Media Equivalency

Equivalent Media
1X PBS, pH 7.4
Saline (0.85%)
Saline (0.90%)
Transport Medium*

*Corning Transport Medium or medium of similar formulation

10.3.2 Instrument Equivalency (96-Well)

An instrument equivalency study was performed to verify equivalent performance between three 96-well instruments as compared to the 96-Well Bio-Rad Opus instrument on which the analytical performance studies were executed. 20 replicates of heat-inactivated SARS-CoV-2 virus was tested in negative sample matrix to verify the LoD for each instrument. All instruments tested demonstrated $\geq 95\%$ detection at LoD and 0% detection of negative samples with the LumiraDx Dual-Target SARS-CoV-2 STAR Complete. A summary of equivalent instruments is shown in **Table 27**.

Table 27. LumiraDx Dual-Target SARS-CoV-2 STAR 96-Well Instrument Equivalency

Instrument	Manufacturer
CFX Opus	Bio-Rad
QuantStudio5 (96-Well)	Applied Biosystems
QuantStudio 7 Flex (96-Well)	Applied Biosystems
qTOWER ³ (96-Well)	Analytik Jena

11 Contact Information, Ordering, and Product Support

For ordering, contact LumiraDx at:

Website: www.LumiraDx.com.

Email (US): CustomerServices.US@LumiraDx.com.

Email (International): CustomerServices@LumiraDx.com.

For product information, contact LumiraDx at:

Email: CustomerServices.US@LumiraDx.com. Include "LumiraDx Dual-Target SARS-CoV-2 STAR Complete" in the subject line.

Phone: 1-888-586-4721.

For technical support, contact LumiraDx at:

Email: TechnicalServices@LumiraDx.com. Include "LumiraDx Dual-Target SARS-CoV-2 STAR Complete" in the subject line.

For return policy, contact LumiraDx at:

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

Intellectual property

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

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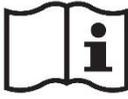
Limited Warranty

LumiraDx Dual-Target SARS-CoV-2 STAR Complete – As per shelf life.

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

LumiraDx Dual-Target SARS-CoV-2 STAR Complete Kit to physical abuse, misuse, abnormal use, use inconsistent with the LumiraDx instructions for use, fraud, tampering, unusual physical stress, negligence or accidents. Any warranty claim by Customer pursuant to the Limited Warranty shall be made in writing within the applicable Limited Warranty period.

12 Glossary of Symbols

	Temperature limitation		Use-by Date – The date after which the unopened reagent cannot be used.
	In vitro diagnostic medical device		Contains sufficient reagent for 'n' reactions
	Catalog Reference Number		Refer to www.lumiradx.com for the electronic form of the instructions for use.
	Lot Number/Batch Code		Manufacturer
	Negative Control Media		CE Mark of Conformity
	Positive Control Media		Authorized representative in the European Community
	Uncontaminated recycled content packaging, kit box, Instructions for Use is recyclable if it can be collected, separated, or otherwise recovered from the waste stream through an established recycling program.		European Importer



LumiraDx UK Ltd. Unit 50, Yorkshire Way Doncaster DN3 3FT, UK
Company Number 09206123



LumiraDx AB Västra Vägen 5A 16961 Solna, Sweden



LumiraDx B.V. Looskade 20 6041 LE Roermond, The Netherlands



LumiraDx 6650 Nancy Ridge Drive, San Diego, CA 92121, USA

13. Appendix A: Detailed Instructions for Instrument Setup (96-Well Format)

This appendix is intended to assist the user with the programming of instruments for use with the LumiraDx Dual-Target SARS-CoV-2 STAR Complete.

13a. Instrument Setup for Applied Biosystems™ QuantStudio 5 (96-Well Format)

Refer to User Manual Part Number MAN0010407 for additional information. The instrument programming instructions are intended for setup of an entire 96-well plate.

13a.1 Programming Instructions for PC/Laptop-connected Instrument

It is recommended to setup the instrument up to Step 12, below before preparing the Reagent Mix as defined in **Section 8.3 qSTAR Reagent Preparation and Plate Setup**. The 96-well plate should be recently centrifuged to guarantee all reagents are at the bottom of the plate and kept on a cold block. Use only 96-well plates and seals that are compatible with the instrument manufacturer.

