PlexPCR® SARS-CoV-2

Multiplex real-time RT-PCR assay for the detection of SARS-CoV-2

CE 0123 IVD UKCA

Product	Platform	Size (reactions)	Catalogue no.
PlexPCR® SARS-CoV-2	LC480 II CFX96™ Dx CFX96 Touch™	384	REF **1301384**

Accessory products – Analysis software

PlexPCR® SARS-CoV-2 (LC480)	REF **99021**
PlexPCR® SARS-CoV-2 (CFX)	REF **99022**

EC REP

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Not for sale in the USA

Contents

1 Product description 4
2 Intended use 4
3 Pathogen information 4
4 Kit contents 4
5 Shipping and storage 5
6 Warnings and precautions 5
6.1 General 5
6.2 Laboratory 5
6.3 Specimen handling 5
6.4 Assay 5
6.5 Safety precautions 5
6.6 Assay Plugins: Warnings/Precautions/Limitations 5
7 Associated Products and Consumables 6
8 Principle of the technology 8
9 Procedure overview 9
10 Detailed procedure 10
10.1 Sample collection, transport, and storage 10
10.2 Sample processing 10
10.2.1 Reagent volumes for MGISP-960 10
10.2.2 Reagent volumes for KingFisher Flex and PurePrep 11
10.3 Internal Control (IC) 11
10.3.1 Internal Control on the MagNA Pure 96, KingFisher Flex, PurePrep 96 and MGISP-960 11
10.4 Preparation of real-time PCR 12
10.4.1 Master Mix preparation 12
11 Programming and analysis 12
12 Interpretation of results 12
13 Limitations 13
14 Quality control 13
15 REDx™ FLOQ SARS-CoV-2 Positive Control instructions 13
15.1 Instructions for use 13
16 Performance characteristics 14
16.1 Clinical performance 14
16.1.1 Clinical Study 1 14
16.2 Analytical performance 15
16.2.1 Repeatability and Reproducibility 15
16.2.1.1 LightCycler® 480 Instrument II 15
16.2.1.2 CFX96™ Dx Real-Time PCR Detection and CFX96 Touch™ Real-Time PCR Detection Systems 16
16.2.2 Analytical sensitivity 17
16.2.2.1 LightCycler® 480 Instrument II 17
16.2.2.2 Workflow with the MGISP-960 & LightCycler® 480 Instrument II 18
16.2.3 Analytical specificity 19
16.2.4 *In silico* analysis 20
16.2.5 Inclusivity 20

16.2.6 Potentially interfering substances 20
17 Customer and technical support 21
18 References 21
19 Appendix 1: LightCycler® 480 Instrument II 22
19.1 Programming the LightCycler® 480 Instrument II (LC480 II) 22
19.2 Setting up a Macro Template for the LightCycler® 480 Instrument II 27
19.3 Colour Compensation for LightCycler® 480 Instrument II 33
19.4 Interpretation of results 33
20 Appendix 2: Bio-Rad CFX96™ Dx and CFX96 Touch™ Real-Time PCR System 35
20.1 Programming the CFX96™ Dx and CFX96 Touch™ Real-Time PCR Detection System (CFX96 Dx, CFX96 Touch) 35
20.2 Interpretation of results using onboard CFX software 37
20.3 Exporting results from onboard CFX analysis 41
20.4 Interpretation of results with the PlexPCR SARS-CoV-2 (CFX) analysis software 43
21 Appendix A: Result Interpretation 44
21.1 FastFinder platform – Minimum IT requirements 44
21.2 Device set up (new user or device) 45
21.2.1 Colour Compensation 45
21.3 Assay plug-in (new user) 46
21.4 Sample naming 46
21.5 Adding mix lot numbers 47
21.6 Analysis 47
21.7 Results 48
21.8 Reference curve 48
21.9 Overview of results 49
21.10 Exporting results 49
22 Glossary 51

1 Product description

The ***Plex****PCR*® SARS-CoV-2 kit is a 1-well qPCR multiplex for the detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The assay gives 3 readouts; Readout 1 indicates the presence or absence of SARS-CoV-2 through detection of the Open Reading Frame (ORF1ab) gene; Readout 2 indicates the presence or absence of SARS-CoV-2 through detection of the RdRp (RNA-dependent RNA polymerase) gene; Readout 3 is an RNA internal control (IC) to monitor extraction efficiency and qPCR inhibition. The ***Plex****PCR*® SARS-CoV-2 kit utilises ***Plex****Zyme*® technology for specificity and superior multiplexing capability.

This assay is validated on samples extracted using the MagNA Pure 96 System (Roche), MGISP-960 (MGI), PurePrep 96 (Molgen), and KingFisher™ Flex Sample Purification System (ThermoFisher), liquid handling using the ***Plex****Prep*™ (SpeeDx), and real-time detection on the LightCycler® 480 II Instrument (LC480 II, Roche), the CFX96™ Dx Real-Time PCR Detection System (CFX96 Dx, Bio-Rad), and the CFX96 Touch™ Real-Time PCR Detection System (CFX96 Touch, Bio-Rad).

2 Intended use

The ***Plex****PCR*® SARS-CoV-2 kit is an *in vitro* diagnostic reverse transcriptase real-time PCR (RT-qPCR) test for the qualitative detection of SARS-CoV-2.

The ***Plex****PCR*® SARS-CoV-2 kit is intended to aid in the diagnosis of SARS-CoV-2 and should be used in conjunction with clinical and other laboratory information.

The ***Plex****PCR*® SARS-CoV-2 kit may be used with nasopharyngeal swab specimens only.

The ***Plex****PCR*® SARS-CoV-2 kit is intended to be used in professional settings such as hospitals, or reference, or state laboratories. It is not intended for self-testing, home use, or point-of-care use.

The target population intended for the ***Plex****PCR*® SARS-CoV-2 kit are symptomatic patients suspected of having severe acute respiratory syndrome-associated coronavirus (SARS-CoV2) infection by their healthcare provider based on clinical presentation and/or history.

3 Pathogen information

An outbreak of respiratory illness of unknown aetiology in Wuhan City, Hubei Province, China was initially reported to the World Health Organisation (WHO) on December 31, 2019.[1] A novel coronavirus was subsequently identified and named SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2), causing the communicable disease COVID-19 (coronavirus disease 2019).[2] SARS-CoV-2 has since been responsible for a global pandemic resulting in over 75 million confirmed cases and greater than 1.5 million deaths as of the end of September 2020.[3]

4 Kit contents

Number of tests: 384 reactions

Table 1. Kit content for *Plex*PCR® SARS-CoV-2 (Cat no 1301384)

Cap colour	Contents	Description	Quantity
Brown	SARS-CoV-2 Mix, 20x	Mix containing oligonucleotides^ for amplification and detection of SARS-CoV-2 and Internal control for LC480 II and CFX	2 x 150 µl
Green	***Plex*** Mastermix, 2x	Mastermix containing components necessary for qPCR including dNTPs, $MgCl_2$, DNA polymerase and buffer	2 x 1.2 ml
Neutral	RTase, 100x	Reverse transcriptase enzyme for generating complementary DNA (cDNA) from RNA template	1 x 90 µl
Black	RNase Inhibitor, 50x	RNase inhibitor	1 x 135 µl
Purple	Internal Control RNA#	Internal control cells containing internal control RNA template to monitor extraction, reverse transcription, and amplification efficiency	1 x 200 µl
Blue	Nuclease Free Water	PCR grade water	1 x 1 ml

Store template tubes separately from oligo mixes, i.e., template or nucleic acid handling room

^ Oligonucleotides are PCR primer pairs, ***Plex****Zyme*® enzymes and fluorescent probe

* Sufficient for 384 x 10µl tests. Additional volume supplied for compatibility with liquid handling instrumentation, validated with ***Plex****Prep*™ (SpeeDx).

5 Shipping and storage

- The components of the ***Plex****PCR*® SARS-CoV-2 kits are shipped on dry ice or ice gel packs. All components should be stored between -25°C to -15°C upon receipt. It is recommended that freeze/thaw cycles are limited to 10.
- When stored under the recommended conditions and handled correctly, activity of the kit is retained until the expiry date stated on the label. Do not use past expiry date.

6 Warnings and precautions

6.1 General

- For *in vitro* diagnostic use only.
- Carefully read these Instructions for Use prior to use. Closely follow procedures as described to ensure reliability of test results. Any deviation from these procedures may affect test performance.
- Users should be adequately trained in the use of the ***Plex****PCR*® SARS-CoV-2 assay.
- Any serious incident shall be reported to the manufacturer and competent authority of the Member State in which user and/or patient is established

6.2 Laboratory

- It is recommended to perform sample preparation/extraction, mastermix preparation, sample addition and thermocycling in spatially separated spaces. At a minimum the PCR instrument should ideally be in a separate room to areas where reactions are prepared.
- It is recommended to follow routine laboratory precautions. Wear appropriate personal protective equipment such as gloves, protective eye wear and laboratory coat when handling reagents.
- Pathogenic organisms may be present in clinical specimens. Treat all biological specimens as potentially infectious and follow your institution's safety procedures for handling chemicals and biological samples.
- Follow your institution's hazardous waste disposal procedures for proper disposal of specimens, reagents and other potentially contaminated materials.

6.3 Specimen handling

- Specimens should be collected, transported and stored using standard laboratory techniques or according to collection kit instructions.

6.4 Assay

- Basic precautions for preventing contamination of PCR reactions include the use of sterile filter pipette tips, use of a new pipette tip for every pipetting action, and separation of workflow.
- PCR tests are prone to contamination from previous PCR products. Never open reaction vessels after the completion of PCR.

6.5 Safety precautions

- Safety Data Sheets (SDS) are available on request. Please contact tech@speedx.com.au for more information.

6.6 Assay Plugins: Warnings/Precautions/Limitations

- SpeeDx software can only control the analysis of raw data generated from the test kit when used with its respective PCR instrument. It does not control the preparation of samples, reactions, programming of equipment or delivery of treatment.
- Users should be adequately trained in the use of the ***Plex****PCR*® SARS-CoV-2 analysis software and the access should be limited to each assigned single user
- It is recommended to implement user authentication access and cybersecurity controls such as anti-virus software or use of a firewall within the IT system and infrastructure which uses the software
- Upon detection of a cybersecurity incident such as unauthorised access and ransomware attacks, please contact tech@speedx.com.au for further support.

7 Associated Products and Consumables

Positive Control Material

- REDx™ FLOQ SARS-CoV-2 Swab Positive Control (Microbix, Cat no RED-S-19-01)

General lab consumables

- Gloves and clean lab coats
- Vortex mixer
- Benchtop centrifuge for 0.5 ml and 1.5 ml tubes
- Micropipettors
- Multichannel pipettors
- Sterile aerosol-resistant pipette tips
- 0.5 ml tubes and 1.5 ml tubes (PCR-grade)
- Adhesive plate seal
- 2.0 ml tubes (for pre-dilution of internal control cells)

For MagNA Pure 96 Instrument

- 1x Phosphate Buffered Saline (PBS)
- MagNA Pure 96 Internal Control Tube (Roche, Cat no 00374905001)
- MagNA Pure 96 DNA and Viral NA Small Volume Kit (Roche, Cat no 06543588001)
- MagNA Pure 96 System Fluid (external) (Roche, Cat no 06640729001)
- MagNA Pure 96 Processing Cartridge (Roche, Cat no 06241603001)
- MagNA Pure 96 Pure tip 1000uL (Roche, Cat no 6241620001)
- MagNA Pure 96 Output Plate (Roche, Cat no 06241611001)
- MagNA Pure Sealing Foil (Roche, Cat no 06241638001)

For MGISP-960 Instrument

- Nucleic Acid Extraction Kit 96 prep (MGI, Cat. No. 1000022201(ARTG-IVD)) or Nucleic Acid Extraction Kit 96 prep (MGI, Cat. No.1000021042 (CE-IVD))
- 4 x 250 µl automated filter tips (MGI, Cat No. 1000000723)
- 5 x 1.3 mL U-bottom deep-well plate (MGI, Cat. No. 1000004644)
- 1 x Hard-shell thin-wall 96-well skirted PCR plate, white shell/clear well (MGI, Cat. No. 1000012059)
- 50 mL tube, DNase-free, RNase-free
- Absolute ethanol (100%)
- Plate centrifuge

For PurePrep 96 Instrument

- 1x Phosphate Buffered Saline (PBS)
- Molecular grade water
- PurePrep Deep well plate 2mL (Molgen Cat no MG96020050)
- PurePrep 96 Elution plate 200uL (Molgen Cat no MG96010050)
- PurePrep 96 Tip combs (Molgen Cat no MG96030050)
- Molgen PurePrep Pathogens 1x96 kit (Molgen Cat no OE00290096) OR 10x96 kit (Molgen Cat no OE00290960)
- Microplate shaker (minimum speed 1000 RPM)
- 50mL Reagent Reservoirs for 8 channel pipettes
- 50mL Falcon Tubes

For KingFisher Flex

- 1x Phosphate Buffered Saline (PBS)
- Thermofisher MagMAX Viral and Pathogen Nucleic Acid Isolation Kit (Thermofisher Cat no A42352)
- KingFisher 96 deep-well plate, v-bottom, polypropylene (Thermofisher Cat no 95040450)
- KingFisher 96 tip comb for deep-well magnets (Thermofisher Cat no 97002534)
- KingFisher 96 microplate (200µL) (Thermofisher Cat no 97002540)
- 80% ethanol
- 50mL Reagent Reservoirs for 8 channel pipettes
- 50mL Falcon Tubes

*For SpeeDx **Plex**Prep™ liquid handling instrument*

- ***Plex**Prep*™ 8 position deck equipped with 2 independent channels and an 8-Probe Head (Part no 6600200-01)
- 4x Framed tip rack modules (Cat no HMT-6600533-01)
- 4x 24 position tube module (Cat no HMT-6600555-01)
- 1x 24 position small tube module (Cat no HMT6600409-01)
- 50uL conductive filtered tips (Cat no HMT-235948)
- 300uL conductive filtered tips (Cat no HMT-235903)
- 1000uL conductive filtered tips (Cat no HMT-235905)

For LightCycler® 480 Instrument II

- ***Plex**PCR*® Colour Compensation (CC) kit (SpeeDx, Cat no 90001)
- LightCycler® 480 Multiwell Plate 96 (Roche, Cat no 04729692001)
- LightCycler® 480 Multiwell Plate 384 (Roche, Cat no 04729749001)
- LightCycler® 480 Sealing Foil (Roche, Cat no 04729757001)

For CFX96™ Dx Real-Time PCR Detection and CFX96 Touch™ Real-Time PCR Detection Systems

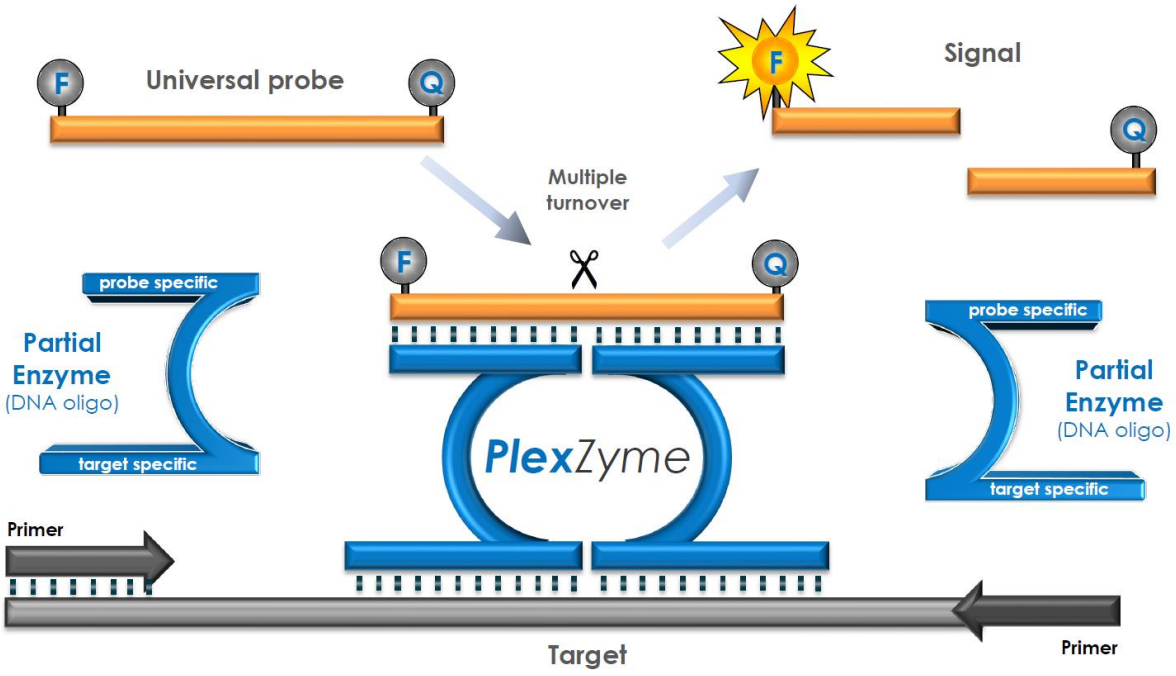
- Hard-Shell® 96-Well PCR Plates, low profile, semi skirted, clear shell/clear well (Bio-Rad, Cat no HSL9901 or HSL9601)
- Microseal® 'B' PCR Plate Sealing Film, adhesive, optical (Bio-Rad, Cat no MSB1001)

