PlexPCR® SARS-CoV-2

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

IVD

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

Accessory products – Analysis software

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

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FOR PROFESSIONAL USE ONLY
Not for sale in the USA

Contents

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).

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 676 million confirmed cases and greater than 6.8 million deaths as of October 2023.[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	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 10 µL 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 1000 µL (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 2 mL (Molgen Cat no MG96020050)
- PurePrep 96 Elution plate 200 µL (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)
- 50 mL Reagent Reservoirs for 8 channel pipettes
- 50 mL 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 (200 µL) (Thermofisher Cat no 97002540)
- 80% ethanol
- 50 mL Reagent Reservoirs for 8 channel pipettes
- 50 mL 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)
- 50 µL conductive filtered tips (Cat no HMT-235948)
- 300 µL conductive filtered tips (Cat no HMT-235903)
- 1000 µL conductive filtered tips (Cat no HMT-235905)

For LightCycler® 480 Instrument II

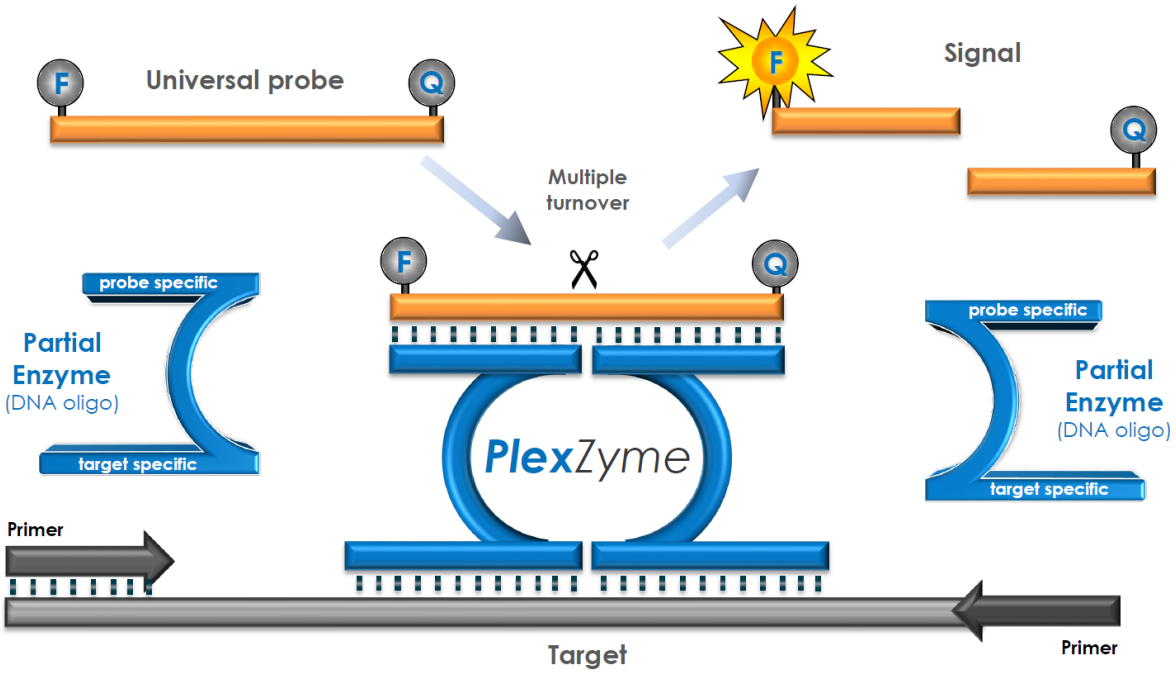
- ***Plex**PCR®* Colour Compensation (CC) kit (SpeeDx, Cat no 90001)
- LightCycler® 480 Multiwell Plate 96 (Roche, Cat no 04729692001)
- LightCycler® 480 Multiwell Plate 384 (Roche, Cat no 04729749001)
- LightCycler® 480 Sealing Foil (Roche, Cat no 04729757001)

8 Principle of the technology

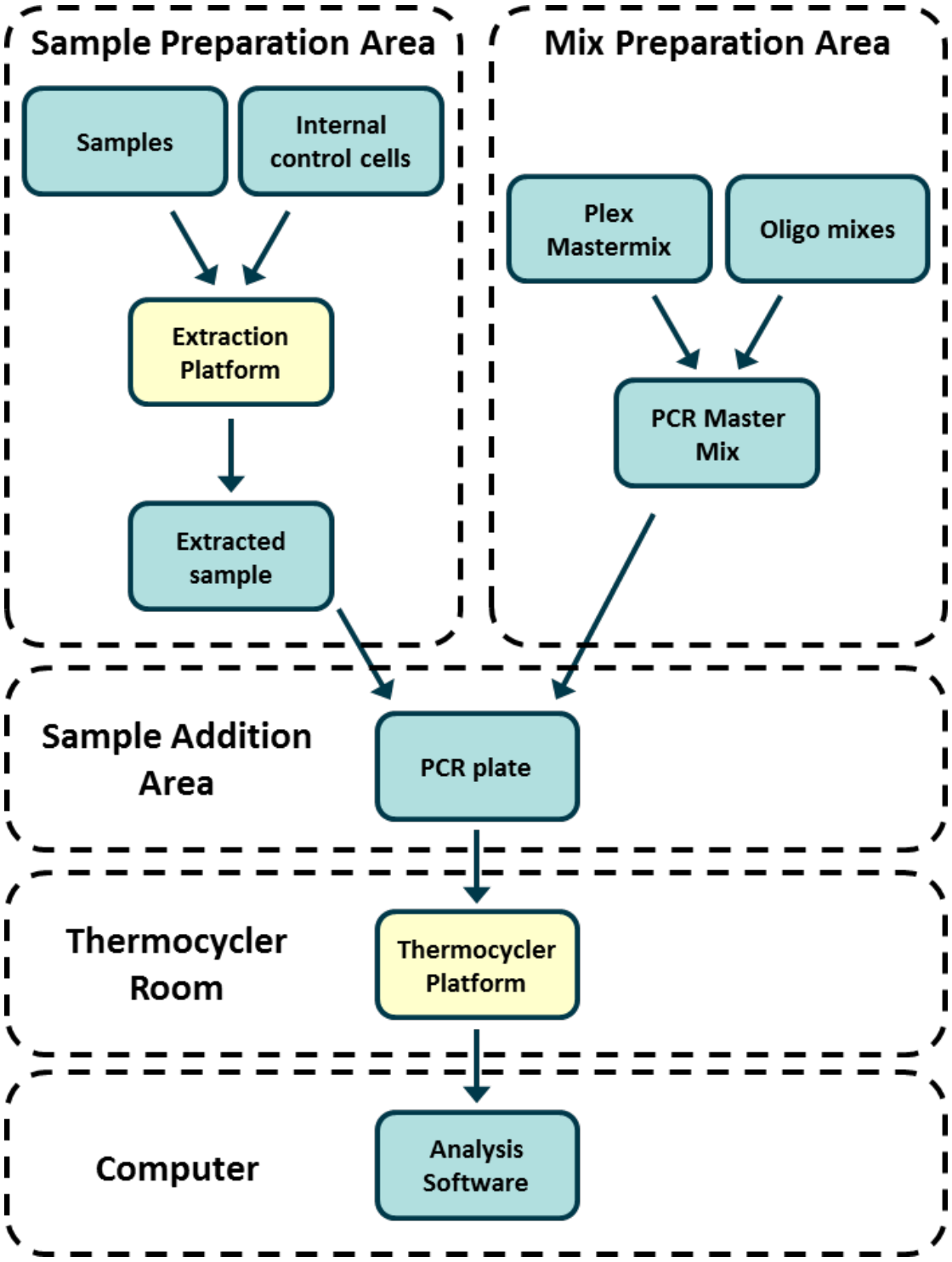
Real-time PCR (qPCR) can be used to amplify and detect specific target nucleic acids from pathogens. ***Plex****PCR*® is a qPCR technology utilising ***Plex****Zyme*® enzymes that detect and report the amplified product through the generation of a fluorescent signal (**Figure 1**).

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	160 µL	U-bottom deep-well plate (Prepared Buffer Mixture)
Absolute Ethanol*	200 µL	U-bottom deep-well plate (Prepared Buffer Mixture)
Magnetic Beads M	15 µL	U-bottom deep-well plate (Prepared Buffer Mixture)
Enhancer Buffer	1 µL	U-bottom deep-well plate (Prepared Buffer Mixture)
RNase Free Water	15 µL	U-bottom deep-well plate (Prepared Buffer Mixture)
RNase Free Water	50 µL	U-bottom deep-well plate
Buffer MW1	170 µL	U-bottom deep-well plate
Buffer MW2	340 µL	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	265 µL	KingFisher 96 deep-well plate (sample plate)
MagMax Total Nucleic Acid Binding Beads	10 µL	KingFisher 96 deep-well plate (sample plate)
MagMax Proteinase K	5 µL	KingFisher 96 deep-well plate (sample plate)
MagMax Wash buffer	500 µL	KingFisher 96 deep-well plate
Wash 2* (80% Ethanol)	500 µL	KingFisher 96 deep-well plate
Wash 3* (80% Ethanol)	250 µL	KingFisher 96 deep-well plate
MagMax Elution Solution	50 µL	KingFisher 96 microplate 200 µL

* Not supplied

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

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, 20 µL 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. 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 19-20**.

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

12 Interpretation of results

Data interpretation may be performed using the LC480 II 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 20**.

See **Table 9** for the analysis software details. 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

* 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.
- Clinical samples with Cq value < 3 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.

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 3 mL 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

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 *Plex*PCR® 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

16.2.2 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 ***Plex****PCR*® 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 ***Plex****PCR*® 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 12**) was 764 copies/ mL (95% CI: 565.69 – 1193.50 copies/mL).

Table 12. LoD of the *Plex**PCR*® SARS-CoV-2 assay**

Positive reference material	Strain	SARS-CoV-2 concn. (genomes per mL)	***Plex****PCR*® 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

* 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 ***Plex****PCR*® 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 13 .** The mean Cq value, standard deviation, and coefficient of variation (%) of each target (ORF1ab, RdRp, and IC) for each extraction kit is detailed in **.** 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 ***Plex****PCR*® SARS-CoV-2 assay when used with samples extracted using the MGISP-960 instrument (MGI).