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

1. Launch the 'QuantStudio Design & Analysis' Desktop Software (version v1.5.1)
2. Select 'Create New Experiment'. A window will open with the 'Properties' tab selected.
3. Enter the 'Experiment Properties' as follows:
 - a. Name the experiment "Dual-Target SARS-CoV-2 STAR Complete Template"
 - b. Set 'Instrument type' to 'QuantStudio 5 System'
 - c. Set 'Block type' to '96-Well 0.2mL Block'
 - d. Set 'Experiment type' to 'Standard Curve'
 - e. Set 'Chemistry' to 'Other'
 - f. Set 'Run mode' to 'Standard'
4. Proceed to the 'Method' tab.
 - a. Click 'Action'
 - b. Select 'Optical filter settings' from the drop-down menu.
 - c. In the 'PCR Filter' table, uncheck all filter combinations except the following:
 - x1(470±15), m1(520±15) FAM
 - x4(580±10), m4(623±14) ROX
 - x5(640±10), m5(682±14) Cy5/Mustang Purple
 - d. Click 'Close' to return to the 'Method' tab.
 - e. Set the 'Volume' to '60 µL'.
5. In the 'Hold Stage' section, change 'Step 1' settings as follows:
 - a. Set ramp rate to '2°C/s'
 - b. Set temp to '30°C'
 - c. Set time to '01:00'
6. In the 'Hold Stage' section, change 'Step 2' settings as follows:
 - a. Set ramp rate to '2°C/s'
 - b. Set temp to '51°C'
 - c. Set time to '03:00'
7. In the 'PCR Stage' section, change 'Step 1' settings as follows:
 - a. Set ramp rate to '2°C/s'
 - b. Set temp to '51°C'
 - c. Set time to '00:25'
 - d. Set the data collection for this stage by ensuring that the camera icon is active (not grayed out)

8. In the 'PCR Stage' section, change 'Step 2' settings as follows:
 - a. Set ramp rate to '2°C/s'
 - b. Set temp to '61°C'
 - c. Set time to '00:01'
9. At the bottom of the 'PCR Stage' section, set 'Cycles' to '20'.
10. Proceed to the 'Plate' tab.
 - a. Change 'Passive Reference' to 'None' in the 'Plate Attributes' window.
 - b. Select all the wells from the plate layout.
11. Proceed to the 'Advanced Setup' tab to set up the targets and controls.

COVID Target 1

- a. Under the 'Targets' section, rename 'Target 1' to '**COVID Target 1**'.
- b. Change the default color to blue .
- c. Select '**FAM**' as the 'Reporter' (the 'Quencher' is automatically entered as 'NFQ-MGB').
- d. Checkmark the empty box to the left of the colored box to apply it to the wells.

COVID Target 2

- a. 'Add' a second target then rename to '**COVID Target 2**'
- b. Change the default color to purple
- c. Select '**Mustang Purple**' as the 'Reporter' (the 'Quencher' is automatically entered as 'NFQ-MGB')
- d. Checkmark the empty box to the left of the colored box to apply it to the wells.

Internal Control

- a. 'Add' a third target then rename to '**IC**'
- b. Change the default color to red
- c. Select '**ROX**' as the 'Reporter' (the 'Quencher' is automatically entered as 'NFQ-MGB')
- d. Checkmark the empty box to the left of the colored box to apply it to the wells.

Optional: Under the 'Samples' section, the 'Sample Name' can be added individually or pasted into the 96-well plate layout from an Excel file.

12. Proceed to the 'Run' tab.
 - a. Save the experiment as a template for subsequent runs by clicking the 'Save' down arrow and selecting the 'Save As' option.
 - b. Name the template "Dual-Target SARS-CoV-2 STAR Complete CE Template" and click 'Save'.
 13. Load the plate as follows:
 - a. Press the 'eject' icon on the QuantStudio 5 instrument touchscreen at the top right of the window.
 - b. 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.
 - c. Press the 'eject' icon on the instrument touchscreen to close the drawer.
 14. 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.
 15. Select the instrument number to initiate the run.
 16. Change the 'File Name' to "LumiraDx Dual-Target SARS-CoV-2 STAR Complete [YYMMDD_Plate#]" and click 'Save'.
 17. Following the run, the instrument touchscreen indicates when the run is 'Complete'. Close the run screen by pressing 'Done'.
 18. Remove the plate and discard the plate in a sealable waste bag or container.
- NOTE:** Wells containing PCM as well as those which were positive for a target will contain high levels of amplicon which can cause false positive results in future tests if allowed into the laboratory environment. Never remove the optical seal from the plate after amplification.

13a.2 Analysis Instructions

1. Modify the analysis settings using the 'Results' tab in the 'QuantStudio Design & Analysis' Desktop Software (version v1.5.1).
2. 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:
 - a. Set 'Plot Type' to ' ΔR_n vs Cycle'
 - b. Set 'Graph Type' to 'Log'
 - c. Set 'Plot Color' to 'Target'
 - d. Click out of the window to accept changes (All other content remains unchanged)
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.