8 Principle of the technology

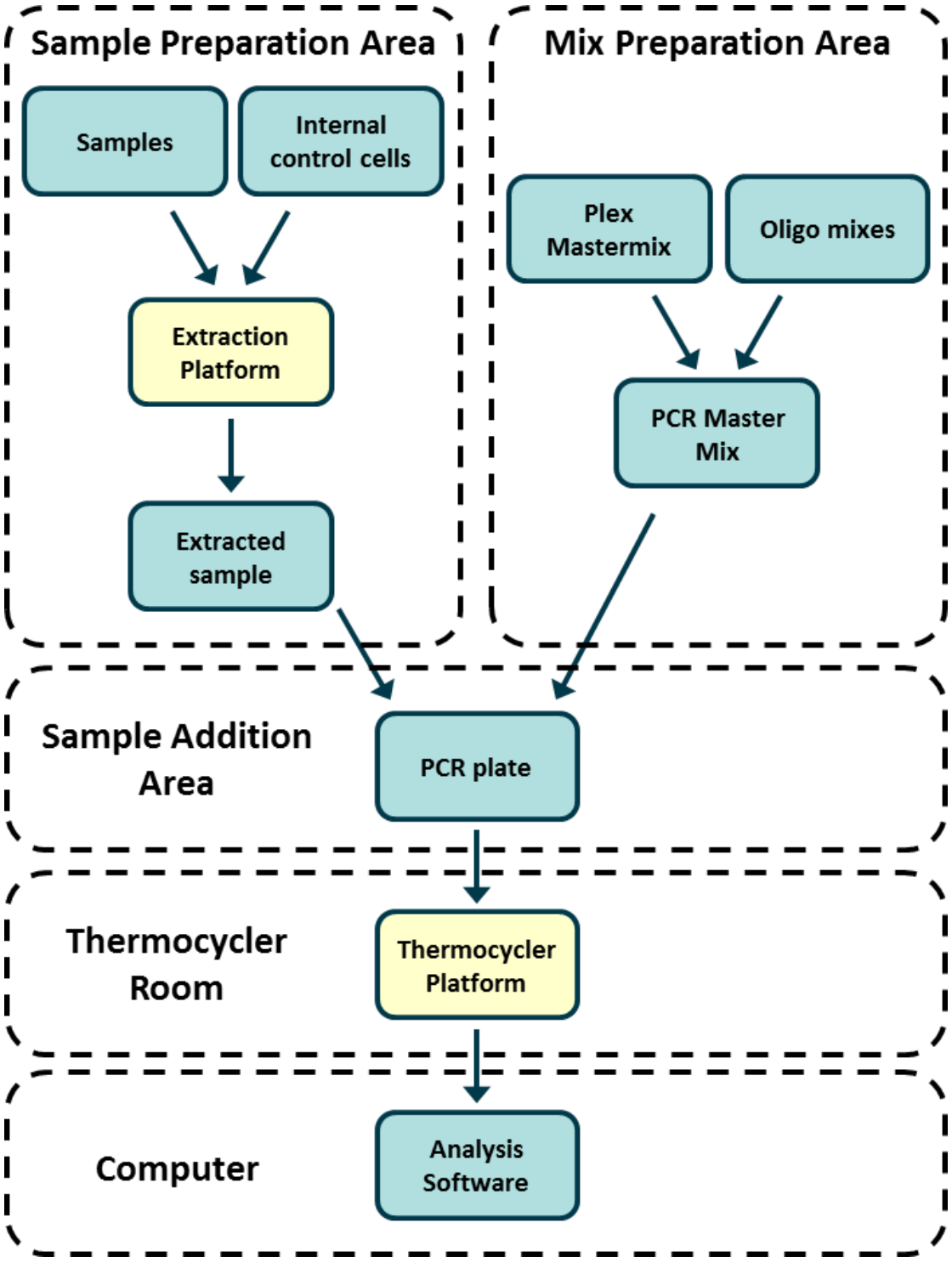
Real-time PCR (qPCR) can be used to amplify and detect specific target nucleic acids from pathogens. ***Plex****PCR*® is a qPCR technology utilising ***Plex****Zyme*® enzymes that detect and report the amplified product through the generation of a fluorescent signal (**Figure 1**).

Plex*Zyme*® enzymes are catalytic DNA complexes composed of two DNA oligos referred to as "Partial Enzymes". Each Partial Enzyme has a target-specific region, a catalytic core and a universal probe binding region. When the target product is present, the two Partial Enzymes bind adjacently to form the active ***Plex****Zyme*® which has catalytic activity to cleave a labelled probe. Cleavage separates the fluorophore and quencher dyes, producing a fluorescent signal that can be monitored in real time. ***Plex****Zyme*® enzymes have additional specificity compared to alternate detection technologies, since two Partial Enzymes are required to bind for detection. ***Plex****Zyme*® enzymes are also multiple turnover enzymes, and multiple probes can be cleaved during each PCR cycle, resulting in a strong and sensitive signal. ***Plex****Zyme*® assays are highly sensitive and specific and are ideally suited for the multiplexed detection of pathogens.

Figure 1. Schematic representation of *PlexZyme*® detection and universal signalling



9 Procedure overview



10 Detailed procedure

Note: Provided reagents are named in italics and colour of the tube cap follows in brackets.

10.1 Sample collection, transport, and storage

Inadequate or inappropriate specimen collection, storage and transport are likely to yield false test results. Proper training in specimen collection is highly recommended to ensure specimen quality and stability.

Follow specimen collection device manufacturer instructions for proper collection methods.

Prior to any collection method, trained staff must ensure proper understanding of the device and methodology. At minimum, review the test description for the following: indication of specimen type, sufficient volume, procedure(s), necessary collection materials, patient preparation, and proper handling and storage instructions.

Nasopharyngeal swabs should be collected and transported according to collection kit instructions. We recommend that nasopharyngeal swab samples are tested immediately or stored between -25º C and -15º C upon arrival and can be freeze thawed during use, no more than 3 times.

10.2 Sample processing

The ***Plex****PCR*® SARS-CoV-2 kit has been validated on the following extraction instruments in **Table 2.**

See **Section 10.3** for instructions to use the Internal Control.

See **Section 15** for instructions to use the REDx™ FLOQ SARS-CoV-2 Swab Positive Control kit.

Table 2. Validated extraction protocols

Instrument	Extraction kit	Sample volume	Protocol	Elution volume
MagNA Pure 96[a b]	MagNA Pure 96 DNA and Viral NA Small Volume Kit	200 µl	Pathogen Universal 200	50 µl
MGISP-960[a b]	Nucleic Acid Extraction Kit	180 µL	MGISP-960 Automated Extraction Standard Workflow	30 µl
KingFisher Flex[a b]	MagMAX Viral/Pathogen Nucleic Acid Isolation Kit	200 µl	MVP_Flex_200uL	50 µl
PurePrep 96[a b]	PurePrep Pathogen kit	200 µl	PP v.3	50 µl

[a] See **10.3.1** for how to use the internal control on the MagNA Pure 96, MGISP-960, KingFisher Flex and PurePrep 96

[b] Samples should be added to the Mastermix within 30 minutes following extraction.

10.2.1 Reagent volumes for MGISP-960

Table 3. MGISP-960 reagent volumes per sample

Reagent	Volume per sample	Plate
Buffer MLB	160µL	U-bottom deep-well plate (Prepared Buffer Mixture)
Absolute Ethanol*	200µL	U-bottom deep-well plate (Prepared Buffer Mixture)
Magnetic Beads M	15µL	U-bottom deep-well plate (Prepared Buffer Mixture)
Enhancer Buffer	1µL	U-bottom deep-well plate (Prepared Buffer Mixture)
RNase Free Water	15µL	U-bottom deep-well plate (Prepared Buffer Mixture)
RNase Free Water	50µL	U-bottom deep-well plate
Buffer MW1	170µL	U-bottom deep-well plate
Buffer MW2	340µL	U-bottom deep-well plate

* Not supplied

10.2.2 Reagent volumes for KingFisher Flex and PurePrep

Table 4. KingFisher reagent volumes		
Reagent	**Volume per sample**	**Plate**
MagMax Binding Solution	265µL	KingFisher 96 deep-well plate (sample plate)
MagMax Total Nucleic Acid Binding Beads	10µL	KingFisher 96 deep-well plate (sample plate)
MagMax Proteinase K	5µL	KingFisher 96 deep-well plate (sample plate)
MagMax Wash buffer	500µL	KingFisher 96 deep-well plate
Wash 2* (80% Ethanol)	500µL	KingFisher 96 deep-well plate
Wash 3* (80% Ethanol)	250µL	KingFisher 96 deep-well plate
MagMax Elution Solution	50uL	KingFisher 96 microplate 200µL

* Not supplied

Table 5. PurePrep96 reagent volumes		
Reagent	**Volume per sample**	**Plate**
Molgen Lysis buffer PA1	200µL	PurePrep Deep well plate 2mL (Sample plate)
Molgen Poly-A-RNA 2.5mg/mL solution	1µL	PurePrep Deep well plate 2mL (Sample plate)
Molgen Proteinase K 20mg/mL solution	10µL	PurePrep Deep well plate 2mL (Sample plate)
Molgen MagSi-PA VII (Magnetic beads)	20µL	PurePrep Deep well plate 2mL (Sample plate)
Molgen Binding buffer U1	400µL	PurePrep Deep well plate 2mL (Sample plate)
Molgen Wash Buffer I	800µL	PurePrep Deep well plate 2mL
Molgen Wash Buffer I	800µL	PurePrep Deep well plate 2mL
Molgen Wash Buffer II	800µL	PurePrep Deep well plate 2mL
Molgen Elution Buffer	50µL	PurePrep 96 Elution plate 200uL

10.3 Internal Control (IC)

The kit includes an internal control to monitor extraction efficiency and qPCR inhibition. The internal control assay is provided within the assay mix and will amplify the *Internal Control RNA* (PURPLE). The *Internal Control RNA* is diluted and processed as below for specific extraction instruments. The internal control template is therefore co-extracted with the sample and co-amplified in the reaction.

10.3.1 Internal Control on the MagNA Pure 96, KingFisher Flex, PurePrep 96 and MGISP-960

Dilute the *Internal Control RNA* (PURPLE) 1 in 100 in 1x PBS (**Table 6**). Adjust volume as required using the same dilution factor (see extraction kit manual for minimum volume for required number of samples). The diluted internal control RNA is loaded into the Internal Control Tube on the MagNA Pure 96 and 20 µl is automatically added to each sample (default). For extractions on the MGISP-960, PurePrep 96 and KingFisher, 20uL of the diluted internal control RNA is manually added to the sample plate.

Note: Do NOT store diluted Internal Control RNA

Table 6. Dilution of Internal Control RNA for MagNA Pure 96, KingFisher, PurePrep 96 and MGISP-960 (1 in 100 dilution)			
***Internal Control RNA* (PURPLE) (µl)**	**1x PBS (µl)**	**Total volume (µl)**	**Volume added to sample (µl)**
36	3564	3600	20

10.4 Preparation of real-time PCR

Note: Before use of reagents, thaw completely, and mix thoroughly by briefly vortexing.

The ***Plex****PCR*® SARS-CoV-2 kit is tested at a final volume of 10 µl in 96-well or 384-well plates on the LC480 II; a final volume of 10 µl in 96-well plates on the CFX96 Dx and CFX96 Touch. The ***Plex****PCR*® SARS-CoV-2 kit has appropriate dead volume for use with liquid handling systems and has been validated with the SpeeDx ***Plex****Prep*™. Contact tech@speedx.com.au for assistance with protocols.

Refer to **Table 1 -** for description of kit contents.

10.4.1 Master Mix preparation

- For a 10 µl reaction volume, 7.5 µl of Master Mix and 2.5 µl extract is required. Prepare Master Mix as outlined in **Table 7**. Pipette the Master Mix into the PCR plate and then add extracted sample to the reaction.
- Positive and negative controls should be run on each plate.
- Seal, then centrifuge the plate and transfer to thermocycler.

Table 7. Master Mix

Reagent	Concentration	Volume per 10 µl reaction (µl)
Nuclease Free Water (**BLUE**)	N/A	1.7
Plex Mastermix (**GREEN**)	2x	5.0
SARS-CoV-2 Mix (**BROWN**)	20x	0.5
RTase (**NEUTRAL**)	100x	0.1
RNase inhibitor (**BLACK**)	50x	0.2
Total volume (µl)		7.5
Add 2.5 µl sample for a final volume of 10 µl		

11 Programming and analysis

Details for programming and analysis are described in the **Sections 19-21**.

The ***Plex****PCR*® SARS-CoV-2 kit uses 3 channels for detection of SARS-CoV-2 via the Open Reading Frame (ORF1ab) and RNA-dependent RNA polymerase (RdRp) genes and Internal Control (**Table 8**).

Table 8. Channels for *PlexPCR*® SARS-CoV-2 targets

qPCR Instrument	ORF1ab	RdRp gene	Internal Control
LC480 II	465-510	533-580	533-610
CFX96 Dx & **CFX96 Touch**	FAM	HEX	Texas Red

12 Interpretation of results

Data interpretation may be performed using the LC480 II on board software, CFX96™ Dx and CFX96™ Touch on board software, or the ***Plex****PCR*® SARS-CoV-2 analysis software. The ***Plex****PCR*® SARS-CoV-2 analysis software automates the data interpretation of amplification results and streamlines workflow. Instructions for how to use the analysis software are described in **Section 21**.

See **Table 9** for the appropriate analysis software for each real-time PCR instrument. The analysis software can be supplied on request. Please contact tech@speedx.com.au for more information.

Table 9. *PlexPCR*® SARS-CoV-2 analysis software		
Cat no	**Analysis software***	**Real-time PCR instrument**
99021	***Plex****PCR*® SARS-CoV-2 (LC480)	LC480 II
99022	***Plex****PCR*® SARS-CoV-2 (CFX)	CFX96 Dx & CFX96 Touch

* Refer to the website https://plexpcr.com/products/respiratory-infections/plexpcr-sars-cov-2/ to ensure you are using the most current version of analysis software.

13 Limitations

- The ***Plex****PCR*® SARS-CoV-2 assay should only be performed by personnel trained in the procedure and should be performed in accordance with the Instructions for Use.
- Reliable results are dependent on adequate specimen collection transport, storage, and processing. Failure to observe proper procedures in any one of these steps can lead to incorrect results.
- The ***Plex****PCR*® SARS-CoV-2 assay is a qualitative assay and does NOT provide quantitative values or information about organism load.
- Results from the test must be correlated with the clinical history, epidemiological data, laboratory data and any other data available to the clinician.
- Prevalence of viral targets will affect the positive and negative predictive values for the assay.
- Negative results do not exclude the possibility of infection due to improper specimen collection, technical error, presence of inhibitors, specimen mix up, or low numbers of organisms in the clinical specimen.
- False positive results may occur due to cross-contamination by target organisms, their nucleic acids or amplified product.

Clinical samples with Cq value < 3 may not give a valid result. These samples will be flagged by the ***Plex****PCR*® SARS-CoV-2 analysis software with the following message “Error: Abnormal change in fluorescence level”. This is indicative of high load SARS-CoV-2 sample above the detection limit, and such samples should be diluted and repeated.

These samples will also be flagged when analysing on the on-board LC480 II software with the following message “Some samples exceed the noiseband value in the background calculation region”. This is indicative of high load SARS-CoV-2 sample above the detection limit, and such samples should be diluted and repeated.

Clinical samples may appear invalid if they are of a high viral load, this is not flagged by the on-board CFX software, so the user must check all curves before proceeding. When a high load SARS-CoV-2 sample exceeds the detection limit, samples should be diluted and repeated.