Table 13. 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 14. 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 15**. 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 15. 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
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 16.**

Table 16. 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 4170, 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 19.4**). 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 17. 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

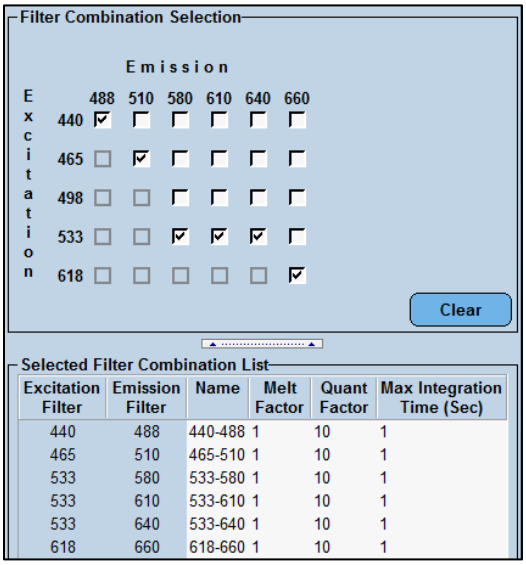
Set the **Selected Filter Combination List for all channels as:**

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

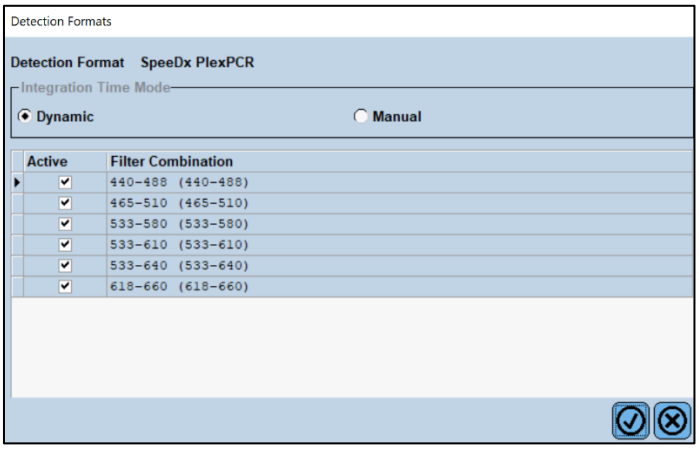
For **Detection Format** select the custom **'SpeeDx PlexPCR'** (**Figure 3**)

Select **Customize** >

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

Select all Active **Filter Combinations** shown in **Figure 3.**

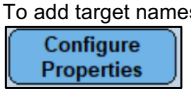
Figure 3. Customise Detection Format



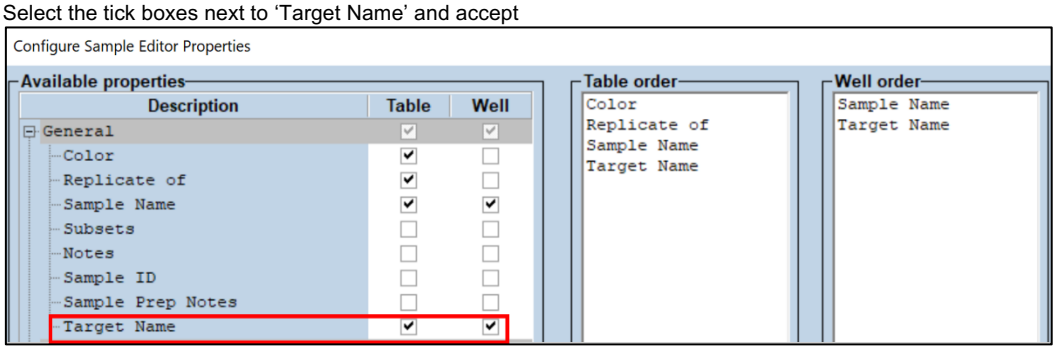
To enable automated sample detection in the analysis software, add target name and assign nametags to the wells on the plate

Open the **Sample Editor** module

To add target names, select **Configure Properties**



Select the tick boxes next to 'Target Name' and accept



Edit the **Target Name** for each channel to match the Target Instrument Reference defined in Lab Configuration > Assays menu of the analysis software and shown in **Table 18**.

Table 18. Channels for *PlexPCR*® SARS-CoV-2 targets

Channel	465-510	533-580	533-610
SARS-CoV-2 target name	ORF1ab	RdRp	IC

Edit **Sample Name** to match the nametag defined in Lab Configuration > Assays menu of the analysis software (see **Section 20.3**)

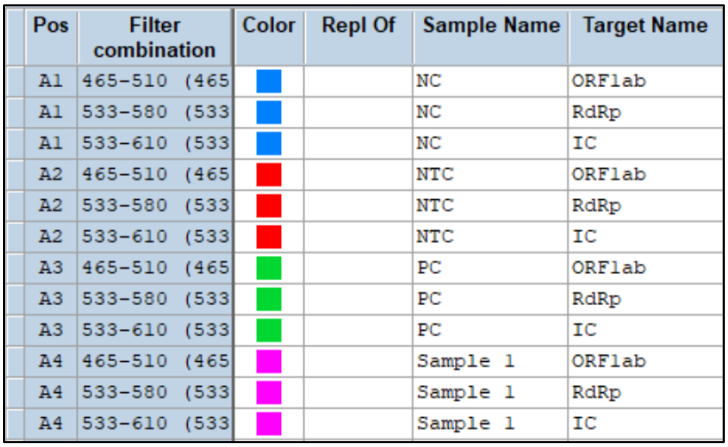
Samples should be labelled with the nametag as a Prefix. Default nametags are provided for the control reactions (as shown in **Table 19** and **Figure 4**). Additional nametags can be defined for both regular samples and controls within the analysis software.

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

Table 19. Sample nametags for analysis software

Sample type	Default Prefix_ (in analysis software)
Regular sample	No default – user defined
Negative control	NC
No Template Control	NTC
Positive control	PC

Figure 4. Sample Editor – Assigning nametags to wells



Pos	Filter combination	Color	Repl Of	Sample Name	Target Name
A1	465-510 (465			NC	ORFlab
A1	533-580 (533			NC	RdRp
A1	533-610 (533			NC	IC
A2	465-510 (465			NTC	ORFlab
A2	533-580 (533			NTC	RdRp
A2	533-610 (533			NTC	IC
A3	465-510 (465			PC	ORFlab
A3	533-580 (533			PC	RdRp
A3	533-610 (533			PC	IC
A4	465-510 (465			Sample 1	ORFlab
A4	533-580 (533			Sample 1	RdRp
A4	533-610 (533			Sample 1	IC

Set **Reaction Volume** > 10 µL

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

Table 20. 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[δ]: Step down -0.5°C/cycle	10	95°C	5 s	4.4	4.8
		61°C – 56.5°C[δ]	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)*

