For use under the Emergency Use Authorization (EUA) only

For in vitro diagnostic use

Rx Only

Lyra® Direct SARS-CoV-2 Assay Instructions for Use

For the qualitative detection of human coronavirus SARS-CoV-2 viral RNA extracted from nasal, nasopharyngeal and oropharyngeal direct swab specimens.

Contents

Intended Use

The Lyra® Direct SARS-CoV-2 Assay is a real-time RT-PCR assay intended for the qualitative detection of nucleic acid from SARS-CoV-2 in nasal (NS), nasopharyngeal (NP), or oropharyngeal (OP) direct swab specimens from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high complexity tests.

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

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

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

Summary and Explanation

SARS-CoV-2, also known as the COVID-19 virus, was first identified in Wuhan, Hubei Province, China December 2019. This virus, as with the novel coronavirus SARS-1 and MERS, is thought to have originated in bats, however the SARS-CoV-2 may have had an intermediary host such as pangolins, pigs or civets.[1] By the start of April 2020, human infection has spread to over 180 countries, infected over 846,000 people and has killed over 41,400 people.[1] On March 11, the WHO had declared the SARS-CoV-2 as a global pandemic.

The median incubation time is estimated to be 5.1 days with symptoms expected to be present within 12 days of infection.[2] The symptoms of COVID-19 are similar to other viral respiratory diseases and include fever, cough and shortness of breath.[3]

The Lyra Direct SARS-CoV-2 Assay has been designed to specifically detect SARS-CoV-2 RNA.

Principle of the Procedure

The Lyra Direct SARS-CoV-2 Assay detects SARS-CoV-2 viral RNA that has been extracted from a patient sample using a simple heat step. A multiplex real-time RT-PCR reaction is carried out under optimized conditions in a single tube generating amplicons for the targeted virus (if present) and the Process Control (PRC) present in the sample. This reaction is performed utilizing one of seven thermocyclers: Applied Biosystems 7500 Fast Dx, Applied Biosystems 7500 Standard, Roche LightCycler 480, Roche Cobas z480, Qiagen Rotor-Gene Q, Bio-Rad CFX96 Touch, Thermofisher QuantStudio 7 Pro.

Identification of the SARS-CoV-2 virus occurs by the use of target specific primers and fluorescent-labeled probes that hybridize to a conserved region of the non-structural polyprotein of the SARS-CoV-2 virus.

Table 1. Lyra® Direct SARS-CoV-2 Assay Probe Labels

Target	Dye
Non-structural polyprotein (pp1ab)	FAM
Process Control (PRC)	Quasar® 670 or Cy5

The following is a summary of the procedure:

1. **Sample Collection:** Obtain nasal, nasopharyngeal, or oropharyngeal swabs using standard techniques. These specimens are transported, stored, and processed according to instructions below.

2. **Nucleic Acid Extraction:** Extract nucleic acids by adding the swab specimen to 400-µL of the Process Buffer and heating at 95°C for 10-minutes. The Process Control (PRC) is in the Process Buffer and serves to monitor inhibitors in the extracted specimen and assures that adequate amplification has taken place.

3. **Rehydration of Master Mix:** Rehydrate the lyophilized Master Mix using 135µL of Rehydration Solution. The Master Mix contains oligonucleotide primers, fluorophore and quencher-labeled probes targeting conserved regions of the SARS-CoV-2 as well as the process control sequence. The probes are dual labeled with a reporter dye attached to the 5' end and a quencher attached to the 3' end. The rehydrated Master Mix is sufficient for eight reactions.

4. **Nucleic Acid Amplification and Detection:** Add 15 µL of the rehydrated Master Mix to each plate well (Applied Biosystems 7500 Fast Dx, Applied Biosystems 7500 Standard, Roche LightCycler 480, Roche Cobas Z480, Bio-Rad CFX96 Touch, Thermofisher QuantStudio 7 Pro) or tube (Qiagen Rotor-Gene Q). 5 µL of extracted nucleic acids (specimen with PRC) is then added to the plate well or tube. Place the plate or tube into the appropriate instrument.

   Once the reaction plate or tubes are added to the instrument, the assay protocol is initiated. This protocol initiates reverse transcription of the RNA targets generating complementary DNA, and the subsequent amplification of the target sequences occurs. The Lyra Direct SARS-CoV-2 Assay is based on TaqMan® chemistry, and uses an enzyme with reverse transcriptase, DNA polymerase, and 5'-3' exonuclease activities. During DNA amplification, this enzyme cleaves the probe bound to the complementary DNA sequence, separating the quencher dye from the reporter dye. This step generates an increase in fluorescent signal upon excitation by a light source of the appropriate wavelength. With each cycle, additional dye molecules are separated from their quenchers resulting in additional signal. If sufficient fluorescence is achieved, the sample is reported as positive for the detected target sequence.

Materials Provided

SKU # M124

Table 2. Detection Kit (96 Reactions) – Store at 2°C to 8°C		
#	**Component**	**Quantity**
❶	**Rehydration Solution** Part M5287	1 vial/kit 1.9 mL
❷	**Lyra Direct SARS-CoV-2 Master Mix** Part M5150 Lyophilized Contents: DNA polymerase enzyme with reverse transcriptase activity Oligonucleotide primer pairs; Oligonucleotide probes dNTPs (dATP, dCTP, dGTP, dUTP, dTTP) Stabilizers	12 vials/kit, 8 reactions/vial
❸	**Process Buffer** Part M5281	1 tube/kit 40 mL
CONTROL+	**Positive Control** containing synthetic SARS-CoV-2 RNA, Part M5274	1 vial/kit 1.0 mL
CONTROL−	**Negative Control** Part M5031	1 vial/kit 1.0 mL

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Materials Required But Not Provided

- Nasopharyngeal flocked swabs for collection of NP Specimens
- Nasal flocked or spun polyester swab for collection of NS Specimens
- Regular flocked or spun polyester swab for collection of OP Specimens
- Swab transport tube
- Micropipettors (range between 1 to 10 µL and 100 to 1000 µL)
- Non-aerosol filtered-barrier pipette tips
- Applied Biosystems® 7500Fast Dx, software version 1.4, or later
- Applied Biosystems® Standard, software version 2.0.6, or later
- Roche LightCycler® 480 Instrument II, software version 1.5.0.39, or later
- Roche Cobas z480 Instrument, software version 1.5.1.62 SP2-, or later
- Qiagen Rotor-Gene Q, software version 2.0.2.4, or later
- Bio-Rad CFX96 Touch, software version 3.1, or later
- Thermofisher QuantStudio 7 Pro, software version 2.4, or later
- 96 well PCR plate #:
  - Applied Biosystems®7500Fast Dx: 4346906
  - Applied Biosystems® Standard: N8010560
  - Roche LightCycler® 480 and Cobas z480: 04729692001, foil included
  - Bio-Rad CFX96 Touch: HSP9631, seals MSB1001
  - Thermofisher QuantStudio 7 Pro: 4483354

- Optical plate films
- Qiagen 72-Well Rotor (Cat No 9018903)
- Qiagen Locking Ring 72-Well Rotor (Cat no 9018904)
- Qiagen Strip Tubes and Caps, 0.1 ml (250) (Cat no 981103)

- Plate centrifuge for 96 well plate
- Dry heating block, capable of heating 1.5 mL tubes at 95°C±1° for 10 minutes
- 1.5 mL microcentrifuge tubes
- Dry heating block, capable of deep well microtiter plate at 95°C±1° for 10 minutes (Eppendorf ThermoMixer® C, with Deep Well insert Part Numbers 5382000023, 531000002)
- Deep Well Microtiter Plate (Eppendorf 951033103 or equivalent)

Warnings and Precautions

- For *In Vitro* Diagnostic Use under Emergency Use Authorization only.
- Positive results are indicative of the presence of SARS-CoV-2 RNA.
- Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.
- Performance characteristics of this test have been established with the specimen types listed in the **Intended Use Section** only. The performance of this assay with other specimen types or samples has not been evaluated.
- Use of specimens in transport media will adversely impact the sensitivity of the assay; they should not be used with the assay.
- The assay has been validated using Applied Biosystems 7500Fast Dx software version 1.4, or later. Please contact Quidel Technical Support prior to modifying or upgrading beyond this version of software.
- The assay has been validated using Applied Biosystems Standard software version 2.0.6, or later. Please contact Quidel Technical Support prior to modifying or upgrading beyond this version of software.
- The assay has been validated using Roche LightCycler® 480 Instrument II, software version 1.5.0.39, or later. Please contact Quidel Technical Support prior to modifying or upgrading beyond this version of software.
- The assay has been validated using Roche Cobas z480 Instrument, software version 1.5.1.62 SP2-, or later. Please contact Quidel Technical Support prior to modifying or upgrading beyond this version of software.
- The assay has been validated using Qiagen Rotor-Gene Q, software version 2.0.2.4, or later. Please contact Quidel Technical Support prior to modifying or upgrading beyond this version of software.
- The assay has been validated using Bio-Rad CFX96 Touch, software version 3.1, or later. Please contact Quidel Technical Support prior to modifying or upgrading beyond this version of software.
- The assay has been validated using Thermofisher QuantStudio 7 Pro, software version 2.4, or later. Please contact Quidel Technical Support prior to modifying or upgrading beyond this version of software.
- Use of this product should be limited to personnel with sufficient training in PCR and RT-PCR techniques.
- Treat all specimen/samples as potentially infectious. Follow universal precautions when handling samples, this kit and its contents.
- Proper sample collection, storage and transport are essential for correct results.
- Store assay reagents as indicated on their individual labels.
- Wear suitable protective clothing, gloves, eye and face protection when using this kit.
- For accurate results, pipette carefully using only calibrated equipment.
- Thoroughly clean and disinfect all surfaces with a 10% bleach solution followed by molecular grade water.
- Use micropipettes with an aerosol barrier or positive displacement tips for all procedures.
- Avoid microbial and cross contamination of the kit reagents. Follow Good Laboratory Procedures.
- Do not mix reagents from kits with different lot numbers.

- Do not use reagents from other manufacturers with this kit.
- Do not use product after its expiration date.
- Proper workflow planning is essential to minimize contamination risk. Always plan laboratory workflow in a uni-directional manner, beginning with pre-amplification and moving through amplification and detection.
- Use dedicated supplies and equipment in pre-amplification and amplification areas.
- Do not allow cross movement of personnel or equipment between areas.
- Keep amplification supplies separate from pre-amplification supplies at all times.
- Do not open sample tubes or unseal plates post amplification.
- Dispose of amplified material carefully and in accordance with local laws and regulations in order to minimize the risk of amplicon contamination.
- Do not use supplies dedicated for reagent or sample preparation for processing target nucleic acid.
- MSDS is available upon request or can be accessed on the product website.