14 Quality control

The ***Plex****PCR*® SARS-CoV-2 kit includes an internal control to monitor extraction efficiency and qPCR inhibition (**Section 10.3**).

The REDx™ FLOQ SARS-CoV-2 Swab Positive Control (Microbix, Cat no RED-S-19-01) is recommended as positive control material for nucleic acid amplification. Refer to **Section 15** for instructions to use REDx™ FLOQ SARS-CoV-2 Swab Positive Control. A known negative specimen is recommended to be used as a negative control.

15 REDx™ FLOQ SARS-CoV-2 Positive Control instructions

The REDx™ FLOQ SARS-CoV-2 Swab Positive Control (Microbix, Cat no RED-S-19-01) contains positive control material for SARS-CoV-2.

The REDx™ SARS-CoV-2 Positive Controls should be stored at 2-8°C until use. Once opened the REDx™ SARS-CoV-2 Positive Control should not be reused.

Please see the REDx™ SARS-CoV-2 Positive Control package insert for further information on storage and limitations.

15.1 Instructions for use

Dilute the REDx™ SARS-CoV-2 Positive Control in 3mL of Universal Transport Media (UTM) or Viral Transport Media (VTM).

Prepare qPCR reactions as described in **Section 10.4** using positive control material as sample.

16 Performance characteristics

16.1 Clinical performance

16.1.1 Clinical Study 1

A retrospective clinical study was conducted at Queensland Paediatric Infectious Diseases Laboratory (QPID), South Brisbane, QLD, Australia, on archived nasopharyngeal swab samples (n=165) previously tested with the Abbott m2000 SARS-CoV-2 assay. Samples were extracted on the MagNA Pure 96 (Roche) extraction platform using the Pathogen Universal 200 protocol. 200 µl of samples were extracted and eluted in 50 µl. Samples were tested with the ***Plex****PCR*® SARS-CoV-2 kit in 10 µl reactions on the LightCycler 480 II.

A composite reference result approach was used as the reference method for the ***Plex****PCR*® SARS-CoV-2 assay. Results of two validated SARS-CoV-2 PCR assays (Abbott m2000 SARS-CoV-2 assay and Real-time fluorescent RT-PCR Kit for detection of SARS-CoV-2 (BGI)) were analysed and samples generating concordant results in the two assays considered as SARS-CoV-2 positive or negative. The SARS-COV-2 status of samples generating discordant results between the two comparator assays (n=22) could not be definitively determined and these samples were excluded from the final analysis. Positive and negative percent agreement between ***Plex****PCR*® SARS-CoV-2 and the composite reference are shown in **Table 10**.

Table 10. Clinical evaluation of the *PlexPCR*® SARS-CoV-2 kit

		Composite Reference Result (n=142)	
		SARS-CoV-2	
		Positive	Negative
Plex*PCR*® **SARS-CoV-2**[1]	**Positive**	83	2
	Negative	6	51
Positive Percent Agreement (PPA)		93.26% (95% CI 85.90 – 97.49%)	
Negative Percent Agreement (NPA)		96.23% (95% CI 87.02 – 99.54%)	
Overall Rate of Agreement (ORA)		94.37% (95% CI 89.20 – 97.54%)	

[1]One sample was repeatedly invalid in the ***Plex****PCR*® SARS-CoV-2 assay and could not be evaluated.

16.2 Analytical performance

16.2.1 Repeatability and Reproducibility

16.2.1.1 LightCycler® 480 Instrument II

A repeatability and reproducibility study was performed across lots, operators, days, and LightCycler® 480 II instruments for the ***Plex****PCR*® SARS-CoV-2 assay, using panels prepared in pooled negative clinical nasopharyngeal swabs collected in Viral Transport Media (VTM). Panel members consisted of SARS-CoV-2 strain USA-WA1/2020 (ZeptoMetrix, NATtrol™ SARS-CoV-2 Stock, Cat no NATSARS(COV2)-ST) reference material spiked into negative nasopharyngeal swabs collected in VTM at 5x LOD, 50x LOD and 100x LOD. Each panel contained six replicates of these panel members.

Testing was performed with two different lots of ***Plex****PCR*® SARS-CoV-2 mix. Panels were tested twice daily over three non-consecutive days by two operators on site, generating a total of 36 observations per panel member (6 replicates x 2 runs x 3 days x 1 site = 36 observations).

Between-lot, between-day, between-instrument, between-operator repeatability and total reproducibility was assessed. Percent agreement was calculated for each panel member based on the expected result in the SARS-CoV-2 detection component of the assay. Percent coefficient of variation (%CV) was calculated from the cycle quantification (C_q) value reported for SARS-CoV-2 detection. Results of repeatability and reproducibility testing are shown in **Table 11**.

Table 11. Repeatability/Reproducibility of the SARS-CoV-2 detection component of the *PlexPCR*® SARS-CoV-2 assay on the LightCycler® 480 Instrument II										
SARS-CoV-2 – ORF1ab										
			Within-run		**Between-run**		**Between-lot**		**Total**	
Panel Member	**N**	**Mean C_q**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**
100x LOD	36	18.6	0.52	2.8	0.31	1.7	0.51	2.7	0.5	2.7
50x LOD	36	19.4	0.53	2.7	0.28	1.5	0.58	3	0.52	2.7
5x LOD	36	22.6	0.91	4	0.53	2.3	0.84	3.7	0.98	4.3
SARS-CoV-2 – RdRp										
			Within-run		**Between-run**		**Between-lot**		**Total**	
Sample ID	**N**	**Mean C_q**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**
100x LOD	36	19.1	0.4	2.1	0.24	1.3	0.31	1.6	0.36	1.9
50x LOD	36	19.9	0.41	2.1	0.19	1	0.36	1.8	0.36	1.8
5x LOD	36	23.2	0.51	2.2	0.31	1.3	0.39	1.7	0.57	2.5
Internal Control										
			Within-run		**Between-run**		**Between-lot**		**Total**	
Sample ID	**N**	**Mean C_q**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**
100x LOD	36	19.3	0.36	1.9	0.45	2.3	0.3	1.6	0.51	2.6
50x LOD	36	19.5	0.42	2.2	0.41	2.1	0.4	1.8	0.52	2.7
5x LOD	36	19.5	0.67	3.4	0.54	2.7	0.5	2.2	0.69	3.4
Negative	36	20.4	0.35	1.7	0.93	4.6	0.2	0.8	0.89	4.4

16.2.1.2 CFX96™ Dx Real-Time PCR Detection and CFX96 Touch™ Real-Time PCR Detection Systems

A repeatability and reproducibility study was performed across lots, operators, days and runs on CFX96™ Touch Real-Time PCR Detection Systems for the ***Plex****PCR*® SARS-CoV-2 assay, using panels prepared in pooled negative clinical nasopharyngeal swabs collected in Viral Transport Media (VTM). Panel members consisted of SARS-CoV-2 strain USA-WA1/2020 (ZeptoMetrix, NATtrol™ SARS-CoV-2 Stock, Cat no NATSARS(COV2)-ST) reference material spiked into negative nasopharyngeal swabs collected in VTM at 5x LOD, 50x LOD and 100x LOD. Each panel contained six replicates of these panel members.

Testing was performed with two different lots of ***Plex****PCR*® SARS-CoV-2 mix. Panels were tested three times daily over three non-consecutive days by two operators on site, generating a total of 108 observations per panel member.

Within-run, between-run, between-lot, between-operator, between-instrument and total reproducibility was assessed. Percent agreement was calculated for each panel member based on the expected result in the SARS-CoV-2 detection component of the assay. Percent coefficient of variation (%CV) was calculated from the cycle quantification (C_q) value reported for SARS-CoV-2 detection. Results of repeatability and reproducibility testing are shown in **Table 12**.

Table 12. Repeatability/Reproducibility of the SARS-CoV-2 detection component of the *PlexPCR*® SARS-CoV-2 assay on the CFX96 Touch™ Real-Time PCR Detection System														
SARS-CoV-2 – ORF1ab														
			Within-run		**Between-run**		**Between-lot**		**Between-operator**		**Between-instrument**		**Total**	
Panel Member	**N**	**Mean C_q**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**
100x LOD	108	19.18	0.27	1.5	0.41	2.2	0.65	3.4	0.85	4.4	0.17	0.9	1.14	5.9
50x LOD	108	20.20	0.05	0.2	0.42	2.1	0.67	3.3	0.82	4.0	0.13	0.6	1.18	5.9
5x LOD	108	22.78	0.37	1.7	0.45	2.0	0.41	1.8	0.72	3.2	0.28	1.2	1.19	5.2
SARS-CoV-2 – RdRp														
			Within-run		**Between-run**		**Between-lot**		**Between-operator**		**Between-instrument**		**Total**	
Sample ID	**N**	**Mean C_q**	SD	%CV	SD	%CV	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**
100x LOD	108	19.80	0.12	0.6	0.35	1.8	0.63	3.2	0.85	4.3	0.16	0.8	1.15	5.8
50x LOD	108	20.73	0.22	1.1	0.22	1.1	0.67	3.2	0.85	4.1	0.18	0.9	1.23	5.9
5x LOD	108	23.18	0.39	1.7	0.24	1.0	0.53	2.3	0.61	2.6	0.07	0.3	1.09	4.7
Internal Control														
			Within-run		**Between-run**		**Between-lot**		**Between-operator**		**Between-instrument**		**Total**	
Sample ID	**N**	**Mean C_q**	SD	%CV	SD	%CV	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**
100x LOD	108	20.34	0.24	1.2	0.51	2.5	0.28	1.4	0.23	1.1	0.06	0.3	0.79	3.9
50x LOD	108	20.75	0.29	1.4	0.75	3.6	0.20	0.9	0.18	0.9	0.01	0.0	0.74	3.6
5x LOD	108	20.98	0.26	1.2	0.76	3.6	0.11	0.5	0.12	0.6	0.05	0.2	0.69	3.3
Negative	108	21.32	0.22	1.0	0.80	3.7	0.10	0.4	0.14	0.6	0.04	0.2	1.01	4.8

16.2.2 Analytical sensitivity

16.2.2.1 LightCycler® 480 Instrument II

SARS-CoV-2 strain USA-WA1/2020 (ZeptoMetrix, NATtrol™ SARS-CoV-2 Stock, Cat no NATSARS(COV2)-ST) was used as the representative strain to assess the limit-of-detection (LoD) of the ***PlexPCR***® SARS-CoV-2 assay on the LightCycler® 480 II Instrument. Quantitated preparations of positive reference material of SARS-CoV-2 were serially diluted into negative nasopharyngeal swabs in VTM. A total of 7 concentration levels were tested over multiple days using 2 independent lots of ***PlexPCR***® SARS-CoV-2 assay reagents for a total of 40 replicates per concentration. The LoD was determined using logistic regression analysis (Probit model) as the lowest concentration (expressed as copies / mL) generating a minimum of ≥ 95% positive replicates.

The LoD value (determined from the data shown in **Table 13**) was 764 copies/mL (95% CI: 565.69 – 1193.50 copies/mL)**.**

Table 13. LoD of the *PlexPCR*® SARS-CoV-2 assay$^{\phi}$					
Positive reference material	**Strain**	**SARS-CoV-2 concn. (genomes per mL)**	***PlexPCR*® SARS-CoV-2 Result**		
			Positive	**Total**	**% Positive**
SARS-CoV-2	USA-WA1/2020	2500	40	40	100.00
		1875	40	40	100.00
		1250	40	40	100.00
		625	36	40	90.00
		313	27	38*	71.05
		156	22	40	55.00
		78	10	40	25.00

$^{\phi}$ Equivalent analytical sensitivity was obtained using the CFX96 systems
* For the 312.5 copies/mL concentration, 2 replicates were reported invalid by the analysis software due to IC failure and thus were excluded from analysis.

16.2.2.2 Workflow with the MGISP-960 & LightCycler® 480 Instrument II

A study was conducted at Queensland Paediatric Infectious Diseases Laboratory (QPID), South Brisbane, QLD, to demonstrate that the analytical performance of the ***PlexPCR***® SARS-CoV-2 assay when samples are extracted using the MGISP-960 instrument (MGI) with the MGIEasy Nucleic Acid Extraction kit (PID: 1000020471; MGI) is equivalent to the analytical performance of the assay when samples are extracted using the MagNa Pure 96 (MP96) instrument (Roche) with the MagNA Pure 96 DNA and Viral NA Small Volume Kit (PID: 06543588001; Roche). Negative reference material consisted of pooled negative nasopharyngeal (NP) swabs in viral transport media (VTM) collected from SARS-CoV-2 negative individuals (**FDA Emergency Use Authorization COVID-19 Molecular Diagnostic Template for Commercial Manufacturers**). Positive reference material consisted of SARS-CoV-2 strain USA-WA1/2020 (ZeptoMetrix, NATtrol™ SARS-CoV-2 Stock, Cat no NATSARS(COV2)-ST) spiked into negative matrix at 2x LOD.

For each MGIEasy Nucleic Acid Extraction kit tested, the percentage hit rate of correctly identified samples was calculated. The results are summarised in **Table 14 .** The mean Cq value, standard deviation, and coefficient of variation (%) of each target (ORF1ab, RdRp, and IC) for each extraction kit is detailed in **Table 15.** The IC was valid for all samples. The hit rate for each MGIEasy Nucleic Acid Extraction kit was ≥95%, which confirms the LOD of the ***PlexPCR***® SARS-CoV-2 assay when used with samples extracted using the MGISP-960 instrument (MGI).

Table 14. Hit Rate (%) Samples Extracted with MGISP-960					
Samples	**Total number of replicates**	**Extraction kit 1**		**Extraction kit 2**	
		Number of correctly identified replicates	**Hit rate (%)**	**Number of correctly identified replicates**	**Hit rate (%)**
SARS-CoV-2 Positive Samples (2X LOD)	30	30	100	30	100
SARS-CoV-2 Negative Samples	60	60	100	60	100

Table 15. Summary table for Mean Cq values, Standard deviations and %CV for all targets.									
	Extraction Lot 1								
	ORF1ab (465-510)			RdRp (533-580)			IC (533-610)		
Sample Type	**Mean Cq**	**SD**	**%CV**	**Mean Cq**	**SD**	**%CV**	**Mean Cq**	**SD**	**%CV**
SARS Positive	21.06	0.34	1.61	22.19	0.39	1.76	21.38	0.32	1.51
SARS Negative	--	--	--	--	--	--	21.62	0.44	2.05
	Extraction Lot 2								
	ORF1ab (465-510)			RdRp (533-580)			IC (533-610)		
Sample Type	**Mean Cq**	**SD**	**%CV**	**Mean Cq**	**SD**	**%CV**	**Mean Cq**	**SD**	**%CV**
SARS Positive	22.20	0.38	1.70	23.27	0.41	1.76	21.44	0.34	1.60
SARS Negative	--	--	--	--	--	--	21.87	0.23	1.03

16.2.3 Analytical specificity

A panel of 20 microorganisms including organisms commonly found in the human respiratory tract, as well as those closely related to SARS-CoV-2, were evaluated for evidence of cross-reactivity in the ***Plex****PCR*® SARS-CoV-2 assay. This study was carried out on the LightCycler® 480 Instrument II. A list of organisms tested is shown in **Table 16**. Organisms were tested at 1 x 10^6 cfu/mL,1 x 10^5 pfu/mL or 10^5 $TCID_{50}$ per mL unless otherwise indicated, with all dilutions prepared in negative nasopharyngeal swabs in VTM. Testing was performed in triplicate in the absence of the positive reference material (SARS-CoV-2). No positive signals were generated in the ***Plex****PCR*® SARS-CoV-2 assay in any of these experiments in the absence of target and there was no impact observed on the performance of the assay in the presence of high concentrations of any microorganism tested.