[δ] ***Step size:*** *-0.5°C/Cycle,* ***Sec Target:*** *56°C*

[+] ***Analysis mode:*** *Quantification,* ***Acquisition mode****: Single*

> Start Run

Figure 5. Thermocycling Program – Reverse Transcription

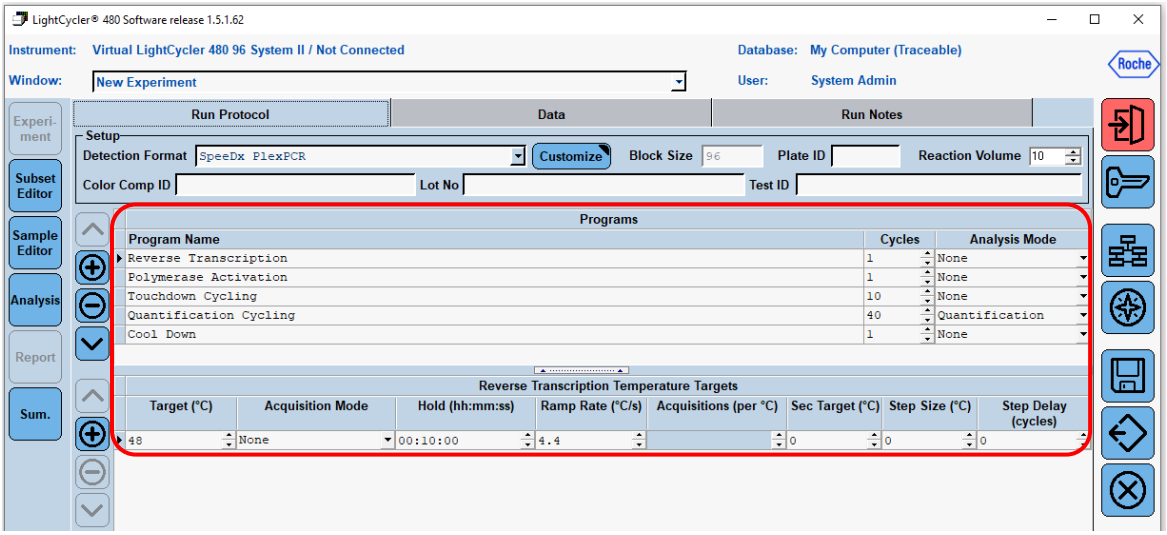


Figure 6. Thermocycling Program – Polymerase Activation

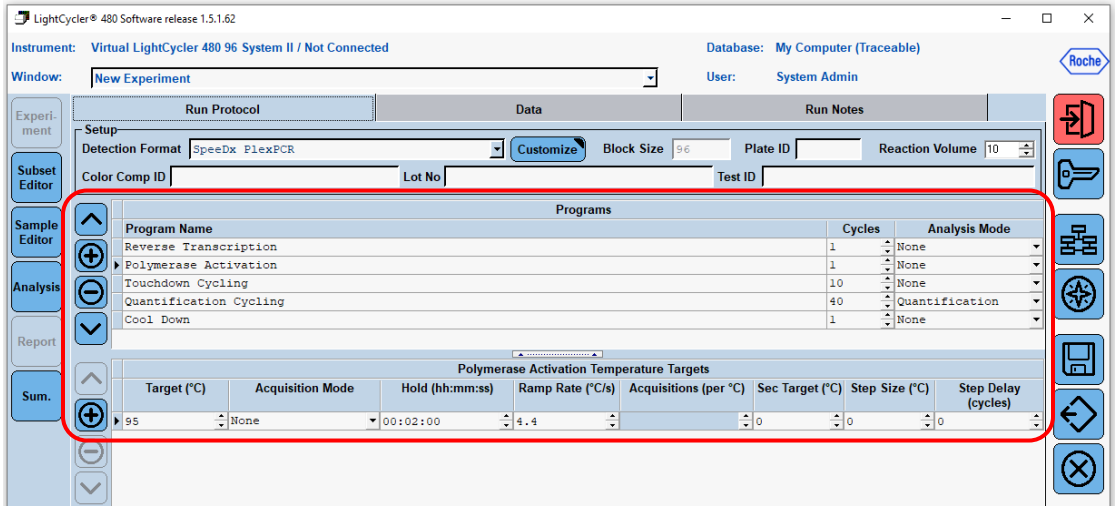


Figure 7. Thermocycling Program – Touchdown Cycling

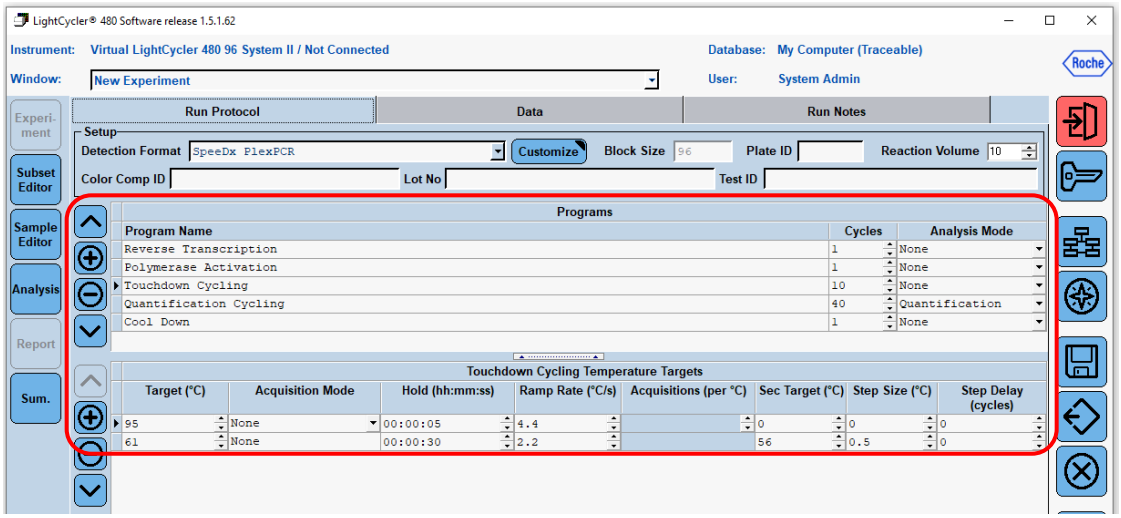


Figure 8. Thermocycling Program – Quantification Cycling

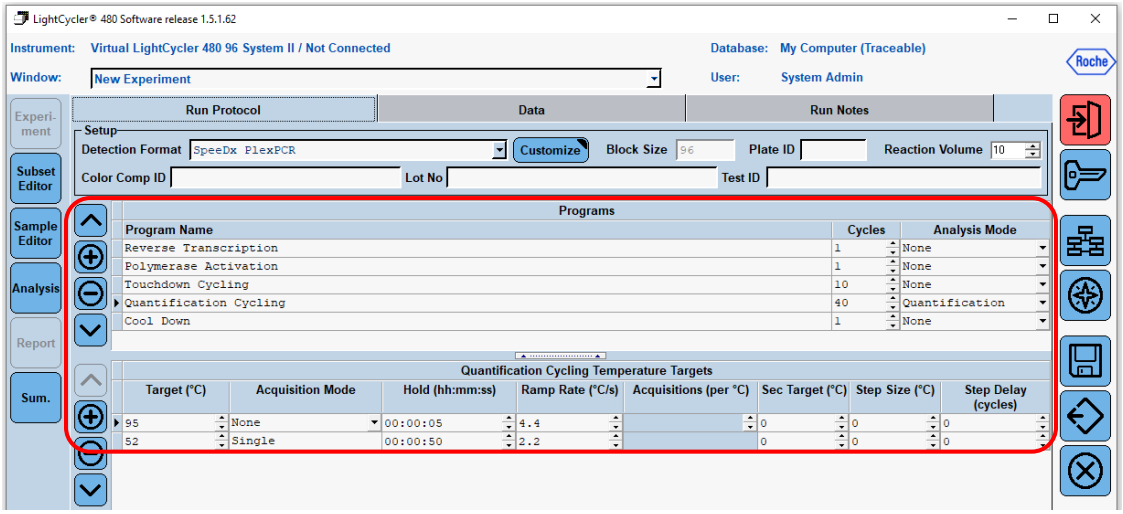
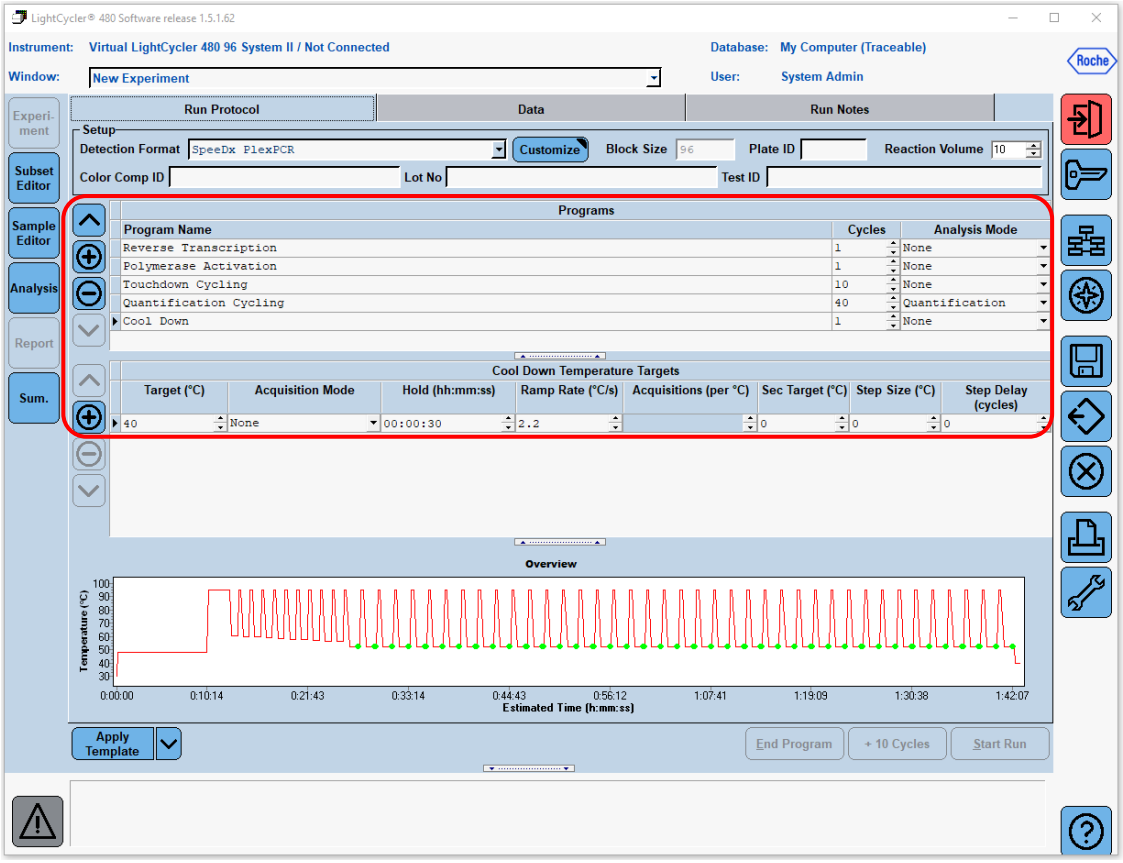


Figure 9. Thermocycling Program – Cooling

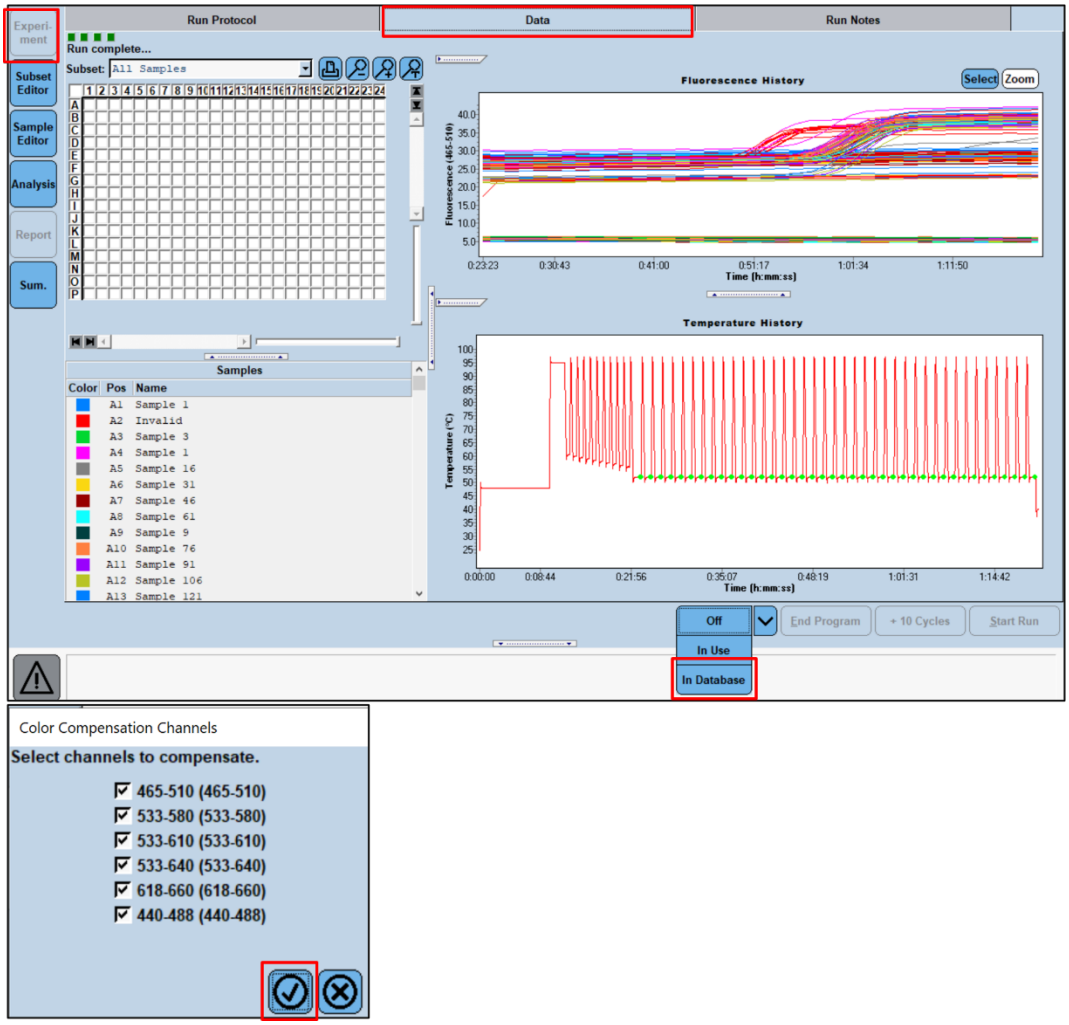


When the cycling program has finished, attach the CC object to the run file as shown in **Figure 10** and export as a .IXO file for analysis in the ***Plex****PCR*® SARS-CoV-2 analysis software. Refer to **Section 19.2** for instructions on how to create the CC Object and store this within the LightCycler 480 software database.

Select **Experiment > Data**

Click the drop-down arrow next to **Colour Comp (Off)** and select **In Database**

Figure 10. Attaching the CC object to the run file



Select the appropriate CC Object, ensure all channels are selected and select the **tick** icon 