Storage and Handling of Kit Reagents

- Store the unopened kit at 2°C to 8°C until the expiration date listed on the outer kit box.
- The rehydrated Master Mix should be used within 2 hours of rehydration and residual master mix may be stored at -20° C for up to 24-hours.

Indications of Instability or Deterioration of Reagents: Cloudiness of the Rehydration Solution, when within expiration, may indicate deterioration of this reagent. Contact Quidel Technical Assistance for a replacement.

Specimen Collection, Storage and Handling

Nasal, nasopharyngeal, or oropharyngeal swab specimens should be collected and placed in a clean, dry transport tube. Specimens should be transported and tested as soon as possible after collection. Specimens are stable for up to 24-hours at room temperature or up to 72-hours when stored at 2°C to 8°C. If specimens cannot be tested within 72 hours of collection, they should be frozen at -70°C or colder until tested.

Processed Specimen Storage

Specimens processed in Process Buffer may be stored at 2°C to 8°C, –20°C, or –70°C up to 7 days.

Assay Procedure

Run the following procedures at controlled room temperature of 20°C to 25°C.

Specimen Processing Procedure

1. 25 minutes prior to the heat lysis step, warm a heating block to 95°C.
2. Add 400-µL of process buffer to the required number of wells (2 for controls and 1 per patient) in a deep well microtiter plate or microcentrifuge tube.
3. Place the swab in the identified well or tube and vigorously twirl the swab for 10 seconds to elute specimen material. Roll the swab head against the inside of the well as you remove it. Dispose of the used swab in your biohazard waste.
4. Heat the plate or tubes at 95 ± 1°C for 10 minutes:

Note: Do not seal or cover the plate during the heat step.

a. For the deep well plate use a rotation setting of 300 rpm;
b. For tubes vortex for 5 seconds before and after the heat step

***Note**: Begin 10-minute lysis procedure after placing tubes in block and waiting until block returns to 95°C*

5. Remove processed samples from tube or plate heating block and allow to cool between room and refrigerated temperatures. This includes samples in a deep well microtiter plate or a microcentrifuge tube. The sample will appear cloudy.

***Note**: The lysed specimens may be stored at 2°C to 8°C, –20°C, or –70°C up to 7 days.*

Master Mix Rehydration Procedure

1. Determine the number of specimens extracted to be tested and obtain the correct number of eight-test lyophilized Master Mix vials for testing.
2. Return unused reagents to the appropriate storage conditions.
3. Open Master Mix carefully to avoid disruption of the pellet.
4. Add 135 µL of Rehydration Solution to the Master Mix.
5. Place vial at room temperature for 1 to 2 minutes to allow rehydration of pellet.
6. Gently pipette up and down 2 to 3 times avoiding the formation of bubbles prior to dispensing into the first plate well or tube.
   Note: The rehydrated Master Mix is sufficient for 8 reactions.
   Note: The rehydrated Master Mix may be stored at room temperature (20°C to 25°C) for up to 2 hours.

RT-PCR Set-up Procedure:

1. Add 15 µL of the rehydrated Master Mix to each plate well or tube.
2. For microcentrifuge tubes, vortex each tube for 10s just prior to addition to plate. Ensure that all of the precipitate has returned back into solution. Add 5 µL of processed specimen (specimen with the process control) into the PCR plate well or tube. Mixing of reagents is not required.
   Note: Use a new barrier micropipettor tip with each extracted specimen.
   Note: Vortex and 5 µL transfer of tube must be performed individually. Vortexing of tubes prior to transfer cannot be batched.
3. For a deep well microtiter plate, pipette each well up and down three times to mix. The pipette should be set to 150 µL. Immediately transfer 5 µL of processed specimen into the PCR plate or tube.
   Note: Use a new barrier micropipettor tip with each extracted specimen.
   Note: Mixing and 5 µL transfer of each well must be performed individually. Mixing of all wells in the plate prior to transfer cannot be batched.
4. Seal the plate or tubes.
5. Centrifuge the plate for a minimum of 15 seconds. Ensure that all liquid is at the bottom of the plate wells and there are no bubbles present.
   Note: Tubes used in the Qiagen Rotor-Gene Q do not require a centrifugation step before loading into instrument.
6. Turn on the appropriate thermocycler.
7. Insert plate or tubes into the appropriate thermocycler.

NOTE: Refer to Appendix for specific programming and testing protocols of each thermocycler.

Quality Control

The Lyra Direct SARS-CoV-2 Assay incorporates several controls to monitor assay performance.

1. The **Process Control (PRC)** consists of inactivated and stabilized MS2 Bacteriophage that contain an RNA genome and is included in the Process Buffer. The PRC serves to monitor inhibitors in the specimen and assures that adequate amplification has taken place.

2. The **Positive Control** (containing SARS-CoV-2 RNA, Part M5274) must be treated as a patient specimen and be included in every extraction and RT-PCR run. The positive control may be dipped by placing a dry nasopharyngeal swab into the control for ten seconds and then vigorously swirled for 10 seconds into aliquoted process buffer or 50 µL may be transferred to aliquoted process buffer.

3. The **Negative Control** (Part M5275) must be treated as a patient specimen and be included in every extraction and RT- PCR run. The negative control may be dipped by placing a dry nasopharyngeal swab into the control for ten seconds and then vigorously swirled for 10 seconds into aliquoted process buffer or 50 µL may be transferred to aliquoted process buffer

4. Failure of either the **Positive Control** or the **Negative Control** invalidates the RT-PCR run and results should not be reported. The RT-PCR run should be repeated with the extracted controls and specimens first. Re-extract and retest another aliquot of the controls and the specimens or obtain new samples and retest if the controls fail again.

Table 3. Expected Results from Controls (Applied Biosystems 7500 Fast Dx, Applied Biosystems 7500 Standard, Bio-Rad Cfx96, Qiagen Rotor-Gene Q, or Thermofisher QS-7)

Control Type/ Name	Used to Monitor	SARS-CoV-2	Expected Ct Values	PRC	Expected Ct Values
Positive Control	Substantial reagent failure including primer and probe integrity	+	5.0≤ Ct ≤30.0	+/-	NA[1]
Negative Control	Reagent and/or environmental contamination	-	None detected	+	5.0≤ Ct ≤30.0

[1]No Ct value is required for the Process Control to make a positive call.

Table 4. Expected Results from Controls (Roche LightCycler 480 and the Roche Cobas z480)

Control Type/ Name	Used to Monitor	SARS-CoV-2	Expected Ct Values	PRC	Expected Ct Values
Positive Control	Substantial reagent failure including primer and probe integrity	+	5.0≤ Ct ≤40.0	+/-	NA[1]
Negative Control	Reagent and/or environmental contamination	-	None detected	+	5.0≤ Ct ≤40.0

[1]No Ct value is required for the Process Control to make a positive call.

Interpretation of Results from Patient Specimens

Table 5. Interpretation of the Lyra Direct SARS-CoV-2 Assay Results on the Applied Biosystems 7500 Fast Dx, Applied Biosystems 7500 Standard, Bio-rad Cfx96, Qiagen Rotor-Gene Q, or Thermofisher QS7				
Assay Result	**Detector: SARS-CoV-2**	**Detector: Process Control**	**Interpretation of Results**	**Notes and Special Guidance**
Negative	No Ct detected	5.0≤ Ct ≤30.0	No SARS-CoV-2 viral RNA detected; PRC Detected.	
SARS-CoV-2 Positive	5.0≤ Ct ≤30.0	NA[1]	SARS-CoV-2 viral RNA detected.	
Invalid	No Ct detected	No Ct detected	No SARS-CoV-2 viral RNA and no PRC RNA detected.	Invalid test. Retest the same processed sample. If the test is also invalid, obtain a new specimen and retest.
Interpretation of the Lyra Direct SARS-CoV-2 Assay Results on the Roche LightCycler 480 and the Roche Cobas z480				
Assay Result	**Detector: SARS-CoV-2**	**Detector: Process Control**	**Interpretation of Results**	**Notes and Special Guidance**
Negative	No Ct detected	5.0≤ Ct ≤40.0	No SARS-CoV-2 viral RNA detected; PRC Detected.	
SARS-CoV-2 Positive	5.0≤ Ct ≤40.0	NA[1]	SARS-CoV-2 viral RNA detected.	
Invalid	No Ct detected	No Ct detected	No SARS-CoV-2 viral RNA and no PRC RNA detected.	Invalid test. Retest the same processed sample. If the test is also invalid, obtain a new specimen and retest.

[1] No Ct value is required for the Process Control to make a positive call.

Limitations

- Specimens in transport media may not be used in this assay.
- Negative results do not preclude infection with SARS-CoV-2 and should not be the sole basis of a patient treatment decision.

- Negative results should be treated as presumptive and confirmed with an FDA authorized molecular assay that utilizes a chemical lysis step followed by solid phase extraction of nucleic acid, if necessary, for clinical management.
- The performance of this test was assessed using nasopharyngeal and oropharyngeal swab specimens. Nasal swabs and mid-turbinate nasal swabs (self-collected under supervision of or collected by a healthcare provider) are also considered acceptable specimen types for use with the Lyra Direct SARS-CoV-2 Assay.
- Improper collection, storage or transport of specimens may lead to false negative results.
- Inhibitors present in the sample and/or errors in following the assay procedure may lead to false negative results.
- A trained health care professional should interpret assay results in conjunction with the patient's medical history, clinical signs and symptoms, and the results of other diagnostic tests.
- Analyte targets (viral sequences) may persist *in vivo*, independent of virus viability. Detection of analyte target(s) does not imply that the corresponding virus(es) are infectious, nor that they are the causative agents for clinical symptoms.
- There is a risk of false positive values resulting from cross-contamination by target organisms, their nucleic acids or amplified product, or from non-specific signals in the assay.
- There is a risk of false negative values due to the presence of sequence variants in the viral targets of the assay.
- The assay performance was not established in immunocompromised patients.

Conditions of Authorization for the Labs

The Lyra Direct SARS-CoV-2 Assay Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients, and authorized labeling are available on the FDA website: https://www.fda.gov/medical-devices/emergency-situations-medical-devices/emergency-use-authorizations#covid19ivd.

However, to assist clinical laboratories using the Lyra Direct SARS-CoV-2 Assay, the relevant Conditions of Authorization are listed below.

- Authorized laboratories[1] using the Lyra Direct SARS-CoV-2 Assay will include with result reports of the Lyra Direct SARS-CoV-2 Assay test, all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.

- Authorized laboratories using the Lyra Direct SARS-CoV-2 Assay will perform the Lyra Direct SARS-CoV-2 Assay as outlined in the Lyra Direct SARS-CoV-2 Assay Instructions for Use. Deviations from the authorized procedures, including the authorized instruments, authorized extraction methods, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to perform the Lyra Direct SARS-CoV-2 Assay are not permitted.