COVID Target 1

- a. In the 'Ct settings' tab, uncheck the 'Default settings' box under the 'Ct settings for COVID Target 1' section.
- b. Uncheck the 'Automatic Threshold' box
- c. Uncheck the 'Automatic Baseline' box
- d. Enter "100,000" for the 'Threshold'
- e. Set 'Baseline Start Cycle' to '1'
- f. Set 'End Cycle' to '2'
- g. Click 'Apply'

COVID Target 2

- a. In the 'Ct settings' tab, uncheck the 'Default settings' box under the 'Ct settings for COVID Target 2' section.
- b. Uncheck the 'Automatic Threshold' box
- c. Uncheck the 'Automatic Baseline' box
- d. Enter "100,000" for the 'Threshold'
- e. Set 'Baseline Start Cycle' to '1'
- f. Set 'End Cycle' to '2'
- g. Click 'Apply'

Internal Control

- a. Select the 'IC' line
 - b. Uncheck the 'Default settings' box
 - c. Uncheck the 'Automatic Threshold' box
 - d. Uncheck the 'Automatic Baseline' box.
 - e. Enter "100,000" for the 'Threshold'
 - f. Set 'Baseline Start Cycle' to '1'
 - g. Set 'End Cycle' to '2'.
 - h. Click 'Apply'.
4. Proceed the 'Export' tab to define the 'File Name' for the exported file ("Dual-Target SARS-CoV-2 STAR Complete [YYMMDD_Plate#]").
 5. Define the 'File name'
 - a. Choose the 'File Type' (default is QuantStudio as an .xlsx file)
 - b. Choose the 'Location' to export the file
 - c. Choose the 'Content' to be exported (the 'Results' box needs to be checked for Ct values).
 - d. Click 'Customize' to proceed to what is to be exported within each Content item.
 6. Go to the 'Results' tab
 - a. Select the 'All Fields' box to uncheck all content
 - b. Check the boxes for the following content:
 - 'Well'

- 'Well Position'
 - 'Sample Name'
 - 'Target Name'
 - 'Reporter'
 - 'CT'
 - 'Ct Threshold'
 - 'Baseline Start'
 - 'Baseline End'
- c. Click 'Close' to return to the 'Export' tab screen.
 7. Click 'Save' to save the modified settings.
 8. Click the 'Export' button to generate the export data file. The exported file will include a 'Results' section which contains the sample Ct values.
 9. Close the software.

13b. Instrument Setup for Applied Biosystems QuantStudio 7 Flex (96-Well Format)

Refer to User Manual Part Number 4489821 for additional information. The instrument programming instructions are intended for setup of an entire 96-well plate.

13b.1 Programming Instructions for PC/Laptop-connected Instrument

It is recommended to setup the instrument up to Step 18, below before preparing the Reagent Mix as defined in **Section 8.3 qSTAR Reagent Preparation and Plate Setup**. The 96-well plate should be recently centrifuged to guarantee all reagents are at the bottom of the plate and kept on a cold block. Use only 96-well plates and seals that are compatible with the instrument manufacturer.

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

1. Launch the 'QuantStudio Real-Time PCR Software' Desktop Software (version v1.3).
2. On the toolbar select 'Tools' then 'Preferences' to open the 'Defaults' tab
3. Confirm that the 'Instrument Type:' is set to 'QuantStudio™ 7 Flex System'
4. Confirm that the 'Block Type:' is set to '96-Well Block (0.2mL)'
5. Confirm that the 'Decimal Places to Show:' is set to '3'.
6. Click the box next to 'Show optical filters for run method' and click on 'OK'.
7. Select 'Create New Experiment'. A window will open with the 'Properties' tab selected.
8. Enter the 'Experiment Properties' as follows:
 - a. Name the experiment "Dual-Target SARS-CoV-2 STAR Complete Template"
 - b. Set 'Instrument type' to 'QuantStudio™ 7 Flex System'
 - c. Set 'Block type' to '96-Well (0.2mL) Block'
 - d. Set 'Experiment type' to 'Standard Curve'
 - e. Set reagents being used to detect target sequences as 'Other'
 - f. Set the properties for the instrument run as 'Standard'
9. Proceed to the 'Define' tab under the 'Targets' section.

COVID Target 1

- a. Under the 'Targets' section, rename 'Target 1' to "**COVID Target 1**"
- b. Select '**FAM**' as the reporter
- c. Change the 'color' to blue
- d. Confirm that the 'Quencher' is automatically entered as 'NFQ-MGB'.

COVID Target 2

- a. 'Add' a second target by clicking 'New' (just above the 'Target Name') then rename to '**COVID Target 2**'
- b. Select '**Cy5**' or '**Mustang Purple**' as the reporter
- c. Change the default color to purple
- e. Confirm that the 'Quencher' is automatically entered as 'NFQ-MGB'.

Internal Control

- a. 'Add' a third target then rename to '**IC**'
- b. Select '**ROX**' as the reporter
- c. Change the default color to red
- d. Confirm that the 'Quencher' is automatically entered as 'NFQ-MGB'.