Select the **Save** icon 

Select the **Export** icon 

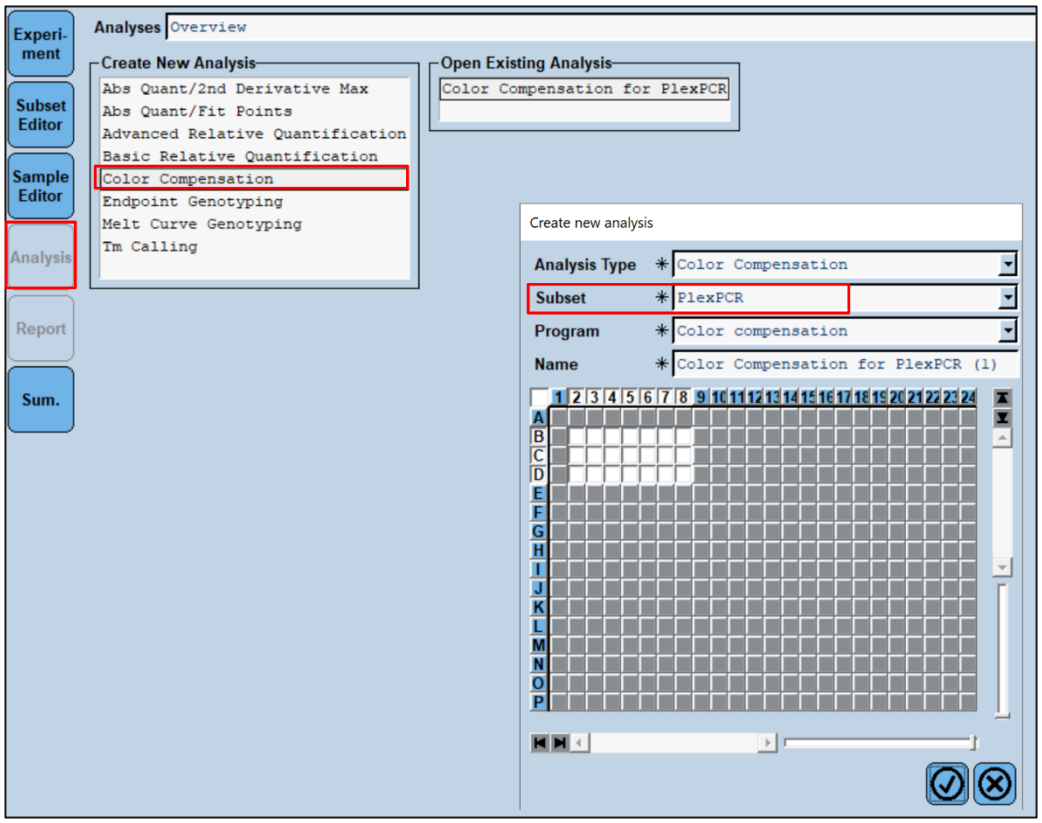
Save in an easily identifiable location

19.2 Colour Compensation for the LightCycler® 480 Instrument II

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

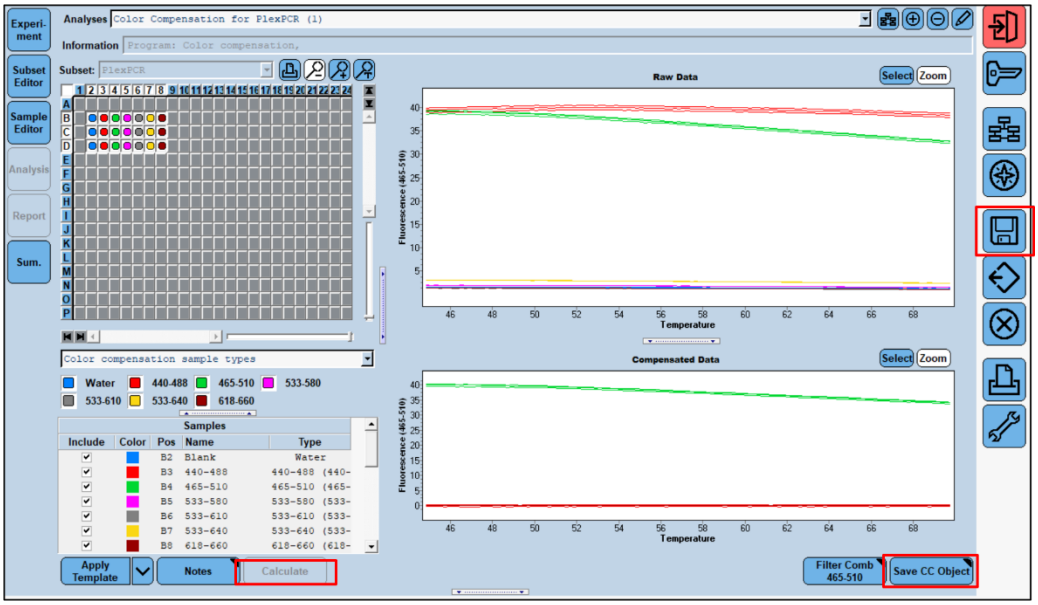
Analyse the Colour Compensation file via **Analysis > Colour Compensation** and select the correct subset, shown in **Figure 11.**

Figure 11. Analysis – Colour Compensation



Select **Calculate** (**Figure 12**).

Figure 12. Calculate and save CC Object



Refer to the PlexPCR Colour Compensation Instructions for Use (IF-IV0001) for further details to ensure the Colour Compensation file has been created correctly.

Select **Save** 

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

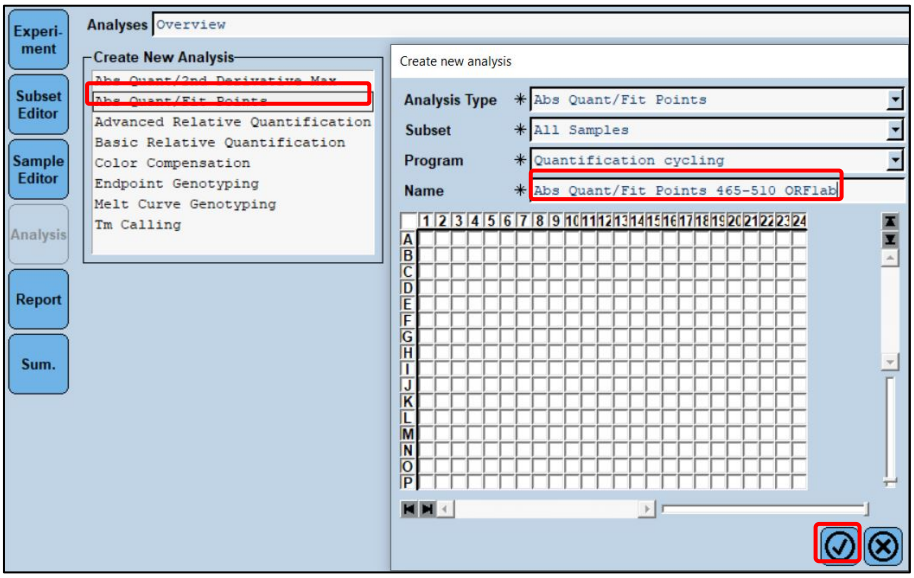
Data interpretation may be performed using the LC480 II onboard software by using a Macro template with the validated parameters provided below. For further assistance, please contact tech@speedx.com.au.

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 13. Abs Quant/Fit Points - 465-510 ORF1ab

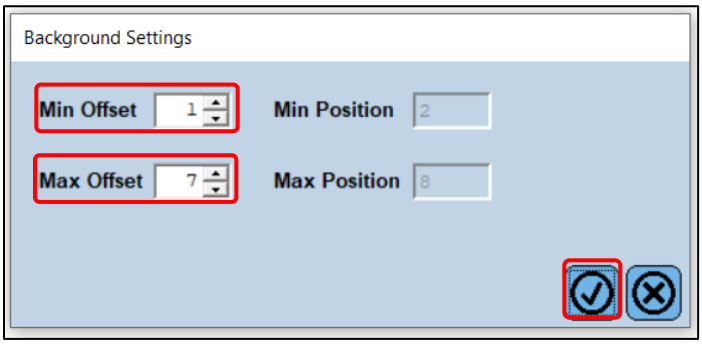


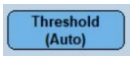
Select **Filter Comb 465 – 510**

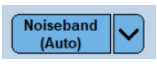
Apply the **Colour Compensation** for all channels > **Ok**

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

Figure 14. Background Settings - 465-510 ORF1ab

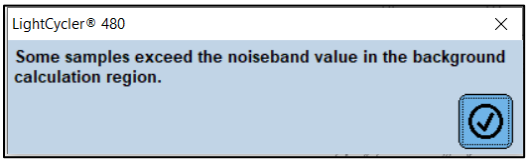


Select the **Analysis** tab and ensure the following setting is selected 

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

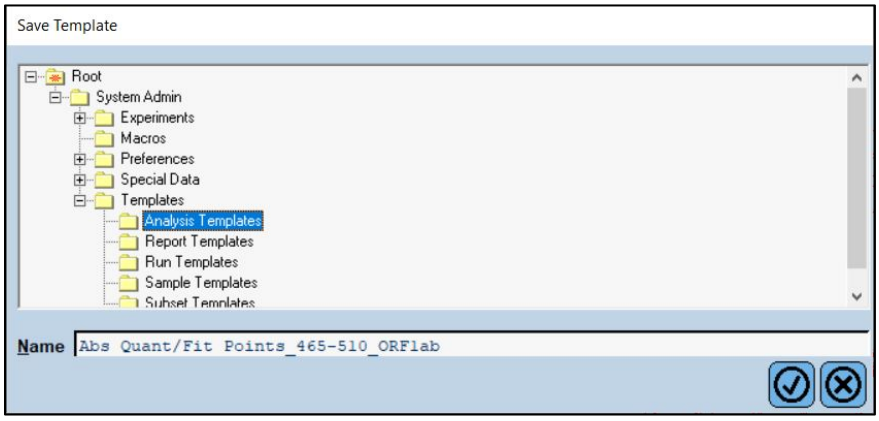
Click **Calculate** (*if a sample curve has crossed the background region the following message shown in **Figure 15** will appear; the user must dilute and re-test the sample)* > **Ok** to continue the analysis

Figure 15. 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 16. Saving Analysis Template Abs Quant/Fit Points - 465-510 ORF1ab

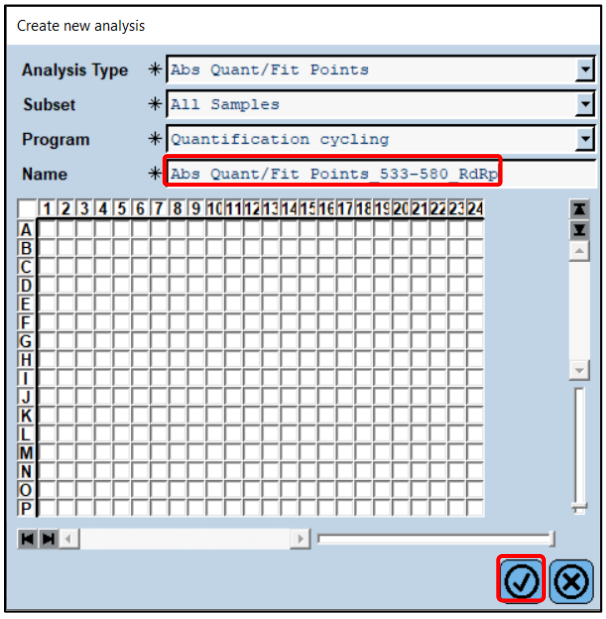


Click on the  icon to save the analysis parameters set for the channel

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 17. Abs Quant/Fit Points 533-580 RdRp

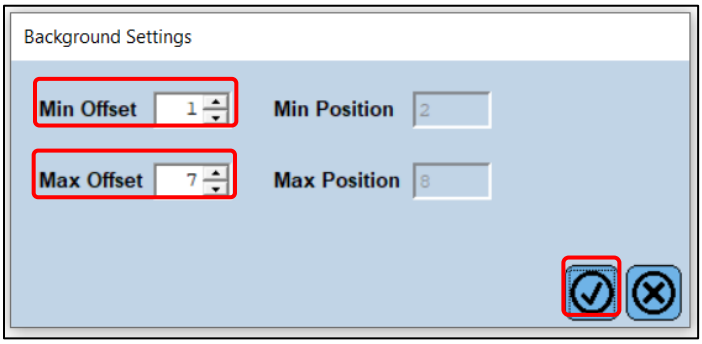


Select **Filter Comb 533 – 580**

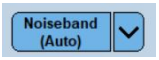
Apply the **Colour Compensation** for all channels > **Ok**

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

Figure 18. Background Settings - 533-6580 RdRp

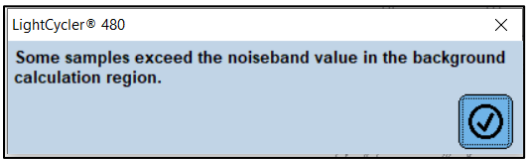


Select the **Analysis** tab and ensure the following setting is selected 

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

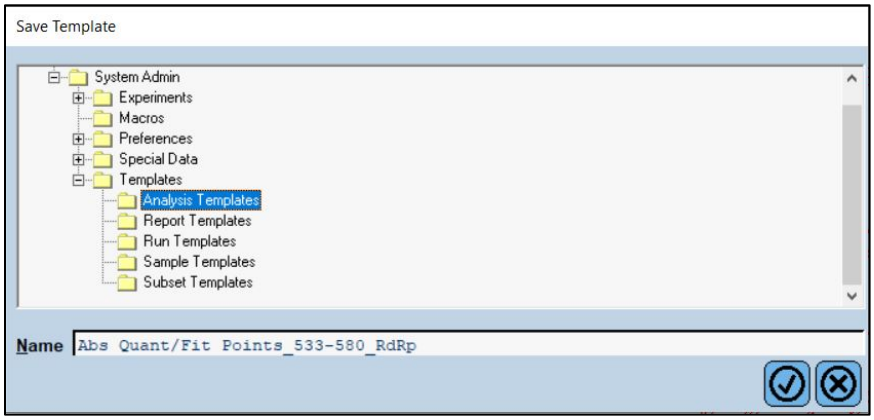
Click **Calculate** (*if a sample curve has crossed the background region the following message in* ***Figure 19*** *will appear; the user must dilute and re-test the sample)* > **Ok** to continue the analysis