Table 16. Microorganisms tested for cross-reactivity	
Organisms	**Concentration tested**
Human coronavirus 229E	5.00E+06 genomes/mL
Human coronavirus OC43	5.00E+06 genomes/mL
Adenovirus 1	1.00E+05 $TCID_{50}$/mL
Parainfluenza Virus 3	1.00E+05 $TCID_{50}$/mL
Influenza A Virus	1.00E+05 PFU/mL
Influenza B Virus	1.00E+05 PFU/mL
Enterovirus A71	1.00E+05 $TCID_{50}$/mL
Respiratory Syncytial Virus A	1.00E+05 PFU/mL
Rhinovirus 17	1.00E+05 $TCID_{50}$/mL

Table 16. Microorganisms tested for cross-reactivity	
Organisms	**Concentration tested**
Chlamydophila pneumoniae	1.00E+06 CFU/mL
Haemophilus influenzae	5.00E+06 genomes/mL
Streptococcus pneumoniae	1.00E+06 CFU/mL
Streptococcus pyogenes	1.00E+06 CFU/mL
Bordetella pertussis	1.45E+05 genomes/mL
Mycoplasma pneumoniae	1.00E+06 CFU/mL
Pooled human nasal wash	neat
Candida albicans	1.00E+06 CFU/mL
Pseudomonas aeruginosa	1.00E+06 CFU/mL
Staphylococcus epidermidis	1.00E+06 CFU/mL
Streptococcus salivarius	2.51E+08 genomes/mL

16.2.4 *In silico* analysis

In silico analysis was performed to evaluate the potential for cross-reactivity of primers and probes included in the ***Plex****PCR*® SARS-CoV-2 assay with additional human and non-human coronaviruses. The ***Plex****PCR*® SARS-CoV-2 assay did not have any predicted cross-reactivity with non-coronavirus or other human coronavirus sequences based on a homology threshold of >80%.

Specificity against non-coronavirus sequences

The ORF1ab and RdRp assay oligo sequences were used to search for non-coronavirus sequences that closely matched the target region to assess the potential for cross-reactivity. No significant cross-reactivity with non-coronavirus organisms was observed with any of the assay oligos.

Specificity against other coronaviruses

The BLAST run with the RdRp assay amplicon resulted in 3,027 coronavirus sequences. When analysed with CLC main workbench 20.0.4, the only sequences where the assay oligos are able to bind are synthetic SARS-CoV-2 constructs and two bat coronavirus sequences (MN996532.1 and KP876546.1). Thus, no cross-reactivity with other human coronavirus sequences was observed.

The BLAST run with the ORF1ab assay amplicon resulted in 272 coronavirus sequences. When analysed with CLC main workbench 20.0.4, the only sequences where the assay oligos are able to bind are synthetic SARS-CoV-2 constructs. Thus, no cross-reactivity with other human coronavirus sequences was observed.

16.2.5 Inclusivity

The GISAID EpiCoV database was queried on 1 June 2020. The resulting dataset contained 24462 SARS-CoV-2 genome sequences for the ORF1ab assay and RdRp assay.

To demonstrate inclusivity of the ***Plex****PCR*® SARS-CoV-2 assay, the GISAID EpiCoV was interrogated independently with each of the oligonucleotide primers and probes included in the assay. Less than 0.2% of SARS-CoV-2 sequences in the database (n >24,0000 as of June 1, 2020) had more than 1 mismatch with any of the primers and probes included in the ***Plex****PCR*® SARS-CoV-2 assay. Monitoring is ongoing to ensure continued inclusivity to current strains and reported variants. Please contact tech@speedx.com.au for more information.

16.2.6 Potentially interfering substances

Potentially interfering endogenous and exogenous substances that might be present in respiratory specimens were assessed for their impact on the performance of the ***Plex****PCR*® SARS-CoV-2 assay. This study was carried out on the LightCycler® 480 Instrument II. All substances were tested in triplicate using negative nasopharyngeal swabs in VTM in the presence and the absence of the target. There was no evidence of a negative impact on assay performance when contrived samples containing the potential interferents at the indicated concentrations were tested. Results are summarised in **Table 17.**

Table 17. Potentially interfering substances in respiratory samples	
Potential Interferent	**Test concentration**
Phenylephrine	15% w/v
Beclomethasone dipropionate	5% v/v
Zanamivir	3.3 mg/mL
Ribavirin	2% w/v
Mupirocin	6.6 mg/mL
Tobramycin, aminoglycoside antibiotic	4.4 µg/mL
Menthol	6.9 mg/mL

17 Customer and technical support

Please contact Technical Support for questions on reaction setup, cycling conditions and other enquiries.

Tel: +61 2 9209 4169, Email: tech@speedx.com.au

18 References

1. Novel Coronavirus (2019-nCoV) Situation Report – 1, 21 January 2020. World Health Organisation. Found at: https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200121-sitrep-1-2019-ncov.pdf.
2. Naming the coronavirus disease (COVID-19) and the virus that causes it. World Health Organisation. Found at: https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance/naming-the-coronavirus-disease-(covid-2019)-and-the-virus-that-causes-it.
3. COVID-19 Dashboard by the Center for Systems Science and Engineering (CSSE) at Johns Hopkins University. Found at: https://coronavirus.jhu.edu/map.html.

19 Appendix 1: LightCycler® 480 Instrument II

The following information is based on LightCycler 480 software (version 1.5).

The ***Plex****PCR*® SARS-CoV-2 kit contains dyes for the LightCycler® 480 Instrument II. The ***Plex****PCR*® Colour Compensation kit (Cat no 90001) must be run and applied for LC480 II analysis (see **Section 19.3**). This kit can be supplied on request.

19.1 Programming the LightCycler® 480 Instrument II (LC480 II)

Detection Format

Create a custom **Detection Format**

Open Tools > Detection Formats

Create a New Detection Format, and name '**SpeeDx Plex PCR**' (may be created during the generation of SpeeDx Colour Compensation file) (see **Figure 2**).

For **Filter Combination Selection** select the following (Excitation-Emission):

Table 18. Filter combinations^

LC480 II	440-488	465-510	533-580	533-610	533-640	618-660

^These Filter Combinations are the default names for the channels

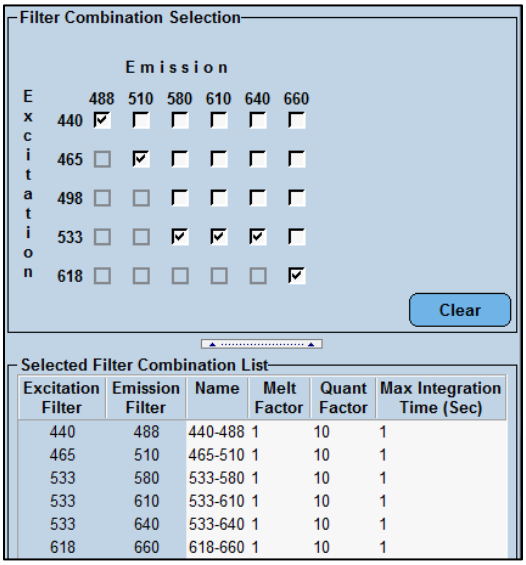
Set the **Selected Filter Combination List for all channels as:**

Melt Factor: 1

Quant Factor: 10

Max Integration Time (sec): 1

Figure 2. Custom SpeeDx Detection Format



Instrument Settings

Create a custom **Detection Format**

Open Tools > **Instruments**

For **Instrument Settings** > select **Barcode Enabled**

Experiment setup

Select **New Experiment**

In the **Run Protocol** tab

For **Detection Format** select the custom **'SpeeDx PlexPCR'** (**Figure 3**)

Select **Customize** >

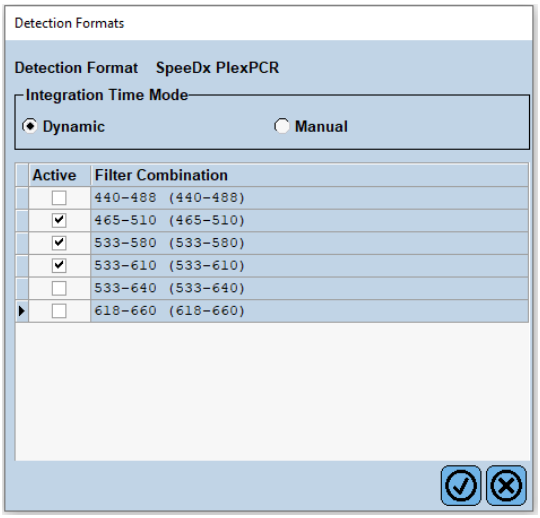
Select **Integration Time Mode** > **Dynamic**

Select the following Active **Filter Combinations** shown in **Table 19**

Table 19. Channels for *PlexPCR*® SARS-CoV-2 targets

Channel	465-510	533-580	533-610
SARS-CoV-2	ORF1ab	RdRp	Internal Control

Figure 3. Customise Detection Format



To enable automated sample detection in the analysis software, assign nametags to the wells on the plate (see **Section 21.4**)

Open the **Sample Editor** module

Select well

Edit **Sample Name** to match nametag defined in Assays module of the analysis software (see **Section 21.4**)

Samples are labelled as *Prefix_Suffix* (as shown in **Table 20** and **Figure 4**) e.g. NEG_CoV

NOTE: Sample nametags are case sensitive. The nametag must match exactly to those assigned in the run file.

Table 20. Sample nametags for analysis software

Sample type	Prefix_ (in analysis software)	Suffix_ (in analysis software)	Sample name (in analysis software)
Regular sample	Sample	_CoV	Sample_CoV
Negative control	N	_CoV	N_CoV
Positive control	Pa	_CoV	Pa_CoV

Figure 4. Sample Editor – Assigning nametags to wells

Pos	Filter combination	Color	Repl Of	Sample Name
A12	465-510 (465-510)			S_MG
A12	533-580 (533-580)			S_MG
A12	533-640 (533-640)			S_MG
B12	465-510 (465-510)			Pa_MG
B12	533-580 (533-580)			Pa_MG
B12	533-640 (533-640)			Pa_MG
C12	465-510 (465-510)			Pb_MG
C12	533-580 (533-580)			Pb_MG
C12	533-640 (533-640)			Pb_MG
G8	465-510 (465-510)			N_MG
G8	533-580 (533-580)			N_MG
G8	533-640 (533-640)			N_MG

Set **Reaction Volume** > 10µl

Create the following program (shown in more detail in **Figure 5 – Figure 9**)

Table 21. Thermocycling Program

Program Name	Cycles	Target °C	Hold	Ramp Rate (°C/s)≠	Ramp Rate (°C/s)§
Reverse transcription	1	48°C	10 min	4.4	4.8
Polymerase activation	1	95°C	2 min	4.4	4.8
Touch down cycling$^{\delta}$: Step down -0.5°C/cycle	10	95°C	5 s	4.4	4.8
		61°C – 56.5°C$^{\delta}$	30 s	2.2	2.5
Quantification cycling^{+}: Acquisition/Detection	40	95°C	5 s	4.4	4.8
		52°C^{+}	50 s	2.2	2.5
Cooling	1	40°C	30 s	2.2	2.5