Passive Reference Dye:

- a. Use the drop-down menu in the 'Passive Reference' section to change the passive reference dye to 'None'

Optional:

- a. Under the 'Samples' section, the 'Sample Name' can be added individually.
10. Proceed to the 'Assign' tab.
 - a. Select all wells from the plate layout by clicking the box between 'A' and '1'.
 - b. On the left of the screen in the 'Targets' section, check the boxes next to 'COVID Target 1', 'COVID Target 2', and 'IC' to apply these targets to the wells.
 - c. If 'Samples' have been defined for each well, select individual wells and check the box next to the appropriate 'Name' in the 'Samples' section.
11. Proceed to the 'Run Method' tab.
 - a. Under the 'Run Method' section, define the 'Reaction Volume per Well' to "60" (µL).
12. Change the 'Hold Stage' 'Step 1' settings as follows:
 - a. Set the ramp rate to '1.946°C/s'
 - b. Set the temp to '30.0°C'
 - c. Set time to '01:00'.
13. Change the 'Hold Stage' 'Step 2' settings as follows:
 - d. Set the ramp rate to '1.946°C/s'
 - e. Set the temp to '51.0°C'
 - f. Set time to '03:00'.
14. Change 'Step 1' in 'PCR Stage' as follows:
 - a. Set the ramp rate (middle) to '**1.645°C/s**'
 - b. Set the temp (upper middle) to '52°C'
 - c. Set the time (lower middle) to '00:25'.
15. Change 'Step 2' in 'PCR Stage' as follows:
 - a. Set the ramp rate (left) to '1.946°C/s'
 - b. Set the temp (upper right) to '61°C'
 - c. Set the time (lower right) to '00:01'
16. 'Step 1' in the 'PCR Stage' is the data collection step. At the top of the 'PCR Stage', set 'Number of Cycles' to '20'.
17. Near the top of the window net to the 'Graphical View' tab select 'Optical Filters'. Just below in the 'PCR Filter' section, uncheck all 'Emission Filter' combinations except the following:
 - x1(470±15), m1(520±15) FAM
 - x4(580±10), m4(623±14) ROX
 - x5(640±10), m5(682±14) Cy5/Mustang Purple
18. Save the experiment as a template for subsequent runs as follows:
 - a. Click the 'Save' drop-down menu and select 'Save As Template'.

- b. Name the template “Dual-Target SARS-CoV-2 STAR Complete Template” and click ‘Save’. The experiment must be saved again to connect to the instrument.
 - c. Click the drop-down menu next to ‘Save’ and select ‘Save As’.
 - d. Save the ‘File Name’ as “LumiraDx Dual-Target SARS-CoV-2 STAR Complete [YYMMDD_Plate#]” and click ‘Save’.
19. Load the plate as follows:
- a. Press the red ‘Arrow’ icon on the QuantStudio 7 Flex instrument touchscreen at the bottom right of the window.
 - b. 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.
 - c. Press the red ‘Arrow’ icon on the instrument touchscreen to close the drawer.
20. Begin the run as follows:
- a. Return to the desktop software ‘Run’ tab and click the ‘START RUN’ button. The instrument number will appear in a drop-down menu below the ‘START RUN’ button.
 - b. Select the instrument number to initiate the run.
 - c. A pop-up window may appear indicating the ramp rate in Stage 1 and 2 is above the maximum. Select ‘Yes’ to proceed.
21. Following the run, the instrument touchscreen indicates when the run is ‘Complete’. Remove the plate and discard the plate in a sealable waste bag or container.
- NOTE:** Wells containing PCM as well as those which were positive for a target will contain high levels of amplicon which can cause false positive results in future tests if allowed into the laboratory environment. Never remove the optical seal from the plate after amplification.

13b.2 Analysis Instructions

1. Modify the analysis settings using the ‘Analysis’ ‘Amplification Plot’ tab in the ‘QuantStudio Real-Time PCR Software’ Desktop Software (version v1.3).
2. Select the desired wells for analysis in the ‘Plate Layout’
3. Configure the ‘Amplification Plot’ window on the left as follows:
 - a. Set ‘Plot Type’ to ‘ ΔR_n vs Cycle’
 - b. Set ‘Graph Type’ to ‘Log’
 - c. Set ‘Plot Color’ to ‘Target’
4. On the top right of the software to the right of the ‘Analyze’ button, click the ‘Analysis Settings’ to open the ‘Analysis Settings’ window.

COVID Target 1

- a. In the ‘Ct settings’ tab in the bottom right window for ‘CT Settings for COVID Target 1’, uncheck the ‘CT Settings to Use’ box next to ‘Default Settings’. (This will toggle the ‘Automatic Threshold’ and ‘Automatic Baseline’ boxes to be checked).
- b. Uncheck the box to the left of ‘Automatic Threshold’
- c. Uncheck the box to the left of ‘Automatic Baseline’
- d. Define the ‘Threshold’ as “500,000”
- e. Set the ‘Baseline Start Cycle’ to ‘1’
- f. Set ‘End Cycle’ to ‘2’.