- Authorized laboratories that receive the Lyra Direct SARS-CoV-2 Assay must notify the relevant public health authorities of their intent to run the test prior to initiating testing.
- Authorized laboratories using the Lyra Direct SARS-CoV-2 Assay will have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.
- Authorized laboratories will collect information on the performance of the test and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and Quidel (QDL.COV2.test.event.report@quidel.com) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of the test of which they become aware.
- All laboratory personnel using the test must be appropriately trained in RT-PCR techniques and use appropriate laboratory and personal protective equipment when handling this kit, and use the test in accordance with the authorized labeling.
- Quidel, its authorized distributor(s) and authorized laboratories using the Lyra Direct SARS-CoV-2 Assay will ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records will be made available to FDA for inspection upon request.

[1] For ease of reference, the letter of authorization refers to, "United States (U. S.) laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high complexity tests" as "authorized laboratories."

Clinical Performance

The clinical performance of the Lyra Direct SARS-CoV-2 Assay was evaluated using two fully contrived positive specimen studies using nasopharyngeal swab and oropharyngeal specimens.

Study 1

Thirty positive NP contrived samples were created by spiking thirty individual clinical samples determined to be negative for SARS-CoV-2 by the Lyra Direct SARS-CoV-2 Assay. The spiked samples were added to the swabs (approximately 50-µL) and then processed and tested according to the Lyra Direct SARS-CoV-2 Assay package insert. Twenty specimens were spiked with 1x LoD (3.40e+4 cp/mL) of virus. Ten additional specimens were spiked with 5x LoD (1.7E+5 cp/mL) of virus.

Twenty-nine of thirty contrived samples were positive in the Lyra Direct SARS-CoV-2 Assay. The results for the contrived positive specimens are shown in the table below:

Table 6. Clinical evaluation in spiked nasopharyngeal swab specimens

Sample RNA Concentration	# Positives/# Tested	Mean SARS-CoV-2 Ct	%CV
unspiked	0/30	NA	NA
1x LoD	19/20	27.06	6.6
5x LoD	10/10	23.51	4.7

Performance against the expected results are:
Positive Percent Agreement 29/30 = 97% (95% CI: 83.3%-99.4%)
Negative Percent Agreement 30/30 = 100% (95% CI: 88.6%-100%)

Study 2

Fifteen positive OP contrived samples were created by spiking fifteen individual clinical samples determined to be negative for SARS-CoV-2 by the Lyra SARS-CoV-2 Assay. The spiked samples were added to the swabs (approximately 50-µL) and then processed and tested according to the Lyra Direct SARS-CoV-2 Assay package insert. Seven specimens were spiked with 1x LoD (3.40e+4 cp/mL), four specimens were spiked with 10x LoD (3.4e+5cp/mL), and four specimens were spiked with 100x LoD (3.4e+6cp/mL) of virus. Eight additional negative OP samples, by the Lyra SARS-CoV-2 Assay, were tested according to the Lyra Direct SARS-CoV-2 Assay package insert.

Fifteen of fifteen contrived samples were positive in the Lyra Direct SARS-CoV-2 Assay. The results for the OP specimens are shown in the table below:

Table 7. Clinical evaluation in spiked OP swab specimens

Sample RNA Concentration	# Positives/# Tested	Mean SARS-CoV-2 Ct	%CV
unspiked	0/8	NA	NA
1x LoD	7/7	23.2	5.1
10x LoD	4/4	20.1	0.6
100x LoD	4/4	17.1	0.5

Performance against the expected results are:
Positive Percent Agreement 15/15 = 100% (95% CI: 79.6%-100%)
Negative Percent Agreement 8/8 = 100% (95% CI: 67.6%-100%)

ANALYTICAL PERFORMANCE

Level of Detection

Study 1
The Limit of Detection of the Lyra Direct SARS-CoV-2 Assay utilized limiting dilutions of gamma-irradiated SARS-Related Coronavirus 2 (SARS-CoV-2) spiked into negative nasopharyngeal matrix in buffer. Each dilution was added to the swabs (approximately 50-µL) and then processed according to the Assay's package insert and tested on Applied Biosystems 7500 Fast Dx, Applied Biosystems 7500 Standard, Roche LightCycler 480, Roche Cobas z480, Qiagen Rotor-Gene Q, Bio-Rad CFX96 Touch, or Thermofisher QuantStudio 7 Pro. Analytical sensitivity (LoD) is defined as the lowest concentration at which at least 95% of all replicates tested positive.

This study established the LoD for the Lyra Direct SARS-CoV-2 Assay as noted below, subsequently confirmed by testing 20 replicates.

Table 8. LoD Confirmation Results testing was performed on Applied Biosystems 7500 Fast Dx, Applied Biosystems 7500 Standard, Roche LightCycler 480 and Cobas z480, Qiagen Rotor-Gene Q, Bio-Rad CFX96 Touch, or Thermofisher QuantStudio 7 Pro

	NP Swabs - 7500 Fast Dx	NP Swabs - 7500 Standard	NP Swabs - LC-480[2]	NP Swabs - Cobas z480[2]	NP Swabs - Bio-Rad	NP Swabs - Rotor-Gene	NP Swabs - QS7
Concentration[1]	3.40E+04	3.40E+04	3.40E+04	3.40E+04	3.40E+04	3.40E+04	6.80E+04
COVID-19							
Overall AVE	25.85	28.48	33.87	33.55	25.10	26.89	26.47
Overall STDEV	1.25	0.95	1.55	1.19	1.06	1.24	0.77
Overall %CV	4.8%	3.3%	4.6%	3.6%	4.2%	4.6%	2.9%
Detection	20/20	20/20	20/20	20/20	20/20	20/20	20/20
PRC							
Overall AVE	21.69	20.03	21.96	23.98	17.85	19.79	23.20
Overall STDEV	0.68	0.20	1.13	1.07	1.29	0.33	0.75
Overall %CV	3.1%	1.0%	5.1%	4.5%	7.2%	1.6%	3.2%
Detection	20/20	20/20	20/20	20/20	20/20	20/20	20/20

[1] Concentration is presented in RNA copies/mL

[2] Results include 10 cycles not captured by the other instruments

Study 2 – Comparative LoD Study for the Lyra Direct SARS-CoV-2 Assay and the Lyra SARS-CoV-2 Assay

A second LoD study was performed to compare the limit of detection (LoD) of the Lyra Direct SARS-CoV-2 Assay and the Lyra SARS-CoV-2 Assay on the ABI 7500 Fast Dx using limiting dilutions of gamma-irradiated SARS-CoV-2 virus. In this study a 1x LoD (based on preliminary testing) concentration of the virus in negative NP Matrix were inoculated onto NP swab. Twenty (20) replicates of the inoculated swabs were tested directly according to the PI for the Lyra Direct SARS-CoV-2 Assay or were added to 3.0-mL of UTM for testing in the Lyra SARS-CoV-2 Assay (Forty total swab replicates). The testing was performed using the ABI 7500 Fast Dx.

LoD Confirmation Study Results

Lyra Extracted Assay	1.28E+04 cp/mL	
Replicate	SARS-CoV-2	PRC
1	26.03	18.62
2	24.02	18.41
3	23.58	18.59
4	24.00	18.48
5	23.70	18.63
6	25.27	18.71
7	24.70	19.13
8	24.42	19.19
9	23.99	19.26
10	26.63	19.21
11	25.29	19.65
12	24.73	19.84
13	25.28	19.56
14	25.01	19.56
15	25.66	19.44
16	26.34	19.57
17	26.23	19.29
18	24.12	19.43
19	25.30	19.24
20	24.40	19.47
% Detected	100%	100%

Lyra Extracted Assay	1.28E+04 cp/mL	
Replicate	SARS-CoV-2	PRC
Average of Pos	24.93	19.16
STDEV of Pos	0.92	0.44
%CV	3.7%	2.3%

Lyra Direct Assay	1.28E+04 cp/mL	
Replicate	SARS-CoV-2	PRC
1	24.93	18.37
2	24.02	17.71
3	27.80	18.21
4	24.62	17.75
5	24.75	18.07
6	23.67	18.03
7	23.32	17.83
8	22.53	17.64
9	24.15	17.91
10	22.93	18.47
11	24.07	17.59
12	24.38	18.45
13	25.80	18.01
14	24.99	17.87
15	23.11	17.67
16	24.52	18.42
17	24.72	18.35
18	24.26	18.30
19	24.70	18.45
20	26.59	18.31
% Detected	100%	100%
Average of Pos	24.49	18.07
STDEV of Pos	1.23	0.31
%CV	5.0%	1.7%

Based on this study design, the LoD for the 2 versions of the Lyra Assay (Lyra SARS-CoV-2 Assay and Lyra Direct SARS-CoV-2) have an input LoD of 1.28×10^4 genome equivalents/mL. It should be noted that the published LoD for the Lyra SARS-CoV-2 Assay (8.00E-01 genomic RNA copies/µL) is accurate. The final concentration of virus tested in the assay, after dilution in 3.0 mL of UTM and concentration during the extraction process, is approximately 800 cp/mL.

Analytical Reactivity (Inclusivity)

The inclusivity of the Lyra Direct SARS-CoV-2 Assay was established by testing gamma-irradiated SARS-related coronavirus 2 (SARS-CoV-2), isolate USA-WA1/2020, and via *in-silico* analysis. The *in-silico* analysis demonstrated the Lyra Direct SARS-CoV-2 primers are >95% conserved to 998 and 11,708 SARS-CoV-2 sequences available from NCBI and GISAID, respectively, as of April 24, 2020.

Analytical Specificity (Cross-Reactivity)

The Analytical Specificity of the assay was established for the Lyra SARS-CoV-2 Assay by both direct testing of organisms in the assay ("wet" testing) and *in silico* analysis. The wet testing used 25 micro-organisms, in high concentrations, identified by the FDA as high priority for evaluation due to the reasonable likelihood they may be present in upper respiratory samples. All micro-organisms were undetectable with the Lyra SARS-CoV-2 Assay when wet tested as shown below. NOTE: the primers and probes used in the Lyra Direct SARS-CoV-2 are the same as in the Lyra SARS-CoV-2 Assay.

