Instruction for Use

Color Compensation Kit SARS-CoV-2 seqc

Color Compensation for the use of specific PCR kits (e.g. virellaSARS-CoV-2 seqc real time RT-PCR) on LightCycler® 480 II Instruments.

G070MP2-CC

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1 Intended Use

Color Compensation Kit SARS-CoV-2 seqc is intended for generating a Color Compensation File for the multiplex real-time RT-PCRs virellaSARS-CoV-2 seqc and other specific PCR kits on the LightCycler® 480 II instruments.

2 Principle of the Kit

Due to the emission spectra overlap of FAM (Green), HEX (Yellow), ROX (Orange) and Cy5 (Red) the Roche LightCycler® 480 II can pick up signals from dyes measured by another channel. This so called 'crosstalk' can lead to incorrect results. To correct the crosstalk impact, Roche recommends to create a Color Compensation File. This File permits the LightCycler® 480 software to subtract fluorescence crosstalk from a reporter dye into inappropriate channels.

3 Package Contents

The reagents supplied are sufficient for 3 reactions (one run).

Table 1: Components of the Color Compensation Kit SARS-CoV-2 seqc.

Label	Lid Color	Content 3 Reactions
Blank Mix	clear	55,2 µl
Green Mix	green	55,2 µl
Orange Mix	orange	55,2 µl
Red Mix	red	55,2 µl
Yellow Mix	yellow	55,2 µl
Blank Control	clear	24 µl
Positive Control	violett	96 µl

4 Equipment and Reagents to be Supplied by User

- LightCycler® 480 II
- Sterile microtubes
- Disposable powder-free gloves
- Pipets (adjustable volume)
- Sterile pipet tips with filters
- Plate centrifuge
- Vortexer
- LightCycler® 480 II 96 well plate and plate sealer (optical foil)

5 Transport, Storage and Stability

Color Compensation Kit SARS-CoV-2 seqc is shipped on dry ice or cool packs. All components must be stored at ≤-18°C in the dark immediately after receipt. Do not use reagents after the date of expiry printed on the package.
For convenience, opened reagents can be stored at +2-8°C for up to 6 months.
Protect kit components from direct sunlight during the complete test run.

6 Important Notes

- Color Compensation Kit SARS-CoV-2 seqc must be performed by qualified personnel only.
- Good Laboratory Practice (GLP) has to be applied.
- Stick to the protocol described in the Instruction for Use.
- Regularly decontaminate equipment and benches with ethanol-free decontaminant.
- Do not combine Color Compensation Kit SARS-CoV-2 seqc components of different lot numbers.

7 Creating a Color Compensation File for the LightCycler® 480 II

7.1 Important Points Before Starting

- Please pay attention to the chapter 6 ‚Important Notes'.
- Before setting up the real time PCR familiarise yourself with the real time PCR instrument and read the user manual supplied with the instrument.
- The programming of the thermal profile should take place before the PCR setup.
- Before each use, all reagents – except the enzyme - should be thawed completely at room temperature, thoroughly mixed and centrifuged briefly.

7.2 Preparation of the Color Compensation Reaction Mixes

Before starting the Color Compensation experiments it is important to prepare the Reaction Mixes. These Mixes must be combined in the corresponding tubes according to Table 2.

Table 2: Prepare mixes of Color Compensation Kit SARS-CoV-2 seqc.

Blank	n=1	n=3 (3+1)
Blank Mix	13,8 µl	55,2 µl
Blank Control	6 µl	24 µl
Total	19,8 µl	79,2 µl

Green	n=1	n=3 (3+1))
Green Mix	13,8 µl	55,2 µl
Positive Control	6 µl	24 µl
Total	19,8 µl	79,2 µl

Orange	n=1	n=3 (3+1)
Orange Mix	13,8 µl	55,2 µl
Positive Control	6 µl	24 µl
Total	19,8 µl	79,2 µl

Red	n=1	n=3 (3+1)
Red Mix	13,8 µl	55,2 µl
Positive Control	6 µl	24 µl
Total	19,8 µl	79,2 µl

Yellow	n=1	n=3 (3+1))
Yellow Mix	13,8 µl	55,2 µl
Positive Control	6 µl	24 µl
Total	19,8 µl	79,2 µl

Seal the plate with optical foil, centrifuge the plate for 1 min at 1500 x g.