≠ *Default ramp rate (96 well plate)*

§ *Default ramp rate (384 well plate)*

$^{\delta}$ ***Step size:*** *-0.5°C/Cycle,* ***Sec Target:*** *56°C*

$^{+}$ ***Analysis mode:*** *Quantification,* ***Acquisition mode****: Single*

> Start Run

Figure 5. Thermocycling Program – Reverse Transcription

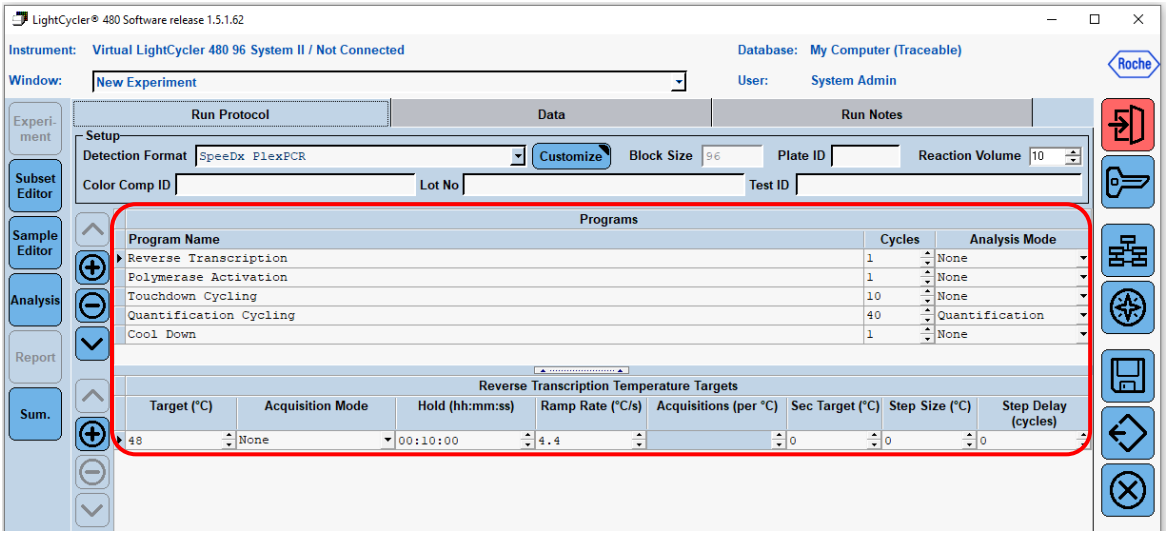


Figure 6. Thermocycling Program – Polymerase Activation

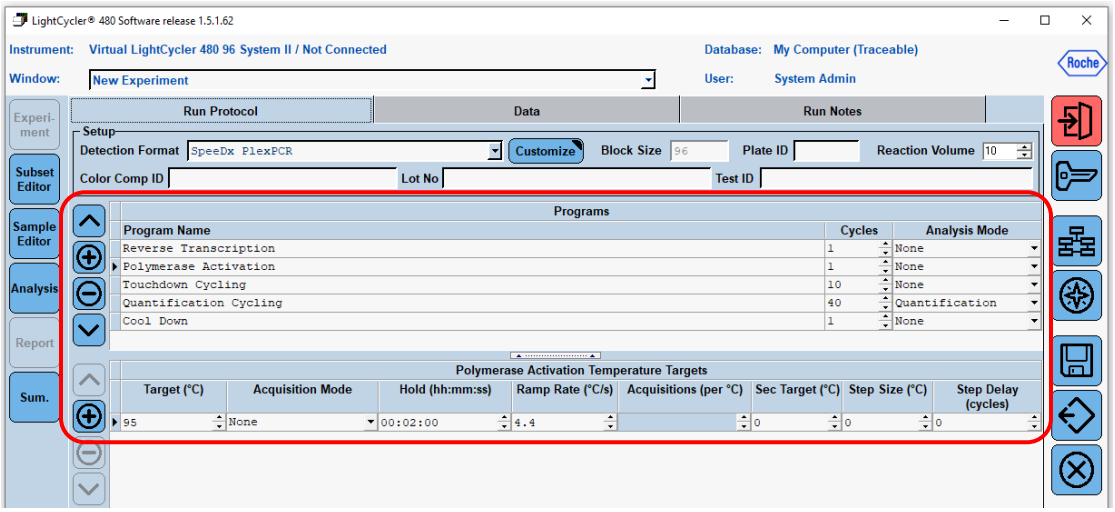


Figure 7. Thermocycling Program – Touchdown Cycling

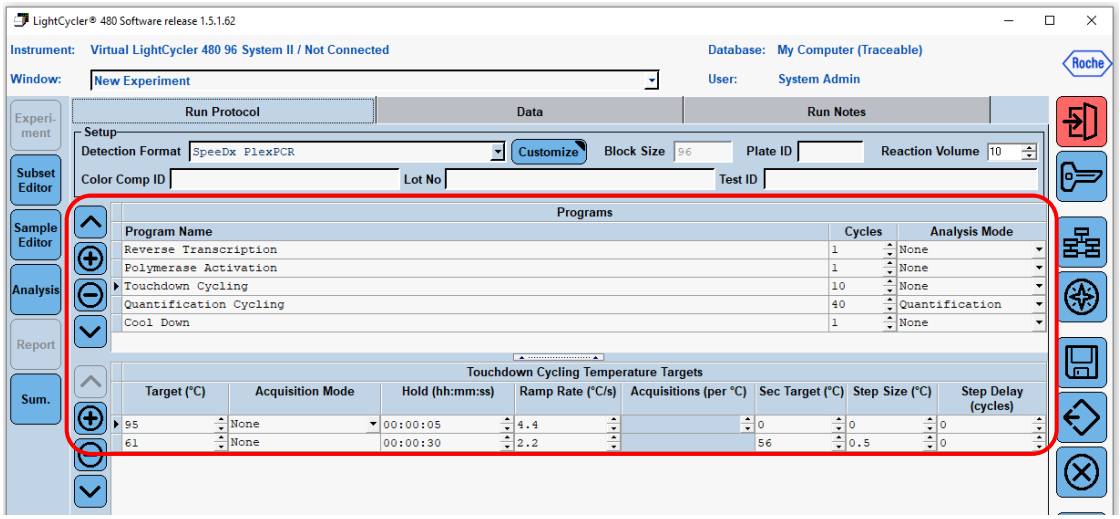


Figure 8. Thermocycling Program – Quantification Cycling

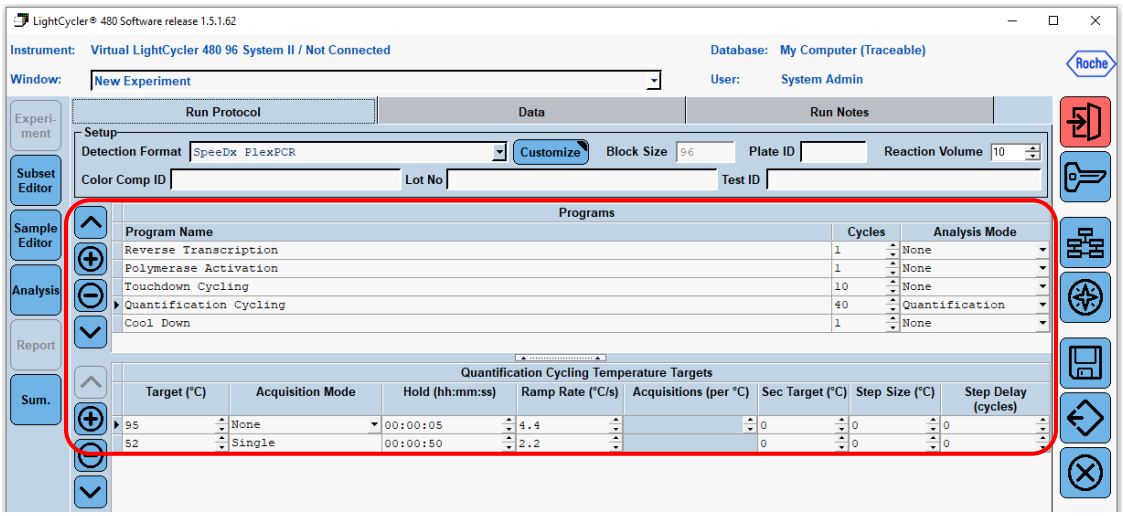
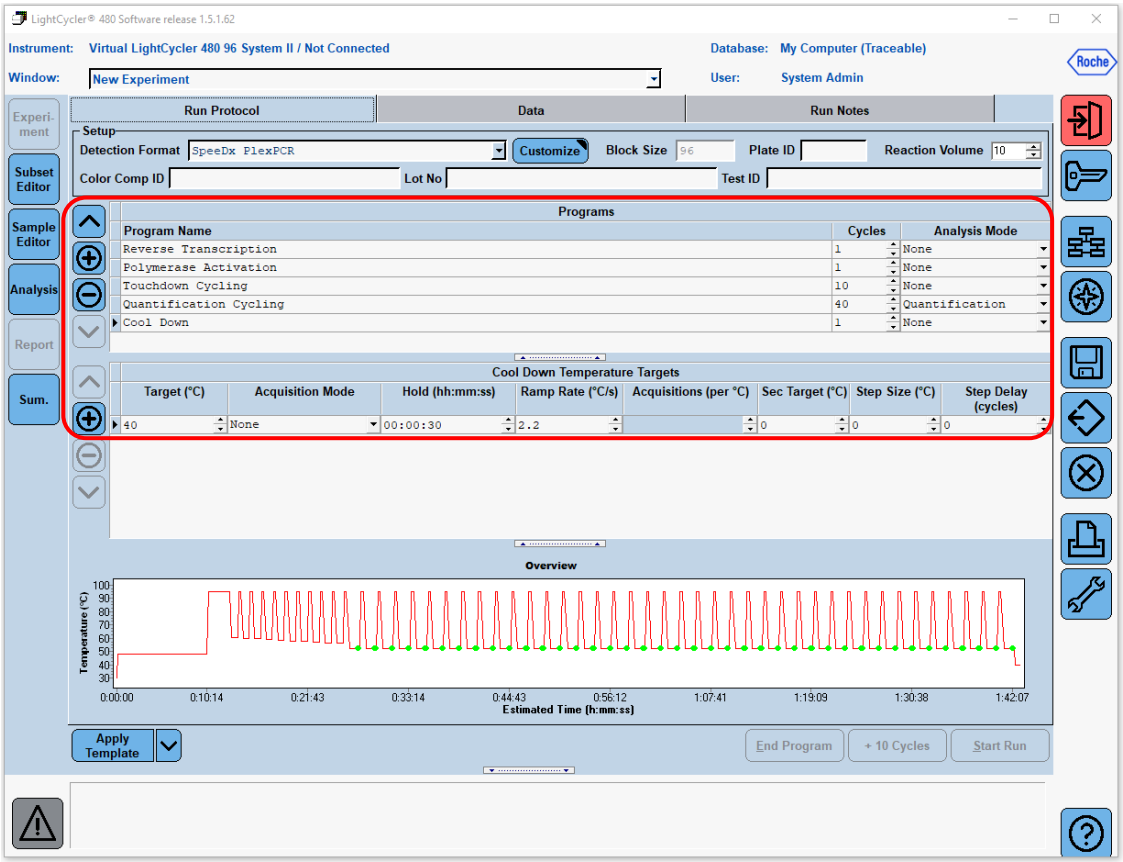


Figure 9. Thermocycling Program – Cooling



When the cycling program has finished, export a .ixo file for analysis in the ***PlexPCR***® SARS-CoV-2 (LC480) analysis software.

Select **Export**

Save in an easily identifiable location

19.2 Setting up a Macro Template for the LightCycler® 480 Instrument II

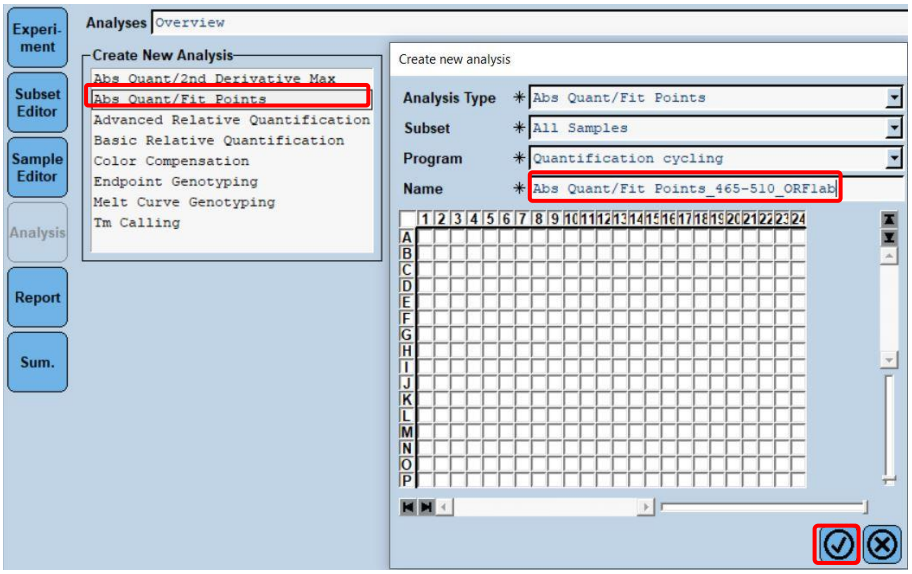
Data interpretation may be performed using the LC480 II onboard software by using a Macro template with the validated parameters provided below. For further assistance, please contact tech@speedx.com.au.

Macro Template settings

Select a run file with the **SpeeDx PlexPCR Cycling** parameters

Select **Analysis** > **Abs Quant/Fit Points** > edit the name to **Abs Quant/Fit Points_465-510_ORF1ab** > **Ok**

Figure 10. Abs Quant/Fit Points - 465-510 ORF1ab

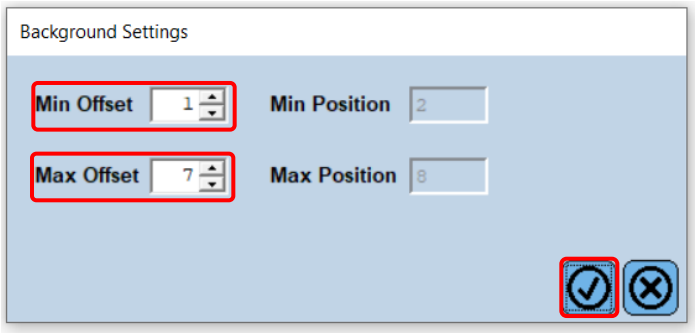


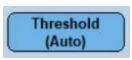
Select **Filter Comb 465 – 510**

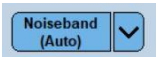
Apply the **Colour Compensation** for all channels > **Ok**

Select the **Cycle Range** tab > **Background settings** > edit the **Min Offset** and **Max Offset** > **Ok**

Figure 11. Background Settings - 465-510 ORF1ab

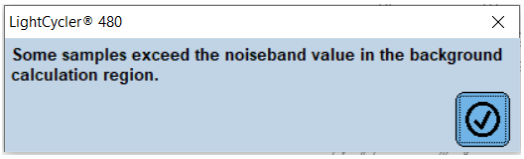


Select the **Analysis** tab and ensure the following setting is selected 

Select the **Noise Band** tab and ensure the following setting is selected 

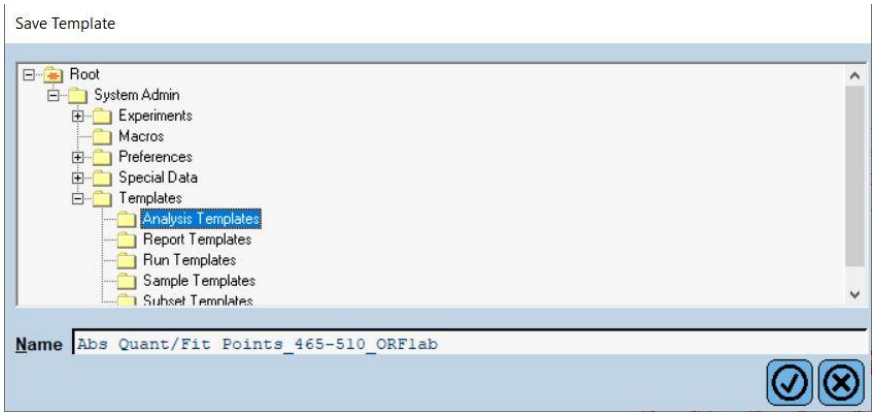
Click **Calculate** (*if a sample curve has crossed the background region the following message shown in **Figure 12** will appear; the user must dilute and re-test the sample)* > **Ok** to continue the analysis

Figure 12. Noiseband Warning Message



Select **Save As Template** using the folder **Templates** > **Analysis Templates** and include the channel and target in the naming format > **Ok**

Figure 13. Saving Analysis Template Abs Quant/Fit Points - 465-510 ORF1ab

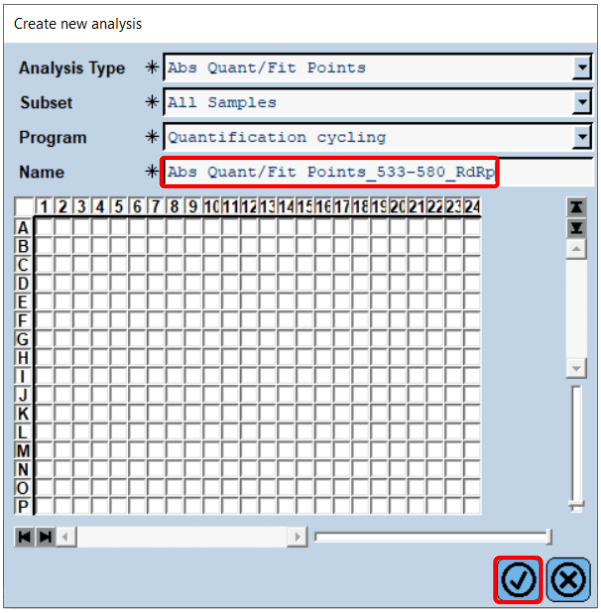


Click on the  icon to save the analysis parameters set for the channel

Click on the  icon to create a **new analysis**

Select **Abs Quant/Fit Points** > edit the name to **Abs Quant/Fit Points_533-580_RdRp** > **Ok**

Figure 14. Abs Quant/Fit Points 533-580 RdRp

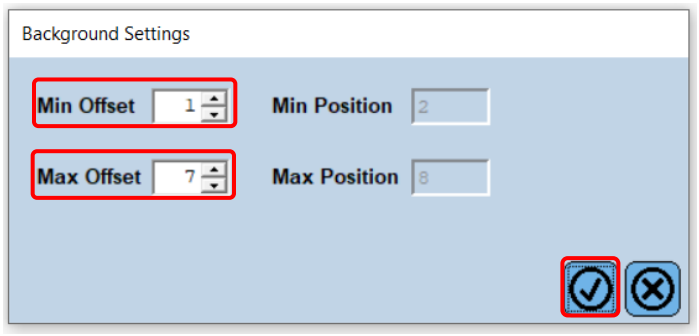


Select **Filter Comb 533 – 580**

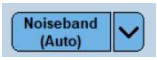
Apply the **Colour Compensation** for all channels > **Ok**

Select the **Cycle Range** tab > **Background settings** > edit the **Min Offset** and **Max Offset** > **Ok**

Figure 15 Background Settings - 533-6580 RdRp

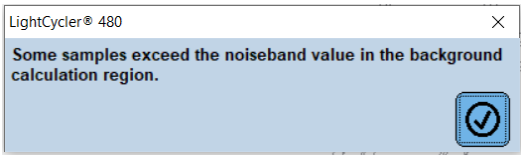


Select the **Analysis** tab and ensure the following setting is selected 

Select the **Noise Band** tab and ensure the following setting is selected 

Click **Calculate** (*if a sample curve has crossed the background region the following message in* ***Figure 16*** *will appear; the user must dilute and re-test the sample)* > **Ok** to continue the analysis

Figure 16. Noiseband Warning Message



Select **Save As Template** using the folder **Templates** > **Analysis Templates** and include the channel and target in the naming format > **Ok**

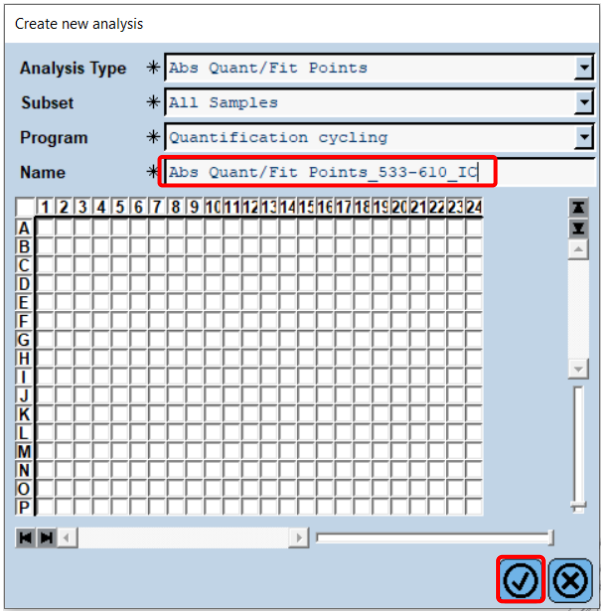
Figure 17. Saving Analysis Template Abs Quant/Fit Points – 533-580 RdRp

Click on the  icon to save the analysis parameters set for the channel

Click on the  icon to create a **new analysis**

Select **Abs Quant/Fit Points** > edit the name to **Abs Quant/Fit Points_533-610_IC** > **Ok**

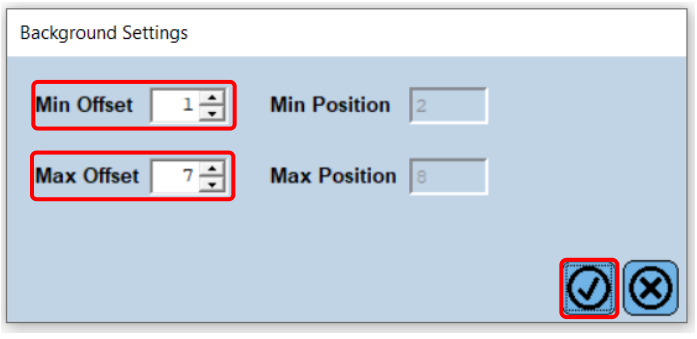
Figure 18. Abs Quant/Fit Points 533-610 Internal Control

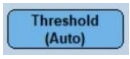


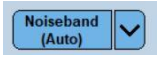
Select **Filter Comb 533 – 610**

Select the **Cycle Range** tab > **Background settings** > edit the **Min Offset** and **Max Offset** > **Ok**