Table 9. Cross-reactivity test results

Virus/Bacteria/Parasite	Strain	Source/ Sample type	Concentration	Results
Adenovirus	Type 1	Isolate	$1 \times 10^{7.53}$ U/mL	Neg, Neg, Neg
Coronavirus	229e	Isolate	$1 \times 10^{6.10}$ U/mL	Neg, Neg, Neg
Coronavirus	OC43	Isolate	9.55×10^{6} $TCID_{50}$/mL	Neg, Neg, Neg
Coronavirus	NL63	Isolate	$1 \times 10^{4.67}$ U/mL	Neg, Neg, Neg
MERS-CoV (heat-inactivated)	Florida/USA-2_Saudia Arabia_2014	Isolate	4.17×10^{5} $TCID_{50}$/mL	Neg, Neg, Neg
SARS -1	2003-00592	Inactivated virus	Not available	Neg, Neg, Neg
Mycoplasma pneumoniae	M129	Isolate	3×10^{7} CCU/mL	Neg, Neg, Neg
Streptococcus pyogenes	Z018	Isolate	3.8×10^{9} cfu/mL	Neg, Neg, Neg
Influenza A H3N2	Brisbane/10/07	Isolate	$1 \times 10^{5.07}$ U/mL	Neg, Neg, Neg
Influenza A H1N1	New Caledonia/20/99	Isolate	$1 \times 10^{6.66}$ U/mL	Neg, Neg, Neg
Influenza B	Brisbane/33/08	Isolate	$1 \times 10^{5.15}$ U/mL	Neg, Neg, Neg
Parainfluenza	Type 1	Isolate	$1 \times 10^{8.01}$ U/mL	Neg, Neg, Neg
Parainfluenza	Type 2	Isolate	$1 \times 10^{6.34}$ U/mL	Neg, Neg, Neg
Parainfluenza	Type 3	Isolate	8.51 x 107 TCID50/mL	Neg, Neg, Neg

Table 9. Cross-reactivity test results				
Virus/Bacteria/Parasite	**Strain**	**Source/ Sample type**	**Concentration**	**Results**
Parainfluenza	Type 4b	Isolate	1 x $10^{7.53}$ U/mL	Neg, Neg, Neg
Enterovirus	Type 68	Isolate	1 x $10^{6.5}$ U/mL	Neg, Neg, Neg
Human Metapneumovirus	A1 (IA10-s003)	Isolate	1 x $10^{5.55}$ U/mL	Neg, Neg, Neg
Respiratory Syncytial Virus	Type A (3/2015 Isolate #3)	Isolate	1 x $10^{5.62}$ U/mL	Neg, Neg, Neg
Human Rhinovirus	N/A	Inactivated virus	Not available	Neg, Neg, Neg
Chlamydophila pneumoniae	AR-39	Isolate	2.9 x 107 IFU/mL	Neg, Neg, Neg
Haemophilus influenzae	Type b; Eagan	Isolate	7.87 x 10^{8} cfu/mL	Neg, Neg, Neg
Legionella pneumophila	Philadelphia	Isolate	6.82 x 10^{9} cfu/mL	Neg, Neg, Neg
Streptococcus pneumoniae	Z022; 19f	Isolate	2.26 x 10^{9} cfu/mL	Neg, Neg, Neg
Bordetella pertussis		Isolate		Neg, Neg, Neg
Pneumocystis jirovecii-S. cerevisiae Recombinant	W303-Pji	Isolate	1.56 x 10^{8} cfu/mL	Neg, Neg, Neg

The *in silico* analysis focused on 32 micro-organisms identified by the FDA as high priority for assessment due to their potential presence in upper respiratory samples.

Table 10. Cross-Reactivity Organisms			
Organism	**Total # Sequences**	**# Complete Genomes**	**# WGS Strains**
Adenovirus	532	532	0
Coronavirus (Seasonal)	288	288	0
Enterovirus [B]	2708	2674	34
Influenza A Virus [A B]	172455	21444 (+39 A/Mexico/4108/2009)	108
Influenza B Virus [A B]	53952	6755 (+16 B/Florida/4/2006)	0
Influenza C Virus [B]	2205	N/A	N/A
Human Metapneumovirus	145	145	0
Human Parainfluenza Virus 1-4	439	439	0
Human Parechovirus	124	124	0
Human Respiratory Syncytial Virus [B]	1275	1275	0
Rhinovirus	214	214	0
SARS-1	236 [C]	232 (+4 pp1ab sequences)	0
Bacillus anthracis	4152	69	86
Candida albicans	1541	59	34
Chlamydia pneumoniae	466	5	20
Chlamydia psittaci	11179	23	45

Table 10. Cross-Reactivity Organisms			
Organism	**Total # Sequences**	**# Complete Genomes**	**# WGS Strains**
Corynebacterium diptheriae	20797	17	194
Coxiella burnetii	419	28	3
Haemophilus influenzae	45267	61	692
Legionella [B]	4843	98	65
Leptospira [B]	64456	133	266
Moraxella catarrhalis [B]	8333	11	184
Mycobacterium tuberculosis	194	194	0
Mycoplasma pneumoniae	808	51	45
Neisseria elongata & *N. meningitidis* [B]	312050	116	1318
Pneumocystis jirovecii	487	15	3
Pseudomonas aeruginosa	195	195	0
Staphylococcus aureus	634	634	0
Staphylococcus epidermidis [B]	61880	23	508
Streptococcus pneumoniae [B]	1633369	107	8526
Streptococcus pyogenes [B]	46153	201	1733
Streptococcus salivarius [B]	9417	18	48
[A] Genome counts for Influenza A and Influenza B were attained for strains that included all 8 segments, except for A/Mexico/4108/2009(H1N1) and B/Florida/4/2006; all available gene sequences were included.			
[B] For BLAST, 'Max Target Seqs' was set to 5000.			
[C] 4 polyprotein cds sequences were also included.			

The *in-silico* analysis demonstrated < 80% homology with all organisms except for the following: three Enterovirus sequences are 80.9% conserved to the reverse primer, however, the forward primer is only 76% conserved and the probe alignment had an overall homology of 56%. The SARS-1 sequences are ≥80% conserved to both primers, however, the last base on the 3' ends of both primers are not conserved. The wet testing of the only available SARS-1 strain using the Lyra SARS-CoV-2 assay was non-detectable.

Interfering Substances

A study was performed to demonstrate that potentially interfering substances that may be found in the upper respiratory tract do not cross-react or interfere with the detection of SARS-CoV-2 RNA in the Lyra Direct SARS-CoV-2 Assay.

Fourteen (14) potential interfering substances in the concentration listed below were tested in the absence or presence of SARS-CoV-2.

Table 11. List of Substances for Interfering Study		
Substances	**Active Ingredient**	**Concentration Tested**
Afrin – nasal spray	Oxymetazoline	5%
Blood (human)	Blood	5%
Chloraseptic, Cepacol	Benzocaine, Menthol	0.7 g/mL
Flonase	Fluticasone	5%
Halls Relief Cherry Flavor	Menthol	0.8 g/mL
Nasocort Allergy 24 hour	Triamcinolone	5.%
Neo-Synephrine	Phenylephrine hydrochloride	5%
Oseltamivir	Oseltamivir	2.2 µg/mL
Purified mucin protein	Mucin protein	2.5 mg/mL
Rhinocort	Budesonide (Glucocorticoid)	5%
Saline nasal spray	Saline	15%
Tobramycin	Tobramycin	1.25 mg/mL
Zanamivir	Zanamivir	282.0 ng/mL
Zicam Cold Remedy	Galphimia glauca, Luffa operculata, Sabadilla	5%

None of the fourteen (14) potential interfering substances tested in the study demonstrated cross-reactivity or interference.

Customer and Technical Assistance

To place an order or for technical support, please contact a Quidel Representative at (800) 874-1517 (toll-free in the U.S.) or (858) 552-1100 (outside of U.S.), Monday through Friday, between 8:00 a.m. and 5:00 p.m., Eastern Time. Orders may also be placed by fax at (740) 592-9820. For e-mail support contact: customer service@dhiusa.com or technical_services@dhiusa.com. For services outside the U.S., please contact your local distributor. Additional information about Quidel, our products, and our distributors can be found on our website quidel.com.

References

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2. Clinical and Laboratory Standards Institute. Viral Culture; Approved Guidelines. CLSI document M41-A [ISBN 1562386239] Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898, USA 2006.
3. Lauer, S.A., et. al. The incubation period of Coronavirus disease 2019 (COVID-19) from publicly reported confirmed cases: estimation and application, Ann Intern Med. 2020
4. www.cdc.gov/coronavirus/2019-ncov/about/symptoms.html

APPENDIX

Applied Biosystems 7500 Fast Dx Programming Instructions

Refer to User Manual Part Number 4406991 for additional information.

1. Launch the 7500 Fast Dx software package.
2. The **Quick Startup document** dialog window will open. Select the **Create New Document** button to start the **New Document Wizard**. Follow each step to initiate the Lyra Direct SARS-CoV-2 Assay protocol.
    a. Define Document: Most of the following should be the default setting. If not, change accordingly.
        i. Confirm or enter the following information.

Assay:	Standard Curve (Absolute Quantitation)
Container:	96-Well Clear
Template:	Blank Document
Run Mode:	Fast 7500
Operator:	*your operator name*
Comments:	SDS v1.4
Plate Name:	'Lyra Direct SARS-CoV-2 Assay'

        ii. Select the **Next** button.

    b. Select Detectors: New detectors for SARS-CoV-2 and the process control (PRC) must be added. For each target, select the **New Detector** button to open the **New Detector** pop-up window. Alternatively, use the **Create Another** button from within the **New Detector** pop-up window for the last two detectors.

        i. Enter the following information for each detector.