7.3 Preparation of the Color Compensation Plate

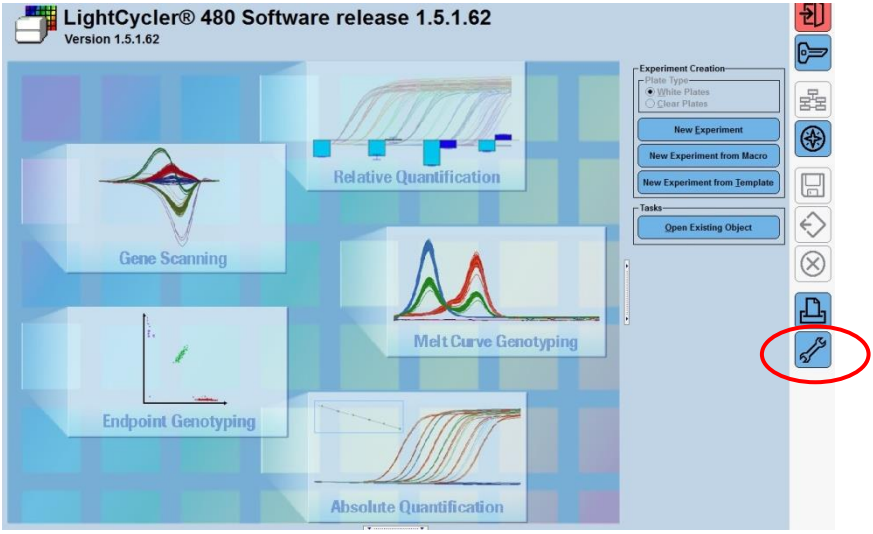
For the Color Compensation experiment pipette 3 reactions with 20 µl of each dye into the microwell plate as shown in Table 3. Seal the plate with the optical foil and centrifuge briefly.

Table 3: Pipetting scheme for Color Compensation experiment.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank		Green		Orange		Red		Yellow			
B												
C												
D												
E												
F												
G												
H												

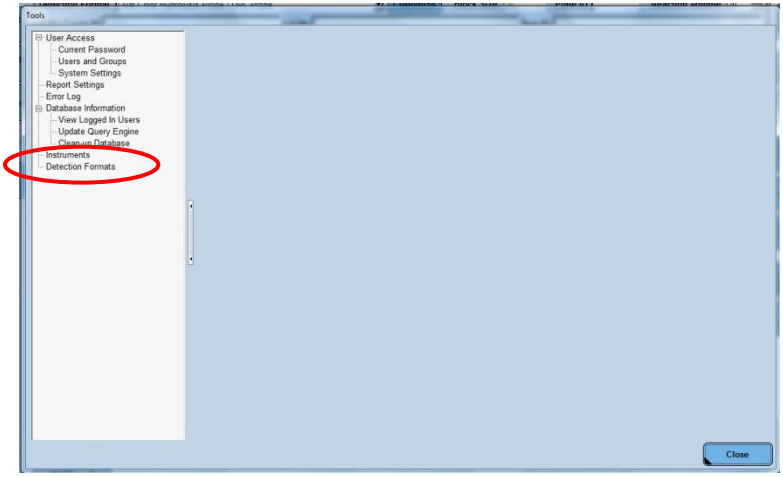
7.4 Run a Color Compensation experiment

Open the LightCycler® 480 Software and click on ‘open Tools’.



Detection Format

Select ‘Detection Formats’ in the new window.



Click ‘New’ in the folder ‘Detection Formats’ and choose a new name, e.g. G070MP2-CC for your new detection format. Use the checkboxes in ‘Filter Combination Selection’ to mark the required wavelengths. Set ‘Melt Factor’ to 1 and ‘Quant Factor’ to 10. ‘Max integration Time’ is set on 1 for 465-510 nm, on 2 for 533–580 nm and 533–610 nm and on 3 for 618–660 nm. Close the window.