Figure 19. 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 20. Saving Analysis Template Abs Quant/Fit Points – 533-580 RdRp

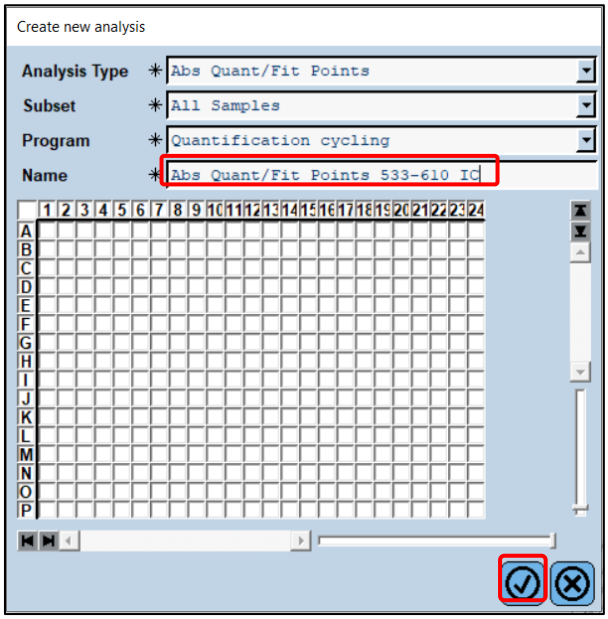


Click on the  icon to save the analysis parameters set for the channel

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**

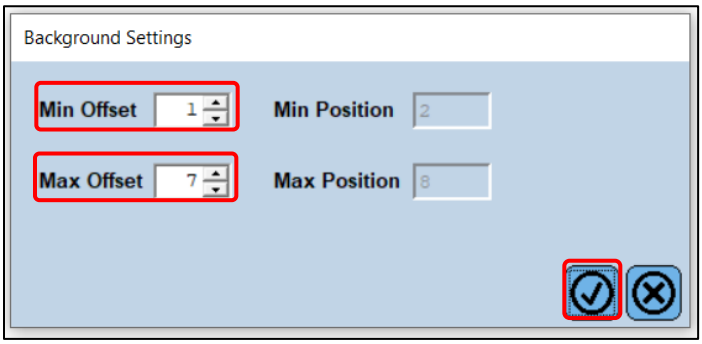
Figure 21. Abs Quant/Fit Points 533-610 Internal Control

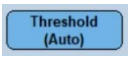


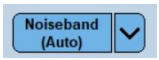
Select **Filter Comb 533 – 610**

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

Figure 22. Background Settings – 533-610 Internal Control



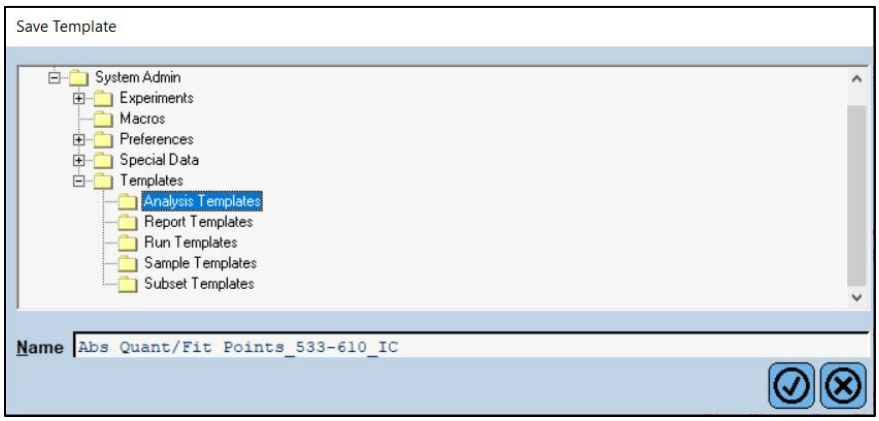
Select the **Analysis** tab and ensure the following setting is selected 

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

Click **Calculate**

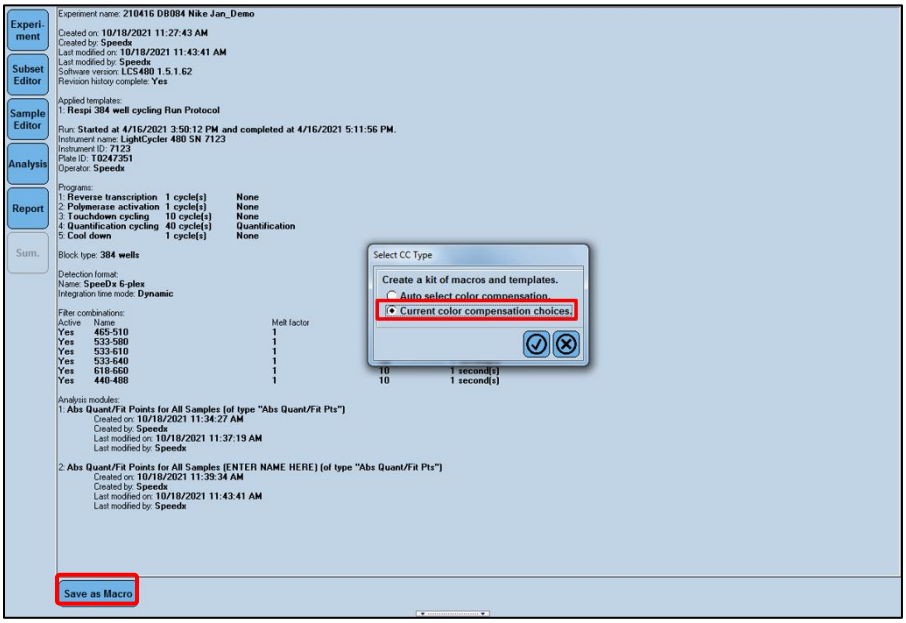
Select **Save As Template** using the folder **Templates** > **Analysis Templates** and include the channel and target in the naming format > **Ok**

Figure 23. Saving Analysis Template Abs Quant/Fit Points – 533-610 Internal Control



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

Figure 24. Selecting CC Type

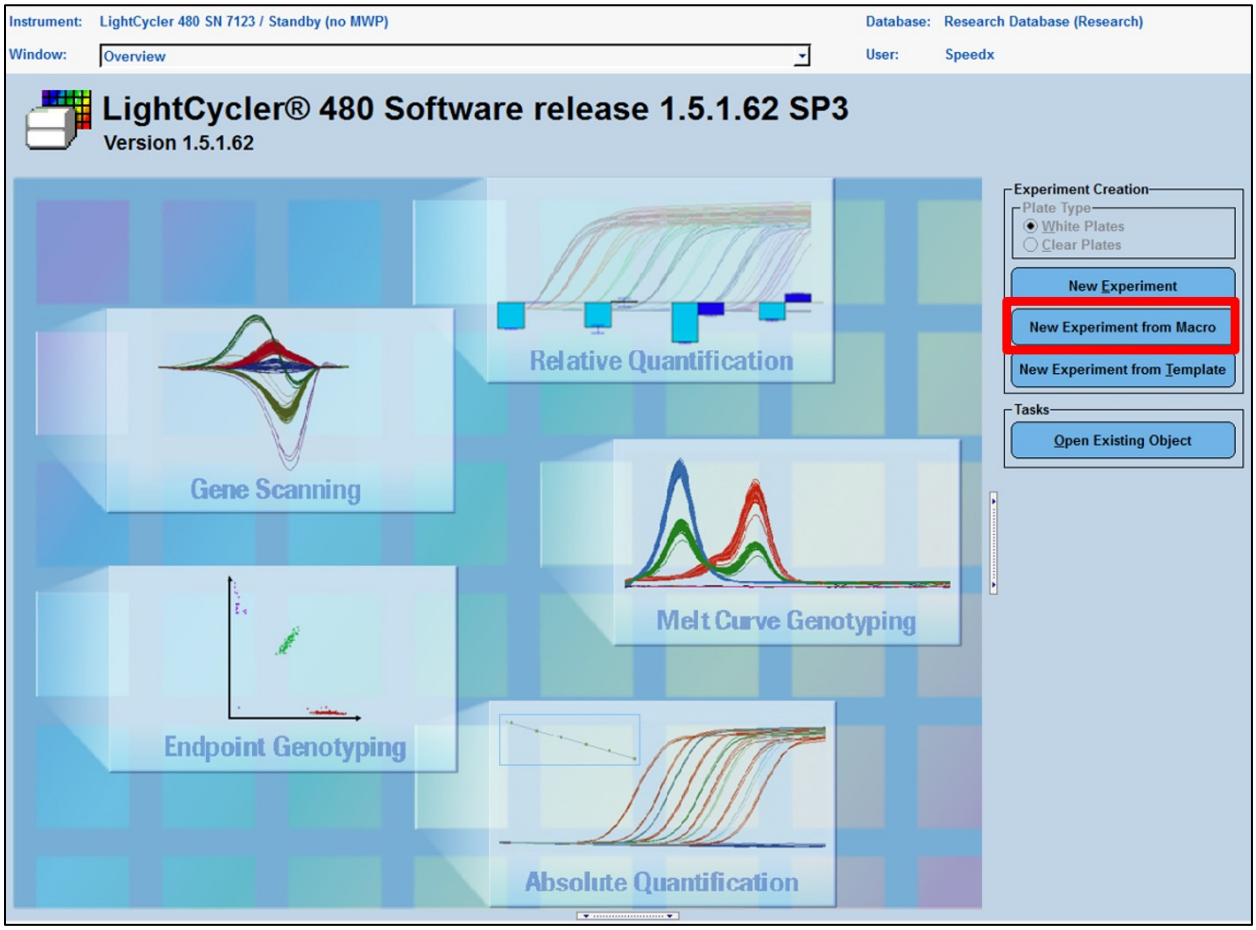


This **Macro template** will now be available to select when setting up for a run.