Name	Reporter Dye	Quencher Dye	Color
SARS-CoV-2	FAM	(none)	(Select)
PRC	Cy5	(none)	(Select)

        ii. Select a unique color to represent each detector.
        iii. Highlight the new detectors and add to the **Detectors in Document** column using the **Add** button.
        iv. Select **(none)** from the **Passive Reference** drop-down menu.
        v. Select the **Next** button.
        vi. Select the **Finish** button without setting any wells.
    c. The wizard will close and the software will open, starting with the **Setup** tab. This will show the sample plate that was set up during the quick start. For the initial set up, nothing needs to be changed here.
    d. Defining the Thermocycler Protocol: Select the **Instrument** tab to set up the Lyra™ Direct SARS-CoV-2 Assay RT-PCR cycling times and temperatures. Under **Thermal Profile** there should be a default 2-stage protocol. Each stage will have 3 user-editable text boxes. The top box value represents the number of reps or cycles for that stage. The middle box value

represents the temperature (˚C) and the lowest box value represents the time (minutes: seconds).

i. Make the following changes to the default **Thermal Cycler Protocol**:
   1. Stage 1
      a. Reps: 1
      b. Temp: 55
      c. Time: 5:00
   2. Select the bar between Stage 1 and Stage 2. Select the **Add Hold** button to add another stage.
   3. Stage 2
      a. Reps: 1
      b. Temp: 60
      c. Time: 5:00
   4. Select the bar between Stage 2 and Stage 3. Select the **Add Hold** button to add another stage.
   5. Stage 3
      a. Reps: 1
      b. Temp: 65
      c. Time: 5:00
   6. Stage 4 (2-Step Dissociation Stage)
      a. Reps: 10
      b. Step 1
         i. Temp: 92
         ii. Time: 0:05
      c. Step 2
         i. Temp: 57
         ii. Time: 0:40
   7. Select the bar to the right of Stage 4. Select the **Add Cycle** button to add another stage.
   8. Stage 5 (2-Step Dissociation Stage)
      a. Reps: 30
      b. Step 1
         i. Temp: 92
         ii. Time: 0:05
      c. Step 2
         i. Temp: 57
         ii. Time: 0:40
   9. If a wrong stage is added the stage can be removed by pressing the **Delete** button after highlighting the stage between the vertical lines

ii. Under **Settings** enter the following:

Sample Volume (µL):	20 (default)
Run Mode:	7500 Fast (default)

Data Collection:	Stage 5, Step 2(57.0 @ 0:40)
NOTE: Do not check the check box next to 'Expert Mode'.	

e. Set threshold for each analyte.
   i. Select the **Results** tab.
   ii. Select the **Amplification Plot** tab.
   iii. Select SARS-CoV-2 from the Detector tab in the top right corner.
   iv. In the **Analysis Settings** block, set the **Threshold** to **7.5e+004**.
   v. Select the **Manual Baseline** radio button.
   vi. Enter "3" for Start and "15" for End.
   vii. Select PRC from the Detector tab in the top right corner.
   viii. In the **Analysis Settings** block, set the **Threshold** to **1.0e+004**.
   ix. Select the **Manual Baseline** radio button.
   x. Enter "3" for Start and "15" for End.

f. Save the new protocol as a template for future use.
   i. At the top of the screen select **File** and then **Save As.**
   ii. **Save In:** D:\Applied Biosystems\7500 Fast System\Templates\
   iii. **File name:** 'Lyra Direct SARS-CoV-2'
   iv. **Save as type:** 'SDS Templates (*.sdt)'

g. Exit the software.

Applied Biosystems® 7500 Fast Dx Thermocycler Test Procedure

1. Launch the Applied Biosystems® 7500 Fast Dx software v1.4 package.
2. The **Quick Startup document** dialog window will open.
3. Click on **Create a new document.**
4. Most of the following should be the default setting. If not, change accordingly.

Assay:	Standard Curve (Absolute Quantitation)
Container:	96-Well Clear
Template:	Lyra Direct SARS-CoV-2
Run Mode:	Fast 7500
Operator:	*your operator name*
Comments:	SDS v1.4
Plate Name:	**YYMMDD-** Lyra Direct SARS-CoV-2

5. Set Up Sample Plate

a. Under the **Setup** and **Plate** tabs the plate setup will appear.

b. Select all wells that will contain sample, right-click and select the **Well Inspector** from the drop-down menu. When the **Well Inspector** pop-up window opens, select the detectors for SARS-CoV-2 and PRC.

c. Use the **Well Inspector** to enter the sample names. Patient IDs can be entered in the Well Inspector window. However, it is recommended that this is done prior to re-suspending the

lyophilized master mix, post run or using the import function to minimize the time the PCR reactions will sit at room temperature prior to starting the run.

d. Save the run as **YYMMDD- Lyra Direct SARS-CoV-2.sds**.

e. A window will open asking for the "Reason for change of entry". Enter "**Setup**" and any other comments relevant to the run.

6. Starting the PCR

a. Select the **Instrument** tab.

b. Insert the 96 well PCR plate into the machine.

c. Under **Instrument Control**, select the **Start** button to initiate the run.

7. Post PCR

IMPORTANT: When the run is finished press OK.

a. Analyze the data by pressing the **"Analyze"** button in the top menu and save the file.

b. Save the file by pressing **Save Document** in the task bar. A window will open asking for the "Reason for change of entry".

c. Enter "**Data analysis post run**" and any other comments relevant to the run.

Applied Biosystems 7500 Standard Programming Instructions

Refer to User Manual Part Number 4387783 rev C for additional information.

1. Launch the ABI 7500 software package.

2. Select the **Advanced Setup** button to open Setup and Experiment Properties. Follow each step to initiate the Lyra Direct SARS-CoV-2 protocol.

a. Experiment Name: Enter Experiment Name as SARS-CoV-2. Leave the Barcode, User Name, and Comments fields blank

b. Define Experiment Setup: Select 7500 (96 Wells), Quantitation- Standard Curve, TaqMan® Reagents, and Standard (~2 hours to complete a run)

3. In the upper left menu select **Plate Setup**

a. Define Targets: New detectors for SARS-CoV-2, and the process control (PRC) must be added.

i. Enter the following information for each detector.

Name	Reporter Dye	Quencher Dye	Color
SARS-CoV-2	FAM	(none)	(Select)
PRC	Cy5	(none)	(Select)

ii. Select **Add New Target** button for each target.

iii. From each drop down menu select reporter, quencher, and color

iv. Select a unique color to represent each detector

b. Assign Targets and Samples: Under this tab in the bottom left corner, select **none** as the Passive Reference.

4. Select **Run Method** from the upper left menu

a. Set the **Reaction Volume** per Well to 20 µL under the **Graphical** or **Tabular View**

b. Define the Thermocycler Protocol: Under the **Graphical** or **Tabular View** the default profile should be 2 holding stages and a 2-step cycling protocol. Each stage will have 3 user-editable text boxes. The first box value represents the Ramp Rate (%) for that stage, the second box value represents the temperature (°C) and the third box value represents the time (minutes:seconds).

i. Make the following changes to the default Thermocycler protocol:

1. Stage 1 First **Holding Stage**
    a. Ramp Rate: 100%
    b. Temp: 55
    c. Time: 5:00
2. Step 1 Second **Holding Stage**.
    a. Ramp Rate: 100%
    b. Temp: 60
    c. Time: 5:00
3. Highlight the second **Holding Stage** and select the **Add Stage** button. In the drop down menu select **Holding**
4. Step 1 **Third Holding Stage**
    a. Ramp Rate: 100%
    b. Temp: 65
    c. Time: 5:00
5. First **2-Step Cycling Stage**
    a. Number of cycles: 10
    b. Do NOT check Enable Auto Delta
    c. Step 1
        i. Ramp Rate: 100%
        ii. Temp: 92
        iii. Time: 0:05
    d. Step 2
        i. Ramp Rate: 100%
        ii. Temp: 57
        iii. Time: 0:40
        iv. Turn data collection "Off" by selecting the **Data Selection** button at the bottom of the step.
6. Highlight step 2 and select the **Add Stage** button. In the drop down menu select **Cycling**
7. Second 2-Step **Cycling Stage**
    a. Number of cycles: 30
    b. Do NOT check Enable Auto Delta
    c. Step 1
        i. Ramp Rate: 100%
        ii. Temp: 92
        iii. Time: 0:05
    d. Step 2
        i. Ramp Rate: 100%
        ii. Temp: 57
        iii. Time: 0:40
        iv. Ensure the data collection has been turned "On" for this step (default setting)
8. If a wrong stage is added the stage can be removed by pressing the **Undo "Add Stage"** button immediately after adding the stage or highlight the stage between the vertical lines and select the **Delete Selected** button

5. Set threshold for each analyte

a. Select the **Analysis** tab in the upper left menu.

b. Select **Analysis Settings** button in the top right corner.

c. Highlight SARS-CoV-2 and deselect the **Use Default Settings** box. De-select **Automatic Threshold** and change threshold to 75,000. Deselect **Automatic Baseline**. Enter 3 for **Baseline Start Cycle** and enter 15 for **End Cycle** by clicking the 'Analysis Settings' button in the top right corner.

d. Highlight PRC and de-select the **Use Default Settings** box. De-select **Automatic Threshold** and change threshold to 10,000. Deselect **Automatic Baseline**. Enter 3 for **Baseline Start Cycle** and enter 15 for **End Cycle** by clicking the 'Analysis Settings' button in the top right corner..

e. At the bottom of the box select **Apply Analysis Settings** button

Target	Threshold	Baseline Start	Baseline End
SARS-CoV-2	75,000	3	15
PRC	10,000	3	15

i. Save the new protocol as a template for future use.

i. At the top of the screen select the drop down menu next to **Save**

ii. Choose **Save as Template**

iii. Save in an appropriate folder

iv. **File name:** 'Lyra Direct SARS-CoV-2'

v. **Save as type: '**Experiment Document Template files (*.edt)'

vi. Exit the software.

Applied Biosystems® 7500 Standard Thermocycler Test Procedure

1. Launch the Applied Biosystems® 7500 Standard software v2.06 package.
2. The **Quick Startup document** dialog window will open.
3. Click on **Create a new document.**
4. Most of the following should be the default setting. If not, change accordingly.

Assay:	Standard Curve (Absolute Quantitation)
Container:	96-Well Clear
Template:	Lyra Direct SARS-CoV-2
Run Mode:	7500 Standard
Operator:	*your operator name*
Comments:	SDS v1.4
Plate Name:	**YYMMDD-** Lyra Direct SARS-CoV-2

5. Set Up Sample Plate
   a. Under the **Setup** and **Plate** tabs the plate setup will appear.
   b. Select all wells that will contain sample, right-click and select the **Well Inspector** from the drop-down menu. When the **Well Inspector** pop-up window opens, select the detectors for SARS-CoV-2 and PRC.
   c. Use the **Well Inspector** to enter the sample names. Patient IDs can be entered in the Well Inspector window. However, it is recommended that this is done prior to re-suspending the

lyophilized master mix, post run or using the import function to minimize the time the PCR reactions will sit at room temperature prior to starting the run.

d. Save the run as **YYMMDD- Lyra Direct SARS-CoV-2.sds**.

e. A window will open asking for the "Reason for change of entry". Enter "**Setup**" and any other comments relevant to the run.

6. Starting the PCR
   a. Select the **Instrument** tab.
   b. Insert the 96 well PCR plate into the machine.
   c. Under **Instrument Control**, select the **Start** button to initiate the run.
7. Post PCR

   IMPORTANT: When the run is finished press OK.

   a. Analyze the data by pressing the **"Analyze"** button in the top menu and save the file.
   b. Save the file by pressing **Save Document** in the task bar. A window will open asking for the "Reason for change of entry".
   c. Enter "**Data analysis post run**" and any other comments relevant to the run.

Bio-Rad CFX96 Touch Thermocycler Programming Procedure

Refer to User Manual Part Number 10010424 Rev D for additional information.