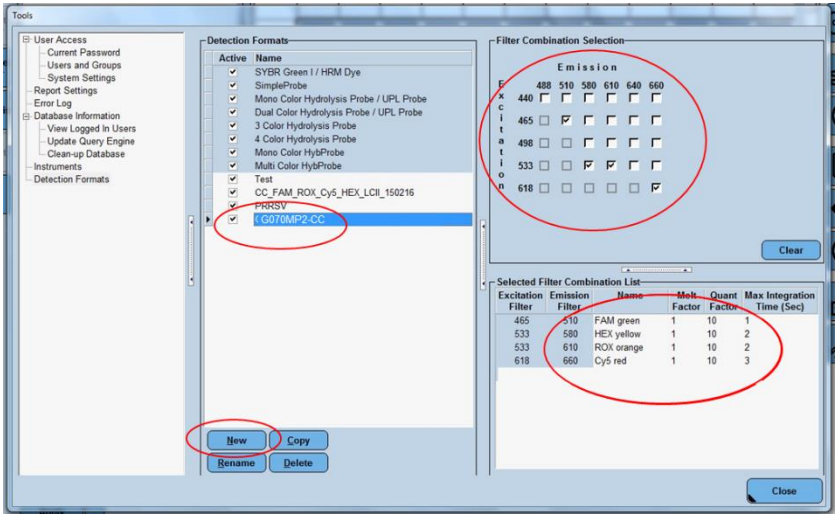
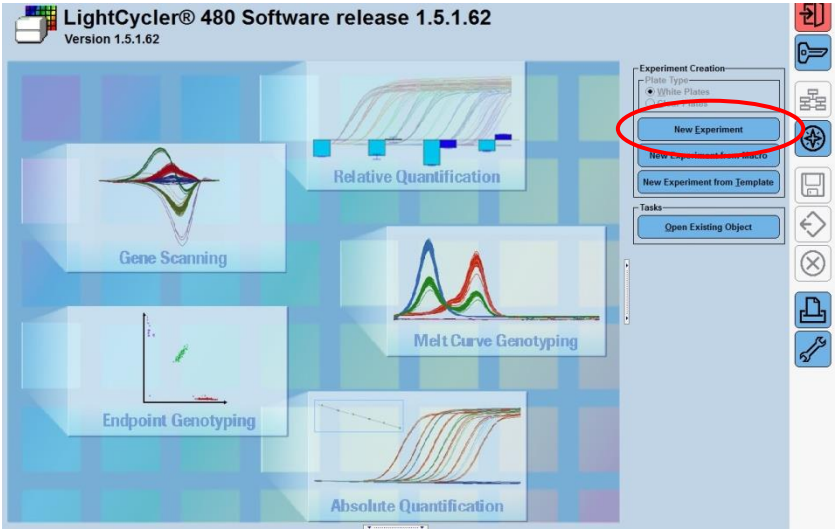


Table 4: Filter Setting and Filter Combination List

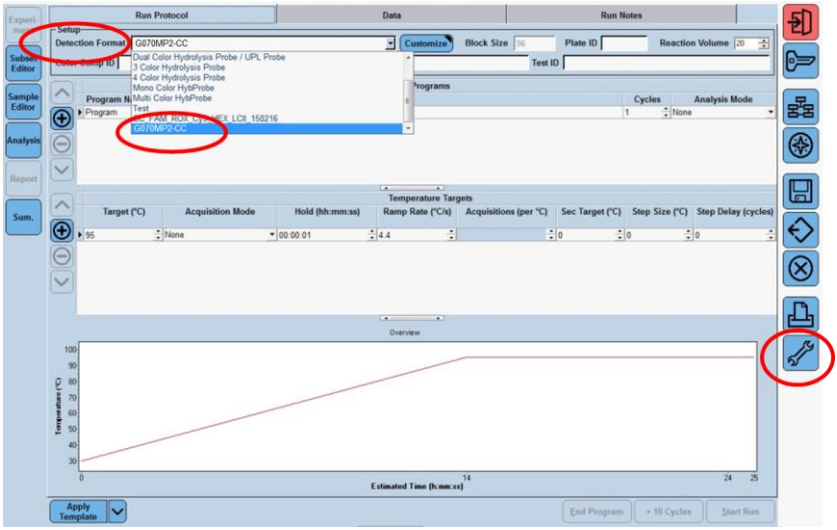
		Emission Filters					
		488	510	580	610	640	660
Excitation Filters	440						
	465		Green				
	498						
	533			Yellow	Orange		
	618						Red

	Name	Melt Factor	Quant Factor	Max integration time
465 – 510 nm	Green	1	10	1
533 – 580 nm	Yellow	1	10	2
533 – 610 nm	Orange	1	10	2
618 – 660 nm	Red	1	10	3

Create the new Color Compensation Experiment. Click on ‘New Experiment’.