Figure 19. Background Settings – 533-610 Internal Control



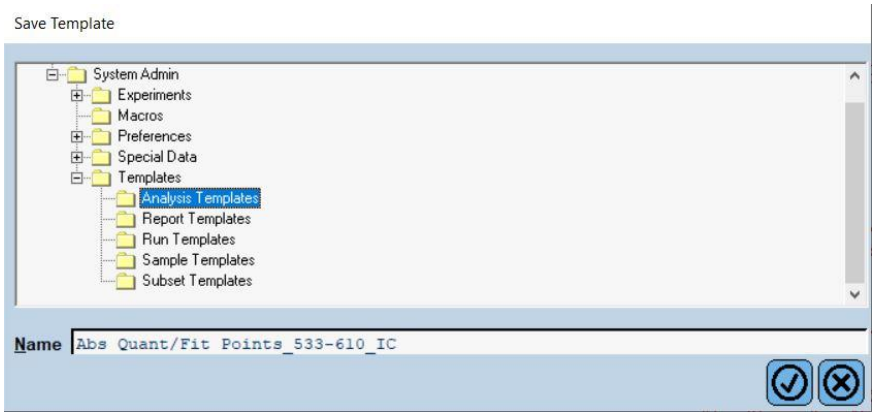
Select the **Analysis** tab and ensure the following setting is selected 

Select the **Noise Band** tab and ensure the following setting is selected 

Click **Calculate**

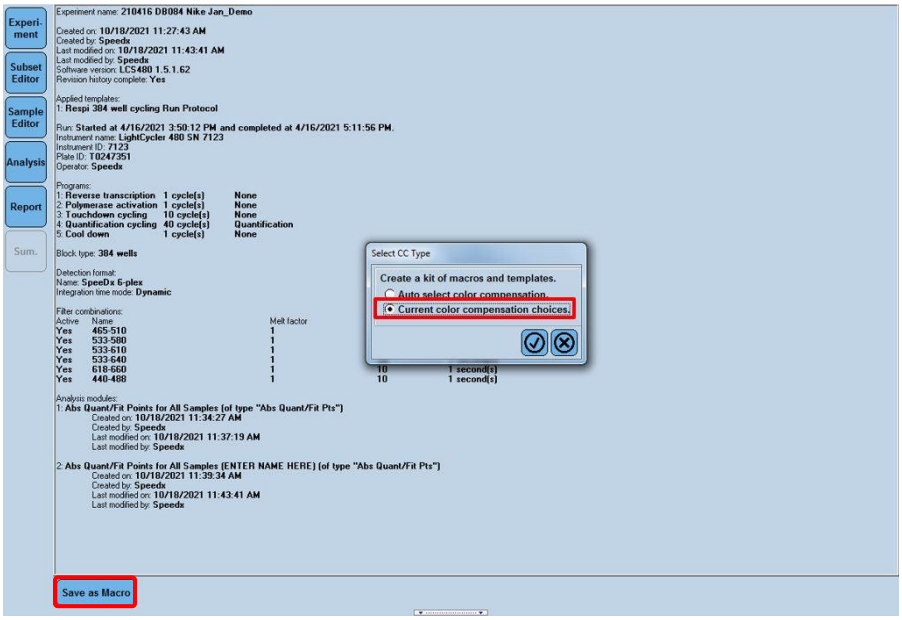
Select **Save As Template** using the folder **Templates** > **Analysis Templates** and include the channel and target in the naming format > **Ok**

Figure 20. Saving Analysis Template Abs Quant/Fit Points – 533-610 Internal Control



Select the **Summary tab** > **Save As Macro** > **Current colour compensation choices**

Figure 21. Selecting CC Type

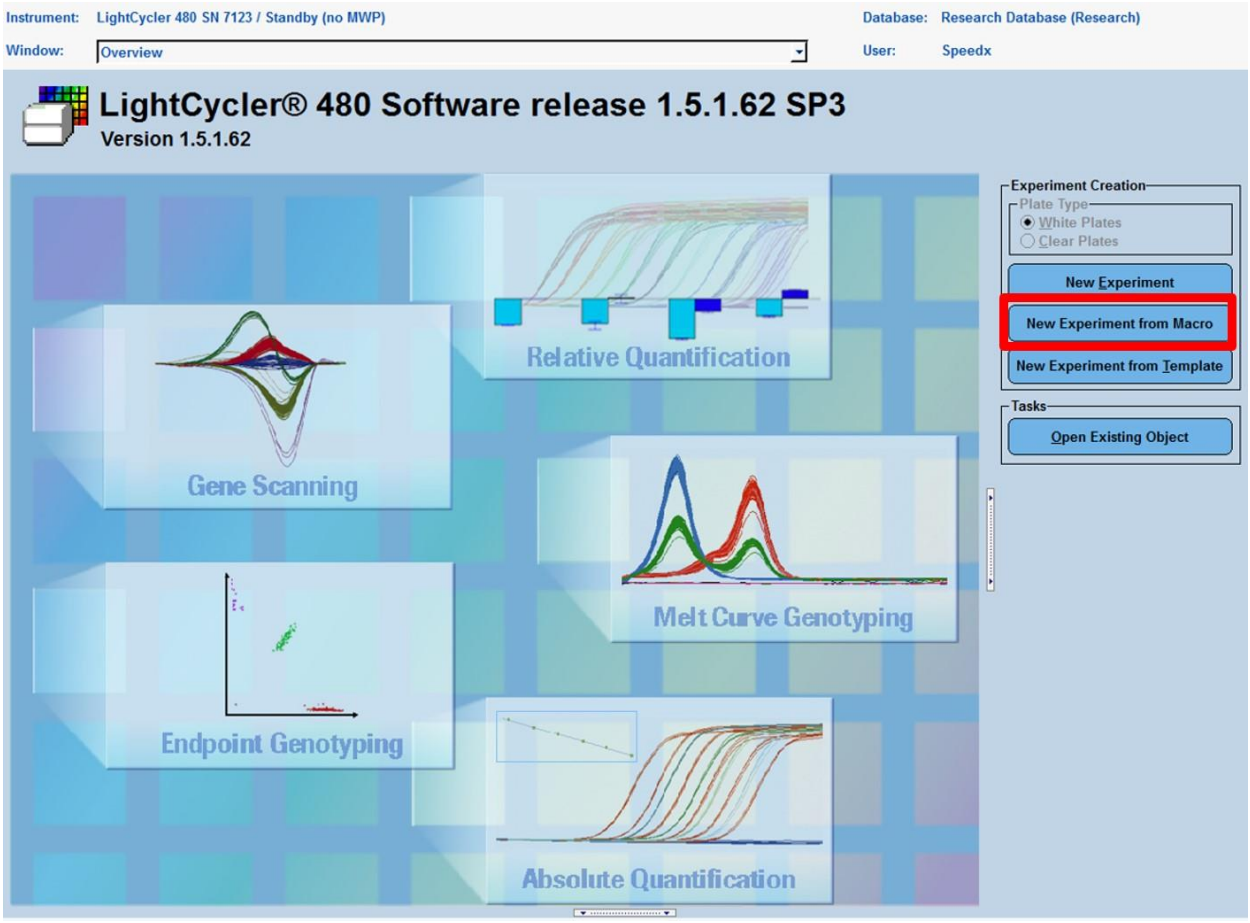


This **Macro template** will now be available to select when setting up for a run.

Macro Template set up

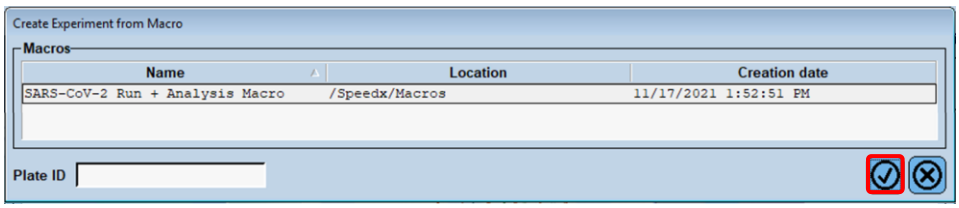
Select **New Experiment from Macro**

Figure 22. Selecting New Experiment from Macro



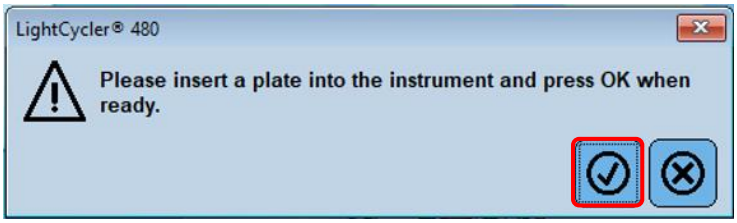
Select the file from the folder **Macros** > **Ok**

Figure 23. Selecting Macro Template



Insert the prepared PCR plate when the following prompt appears > **Ok** and the run will automatically begin

Figure 24. Insert Plate Message



Proceed with using the **Subset Editor** and **Sample Editor** to ensure appropriate labelling for the results output.

19.3 Colour Compensation for LightCycler® 480 Instrument II

Note: The ***Plex****PCR*® Colour Compensation Kit (Cat no 90001) kit must be run and applied for LC480 II analysis. This kit can be supplied on request.

For analysis to proceed, the Sample Name of the colour compensation reactions must be labelled as shown in **Table 22**.

When the cycling program has finished, export a .ixo file for analysis in the ***Plex****PCR*® SARS-CoV-2 (LC480) analysis software.

Select **Export**

Save in an easily identifiable location

Table 22. Sample Name for colour compensation reactions for the analysis software

	Reactions						
	BLANK	**488 mix**	**510 mix**	**580 mix**	**610 mix**	**640 mix**	**660 mix**
Dominant Channel	Water	440-488	465-510	533-580	533-610	533-640	610-660
Sample Name	BLANK	440-488	465-510	533-580	533-610	533-640	610-660

19.4 Interpretation of results

Data interpretation may be performed using the LC480 II onboard software or the ***Plex****PCR*® SARS-CoV-2 (LC480) analysis software. The ***Plex****PCR*® SARS-CoV-2 (LC480) analysis software can be supplied on request. Please contact tech@speedx.com.au for more information.

For interpretation of results without the ***Plex****PCR*® SARS-CoV-2 (LC480) analysis software, each sample must be analysed individually. See **Table 23** for how to interpret signals from different Filter Combinations.

Any Cp registered within the Cut-off, with visual confirmation of amplification curve, is a positive result (**Table 23**). Example amplification curves are shown in **Figure 25.**

Note: NTC sample should not produce a signal in any well:

→ Result is INVALID and PCR should be REPEATED.

Internal Control

The internal control monitors extraction and PCR inhibition. The internal control is valid if the 533-610 channel registers a Cp within the Cut-off (**Table 23**). However, it may be possible to have a positive signal for any target assay (ORF1ab or RdRp) when the Internal Control is negative. For such samples, presence of the target is still interpreted as a valid result.

Note: For samples where target assays are negative, and the internal control assay is also negative:

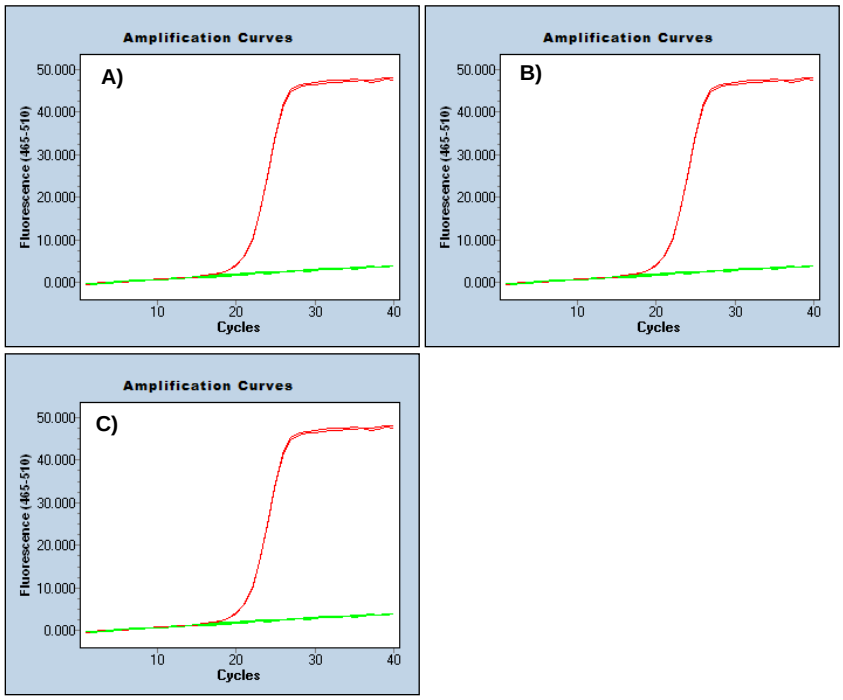
→ Result is INVALID and the extraction and PCR should be REPEATED.

Table 23. Interpretation of Results (LC480 II)

	Target		
Interpretation	**ORF1ab (465-510)**	**RdRp (533-580)**	**Internal Control (533-610)** ^
SARS-CoV-2 detected	< 31	N/A	N/A
SARS-CoV-2 detected	N/A	< 31	N/A
SARS-CoV-2 not detected. IC valid.	≥ 31	≥ 31	≤ 26
IC invalid. Re-extract and re-test sample.	≥ 31	≥ 31	≥ 26

^If the internal control is negative but a target assay is positive, the result is still valid.

Figure 25. Example of amplification curves for A) ORF1ab, B) RdRp, C) Internal control. (Positive (red) and Negative (green)).



Refer to **Appendix A: Result Interpretation** for instructions for using the ***Plex****PCR*® SARS-CoV-2 (LC480) analysis software.

20 Appendix 2: Bio-Rad CFX96™ Dx and CFX96 Touch™ Real-Time PCR System

The following information is based on CFX Manager Dx Software (Version 3.1).

The ***Plex****PCR*® SARS-CoV-2 kit contains dyes for the CFX96 Dx System. Default dye calibrations are used for all channels. Customer calibration is not required.

20.1 Programming the CFX96™ Dx and CFX96 Touch™ Real-Time PCR Detection System (CFX96 Dx, CFX96 Touch)

Select **View** > Open **Run Setup**

In **Run Setup** > **Protocol** tab > Select **Create New**

In the **Protocol Editor** (see **Figure 26**):

Set **Sample Volume** > 10µl

Create the following thermocycling program and save as '**SpeeDx PCR**'. This protocol can be selected for future runs.

For Touch down cycling, select Step 3 and select **Step options** > Increment: -0.5°C/cycle shown in more detail in **Figure 27**).