Programming Instructions:

1. Launch the CFX96 Touch software package
2. In the **Startup Wizard** pop-up window **Select instrument** to be **CFX96** from the drop down menu
3. Under **Select Run Type** press the **User-defined** button
4. Create a new thermocycler protocol by selecting **Create New** from the **Run Setup** window
5. Make the following changes to the cycling conditions in the **Protocol Editor:**
   a. Change the **Sample Volume** to **20ul**
   b. Under **Tools** in the top left toolbar select **Run Time Calculator** and check **96 Wells-All Channels**
   c. **Step 1** (Hold)
      i. Reps: 1
      ii. Temp: 55C
      iii. Time: 5:00
   d. **Step 2** (Hold)
      i. Reps: 1
      ii. Temp: 60C
      iii. Time: 5:00
   e. **Step 3** (Hold)
      i. Reps: 1
      ii. Temp: 65C
      iii. Time: 5:00
      iv. Remove the plate read from this stage by selecting the **Remove Plate Read** button on the lower left
   f. **Step 4** (2-Step Amplification Stage)

i. Highlight **step 3** and go to the lower left of the window and select **Insert Step** for a total of 2 times until step 5 is reached (ensure in the upper left of the window the drop-down menu for **Insert Step** has **After** selected).
ii. Highlight **step 4** and set as follows:
    1. Temp: 92C
    2. Time: 0:05
iii. Highlight **step 5** and set as follows:
    1. Temp: 57C
    2. Time: 0:40
    3. Go to the left of the screen and select **Remove Plate Read** button
iv. Select **step 6**, the **GOTO step**, and change to state **GOTO step 4** and change the times to repeat to **9**

g. **Step 7** (2-Step Amplification Stage)
i. With step 6 highlighted select **Insert Step** button, on the lower left of the window, for a total of 2 times (until step 8 is reached)
ii. Highlight **step 7** and set as follows:
    1. Temp: 92C
    2. Time: 0:05
iii. Highlight **step 8** and set as follows:
    1. Temp: 57C
    2. Time: 0:40
    3. In the left of the window select **Add Plate Read to Step** button
    4. Highlight **step 8** and select **Insert GOTO** button on the lower left of the window
iv. Select **step 9**, the **GOTO step**, and change to **GOTO step 7** and times to repeat to **29**

h. Save the new cycling conditions as protocol for future use
i. At the upper left of the screen select the **Save** button
ii. Save in the **ExpressLoad** folder
iii. **Name** the file 'Lyra Direct SARS-CoV-2'
iv. **Save as type** 'Protocol File (*.prcl)'
v. Select **Save**
vi. Click **Ok** in the protocol editor window

6. Define the plate setup
a. In the **Run Setup** window select the **Plate** tab
b. Under **Express Load** in the drop-down menu select **Quick Plate 96 wells All Channels.pltd**
c. Select the **Edit Selected** button to customize the plate setup
d. In the upper toolbar select **Settings.** The default settings need to be set.
i. **Plate Size** select **96 Wells**
ii. **Plate Type** select **BR Clear**
iii. **Number Convention** select **Scientific Notation**
iv. **Units** select **Copy Number**
e. Leave the **Scan Mode** set to **All Channels** at the top of the window
f. Select the **Select Fluorophores** button on the upper right of the Plate Editor window
i. De-select all default fluorophores

ii. Select **FAM,** and **Cy5** and click Ok

g. In the **Plate Editor** window highlight the whole plate and click the check box in front of all fluorophores: **FAM** and **Cy5**

h. Select the **Experiment Settings** button in order to define the Targets

i. In the lower left of the **Experiment Settings** window in the **New** box type in **SARS-CoV-2** and select **Add**

ii. Repeat this for the **PRC**

iii. Select **Ok**

i. In the **Plate Editor** window next to **FAM** in the drop-down menu under **Target Name** select **SARS-CoV-2** and for Cy5 select **PRC**

j. Save the new plate setup for future use

i. At the upper left of the screen select the **Save** button

ii. Save in the **ExpressLoad** folder

iii. **Name** the file 'Lyra Direct SARS-CoV-2 plate'

iv. **Save as type** 'Plate File (*.pltd)'

v. Select **Save**

vi. Click **Ok** in the **Plate Editor** window

k. Exit the software

Bio-Rad CFX96 Touch Thermocycler Test Procedure

Analysis Instructions:

1. Open the run file that needs to be analyzed
2. In the upper left select the **Quantification Tab**
3. On the Amplification curve check the box in front of **Log Scale**
4. Select **Settings** in the toolbar in the upper left of the screen
    a. For the **Cq Determination Mode** select **Single Threshold**
    b. Under the **Baseline Setting** choose **Baseline Subtracted Curve Fit**
    c. For **Analysis Mode** select **Target**
    d. Under **Cycles to Analyze** choose 1-30 and then click **Ok**
    e. The baseline cycles and the threshold for each target need to be set
        i. Ensure that only the **SARS-CoV-2 box** is checked in the amplification plot
        ii. Go up to **Settings** in the toolbar and select **Baseline Threshold**
            1. At the top of the box select **Auto Calculated** for the **Baseline Cycles**
            2. For the **Single Threshold** at the bottom of the box select **User Defined**
                a. Set this to **164**
                b. Select Ok
        iii. **Uncheck** the **SARS-CoV-2 box** and **check** the **PRC box** in the amplification plot
        iv. Go up to **Settings** in the toolbar and select **Baseline Threshold**
            1. At the top of the box select **Auto Calculated** for the **Baseline Cycles**
            2. For the **Single Threshold** at the bottom of the box select **User Defined**
                a. Set this to **25**
                b. Select Ok

5. Exit the software

Qiagen Rotor-Gene Q Programming Instructions

Refer to User Manual Part Number 1065453EN for additional information.

Programming Instructions:

1. Launch the Rotor-Gene Q software package
2. In the **New Run** pop-up window select the **Advanced** tab on the top of the screen
3. Select **Empty Run** and then **New** on the lower right of the pop-up window to start the **Advanced Run Wizard**
    a. Select the appropriate rotor size in the **Advanced Run Wizard** on the upper left of the screen
    b. Check the box that states the **Locking Ring** is **Attached** and select **Next**
    c. Leave the **Operator** and **Notes** sections empty
    d. Enter **20ul** as the **Reaction Volume** in the lower left of the screen
    e. For the **Sample Layout** choose **1, 2, 3…** and then select **Next**
    f. Under **Channel Setup** select **Create New** to enter information for each detector
        i. Under **Name** enter **SARS-CoV-2**
        ii. **Source** select 470nm
        iii. **Detector** select 510nm
        iv. Do not adjust the default **Gain** setting of 7 as this will be set in a later step
        v. Select **OK**
    g. Repeat the step above by selecting **Create New**
        i. Under **Name** enter **PRC**
        ii. **Source** select 625nm
        iii. **Detector** select 660nm
        iv. Do not adjust the default **Gain** setting of 7 as this will be set in a later step
        v. Select **OK**
    h. Select the **Edit Profile** button in the middle of the window to setup a cycling profile
        i. In the **Edit Profile** window go to the upper left of the screen to **New** and in the drop-down menu select **Cycling**. A hold and three step cycling stage should appear.
        ii. Modify the hold stage to have a temperature at **55°C** and a time of **5:00 minutes**
        iii. Select the **Insert After** button in the middle of the pop-up window and then select **New Hold at Temperature**
        iv. Modify the second hold stage to have a temperature at **60°C** and a time of **5:00 minutes**
        v. Select the **Insert After** button in the middle of the pop-up window and then select **New Hold at Temperature** to insert a third hold stage
        vi. Modify the third hold stage to have a temperature at **65°C** and a time of **5:00 minutes**
        vii. Highlight the first **cycling stage** and modify it as follows:
            1. This cycle repeats **10** time(s)
            2. Select **Timed Step** from the drop-down menu in the middle left of the screen
            3. Do not select **Long Range** or **Touchdown** on the left of the screen

4. The first step:
    a. **92°C**
    b. **5 seconds**
    c. **Not Acquiring**
5. Select step two and set as follows:
    a. **57°C**
    b. **40 seconds**
    c. **Not Acquiring**
6. Highlight step three and delete it by selecting the **"-"** button in the middle of the window
7. Select the **Insert After** button in the middle of the pop-up window and then select **New Cycling**

viii. Highlight the second **cycling stage** and modify it as follows:
1. This cycle repeats **30** time(s)
2. Select **Timed Step** from the drop-down menu in the middle left of the screen
3. Do not select **Long Range** or **Touchdown** on the left of the screen
4. The first step:
    a. **92°C**
    b. **5 seconds**
    c. **Not Acquiring**
5. Select step two and set as follows:
    a. **57°C**
    b. **40 seconds**
    c. Select **Acquiring to Cycling A**
        i. **Under Acquiring Channels** highlight the default channel name (Green) and select the **<** button to move it over to the **Available Channels** list
        ii. In the **Available Channels** list select **SARS-CoV-2** and select the **>** button to move it over to the **Acquiring Channels** list
        iii. Repeat the step above for the **PRC** and then select **OK**
6. Highlight step three and delete it by selecting the **"-"** button in the middle of the window

ix. In the **Edit Profile** window select **OK**

i. In the **New Run Wizard** window select **Gain Optimisation**
i. In the middle of the **Auto-Gain Optimisation Setup** window select the drop-down menu under **Channel Settings** and select **SARS-CoV-2**.
ii. Select the **Add** button on the right
1. In the **Auto-Gain Optimisation Channel Settings** window ensure that the SARS-CoV-2 **Tube Position** is set to **1**. This requires that a positive control, containing SARS-CoV-2 and PRC, be tested with each PCR run and placed in the first tube. Failure to do so may cause the gain to be incorrectly set.
2. Leave the **Target Sample Range** and the **Acceptable Gain Range** set to the defaults, 5-10Fl and -10 to 10 respectively.