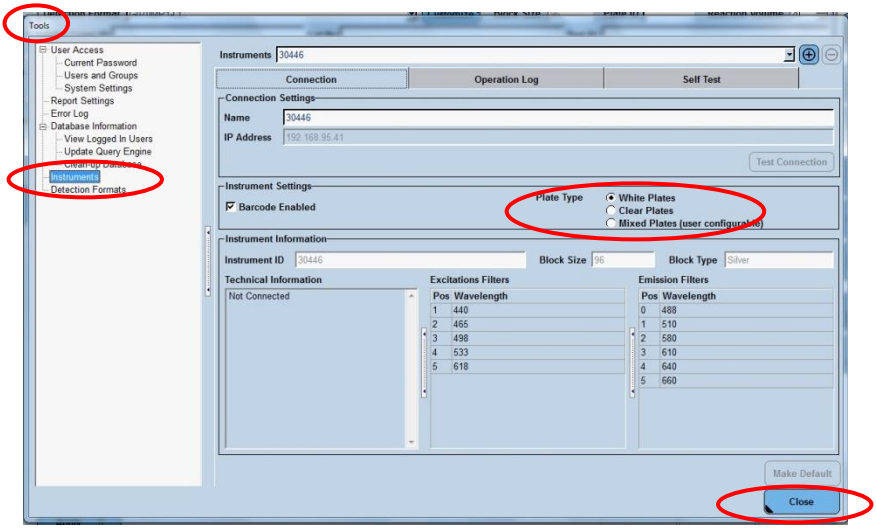


Choose the new detection format in ‘Setup’. Click ‘Open Tools’.



Instrument Settings

Select ‘Instruments’. Make sure to enter the correct plate type (white or clear). The Color Compensation is bound to this kind of plate, if the plate type is changed, an additional Color Compensation experiment will be needed. Close the window.



Program Settings

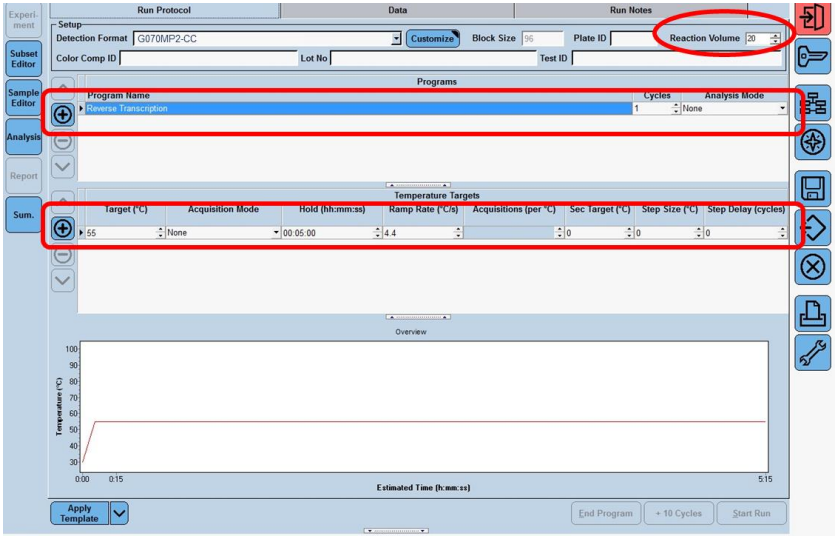
Please refer to screenshots starting from page 12. Press ‘+’ to add further steps to the PCR protocol. Change the ‘Program Name’, ‘Cycles’, ‘Analyses Mode’, ‘Target’, ‘Acquisition Mode’, ‘Hold’, ‘Ramp Rate’ as shown in Table 6.

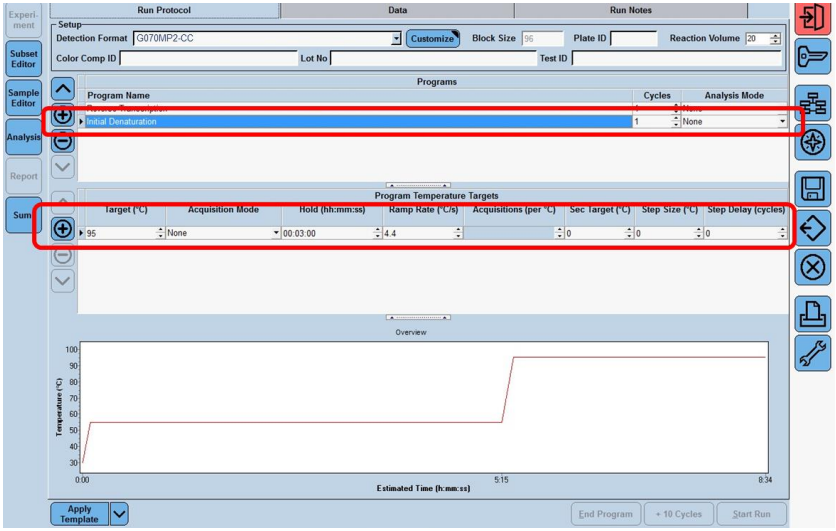
Table 5: Cycler steps for Color Compensation