Table 24. Thermocycling Program

Program name	Cycles	Target °C	Hold
Reverse transcription	1	48°C	10 min
Polymerase activation	1	95°C	2 min
Touch down cycling[δ]: Step down -0.5°C/cycle	10	95°C	5 s
		61°C – 56.5°C[δ]	30 s
Quantification cycling[+]: Acquisition/Detection	40	95°C	5 s
		52°C[+]	50 s

[δ] **Step options** > Increment: -0.5°C/cycle

[+] **Add Plate Read to Step**

Figure 26. Thermocycling Protocol – Graphical view

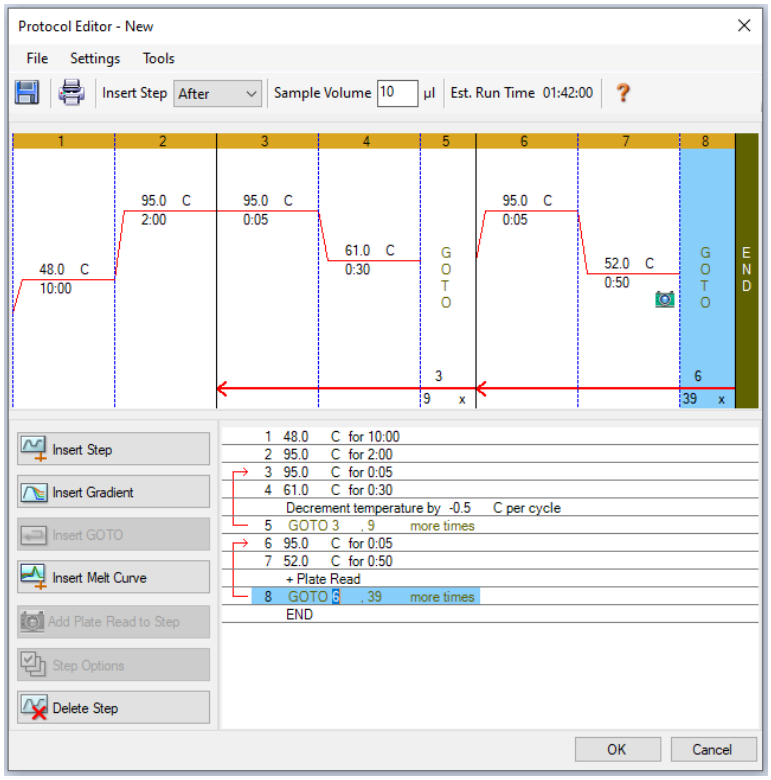
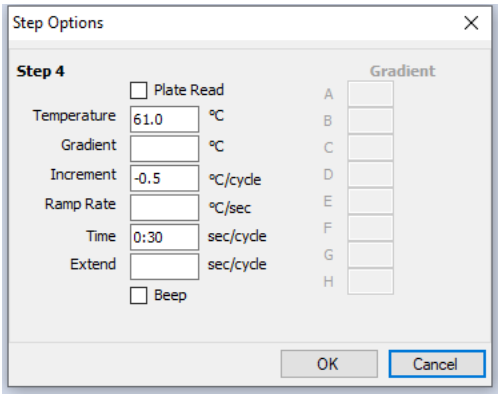


Figure 27. Step options



In **Run Setup** > **Plate** tab

Select **Create New**

Select **Settings** > **Plate Type** > Select **BR clear**

Set **Scan mode** > All channels

Select Fluorophores > FAM, HEX, Texas Red (see **Table 25**)

Select wells containing samples and assign **Sample Type** and check **Load** for fluorophores (FAM, HEX, Texas Red)

Save plate

Table 25. Channels for *PlexPCR*® SARS-CoV-2 targets			
Channel	**FAM**	**HEX**	**Texas Red**
SARS-CoV-2	ORF1ab	RdRp	Internal Control

In **Run Setup** > **Start Run** tab

Select Block

Start Run

To enable automated sample detection in the analysis software, assign nametags to the wells on the plate.

Open the **Plate Setup** module

Select well

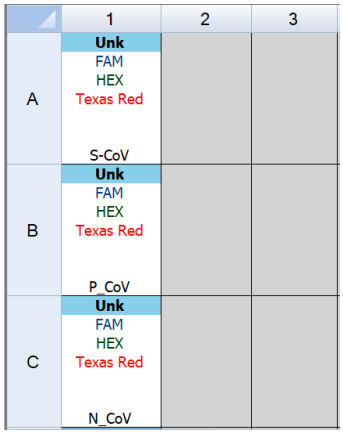
Edit **Sample Name** to match nametag defined in **Assays** module of the analysis software (see **Section 21.4**)

Samples are labelled as *Prefix_Suffix* (as shown in **Table 26** and **Figure 28**) e.g., NEG_CoV

NOTE: Sample nametags are case sensitive. The nametag must match exactly to those assigned in the run file.

Table 26. Sample nametags for analysis software			
Sample type	**Prefix_ (in analysis software)**	**Suffix_ (in analysis software)**	**Sample name (in analysis software)**
Regular sample	Sample	_CoV	Sample_CoV
Negative control	N	_CoV	N_CoV
Positive control	Pa	_CoV	Pa_CoV

Figure 28. Sample editor – Assigning nametags to wells



20.2 Interpretation of results using onboard CFX software

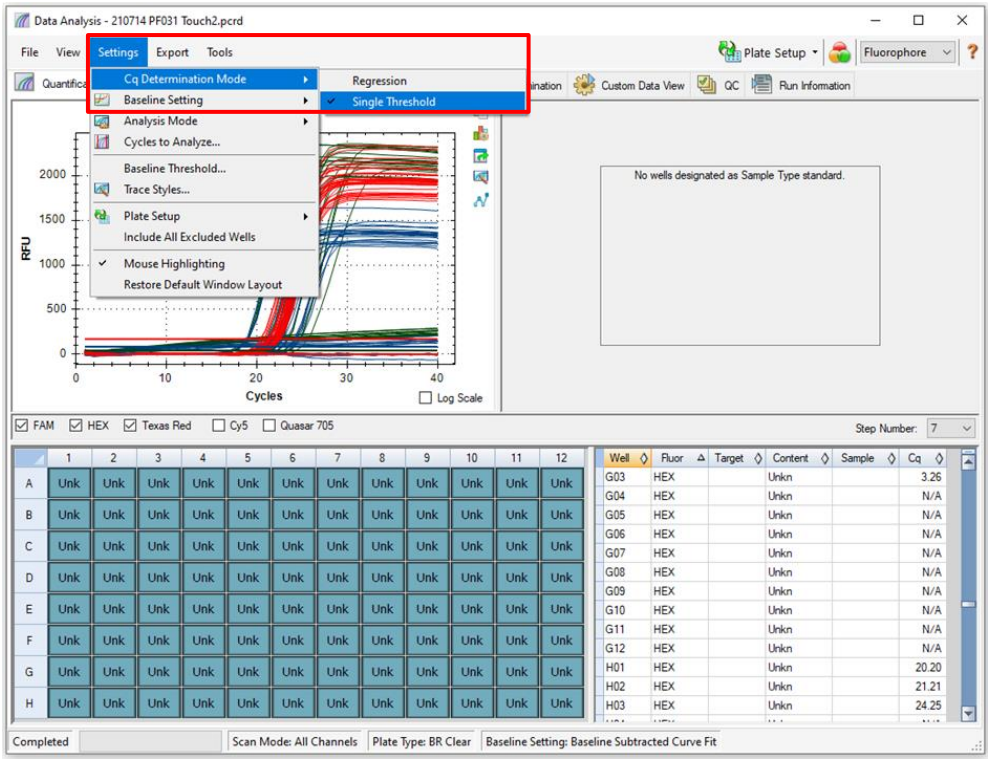
Data interpretation may be performed using the CFX onboard software by using the validated parameters provided below. For further assistance, please contact tech@speedx.com.au.

Select a run file with the **SpeeDx PlexPCR Cycling** parameters

Ensure there are no additional channels selected other than those listed in **Table 25**.

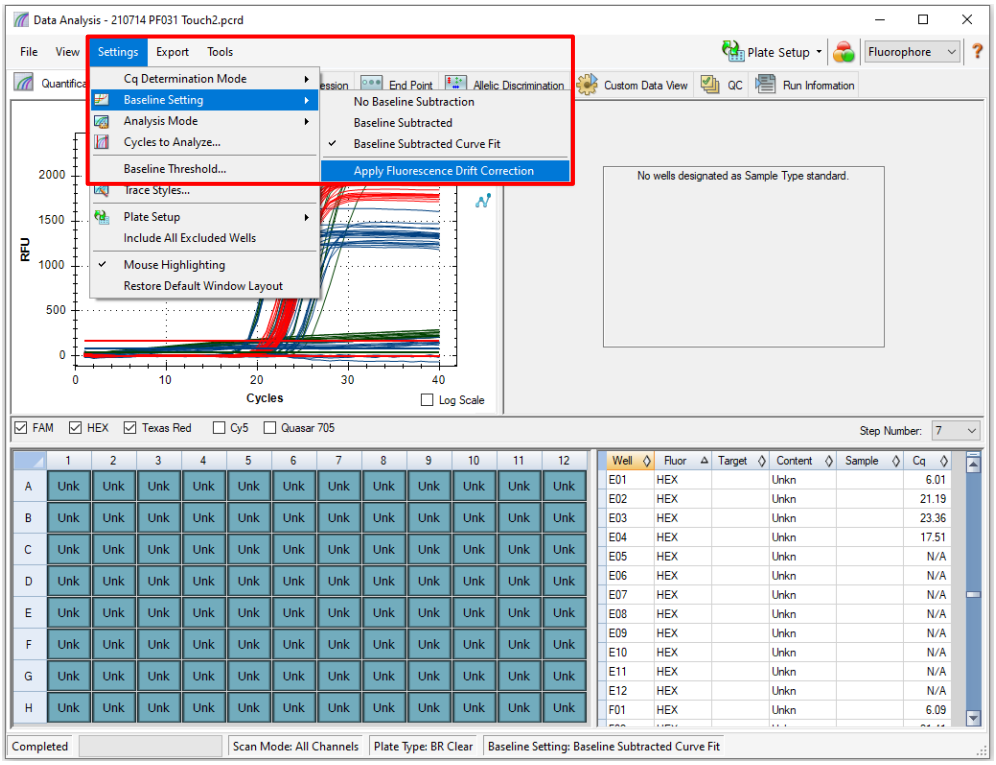
Click on **Settings** > **Cq Determination Mode** and select **Single Threshold** (**Figure 29**)

Figure 29. Cq Determination Mode settings



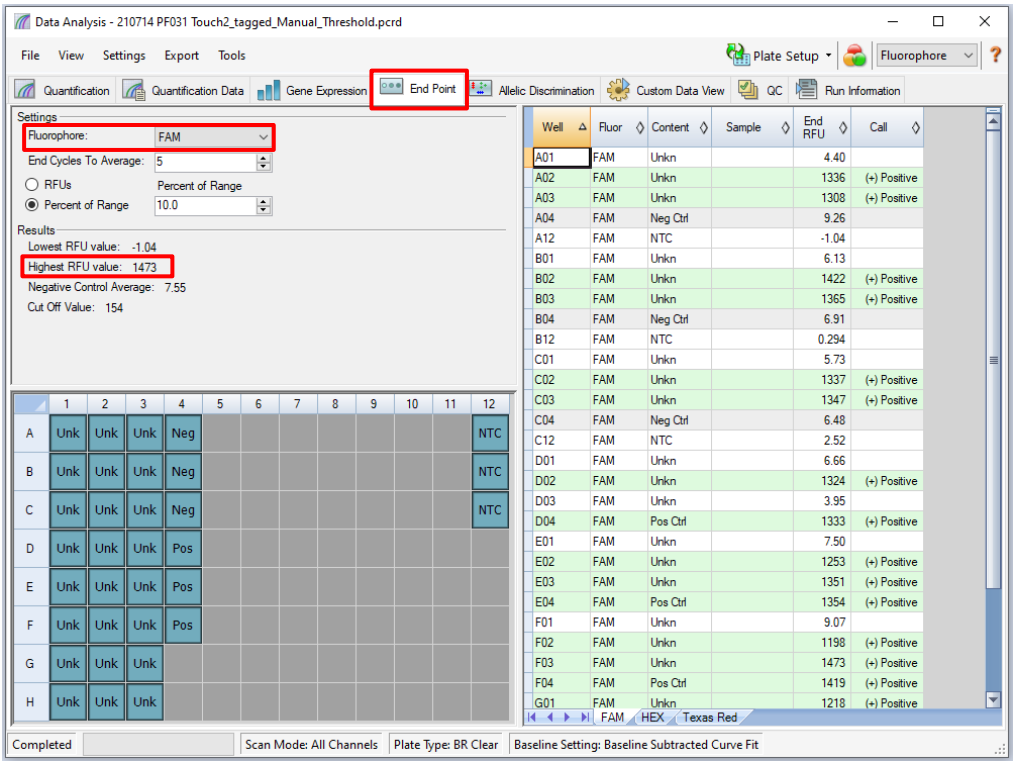
Click on **Settings** > **Baseline Setting** and select **Baseline Subtracted Curve Fit** and enable **Apply Fluorescence Drift Correction** (**Figure 30**)

Figure 30. Baseline settings



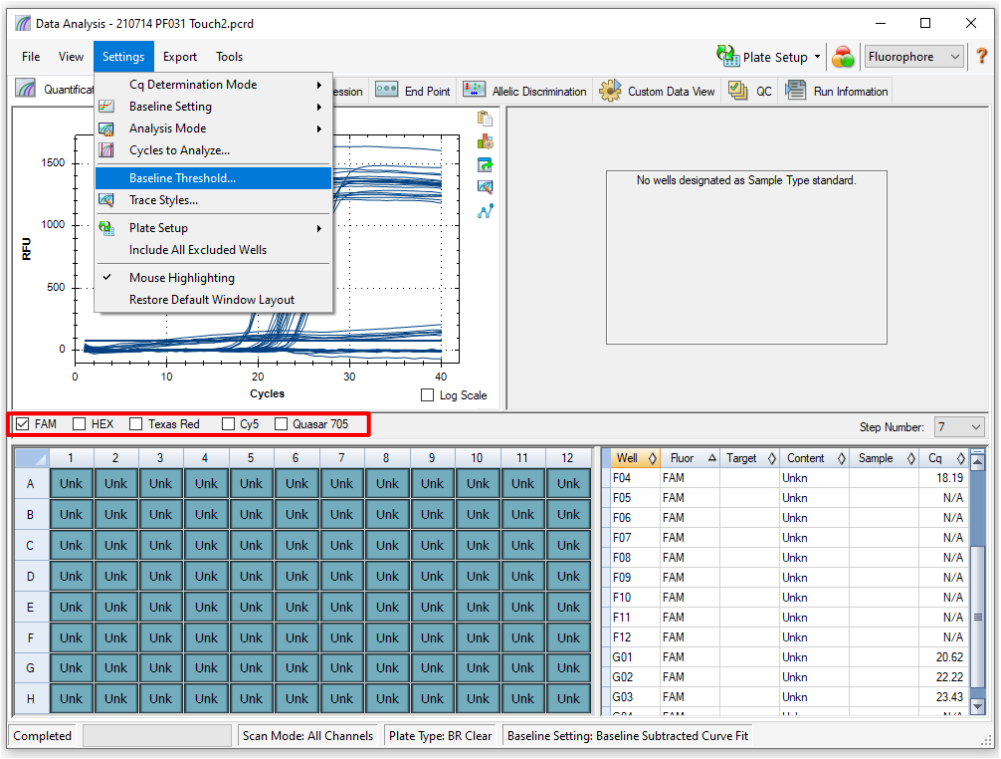
Select the **End Point** tab to view end point fluorescence values and select the **FAM fluorophore** and note the **'Highest RFU value'** (**Figure 31**)

Figure 31. Note the 'Highest RFU value'



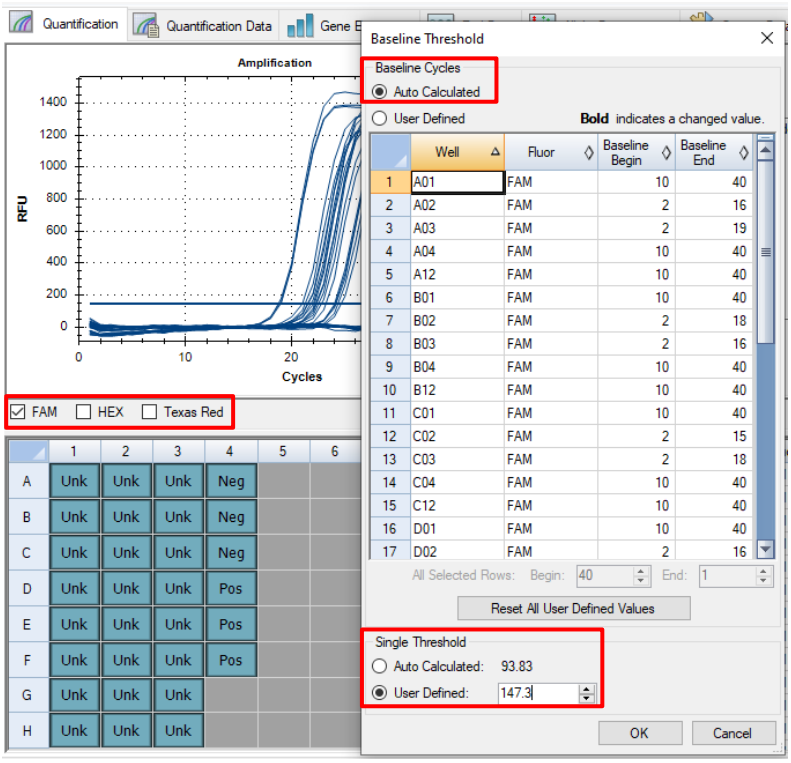
Return to the **Quantification** tab and de-select **HEX** and **Texas Red** fluorophores. Then select **Settings** > **Baseline Threshold** (**Figure 32**)

Figure 32. Check baseline threshold for each channel



Enable **Baseline Cycles** > **Auto Calculated** for all wells and **Single Threshold** > **User Defined** > edit the value to **10%** of the **'Highest RFU value'** for that channel as determined with **Figure 31**. *This step must be performed with one channel selected at a time* (**Figure 33**)