Macro Template set up

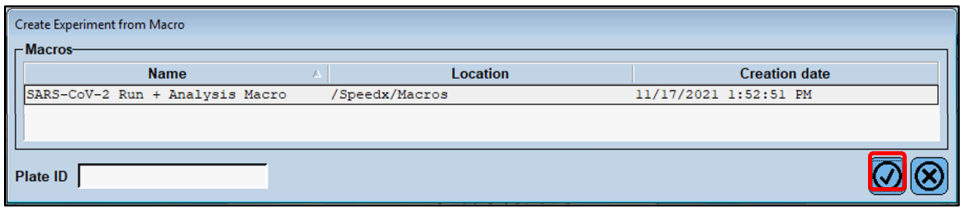
Select **New Experiment from Macro**

Figure 25. Selecting New Experiment from Macro



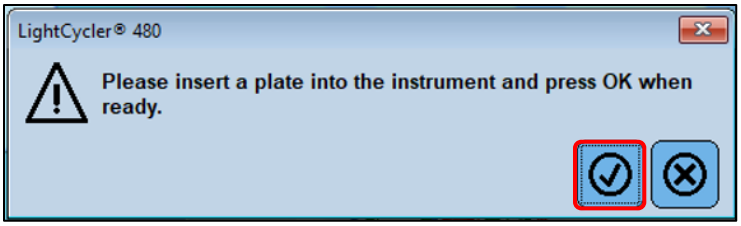
Select the file from the folder **Macros** > **Ok**

Figure 26. Selecting Macro Template



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

Figure 27. Insert Plate Message



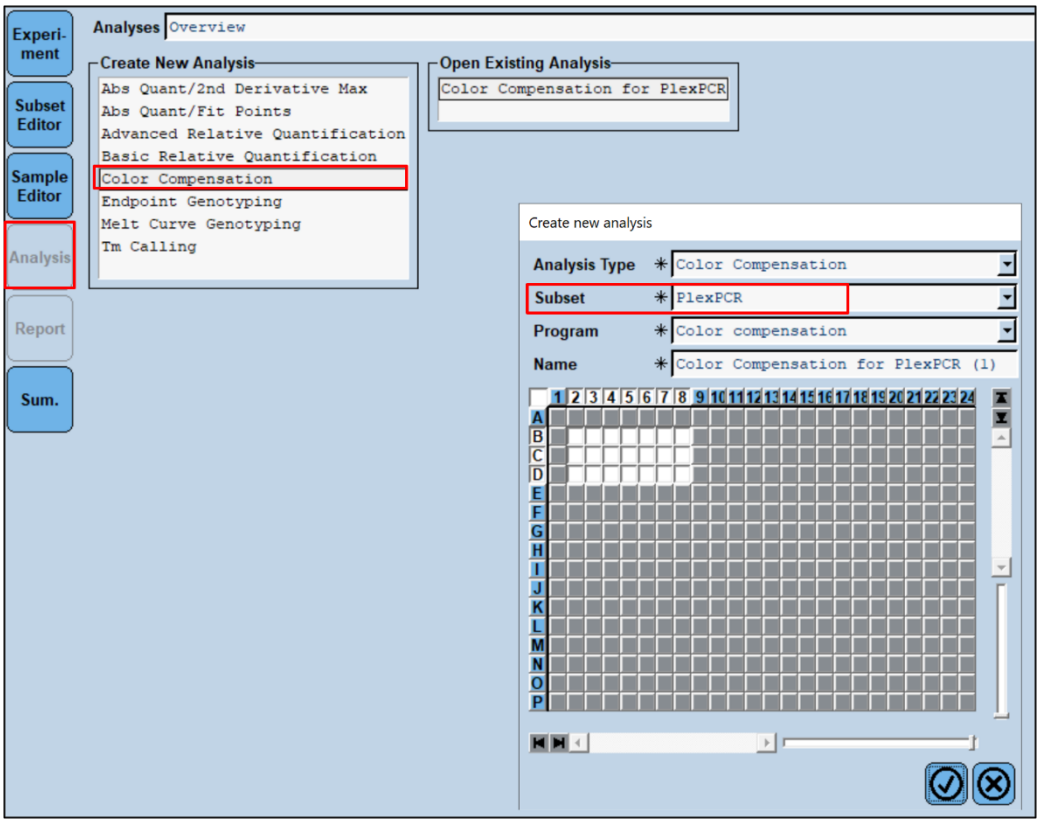
Proceed with using the **Subset Editor** and **Sample Editor** to ensure appropriate labelling for the results output.

19.4 Colour Compensation for LightCycler® 480 Instrument II

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

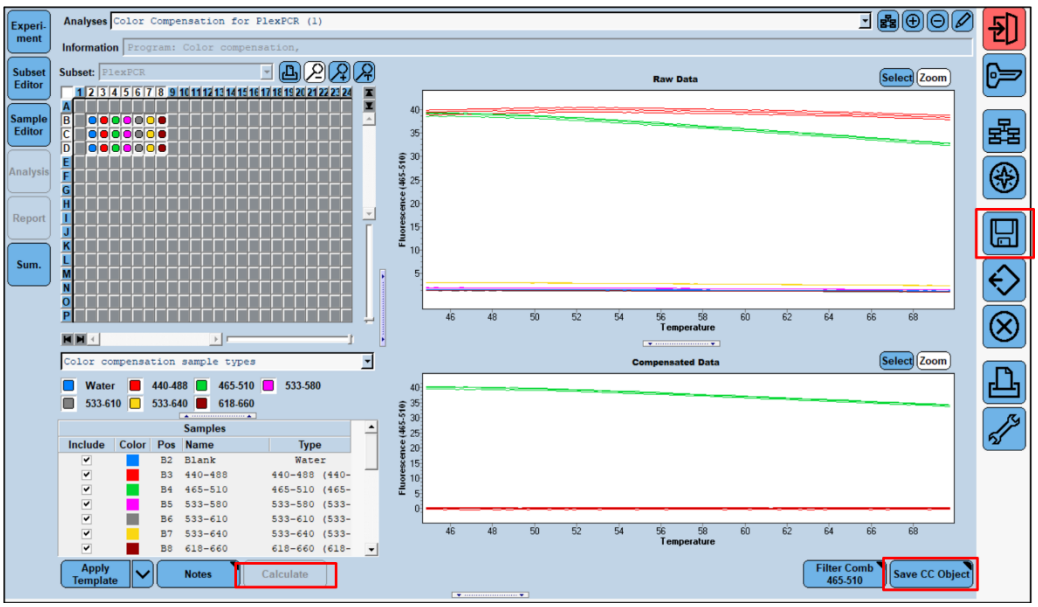
Analyse the Colour Compensation file via **Analysis > Colour Compensation** and select the correct subset, shown in **Figure 28.**

Figure 28. Analysis – Colour Compensation



Select **Calculate** (**Figure 29**).

Figure 29. Calculate and save CC Object



Refer to the PlexPCR Colour Compensation Instructions for Use (IF-IV0001) for further details to ensure the Colour Compensation file has been created correctly.

Select **Save** 

19.5 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 21** 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 21**). Example amplification curves are shown in **Figure 30.**

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 21**). 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.

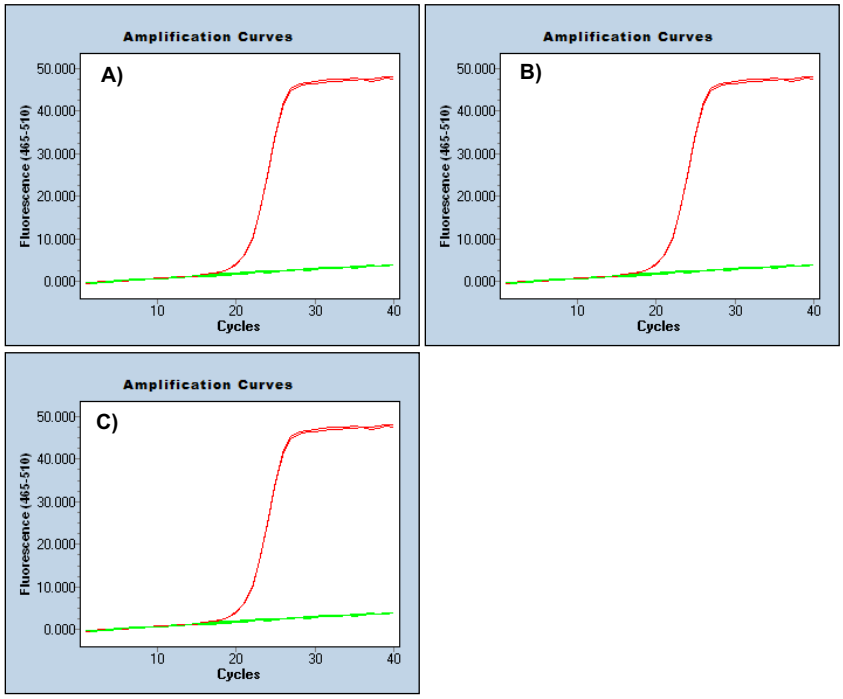
Note: For samples where target assays are negative, and the internal control assay is also negative:

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

Table 21. 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 30. 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 ***PlexPCR***® SARS-CoV-2 (LC480) analysis software.

20 Appendix A: Result Interpretation using *PlexPCR*® SARS-CoV-2 analysis software

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.

See **Table 22** for the analysis software details. The analysis software can be supplied upon request. Please contact tech@speedx.com.au for more information.

Table 22. *PlexPCR*® SARS-CoV-2 analysis software

Cat no	Analysis software*	Real-time PCR instrument
99021	***Plex****PCR*® SARS-CoV-2 (LC480)	LC480 II

* 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.

20.1 FastFinder platform – Minimum IT requirements

The analysis software is available within the FastFinder platform (https://www.ugentec.com/fastfinder/analysis). It is recommended that customers access the software platform from a secure and trusted network and computer. The minimum IT requirements for access and use of the FastFinder platform are listed below.

Hardware requirements

Internet Connection Cable or DSL

Min. screen resolution: 1366x768 pixels, optimal 1920 x 1080 pixels or higher

Supported browsers

- Microsoft Edge 88 or newer
- Firefox 83 or newer
- Google Chrome 88 or newer.

Firewall requirements

The following hosts must be reachable over HTTPS (port 443):

*.ugentec.app

*.fastfinder.app

*.pendo.io

*.fonts.gstatic.com

*.googleapis.com

*.msecnd.net

*.visualstudio.com

*.browser-update.org

*.blob.core.windows.net

*.powerbi.com

*.analysis.windows.net

*.pbideldicated.windows.net

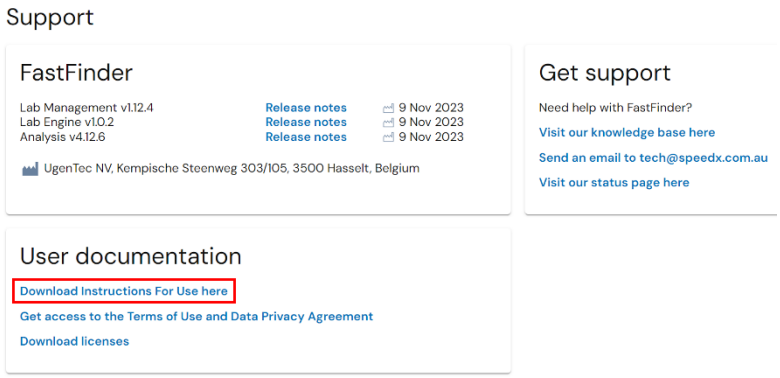
*.content.powerapps.com

If required, firewall exceptions will have to be configured for these hosts. In order to access all content of in-app user guides, the host *.player.vimeo.com must also be reachable.

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

To access the Support menu

- Select Support from the list of menu options on the left-hand side panel
- Select **Download Instructions For Use here** within the **User Documentation** section



20.2 Assay plug-in (new user)

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

FastFinder can be accessed directly through a web browser by logging in with your unique username and password at https://customer.fastfinder.app.