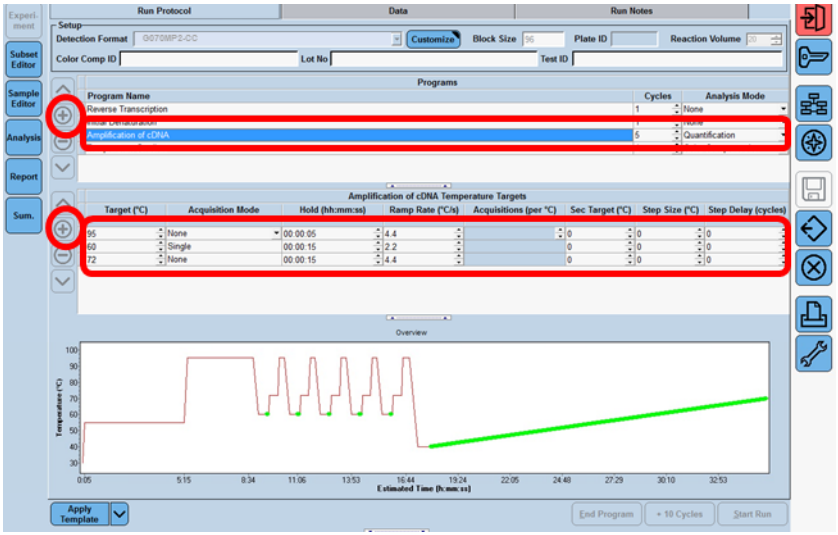
Program Name	Cycles	Analysis Mode
Reverse Transcription	1	None
Initial Denaturation	1	None
Amplification	5	Quantification
Temperature Gradient	1	Color Compensation

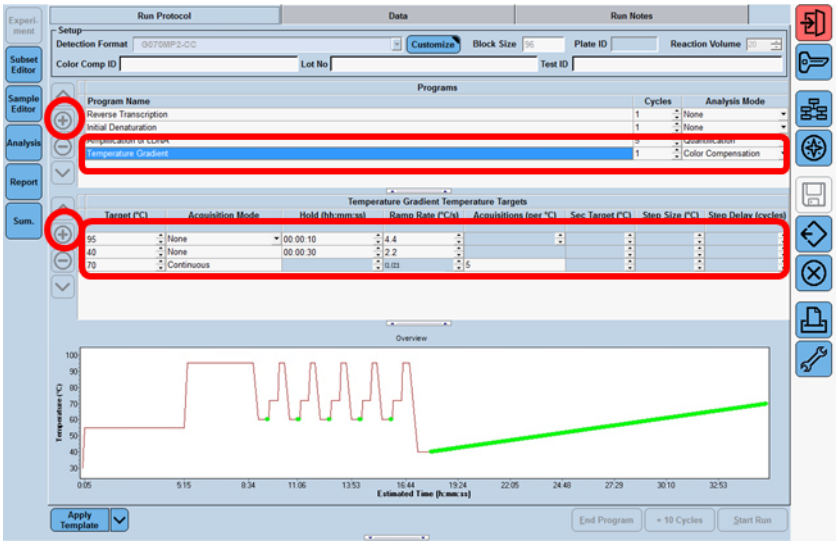
Table 6: Program Temperature Targets

Description	Temp.	Acquisition Mode	Time	Ramp Rate (°C / s)	Acquisition (per °C)
Reverse Transcription	55°C	none	5 min	4.4	
Initial Denaturation	95°C	none	3 min	4.4	
Amplification	95°C	none	5 s	4.4	
	60°C	single	15 s	2.2	
	72°C	none	15 s	4.4	
Temperature Gradient	95°C	none	10 s	4.4	
	40°C	none	30 s	2.2	
	70°C	continuous		0.02	5