COVID Target 2

- a. In the ‘Ct settings’ tab in the bottom right window for ‘CT Settings for COVID Target 2’, uncheck the ‘CT Settings to Use’ box next to ‘Default Settings’. (This will toggle the ‘Automatic Threshold’ and ‘Automatic Baseline’ boxes to be checked).
- b. Uncheck the box to the left of ‘Automatic Threshold’
- c. Uncheck the box to the left of ‘Automatic Baseline’
- d. Define the ‘Threshold’ as “250,000”
- e. Set the ‘Baseline Start Cycle’ to ‘1’

- f. Set 'End Cycle' to '2'.

Internal Control

- a. In the 'Select a Target' window (on the bottom left), select the 'IC'
 - b. In the 'CT Settings for IC' uncheck the 'Default settings'
 - c. In the 'CT Settings for IC' uncheck the 'Automatic Threshold'
 - d. In the 'CT Settings for IC' uncheck the 'Automatic Baseline'.
 - e. Define the 'Threshold' as "500,000"
 - f. Set 'Baseline Start Cycle' to '1'
 - g. Set 'End Cycle' to '2'.
 - h. On the bottom of the window, select 'Apply Analysis Settings' to close out of the 'Analysis Settings' pop-up window.
5. On the left of the screen, proceed to the 'Export' tab:
 - a. Define the 'Export File Name' as "Dual-Target SARS-CoV-2 STAR Complete [YYMMDD_Plate#]"
 - b. Choose the 'File Type' (default is QuantStudio as an .xls file)
 - c. Choose the 'Export File Location' to export the file
 - d. Select the desired tabs below 'Export File Location' to determine what data is exported.

Note: The box next to the 'Results' tab needs to be checked to export CT values.

6. Within the 'Results' tab select the following:
 - a. 'Well'
 - b. 'Well Position'
 - c. 'Sample Name'
 - d. 'Target Name'
 - e. 'Reporter'
 - f. 'Quencher'
 - g. 'CT'
 - h. 'Ct Threshold'
 - i. 'Baseline Start'
 - j. 'Baseline End'
 - k. Click 'Start Export' at the bottom of the screen to generate the export data file. (The exported file will include a 'Results' section which contains the sample Ct values).
7. Click 'Save' to save the modified settings.
8. Close the software.

13c. Instrument Setup for Bio-Rad CFX OPUS (96-Well Format)

Refer to User Manual Part Number 10000119983 for additional information. The instrument programming instructions are intended for setup of an entire 96-well plate.

13c.1 Programming Instructions for PC/Laptop-connected Instrument

It is recommended to set up the instrument up to Step 18, below before preparing the Reagent Mix as defined in **Section 8.3 qSTAR Reagent Preparation and Plate Setup**. The 96-well plate should be recently centrifuged to guarantee all reagents are at the bottom of the plate and kept on a cold block. Use only 96-well plates and seals that are compatible with the instrument manufacturer.

1. Launch the 'Bio-Rad CFX Maestro™' Software (version v2.2). In the 'Startup Wizard' pop-up window, select 'User-defined' as the run type.
2. In the 'Run Setup' pop-up window, select 'Edit Selected...' on the right of the window under the 'Protocol' tab to begin setup.
3. The 'Protocol Editor' pop-up window will open. Set the 'Sample Volume' to "0" µL (the sample volume should not be set higher than this).