- Select **Lab Configuration > Assays** from the left-hand menu
- Select **Add New Assay**
- For LC480 II > Select **PlexPCR SARS-CoV-2 (LC480)** from the list
- Select **Import Selected**

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

> In **General tab**

> Navigate to the Status

> Select Active to activate or deactivate the version of the assay

20.3 Sample naming

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

Select Lab **Configuration > Assays** from the left-hand menu

- In the **General tab**, navigate to the **Sample types** table nametags (prefix), select ⊕ to add a new nametag
  - > Add desired word, acronym or letter to text box
  - > Default nametags are provided for the controls. These can be removed by selecting the × next to the nametag
- 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

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

20.4 Analysis

Select **Analyses** from the left-hand menu to start a new analysis

Select **+ Create New Analysis** from the top right of the screen

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

- Select run (data) file from the relevant folder
  > Select **Open**

The analysis will appear within the **Open Tab** as a new row within the table

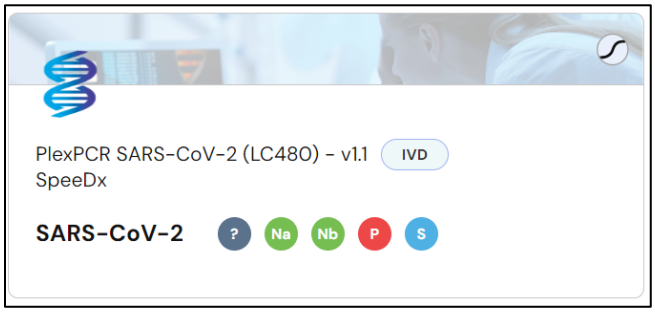
- If all nametags have been applied and read correctly, the status will appear as **Ready for review**
- If the assay information needs to be manually assigned to the wells, the status will appear as **Manual PCR setup required**

Assign the assay information to the plate manually if sample naming has not been set up in the Lab **Configuration > Assays** menu or sample names/targets have not been applied in the instrument software

Select the runfile from the **Open tab** within the **Analyses** menu

The Plate Configuration will be displayed within the **PCR setup tab** for the open analysis

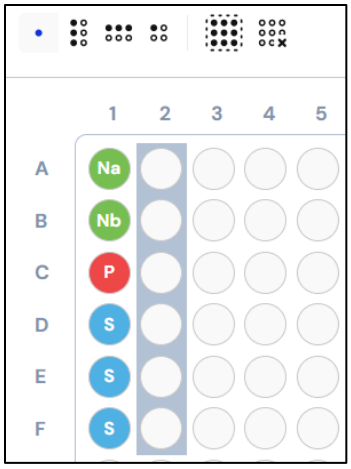
- For **LC480 II** > Select **PlexPCR SARS-CoV-2 (LC480)**



- Select wells and assign as:
  > Regular Sample (S)
  > Negative Control (Na)
  > No Template Control (Nb)
  > Positive Control (P)

To assign wells on the plate, either:

- Click and drag the coloured symbols to place them on the plate
- Select one or multiple wells (use Ctrl and shift keys) and then click the relevant coloured symbols to assign to selection.



- Select **Analyze**

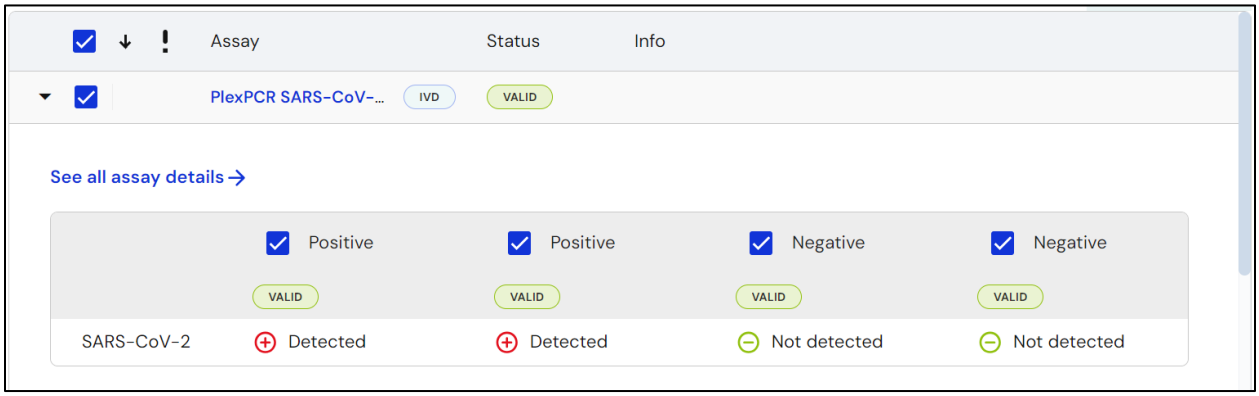
20.5 Results

See **Table 24** for a summary of possible reported sample results.

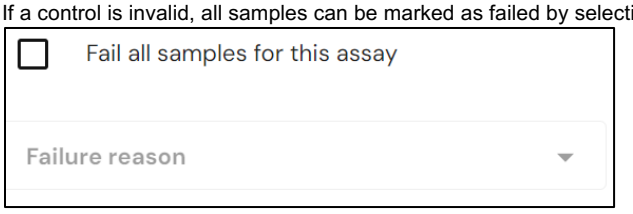
NOTE: It is highly recommended that amplification curves should be visually inspected and confirmed for all positive samples.

20.5.1 Summary Tab

Control results for every assay are shown at the top-left of the Summary tab, allowing evaluation of control validity for the run. More details can be found by expanding this block, displaying the details per control.

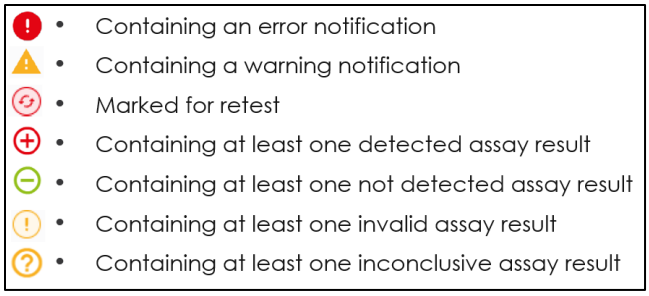


If a control is invalid, all samples can be marked as failed by selecting **Fail all samples for this assay**

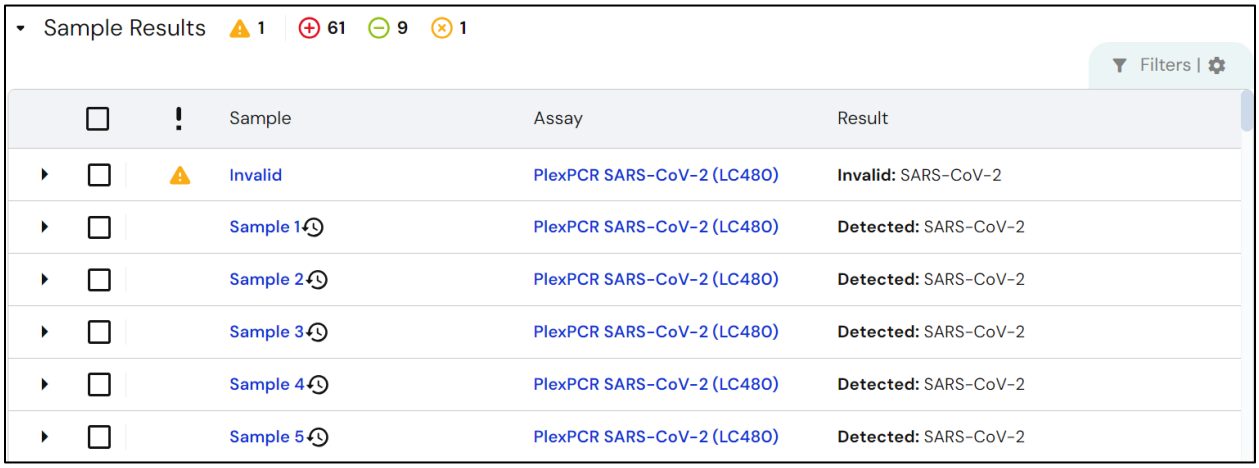


A failure reason needs to be chosen from the dropdown menu

Sample results are shown at the bottom-left of the Summary tab. Next to the header, additional icons may provide a high-level overview of the analysis results as well as indicating the total number of samples corresponding to a particular icon.

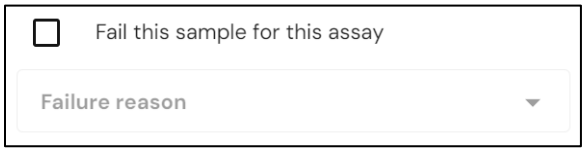


Each sample is displayed as a row within the sample results table.



The drop-down menu offers more details on each target result and Cq per sample (Refer to the examples shown in **Section 20.10**).

Individual samples can be marked as failed if desired (e.g. if the sample is Invalid) by selecting **Fail this sample for this assay**



A failure reason needs to be chosen from the dropdown menu

Fluorescence graphs can be viewed at the top-right of the Summary tab

A plate layout can be viewed at the bottom-right of the Summary tab

Example information and warning notifications are summarized below in **Table 23.**

Table 23. Example information and warning notifications for the PlexPCR® SARS-CoV-2 analysis software*

Sample Type	Error	Notification
Assay target notifications		
Regular Sample	Invalid – IC failure	Warning: IC invalid. Re-extract and re-test sample.
	Valid but control invalid – Invalid control warning on regular sample with valid result	Warning: Invalid control present. Re-extract and re-test the sample.
Negative Control	Invalid - Contamination	Warning: Possible contamination detected.
No Template Control		
Gene target notifications		
Regular Sample	Target Cq outside cut-off	Info: Cq outside cutoff

Table 23. Example information and warning notifications for the PlexPCR® SARS-CoV-2 analysis software*		
Sample Type	**Error**	**Notification**
Positive Control	Invalid – Target not detected	Warning: Expected reaction did not occur in control.
Negative Control	Invalid - Contamination	Warning: Possible contamination
	Invalid – IC not detected	Warning: IC not detected
	Invalid – IC Cq outside cut-off	Warning: Cq outside cutoff
No Template Control	Invalid - Contamination	Warning: Possible contamination
Regular Sample or Control	Uncertain fluorescence signal	Warning: Uncertain fluorescence signal. Review required.
	Cq detected with low fluorescence	dRn end fluorescence below cut-off

*The examples listed here may not be applicable for all assay plug-ins. Refer to the FastFinder Instructions For Use for all possible notifications, accessible from the Support menu

20.5.2 Details Tab

All targets are shown for each sample as separate rows within the table on the left-hand side. Selecting one or more rows will display the corresponding fluorescence curves on the graph at the top-right and will also highlight the wells within the plate layout shown at the bottom-right.

Select **Filters** to display results according to parameters such as assay name, sample type, target and result.

To finalise analysis and prevent further user edits

> Select **Authorize**

> Select **Authorize** again to confirm

- To assign a second review

> Select **Actions**, **Assign label** and **Second Review**

- To assign the analysis to a different user

> Select **Actions** and **Assign User**

> Select the appropriate user from the drop-down list

- To reject the analysis

> Select **Actions** and **Discard Analysis**

> Add a comment and select **Discard** to confirm

20.6 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 **Summary** or **Details** tab
- From the amplification graph menu > Select 

> Select the check box for the curve of interest and select **Mark as reference**

This reference curve will now appear linked to the assay in the **Lab Configuration > Assays** menu within the **PCR tab** and can be inactivated at any time.

20.7 Exporting results

- To export results from an individual authorised run as either a CSV or PDF file:

> Select **Actions > Downloads** in the top-right corner

> Select either of the following report types: **Analysis (CSV)** or **Analysis (PDF)**

- To export results from multiple previously authorised runs as a single CSV file:
  > Navigate to the **Archive > Sample Results** menu
  > Use the filters at the top of the page to display the results of interest (the CSV file is limited to a maximum of 10,000 results)
  > Select **Export CSV** in the top-right corner

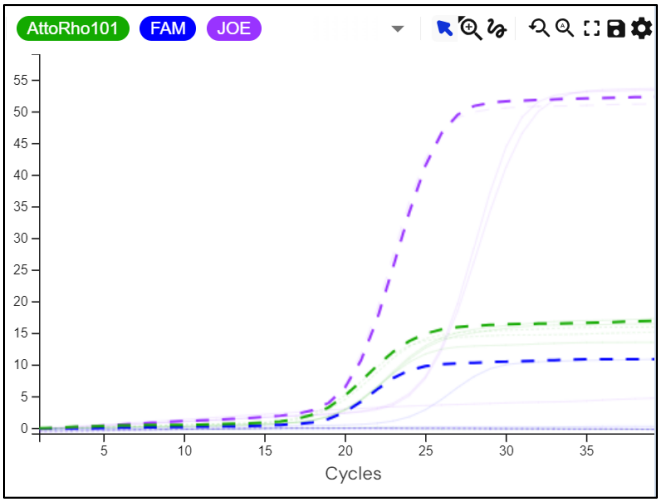
20.8 Retrieving authorized analyses

- All authorized analyses are available by selecting **Archive > Analysis Results**. Select a row to return to the results overview for that particular analysis
- All authorized regular samples are stored within the **Archive > Sample Results** menu. Selecting a sample will display additional information including the analysis name and the result details
- The individual target results for all authorized regular samples and controls are stored within the **Archive > Target Results** menu. Selecting a target will highlight this on the fluorescence graph. Selecting the Analysis Name will return to the results overview for that particular analysis.