3. Select **OK**
4. Repeat steps 3. j. ii. 1-3. for the **PRC**

iii. In the **Auto-Gain Optimisation Setup** window check the box next to **Perform Optimisation Before 1st Acquisition**

iv. Select **Close**

j. In the **New Run Wizard** window select the **Next** button

k. Save the new protocol as a template for future use

i. On the bottom right of the window select the **Save Template** button

ii. **Save In:** C:\Program Files\Rotor-Gene Q Software\Templates

iii. File name: 'Lyra Direct SARS-CoV-2'

iv. Save as type: 'Template (*.ret)'

l. Exit the software

Qiagen Rotor-Gene Q Test Run

Analysis Instructions:

1. In the New Run Wizard load the Direct SARS-CoV-2 Template.
2. Press Start.
3. Open the run file that needs to be analyzed
4. In the upper menu toolbar select the **Analysis** button
   a. Select **Quantitation**, then **Cycling A. SARS-CoV-2**, and **Show**
   b. The threshold needs to be set for SARS-CoV-2
      i. In the far right bottom of the screen under **CT Calculation** enter **0.03** for the **SARS-CoV-2 Threshold**
      ii. In the **Eliminate Cycles before** box ensure the default of **1** is entered
      iii. Ensure the amplification graph is set to **Log Scale** (toggle button on the bottom left of the graph states Linear Scale or Log Scale)
   c. Select **Quantitation**, then **Cycling A. PRC**, and **Show**
   d. The threshold needs to be set for PRC
      i. In the far right bottom of the screen under **CT Calculation** enter **0.05** for the **PRC Threshold**
      ii. In the **Eliminate Cycles before** box ensure the default of **1** is entered
      iii. Ensure the amplification graph is set to **Log Scale** (toggle button on the bottom left of the graph states Linear Scale or Log Scale)

Roche's LightCycler® 480 Instrument II Programming Instructions

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

Creating a LC 480 II Assay Run Template

1. Launch the LightCycler (LC) 480 software package
2. The **Detection Format** must be established to specify the channels in which fluorescence will be read
   a. Select **Tools** in the startup screen in the lower right of the screen

b. Select **Detection Formats** then choose **New**
c. Name the format Lyra Direct SARS-CoV-2
d. In the **Filter Combination Selection** window select 465-510 and 618-660
e. In the **Selected Filter Combination List** window under name type in SARS-CoV-2 for 465-510 and PRC for 618-660
f. Leave all default setting values to 1 under Melt Factor, Quant Factor, and Max Integration Time
g. Select **Close** to save the new detection format and return to startup screen
h. To access this newly created **Detection Format**, the LC 480 software must be closed, then reloaded

3. After closing and reloading the software select **White Plates** and **New Experiment** under Experiment Creation window
4. On the next screen select "Lyra Direct SARS-CoV-2" from the pull-down menu under **Detection Formats**
5. Enter **20ul** as the **Reaction Volume** in the upper right of the screen
6. Enter the names for each of the RT-PCR programs
   a. Under **Program Name** enter **Stage 1**, under **Cycles** enter **1**, and in **Analysis Mode** select **none**
   b. Select the **"+"** icon to add a program
   c. Name the next program **Stage 2**, under **Cycles** enter **1**, and in the **Analysis Mode** select **none**
   d. Select the **"+"** icon to add a program
   e. Name the next program **Stage 3**, under **Cycles** enter **1**, and in the **Analysis Mode** select **none**
   f. Select the **"+"** icon to add a program
   g. Name the next program **Stage 4**, under **Cycles** enter **40**, and in the **Analysis Mode** select **quantification**
7. Set the RT-PCR cycling times and temperatures
   a. Highlight **Stage 1** under **Program Name** and change **Stage 1 Temperature Targets** as follows:
      i. **Target (°C)** set to **55**
      ii. **Acquisition Mode** select **none**
      iii. **Hold** (hh:mm:ss) set to **5:00**
      iv. **Ramp Rate (°C/s)** to 4.4
      v. **Sec Target (°C)**, **Step Size (°C),** and **Step Delay (cycles)** will be left at 0 for stages 1-4.
   b. Highlight **Stage 2** under **Program Name** and change **Stage 2 Temperature Targets** as follows:
      i. **Target (°C)** set to **60**
      ii. **Acquisition Mode** select **none**
      iii. **Hold** (hh:mm:ss) set to **5:00**
      iv. **Ramp Rate (°C/s)** to 4.4
   c. Highlight **Stage 3** under **Program Name** and change **Stage 3 Temperature Targets** as follows:
      i. **Target (°C)** set to **65**
      ii. **Acquisition Mode** select **none**
      iii. **Hold** (hh:mm:ss) set to **5:00**
      iv. **Ramp Rate (°C/s)** to 4.4
   d. Highlight **Stage 4** under **Program Name** and change **Stage 4 Temperature Targets** as follows:
      i. The first step:
         1. **Target (°C)** set to **92**
         2. **Acquisition Mode** select **none**

3. **Hold** (hh:mm:ss) set to **0:05**
4. **Ramp Rate (°C/s)** to 4.4

ii. Select the "+" icon to add a step and set the second step:

1. **Target (°C)** set to **57**
2. **Acquisition Mode** select **single**
3. **Hold** (hh:mm:ss) set to **0:40**
4. **Ramp Rate (°C/s)** to 2.2

8. Save the new protocol as a run template for future use.
   a. In the lower left corner of the screen select the pull-down menu next to the **Apply Template** button
   b. Choose **Save As Template**
   c. Select the **Templates Folder**
   d. Highlight **Run Templates Folder**
   e. Name the template Lyra Direct SARS-CoV-2 run template and click the "check" button
9. Exit the software.

Creating a LC 480 II Assay Test Procedure

1. Load the Lyra Direct SARS-CoV-2 run template.
2. Press Start.
3. The analysis template can only be established after the initial experiment has completed and two templates will be established; one for SARS-CoV-2 detection and one for PRC detection.
4. On the Lyra Direct SARS-CoV-2 run select the **Analysis** button in the module bar
   a. Choose **Abs Quant/Fit Points**
   b. In the **Create New Analysis** pop-up window select your pre-defined subset from the **subset** drop down menu and then select the "check" button
   c. Click **Background** for all analytes
      i. Set **Min Offset** to 1
      ii. Set **Max Offset** to 9
   d. In the center bottom of the screen ensure that **Color Compensation** is off for all analytes
   e. Change the default setting to **First Cycle** 7 and confirm **Last Cycle** 40
5. At the top middle of the screen select **Noise Band**
6. Choos**e Filter Comb** 465-510
7. Choose the pull-down menu next to the **Noise Band** button and select the following:
   a. SARS-CoV-2 Noiseband Fluorescence. Set to 1.5
8. Choose **Calculate** in the bottom left of the screen
9. Save the SARS-CoV-2 analysis protocol as a template for future use
   a. In the lower left corner of the screen select the pull-down menu next to the **Apply Template** button
   b. Choose **Save As Template**
   c. Select the **Templates Folder**
   d. Highlight **Analysis Templates Folder**
   e. Name the template Lyra Direct SARS-CoV-2_465-510 analysis template and click the "check" button

10. Go back to run and choose **Filter Comb** 618-660
11. Choose the pull-down menu next to the **Noise Band** button and select the following:
    a. PRC Noiseband Auto
12. Choose **Calculate** in the bottom left of the screen
13. Save the SARS-CoV-2 analysis protocol as a template for future use
    a. In the lower left corner of the screen select the pull-down menu next to the **Apply Template** button
    b. Choose **Save As Template**
    c. Select the **Templates Folder**
    d. Highlight **Analysis Templates Folder**
    e. Name the template Lyra Direct SARS-CoV-2_618-660 analysis template and click the "check" button
14. Create a report
    a. Select the **Save** icon on the global action bar on the right side of the screen
    b. This will be performed under each channel analyzed
    c. Choose the **Report** button on the module bar on the left of the screen
    d. Select the appropriate settings and press the **Generate** button
15. To apply an Analysis Template to subsequent runs
    a. Once the run has finished select the **Analysis** button in the module bar
    b. Choose **Abs Quant/Fit Points**
    c. In the **Create New Analysis** pop-up window select your pre-defined subset from the **subset** drop down menu and then select the "check" button
    d. Select the **Apply Template** button on the far left of the screen and choose the Lyra Direct SARS-CoV-2_465-510 or Lyra Direct SARS-CoV-2_618-660 analysis template from the **Analysis Templates Folder**
    e. Select yes in the pop-up window
16. Interpretation of results (See Table 4)

Roche Cobas z480 Instrument Programming Instructions

Refer to User Manual Version1.1.2 for additional information.

Creating a Cobas z480 Assay Run Template

10. Launch the Cobas z480 software package
11. The **Detection Format** must be established to specify the channels in which fluorescence will be read
    a. Select **Tools** in the startup screen in the lower right of the screen
    b. Select **Detection Formats** then choose **New**

c. Name the format Lyra Direct SARS-CoV-2
d. In the **Filter Combination Selection** window select 465-510 and 610-670
e. In the **Selected Filter Combination List** window under name type in SARS-CoV-2 for 465-510 and PRC for 610-670
f. Leave all default setting values to 1 under Melt Factor, Quant Factor, and Max Integration Time
g. Select **Close** to save the new detection format and return to startup screen
h. To access this newly created **Detection Format**, the Z480 software must be closed, then reloaded

12. After closing and reloading the software select **White Plates** and **New Experiment** under Experiment Creation window
13. On the next screen select "Lyra Direct SARS-CoV-2" from the pull-down menu under **Detection Formats**
14. Enter **20ul** as the **Reaction Volume** in the upper right of the screen
15. Enter the names for each of the RT-PCR programs
    a. Under **Program Name** enter **Stage 1**, under **Cycles** enter **1**, and in **Analysis Mode** select **none**
    b. Select the **"+"** icon to add a program
    c. Name the next program **Stage 2**, under **Cycles** enter **1**, and in the **Analysis Mode** select **none**
    d. Select the **"+"** icon to add a program
    e. Name the next program **Stage 3**, under **Cycles** enter **1**, and in the **Analysis Mode** select **none**
    f. Select the **"+"** icon to add a program
    g. Name the next program **Stage 4**, under **Cycles** enter **40**, and in the **Analysis Mode** select **quantification**
16. Set the RT-PCR cycling times and temperatures
    a. Highlight **Stage 1** under **Program Name** and change **Stage 1 Temperature Targets** as follows:
        i. **Target (°C)** set to **55**
        ii. **Acquisition Mode** select **none**
        iii. **Hold** (hh:mm:ss) set to **5:00**
        iv. **Ramp Rate (°C/s)** to 4.4
        v. **Sec Target (°C)**, **Step Size (°C),** and **Step Delay (cycles)** will be left at 0 for stages 1-4.
    b. Highlight **Stage 2** under **Program Name** and change **Stage 2 Temperature Targets** as follows:
        i. **Target (°C)** set to **60**
        ii. **Acquisition Mode** select **none**
        iii. **Hold** (hh:mm:ss) set to **5:00**
        iv. **Ramp Rate (°C/s)** to 4.4
    c. Highlight **Stage 3** under **Program Name** and change **Stage 3 Temperature Targets** as follows:
        i. **Target (°C)** set to **65**
        ii. **Acquisition Mode** select **none**
        iii. **Hold** (hh:mm:ss) set to **5:00**
        iv. **Ramp Rate (°C/s)** to 4.4
    d. Highlight **Stage 4** under **Program Name** and change **Stage 4 Temperature Targets** as follows:
        i. The first step:
            1. **Target (°C)** set to **92**
            2. **Acquisition Mode** select **none**
            3. **Hold** (hh:mm:ss) set to **0:05**

4. **Ramp Rate (°C/s)** to 4.4

ii. Select the "+" icon to add a step and set the second step:

1. **Target (°C)** set to **57**
2. **Acquisition Mode** select **single**
3. **Hold** (hh:mm:ss) set to **0:40**
4. **Ramp Rate (°C/s)** to 2.2

17. Save the new protocol as a run template for future use.
    a. In the lower left corner of the screen select the pull-down menu next to the **Apply Template** button
    b. Choose **Save As Template**
    c. Select the **Templates Folder**
    d. Highlight **Run Templates Folder**
    e. Name the template Lyra Direct SARS-CoV-2 run template and click the "check" button
18. Exit the software.