4. Set the lid temperature as follows:
 - a. Click on 'Settings' from the top menu, then 'Lid Settings...' from the drop-down menu.
 - b. Change 'User defined' temperature to '70'.
 - c. Click 'OK'
5. Click 'Insert Step' to add a total of five steps to the protocol.
6. Edit steps '1' through '5' as follows:
 - a. Set step '1' temp to '45.0' C
 - b. Set '1' time to '0:00' (this sets the time to 'Forever')
 - c. Set step '2' temp to '49.5' C, set step '2' time to '3:00'
 - d. Set step '3' temp to '49.0' C, set '3' time to '0:12' and 'Add Plate Read' by clicking the icon on the left.
 - e. Set step '4' temp to '67.5' C, set '4' time to '0:01'
 - f. Set step '5 GO TO' to '3' and '26' x (this defines that step 3 through step 4 will be repeated a total of 25 times).
 - g. Select 'OK' and then select 'Yes' to save the changes to the protocol file.
 - h. Define the 'File name' as "Dual-Target SARS-CoV-2 STAR Complete Template.prcf" in the appropriate file path and select 'Save'.
7. Proceed to the 'Plate' tab by selecting the 'Next' button on the bottom right of the 'Protocol Editor' window.
8. Select 'Edit Selected...' on the right of the window.
9. Select the entire plate by selecting the gray box between position 'A' and '1' on the plate.
10. Ensure that 'Scan Mode' is set to 'All Channels' by using the drop-down menu.
11. Set up the fluorophores by clicking s 'Select Fluorophores...' on the far top right of the window.
 - a. Ensure the only fluorophores selected are 'FAM', 'ROX', and 'Cy5'.
 - b. Change the 'Color' for 'FAM' to blue.
 - c. Change the 'Color' for 'ROX' to red.
 - d. Change the 'Color' for 'Cy5' to purple.
 - e. Select 'OK' to close out of the pop-up window.
12. To define targets select 'Experiment Settings...' near the bottom right of the window to open the 'Experiment Settings' pop-up window and do the following as follows:
 - a. Under the 'Targets' tab, add a new target by defining 'New' as "COVID Target 1" and selecting 'Add'.
 - b. Add a second target and define 'New' as "COVID Target 2" then select 'Add'.
 - c. Add a third target and define 'New' as "IC" then select 'Add'.
 - d. Under the 'Exclude the following sample types from Gene Expression analysis' at the bottom of the pop-up window, deselect all checkboxes and select 'OK' to close out the pop-up window.
 - e. Select the entire plate again by selecting the gray box between position 'A' and '1' on the plate and ensure the check boxes to the left of the 'Load' selection are checked for 'FAM', 'ROX', and 'Cy5'.
 - f. Using the drop-down menu under 'Target Name' define the 'Target Name' for '**FAM**' as 'COVID Target 1'
 - g. Define the 'Target Name' for '**Cy5**' as 'COVID Target 2'.
 - h. Define the 'Target Name' for '**ROX**' as 'IC'.
13. Select the plate type as follows:
 - a. Click 'Settings' from 'Plate Editor' menu bar
 - b. Select 'Plate Type' from the dropdown list.
 - c. Select 'BR White' from the dropdown sub-list.
 - d. Select 'OK' and save the changes by clicking 'Yes'.

14. Define the 'File name' as "Dual-Target SARS-CoV-2 STAR Complete Template.pltd" in the appropriate file path and select 'Save' to save the plate file.
15. The 'Plate Editor' pop-up window will close and the new plate will be loaded in the 'Plate' tab of the 'Run Setup' window. Proceed to the 'Start Run' tab by selecting 'Next >>' on the bottom right of the window.
16. On the 'Start Run' tab, click 'Start Run'
17. Save the experiment by defining the 'File name' as "Dual-Target SARS-CoV-2 STAR Complete [YYMMDD_Plate#].pcrd" in the appropriate file path then selecting 'Save'. The run will begin initializing and lid will begin preheating.

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

18. To load the plate do the following:
 - a. At the bottom left of the screen, select 'Open Lid'. A pop-up window may appear indicating the run will pause to open the lid.
 - b. Click 'OK' to proceed. The lid will automatically open.
 - c. Place the sample plate on the amplification block
 - d. Select 'Close Lid' to close the lid.
 - e. Once the lid has finished closing, the 'Skip Step' button will become available.
 - f. Select 'Skip Step' to initiate the run.
19. When the run finishes, the instrument will become idle and the 'Data Analysis' window will pop up.
20. Refer to the 'Bio-Rad CFX Maestro' Software (version v2.2) and select 'Open Lid'. The lid will automatically open.
21. Remove the plate and discard the plate in a sealable waste bag or container. Select 'Close Lid' to close the lid.

NOTE: Wells containing PCM as well as those which were positive for a target will contain high levels of amplicon which can cause false positive results in future tests if allowed into the laboratory environment. Never remove the optical seal from the plate after amplification.

13c.2 Analysis Instructions

1. In the 'Data Analysis' pop-up window under the 'Quantification' tab, select 'Settings' in the main menu then select 'Baseline Setting' and then 'Baseline Subtracted Curve Fit'.
2. Edit the baseline and cycle threshold settings for **COVID Target 1** by performing the following steps:
 - a. Under the 'Amplification' curves, uncheck all fluorophore boxes **except 'FAM'**.
 - b. From the main menu, select 'Settings'
 - c. Select 'Baseline Threshold...' to open the 'Baseline Threshold' pop-up window.
 - d. Under the 'Baseline Cycles' section, select 'User Defined' then, immediately below this, click the square between 'Well' and '1' to select all the wells.
 - e. Edit 'All Selected Rows: Begin' to "1" and 'End:' to "2".
 - f. In the 'Single Threshold' section, select 'User Defined:' and set this to "5000.00".
 - g. Click 'OK' to proceed.
3. Edit the baseline and cycle threshold settings for **COVID Target 2** by performing the following steps:
 - a. Under the 'Amplification' curves, uncheck all fluorophore boxes **except 'Cy5'**.
 - b. From the main menu, select 'Settings'
 - c. Select 'Baseline Threshold...' to open the 'Baseline Threshold' pop-up window.