Figure 33. Baseline Threshold settings



Repeat steps from **Figure 31** to **Figure 33** for the **HEX channel** and **Texas Red channel**. *Note that these steps must be performed with one channel selected at a time*

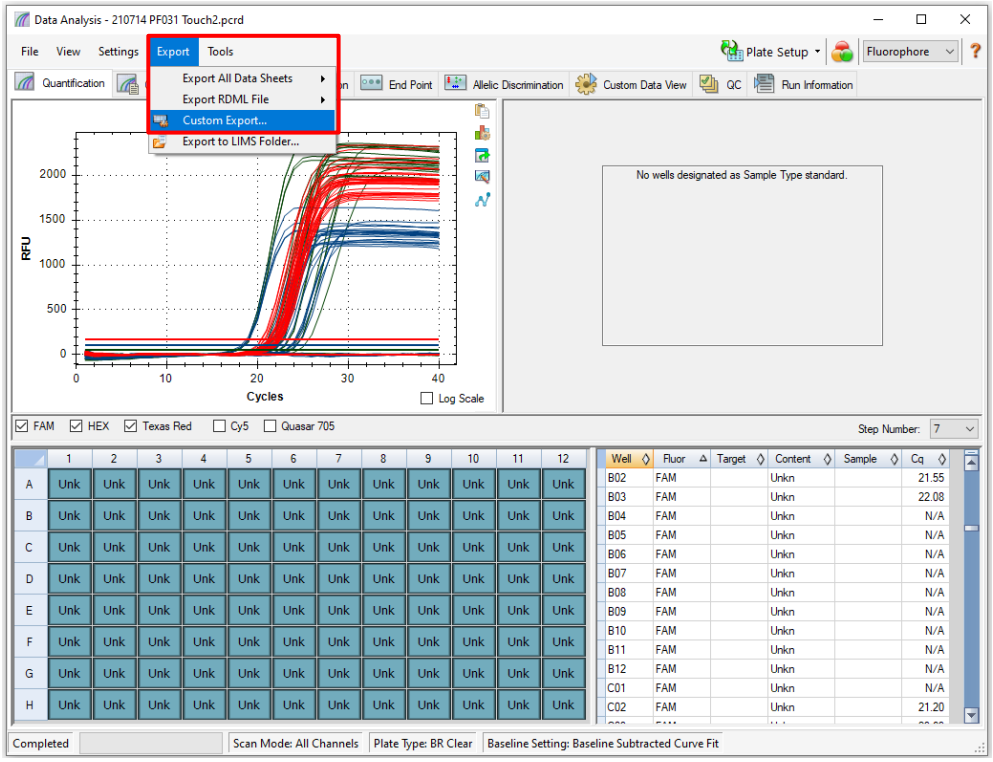
20.3 Exporting results from onboard CFX analysis

Select **Export** > **Custom Export** (**Figure 34**)

For results as a comma-separated values (.csv) file

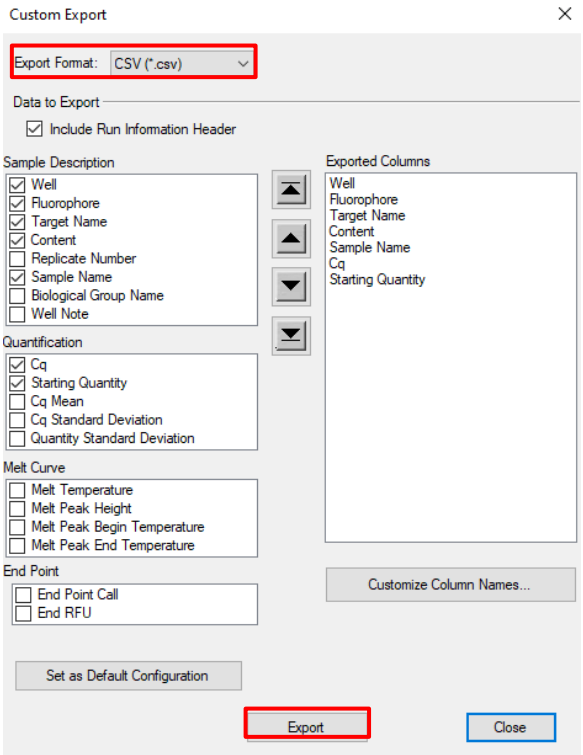
For results as a tab delimited text (.txt) file

Figure 34. Exporting results



Select the desired export format (eg. .csv or .txt), choose the desired fields to export and click **Export** (**Figure 35**)

Figure 35. Custom export settings



20.4 Interpretation of results with the PlexPCR SARS-CoV-2 (CFX) analysis software

Data interpretation may be performed using the ***Plex****PCR*® SARS-CoV-2 (CFX) analysis software. The analysis software can be supplied on request. Please contact tech@speedx.com.au for more information.

Refer to **Appendix A: Result Interpretation** for instructions for using the ***Plex****PCR*® SARS-CoV-2 (CFX) analysis software.

21 Appendix A: Result Interpretation

Data interpretation can be performed using the ***Plex****PCR*® SARS-CoV-2 analysis software. The SARS-CoV-2 analysis software automates the data interpretation of amplification results and streamlines workflow.

For further detailed instructions on the **FastFinder** platform, refer to the **FastFinder Instructions For Use** accessible from the **Help** menu.

See **Table 27** for the appropriate analysis software for each real-time PCR instrument. The analysis software can be supplied upon request. Please contact tech@speedx.com.au for more information.

Table 27. *Plex**PCR*® SARS-CoV-2 analysis software**

Cat no	Analysis software*	Real-time PCR instrument
99021	***Plex****PCR*® SARS-CoV-2 (LC480)	LC480 II
99022	***Plex****PCR*® SARS-CoV-2 (CFX)	CFX96 Dx & CFX96 Touch

* Refer to the website https://plexpcr.com/products/respiratory-infections/plexpcr-sars-cov-2/ to ensure you are using the most current version of analysis software.

NOTE: Follow standard laboratory practices for transfer, reporting and storage of results to prevent loss of sample information.

21.1 FastFinder platform – Minimum IT requirements

The analysis software is available within the FastFinder platform (https://www.ugentec.com/fastfinder/analysis). The minimum IT requirements for installation of the FastFinder platform are listed below.

Hardware requirements

PC (Mac computers are not supported)

Processor: 2 GHz, 2 GB RAM

Disk space: 10Gb

Internet Connection Cable or DSL, proxy not supported

Min. screen resolution: 1366x768 pixels

Supported client operating system

Operating system	Supported editions
Windows 10	32-bit and 64-bit
Windows 8.1	32-bit, 64-bit, and ARM
Windows 8	32-bit, 64-bit, and ARM
Windows 7 SP1	32-bit and 64-bit
Windows Vista SP2	32-bit and 64-bit

Supported browsers

FastFinder Administrator account users require one of the following:

- Internet Explorer 11 or newer
- Microsoft Edge 25 or newer
- Firefox 45 or newer
- Google Chrome 47 or newer.

It may run on older versions, but these are not officially supported.

Software requirements

To use the FastFinder software, at least .NET 4.6.1 is necessary. For more information about the .NET framework, please visit the Microsoft Windows help pages.

Antivirus settings

Your antivirus software might put the FastFinder installer (UgenTec.FastFinder.Installer.exe) in quarantine. Please add this file to the antivirus whitelist. Example: Symantec (Risk: WS.Reputation.1)

Firewall requirements

https Connections should be allowed to *.fastfinderplatform.com:443

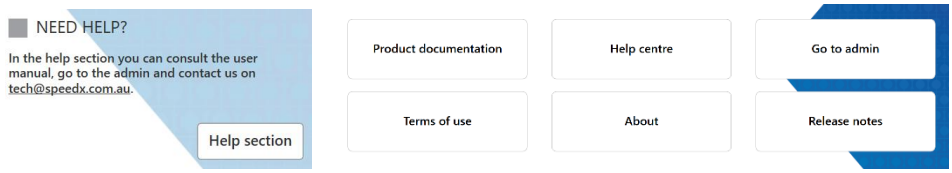
For further details instructions on the **FastFinder** platform, refer to the **FastFinder Instructions for Use** accessible from the **Help** menu.

To access the help menu:

- Open the start menu 

- Select 

or **Help section** and then select **Product Documentation** followed by **Instructions for Use**



21.2 Device set up (new user or device)

Refer to the **FastFinder Instructions For Use** for detailed instructions to set up device, accessible from the **Help** menu

Open **FastFinder**

- Select **Devices** from the workflow bar

> Select **Add**

> Select a file (run file) for the new device

- To change the **Current directory**

> Select **Browse** and select the folder containing relevant files

> Select **Next**

- Add device information

> Select **Save**

21.2.1 Colour Compensation

NOTE: See **Section 19.3** for more information on Colour Compensation

For **LC480 II** devices, a colour compensation file must be added to the device

- Select the LC480 II device
  - > In the **Colour Compensation** section, select 
  - > Select the colour compensation file for the device from the directory
- To change the Current directory
  - > Select **Browse** and select the folder containing relevant files

- Select **Next**
- Select **PlexPCR SARS-CoV-2 (LC480)** from the list to link to this assay
- Select **Save**

New or additional colour compensation files can be added to a device or deactivated as needed.

In the device colour compensation section

- Next to the file name, select 
- Select 

to activate or deactivate a colour compensation file for an assay
- Select **Save**

21.3 Assay plug-in (new user)

Refer to the **FastFinder Instructions For Use** for detailed instructions to set up assays, accessible from the **Help** menu

Open **FastFinder**

- Select **Assays** from the workflow bar
- Select **Add**
  - > For LC480 II > Select **PlexPCR SARS-CoV-2 (LC480)** from the list
  - > For CFX96 Dx and CFX96 Touch > Select **PlexPCR SARS-CoV-2 (CFX)** from the list
- Select **Add**

To activate or de-activate versions of the assay plug-in

- In **General assay information**

> Select  **Versions**

> Select 

to activate or deactivate the version of the assay

> Select **Save**

21.4 Sample naming

Sample nametags can be assigned to an assay plug-in to automate detection of wells and sample types for analysis.

Select **Assays** from the workflow bar

- In the sample type nametags (prefix), select 

> Select  to add a nametag to define sample type nametags (Negative control, Positive control/s, and Regular sample)

> Add desired word, acronym, or letter to text box

> Select **Save**

- In Mix definition nametags (suffix), select 

> Select  to add a nametag to define the mix name

> Add desired word, acronym, or letter to text box

> Select **Save**

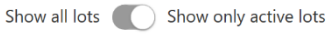
- In the instrument software (before or after run is completed) assign the same nametag to appropriate wells
  - > For **LC480 II** see **Section 19** for instructions on programming sample nametags in the run file
  - > For **CFX96 Dx** and **CFX96 Touch** see **Section 20** for instructions on programming sample nametags in the run file

NOTE: Sample nametags are case sensitive. The nametag must match exactly to those assigned in the run file.

21.5 Adding mix lot numbers

Mix lot numbers can be assigned to the assay to enable traceability of reagents

- Select **Assays** from the workflow bar
  - > In the **Assay Lot:** Select  to add a new lot or select  to edit an existing lot
  - > Once added, lot numbers will become available in the analysis module

Select 

to show all lot numbers or only active numbers

21.6 Analysis

Select **Analyses** from the workflow bar to start a new analysis

1 Select datafile

Search for the file to be uploaded for analysis from a specific directory

- To change the **Current directory**
  - > Select **Browse** and select the folder containing relevant files
- Select run (data) file from the list
  - > Select **Next step**

2 Assign assay(s)

Assign the assay information to the plate manually if sample naming has not been set up in **Assays** module

- For **LC48 II** > Select **PlexPCR SARS-CoV-2 (LC480)**
- For **CFX96 Dx** and **CFX96 Touch** > Select **PlexPCR SARS-CoV-2 (CFX)**
- Select wells and assign as:
  - > Regular sample (S)
  - > Negative control (N)
  - > Positive control (P)
- Select **Next step**

To save the plate layout as a template for future use

- Select wells and assign types of samples
  - > Select  to save template
- Specify template name for future use
  - > Select **Save**

To load a previously saved plate template

- Select  to load plate template
    - > Select template from drop down menu
    - > Check the box to load sample types specified within the plate template
    - > Select **Load**

3 Configure assay(s)

- For **LC480 II** > Select **PlexPCR SARS-CoV-2 (LC480)**
    - > Select **Assay Lot** from the drop-down menu
    - > Select **Analyse**
- For **CFX96 Dx** and **CFX96 Touch** > Select **PlexPCR SARS-CoV-2 (CFX)**
    - > Select **Assay Lot** from the drop-down menu
    - > Select **Analyse**

21.7 Results

See **Table 28** for a summary of possible reported sample results.

NOTE: It is highly recommended that amplification curves should be confirmed for all positive samples.

To finalise analysis and prevent further user edits

- > Select **Authorise Analysis**
- > Select **Yes** to confirm

- To reject analysis or restart the analysis
    - > Select **Restart Analysis** or **Reject Analysis**
    - > Select option to confirm

21.8 Reference curve

A reference curve can be saved and used to compare to samples on the same or across different plates

- Select the sample of interest in either the **Well Details** or **Target Details** menu
- From the amplification graph menu > select 
    - > Select the check box for the channel of interest and add a label
    - > Select **Save** to add signal as reference curve

This reference curve will now appear linked to the assay in the **Assays** menu and can be inactivated at any time.

21.9 Overview of results

Table 28. Result Interpretation of the *PlexPCR*® SARS-CoV-2 analysis software (Results Overview tab)

Well	Name	Assay	Result	Cq values	Overall results
A1	Sample 1_CoV	PlexPCR SARS-CoV-2	Positive	RdRp: 25.94 IC:19.17	**Sample 1 – Positive** SARS-CoV-2 detected.
A2	Sample 2_CoV	PlexPCR SARS-CoV-2	Negative	IC: 18.82	**Sample 2 – Negative** SARS-CoV-2 not detected. IC valid
A3	N_CoV	PlexPCR SARS-CoV-2	Negative	IC: 18.63	**N – Negative** Negative control valid.
A4	Sample 3_CoV	PlexPCR SARS-CoV-2	Invalid		**Sample 3 – Invalid** IC invalid. Re-extract and re-test sample.
A5	Sample 4_CoV	PlexPCR SARS-CoV-2	Positive	ORF1ab: 22.75 RdRp: 23.48 IC: 18.79	**Sample 4 – Positive** SARS-CoV-2 detected.
A6	Sample 5_CoV	PlexPCR SARS-CoV-2	Positive	ORF1ab: 22.75 IC: 18.79	**Sample 5 – Positive** SARS-CoV-2 detected.
A7	N_CoV	PlexPCR SARS-CoV-2	Invalid		**N – Invalid** Negative control invalid.
A8	Sample 6_CoV	PlexPCR SARS-CoV-2	Positive	ORF1ab: 23.08 RdRp: 24.34	**Sample 6 – Positive** SARS-CoV-2 detected.
A9	P_CoV	PlexPCR SARS-CoV-2	Positive	ORF1ab: 18.98 RdRp: 19.97 IC: 18.39	**P – Positive** Positive control valid.
A10	P_CoV	PlexPCR SARS-CoV-2	Invalid		**P – Invalid** Positive control invalid.
A11	Sample 7_CoV	PlexPCR SARS-CoV-2	Invalid	IC: 18.83	**Sample 7_CoV – Invalid** Error: Abnormal change in fluorescence level.
A12	Sample 8_CoV	PlexPCR SARS-CoV-2	Invalid		**Sample 8_CoV – Invalid** **Sample was rejected**

21.10 Exporting results

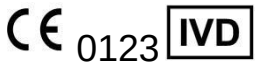
- To export results
  - > Select **Exports** in the workflow bar
  - > Export one or more of the following report types: **Cq values list (CSV), Results (CSV), Generic Amplification CSV** or the appropriate LIS-integration file.
  - > Select **Exports**
- To download exports
  - > Select **Reports** in the workflow bar
  - > Select files and save
- Alternatively export a customised report

- > Export **Amplification Curve Analysis (PDF)**
- > Select desired included information (graphs, audit trail, results overview)
- > Select desired report settings to customise sample order

– Select **Exports**

- > Open in **Report Viewer** to view, save and print

22 Glossary



European Conformity
For *In Vitro* Diagnostic Use

Catalogue number

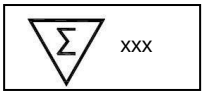
Batch code

Authorised Representative
In the European Community

Manufacturer

Date of manufacture

Temperature limitation



Contains sufficient for
xxx determinations

Use by Date

Importer

United Kingdom Conformity