20.9 Control example graphs

The following examples show the amplification curves (baseline-corrected amplification curves) and the Results overview from the **PlexPCR SARS-CoV-2 (LC480)** analysis software for control sample types.

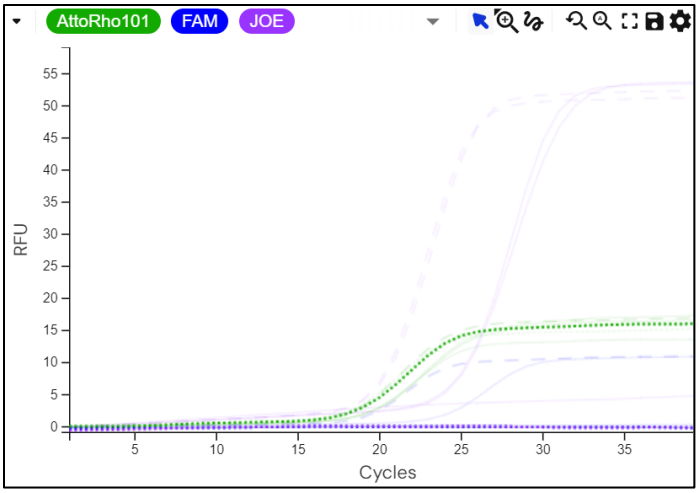
20.9.1 Positive control



ORF1ab **RdRp** **IC**

Sample	Assay	Result
PC	PlexPCR SARS-CoV-2 (LC480)	**Valid**
SARS-CoV-2 ⊕ Detected		

20.9.2 Negative control (negative specimen)



ORF1ab RdRp IC

Sample	Assay	Result
NC	PlexPCR SARS-CoV-2 (LC480)	**Valid**
SARS-CoV-2 ⊖ Not detected		

20.10 Examples

Example results for the ***PlexPCR***® SARS-CoV-2 analysis software are shown in **Table 24.**

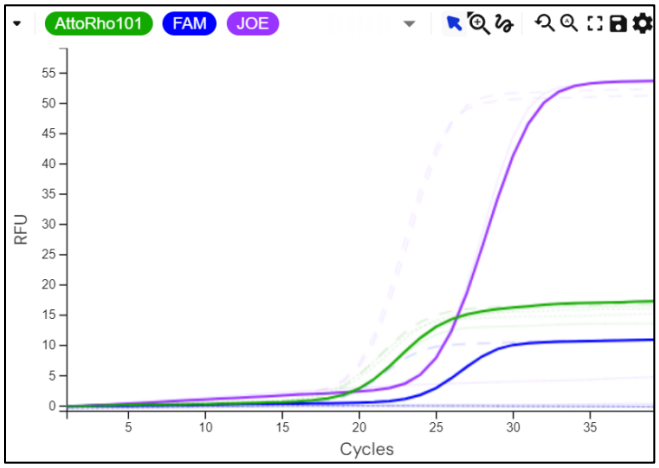
Table 24. Example results for interpretation of the *PlexPCR*® SARS-CoV-2 analysis software

	Sample	Assay	Result
	Sample 101	PlexPCR SARS-CoV-2 (LC480)	**Not detected**
	Sample 102	PlexPCR SARS-CoV-2 (LC480)	**Detected: SARS-CoV-2**
[1]	Sample 103	PlexPCR SARS-CoV-2 (LC480)	**Invalid: SARS-CoV-2**

[1] A sample interpreted as Invalid will be flagged with ⚠

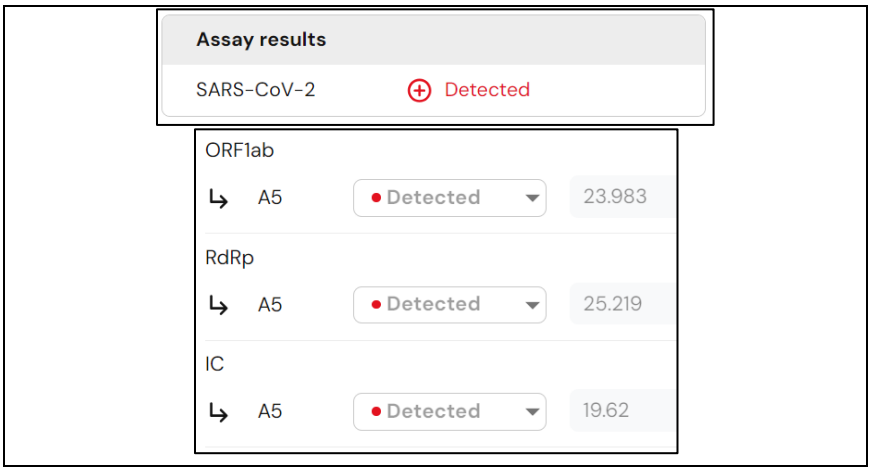
The following examples show the amplification curves (baseline-corrected amplification curves) and the Results overview from the **PlexPCR SARS-CoV-2 (LC480)** analysis software for regular/unknown sample types.

20.10.1 Positive sample 1 (Both SARS-CoV-2 targets detected)

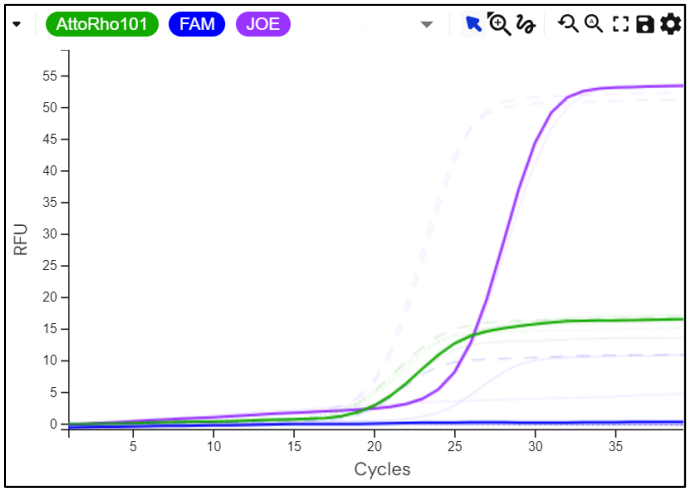


ORF1ab **RdRp** **IC**

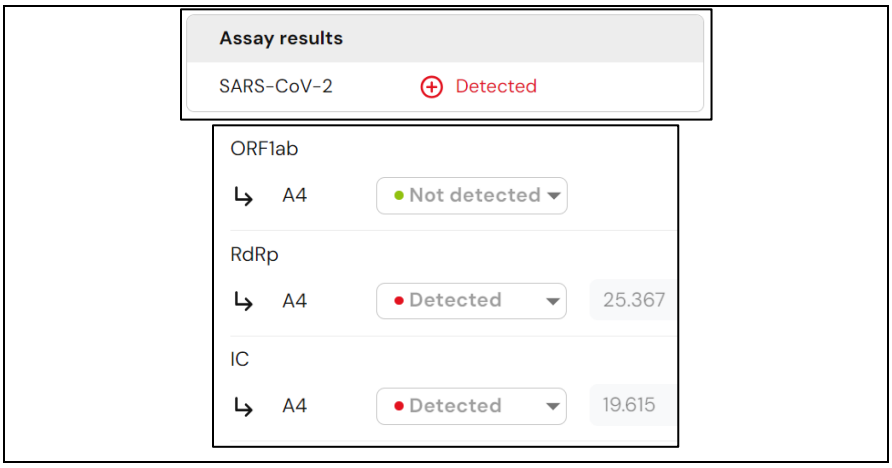
Sample	Assay	Result
Sample 201	PlexPCR SARS-CoV-2 (LC480)	**Detected:** SARS-CoV-2



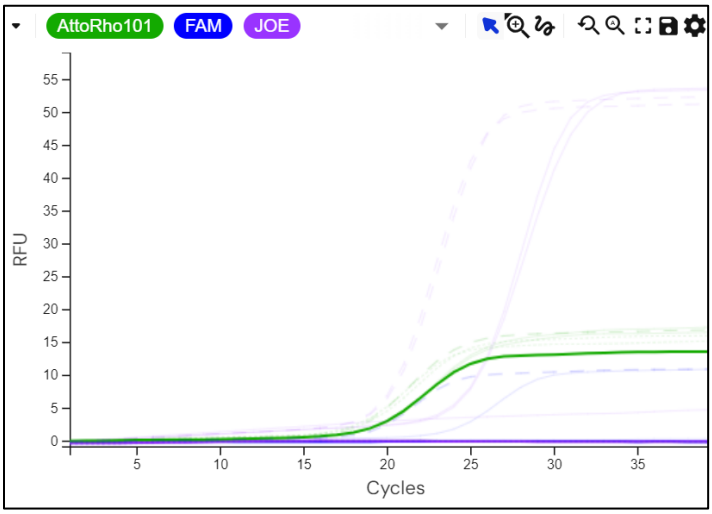
20.10.2 Positive sample 2 (Single SARS-CoV-2 target detected)



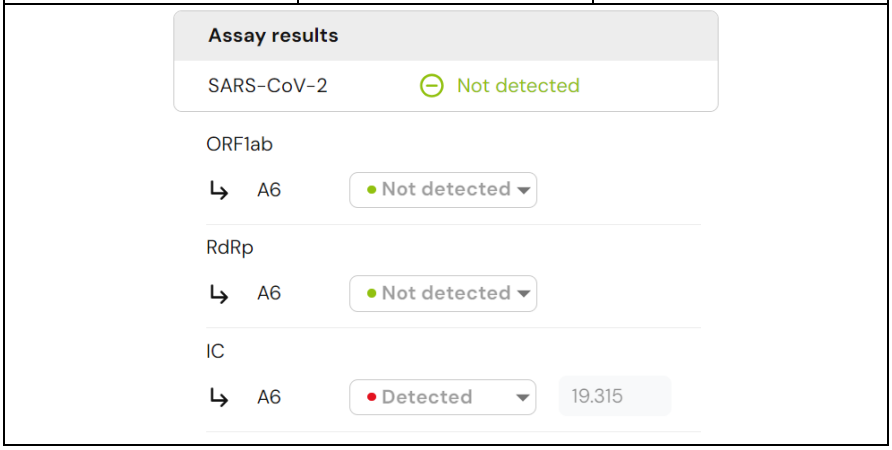
Sample	Assay	Result
Sample 202	PlexPCR SARS-CoV-2 (LC480)	**Detected:** SARS-CoV-2



20.10.3 Negative sample



Sample	Assay	Result
Sample 203	PlexPCR SARS-CoV-2 (LC480)	**Not detected**



21 Glossary

For *In Vitro* Diagnostic Use

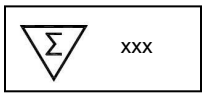
Catalogue Number

Batch Code


Manufacturer


Date of manufacture


Temperature Limitation



Contains sufficient for xxx determinations


Use by Date

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