- d. Under the 'Baseline Cycles' section, select 'User Defined' then, immediately below this, click the square between 'Well' and '1' to select all the wells.
- e. Edit 'All Selected Rows: Begin' to "1" and 'End:' to "2".
- f. In the 'Single Threshold' section, select 'User Defined:' and set this to "5000.00".
- g. Click 'OK' to proceed.
4. Edit the baseline and cycle threshold settings for IC by performing the followings steps:
 - a. Under the 'Amplification' curves, uncheck all fluorophore boxes **except 'ROX'**.
 - b. From the main menu, select 'Settings'
 - c. Select 'Baseline Threshold...' to open the 'Baseline Threshold' pop-up window.
 - d. Under the 'Baseline Cycles' section, select 'User Defined' then, immediately below this, click the square between 'Well' and '1' to select all the wells.
 - e. Edit 'All Selected Rows: Begin' to "1" and 'End:' to "2".
 - f. In the 'Single Threshold' section, select 'User Defined:' and set this to "5000.00".
 - g. Click 'OK' to proceed.
5. After defining baseline and cycle threshold settings for COVID Target 1, COVID Target 2, and IC, check both boxes on the left of all channels then proceed to the 'Quantification Data' tab.
6. Right-click over the 'Results' table, and chose the desired method for exporting the data (e.g. 'Export to Excel...').
7. Save the report by defining the 'File name' as "Dual-Target SARS-CoV-2 STAR Complete Cq Results [YYMMDD_Plate#].xlsx" in the appropriate file path then selecting 'Save'.
8. Close out the software. (If prompted, save the changes to the experiment).

13d. Instrument Setup for Analytic Jena qTOWER³ (96-Well Format)

Refer to User Manual Part Number 10000119983 for additional information. The instrument programming instructions are intended for setup of an entire 96-well plate.

13d.1 Programming Instructions for PC/Laptop-connected Instrument

It is recommended to set up the instrument up to Step 11, below before preparing the Reagent Mix as defined in **Section 8.3 qSTAR Reagent Preparation and Plate Setup**. The 96-well plate should be recently centrifuged to guarantee all reagents are at the bottom of the plate and kept on a cold block. Use only 96-well plates and seals that are compatible with the instrument manufacturer.

1. Launch the 'qPCRsoft' Software (version v4.1).
2. On the toolbar select 'File' then 'New'. This will open the 'Real time pcr project – Unnamed' window 'General' tab.
3. In the 'General' tab, define the 'Title' as "Dual-Target SARS-CoV-2 STAR Complete Template".
4. Select the 'Thermal Cycler' tab and do the following:
 - a. Confirm that the 'Lid temp. °C' is set to '70'
 - b. Confirm that the 'Preheat lid' check box is checked
 - c. From the 'Device' drop-down menu, select your instrument (qTOWER³ or qTOWER³ G)
5. Set up the thermal profile in the table provided in the software as follows:

Step 1:

 - a. Confirm 'scan' is blank
 - b. Set '°C' to '51'
 - c. Set 'm:s' to '03:00'
 - d. Confirm that 'goto' is set to '--'
 - e. Confirm that 'loops' is set to '---'
 - f. Confirm that 'ΔT(°C)' is set to '--.-'
 - g. Confirm that 'Δt(s)' is set to '---'
 - h. Set '↗(°C/s)' to '2'

Step 2:

- a. Set 'scan' to 'Ø' by clicking in the empty box
- b. Set '°C' to '49'
- c. Set 'm:s' to '00:10'
- d. Confirm that 'goto' is set to '--'
- e. Confirm that 'loops' is set to '---'
- f. Confirm that 'ΔT(°C)' is set to '---'
- g. Confirm that 'Δt(s)' is set to '---'
- h. Set '↗(°C/s)' to '2'

Step 3:

- a. Confirm 'scan' is blank
 - b. Set '°C' to '61'
 - c. Set 'm:s' to '00:01'
 - d. Confirm that 'goto' is set to '2'
 - e. Confirm that 'loops' is set to '19'
 - f. Confirm that 'ΔT(°C)' is set to '--.'
 - g. Confirm that 'Δt(s)' is set to '---'
 - h. Set '↗(°C/s)' to '2'
6. Select the 'Scan' tab and change the 'Pos.' '1' settings as follows:
 - a. Confirm that 'Channel' is set to 'Blue'
 - b. Confirm 'Dye' is set to 'FAM'
 - c. Set 'Gain' to '5'
 - d. Set 'Measurement' to 'Ø' by clicking in the empty box
 - e. Confirm 'Pass. Ref.' is left blank.
 7. Change the 'Pos.' '2' settings as follows:
 - a. Confirm 'Channel' is set to 'Purple'
 - b. Confirm 'Dye' is set to 'Cy5'
 - c. Set 'Gain' to '5'
 - d. Set 'Measurement' to 'Ø' by clicking in the empty box
 - e. Confirm that 'Pass. Ref.' is left blank
 8. Change the 'Scan' tab and change the 'Pos.' '3' settings as follows:
 - a. Confirm that 'Channel' is set to 'Red'
 - b. Confirm 'Dye' is set to 'ROX'
 - c. Set 'Gain' to '5'
 - d. Set 'Measurement' to 'Ø' by clicking in the empty box
 - e. Confirm 'Pass. Ref.' is left blank.
 - f. Confirm 'Meas. Repeats:' is set to '3'
 - g. Confirm 'Color compensation' is set to 'Standard 1'.
 - h. Confirm 'Scan region according to layout' is selected.
 9. Assign targets and fluorophores using the 'Samples' tab from the plate layout
 - a. Select all 96 wells
 - b. From the dropdown menu, change 'Sample type:' to 'Unknown'
 - c. In the 'Target:' table under 'Gene'
 - Enter "COVID Target 1" in the box to the right of the 'FAM' 'Dye'
 - Enter "COVID Target 2" in the box to the right of the 'Cy5' 'Dye'
 - Enter "IC" in the box to the right of the 'ROX' 'Dye'
 - d. Click on the execute icon to apply changes to all 96 samples
 10. Assign the positive and negative control locations on the plate as follows:
 - a. From the plate layout, select position 'A1', change 'Sample type:' to 'Negative Control'
 - b. From the drop-down menu, enter "NCM" in the empty box next to 'Sample name:'

- c. Click on the execute icon to apply changes to the sample.
- d. From the plate layout, select position 'A12', change 'Sample type:' to 'Positive Control'
- e. From the drop-down menu, enter "PCM" in the empty box next to 'Sample name:'
- f. Click on the execute icon to apply changes to the sample
11. Save the template for subsequent runs by clicking on 'File' from the top menu bar and selecting 'Save Template' from the drop-down menu
12. Lift the instrument lid and load the plate.
13. In the 'Save As' pop-up window next to 'File Name', name the template "Dual-Target SARS-CoV-2 STAR Complete Template" and click 'Save'

NOTE: Load the plate so that position A1 on the plate is the top, left-most well.

14. Close and lock the lid.
15. Following the run, a pop-up window will read 'PCR run finished successfully'.
16. Click 'Save' to save the file run
17. Define the 'File Name' as "Dual-Target SARS-CoV-2 STAR Complete [YYMMDD_Plate#]" and click 'Save'
18. Remove the plate and discard the plate in a sealable waste bag or container. Select 'Close Lid' to close the lid.

NOTE: Wells containing PCM as well as those which were positive for a target will contain high levels of amplicon which can cause false positive results in future tests if allowed into the laboratory environment. Never remove the optical seal from the plate after amplification.

13d.2 Analysis Instructions

1. Select the desired wells for analysis using the menu option on the far left titled 'Samples'.
2. Open the option by clicking on the 'plus' icon (+). A plate layout will appear where the desired wells can be selected.
3. Modify the analysis settings using the 'Monitoring' tab.
 - a. Under 'Measurement', click on the 'Calculate Ct' to open the 'Measurement (Ct)' window.
 - b. Click on 'Monitoring' in top menu bar and select 'Display options' from the drop-down menu. A Pop-up window titled 'Display options' will appear.
 - c. Change the options as follows:
 - Under 'Smoothing' change the value to 'none' next to 'Points'
 - Under 'Scaling, select 'logarithmic'
 - Under 'Baseline correction' select 'For all samples'
 - Change 'From cycle' to '1'
 - Change 'To cycle' to '6'
 - Confirm 'Intensity' under 'Filter' is disabled (unchecked)
 - Click 'Ok – Fix Thr.'
4. Apply thresholds to the data set as follows:
 - a. In the 'Monitoring' tab under 'Measurement (Ct)' select the 'FAM' tab below the plot and change the 'Threshold' to '1.5'
 - b. Select the 'Cy5' tab and change the 'Threshold' to '5'.
 - c. Select the 'ROX' tab and change the 'Threshold' to '5'.
 - d. Save the analysis by clicking on 'File' from the top menu bar and selecting 'Save project' from the drop-down menu.
5. Export Ct values as follows:
 - a. In the 'Monitoring' tab under 'Measurement (Ct)' select the 'All colors' tab below the plot
 - b. Right click on the table below the plot and save the data in one of the options provided ('Save table as Excel-File', 'Save table as Excel-File')
 - c. Open Excel and save the table as a CSV-File

- d. In the 'Save As' pop-up window define the 'File name:' for the data set
- e. Click 'Save'
- f. Close out the software.