Creating a Cobas z480 Assay Test Procedure

17. Load the Lyra Direct SARS-CoV-2 run template.
18. Press Start.
19. The analysis template can only be established after the initial experiment has completed and two templates will be established; one for SARS-CoV-2 detection and one for PRC detection.
20. On the Lyra Direct SARS-CoV-2 run select the **Analysis** button in the module bar
    a. Choose **Abs Quant/Fit Points**
    b. In the **Create New Analysis** pop-up window select your pre-defined subset from the **subset** drop down menu and then select the "check" button
    c. Click **Background** for all analytes
        i. Set **Min Offset** to 1
        ii. Set **Max Offset** to 9
    d. In the center bottom of the screen ensure that **Color Compensation** is off for all analytes
    e. Change the default setting to **First Cycle** 7 and confirm **Last Cycle** 40
21. At the top middle of the screen select **Noise Band**
22. Choos**e Filter Comb** 465-510
23. Choose the pull-down menu next to the **Noise Band** button and select the following:
    a. SARS-CoV-2 Noiseband Fluorescence. Set to 1.5
24. Choose **Calculate** in the bottom left of the screen
25. Save the SARS-CoV-2 analysis protocol as a template for future use
    a. In the lower left corner of the screen select the pull-down menu next to the **Apply Template** button
    b. Choose **Save As Template**
    c. Select the **Templates Folder**
    d. Highlight **Analysis Templates Folder**
    e. Name the template Lyra Direct SARS-CoV-2_465-510 analysis template and click the "check" button
26. Go back to run and choose **Filter Comb** 610-670

27. Choose the pull-down menu next to the **Noise Band** button and select the following:
    a. PRC Noiseband Auto
28. Choose **Calculate** in the bottom left of the screen
29. Save the SARS-CoV-2 analysis protocol as a template for future use
    a. In the lower left corner of the screen select the pull-down menu next to the **Apply Template** button
    b. Choose **Save As Template**
    c. Select the **Templates Folder**
    d. Highlight **Analysis Templates Folder**
    e. Name the template Lyra Direct SARS-CoV-2_610-670 analysis template and click the "check" button
30. Create a report
    a. Select the **Save** icon on the global action bar on the right side of the screen
    b. This will be performed under each channel analyzed
    c. Choose the **Report** button on the module bar on the left of the screen
    d. Select the appropriate settings and press the **Generate** button
31. To apply an Analysis Template to subsequent runs
    a. Once the run has finished select the **Analysis** button in the module bar
    b. Choose **Abs Quant/Fit Points**
    c. In the **Create New Analysis** pop-up window select your pre-defined subset from the **subset** drop down menu and then select the "check" button
    d. Select the **Apply Template** button on the far left of the screen and choose the Lyra Direct SARS-CoV-2_465-510 or Lyra Direct SARS-CoV-2_610-670 analysis template from the **Analysis Templates Folder**
    e. Select yes in the pop-up window
32. Interpretation of results (See Table 4)

ThermoFisher QuantStudio 7 Pro Programming Instructions

Refer to User Manual Part Number 4489822 Revision A for additional information.

ThermoFisher QS7 Test Run Programming Instructions:

1. Open the Design and Analysis Software
2. Select the "SET UP PLATE" option
3. From the side bar on the screen, select the following properties to filter:
    a. Instrument – QuantStudio 7 Pro
    b. Block – 96-Well 0.2 mL
    c. Run Mode – Fast
    d. Analysis options are left blank
4. From the plate selections present on the screen, select the System Template "Presence/Absence" and the system will automatically navigate to the "Run Method" tab
5. Run Method
    a. Change the Reaction Volume to 20.0uL
    b. The temperature of the enabled heated cover will remain at 105.0 degrees C

c. Scroll over the Hold stage present in the cycling parameters and addition/subtraction buttons will become visible at both the top and bottom of the first stage.
d. Left click the right addition button at the top and a list of Stage choices will become visible. Scroll down and choose Hold.
e. Repeat the previous steps so there are three Hold stages present in the cycling parameters.
f. Scroll over to the PCR stage and addition/subtraction buttons will become visible at both the top and bottom. Left click the right addition button at the top and a list of Stage choices will become visible. Scroll down and choose PCR.

g. Going back to the first stage enter the following parameters:
    i. Stage 1 Hold
        1. 2.63 ramp rate
        2. 55°C
        3. 5 minutes
    ii. Stage 2 Hold
        1. 2.63 ramp rate
        2. 60°C
        3. 5 minutes
    iii. Stage 3 Hold
        1. 2.63 ramp rate
        2. 65°C
        3. 5 minutes
    iv. Stage 4 PCR
        1. Step 1:
            a. 2.63 ramp rate
            b. 92°C
            c. 5 seconds
        2. Step 2:
            a. 2.32 ramp rate
            b. 57°C
            c. 40 seconds
            d. Click on the camera icon under Step 2. A window will pop up asking for confirmation to turn off data collection during this step. Click “Ok”.
    v. Located at the bottom of Stage 4 PCR change the number of cycles to 10
    vi. Stage 5 PCR
        1. Step 1:
            a. 2.63 ramp rate
            b. 92°C
            c. 5 seconds
        2. Step 2:
            a. 2.32 ramp rate
            b. 57°C
            c. 40 seconds
            d. Ensure the camera icon image is bold/on for data collection during the 30 cycles of Stage 5, Step 2.

vii. Located at the bottom of Stage 4 PCR change the number of cycles to 30

h. Scroll up and choose the "Plate Setup" tab near the top of the screen.

6. Plate Setup
   a. Change the Passive Reference to "NONE"
   b. On the lower right side of screen, ensure the Targets Tab is chosen then highlight and press the addition button to add "Target 1". Press again to add "Target 2"
   c. Click on the "Target 1" box and change the name to CoV-2.
   d. Click the associated reporter box below the Reporter tab and, from the drop down menu, choose FAM.
   e. Click on the "Target 2" box and change the name to PRC.
   f. Click the associated reporter box below the Reporter tab and, from the drop down menu, choose CY5.
   g. Highlight the "Actions" button located in the upper right side of the screen and press the drop down button. In the drop down menu choose "Analysis Setting"
   h. Under Analysis Setting, disable the following for all targets:
      i. Use Default Column
      ii. Auto Threshold Column
      iii. Auto Baseline Column
      iv. The Baseline Start and Baseline End should default to 3 and 15
   i. Under "Threshold" click on the box associate with the CoV target and enter 40000.
   j. Under "Threshold" click on the box associated with the PRC target and enter 15000
   k. Click "Save"
   l. Navigate back to the "Actions" button and press the drop down button, choosing "Save As". This will save your template to a location of choice. Save the template as "Lyra Direct SARS Cov-2 Assay".

Creating a ThermoFisher QuantStudio 7 Pro Test Procedure

Note: These instructions are based upon the user not having the QuantStudio 7 Real-Time PCR instrument and the ABI Design and Analysis 2.4 software connected. The user must open the Lyra Direct SARS CoV-2 template created previously with the software and save any newly created sample run template onto a USB and transfer the template to the instrument.

For connectivity related to the software and the instrument please contact your Thermo Fisher/ABI QuantStudio representative.

1) Open the Lyra Direct SARS CoV-2 Assay Template previously generated.
2) Click on the Plate Setup Tab located near the top of the screen.
3) On the right side of the screen ensure the "Samples" tab is highlighted and press the addition button to add the number of samples being tested.
4) Click on the "Sample 1" box to rename the sample. Repeat this step for all subsequent samples being entered.
5) Click the well located in the plate map then check the box next to the sample name from the right side bar to associate the name to the well.

877 a. User also has the option to highlight the well location in the plate map and click on the
878 "Enter sample" box. Enter the sample ID and press tab to continue to the next well in
879 the plate map. This will automatically load the sample name into the sidebar.
880 6) Once samples names have been entered, the wells may be highlighted by left clicking the mouse
881 over starting well and dragging the mouse across all wells associated in run. The targets are then
882 chosen by clicking the check boxes next to each target in the side bar.
883 7) Click on the Actions button located top right of the screen and choose "Save As" in the dropdown
884 menu.
885 a. A pop-up window will appear directing the user to title the file according to information
886 pertaining to the sample run and the location of the file to be saved.
887 b. Save the newly named (.edt) run file to a USB that is inserted into the computer.
888 8) Transfer the USB to the port on the front of the instrument.
889 9) From the options on the instrument's screen press "Load plate file". The QuantStudio 7 is a
890 touchscreen device.
891 10) From the "Run Queue" screen, press "USB drive" on the right side. This will bring up any plate
892 files saved on the USB.
893 11) Press the plate file associated with the run to be performed.
894 12) A new window will appear requesting location of results once the run is complete.
895 a. Press the "USB drive Connected" if the icon is not already highlighted and press "Done".
896 13) Centrifuge the 96-well sample plate to ensure all liquid is toward the bottom of each well.
897 a. Ensure the centrifuge is properly balanced.
898 b. Gently pull the plate from the centrifuge to ensure all liquids remain at the bottom of
899 the wells.
900 14) Press the double-arrow icon located at the top right sided corner of the screen on the
901 instrument.
902 a. The instrument drawer will open from the front.
903 15) Place the centrifuged plate into the plate holder ensuring proper orientation of the plate.
904 a. A1 well should be in the position of the top left corner
905 b. The plate will appear slightly suspended above the block due to two silicone strips
906 above and below this plate. This is to be expected and the instrument lid will press the
907 plate down once the drawer has closed.
908 16) Press "Start Run" on the screen of the instrument.
909 a. A pop-up window will appear asking the user to confirm the plate has been loaded.
910 b. If the plate has been loaded, press "Start Run" again or press "Open Drawer" to place
911 the plate into the block and then press "Start Run"

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M124 – Lyra Direct SARS-CoV-2 Assay kit

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GLOSSARY

 Intended use	 Catalog number
 Contents / Contains	 Contains sufficient for 96 determinations
	 Control
 Batch code	 Use by
	 Consult e-labeling instructions for use
 Manufacturer	 Temperature limitation

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