Run Protocol
Data
Run Notes
Setup
Detection Format G070MP2-CC
Customize
Block Size 96
Plate ID
Reaction Volume 20
Color Comp ID
Lot No
Test ID
Programs
Program Name
Cycles
Analysis Mode
Reverse Transcription
1
None
Temperature Targets
Target (°C)
Acquisition Mode
Hold (hh:mm:ss)
Ramp Rate (°C/s)
Acquisitions (per °C)
Sec Target (°C)
Step Size (°C)
Step Delay (cycles)
55
None
00:05:00
4.4
0
0
0
Overview
Temperature (°C)
Estimated Time (h:mm:ss)
0:00
0:15
5:15
Apply Template
End Program
+ 10 Cycles
Start Run


Run Protocol
Data
Run Notes
Setup
Detection Format G070MP2-CC
Customize
Block Size 96
Plate ID
Reaction Volume 20
Color Comp ID
Lot No
Test ID
Programs
Program Name
Cycles
Analysis Mode
Initial Denaturation
1
None
Program Temperature Targets
Target (°C)
Acquisition Mode
Hold (hh:mm:ss)
Ramp Rate (°C/s)
Acquisitions (per °C)
Sec Target (°C)
Step Size (°C)
Step Delay (cycles)
95
None
00:03:00
4.4
0
0
0
Overview
Temperature (°C)
Estimated Time (h:mm:ss)
0:00
5:15
8:34
Apply Template
End Program
+ 10 Cycles
Start Run

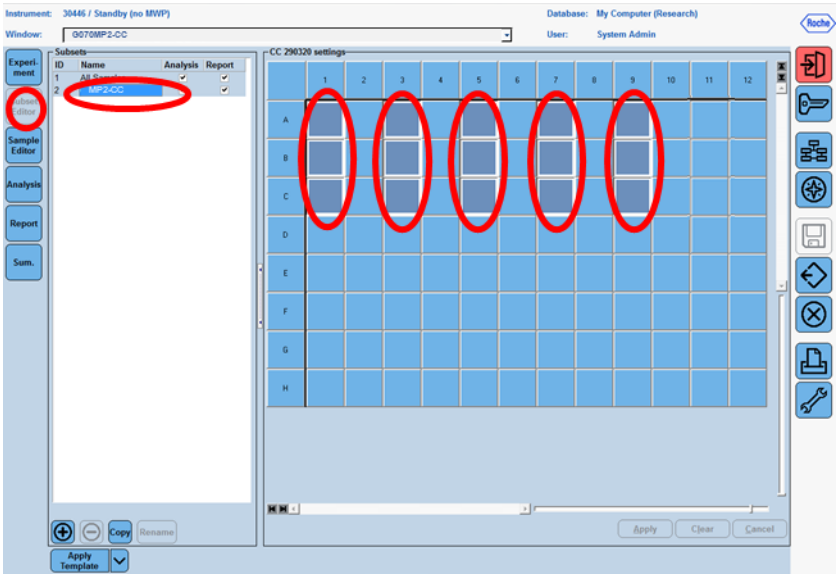

Run Protocol
Data
Run Notes
Setup
Detection Format
Customize
Block Size
Plate ID
Reaction Volume
Color Comp ID
Lot No
Test ID
Programs
Program Name
Cycles
Analysis Mode
Reverse Transcription
Amplification of cDNA
Quantification
Amplification of cDNA Temperature Targets
Target (°C)
Acquisition Mode
Hold (hh:mm:ss)
Ramp Rate (°C/s)
Acquisitions (per °C)
Sec Target (°C)
Step Size (°C)
Step Delay (cycles)
95 None 00:00:05 4.4
60 Single 00:00:15 2.2
72 None 00:00:15 4.4
Overview
Temperature (°C)
Estimated Time (h:mm:ss)
Apply Template
End Program
+ 10 Cycles
Start Run


Run Protocol
Data
Run Notes
Setup
Detection Format
Customize
Block Size
Plate ID
Reaction Volume
Color Comp ID
Lot No
Test ID
Programs
Program Name
Cycles
Analysis Mode
Reverse Transcription
Initial Denaturation
Temperature Gradient
Color Compensation
Temperature Gradient Temperature Targets
Target (°C)
Acquisition Mode
Hold (hh:mm:ss)
Ramp Rate (°C/s)
Acquisitions (per °C)
Sec Target (°C)
Step Size (°C)
Step Delay (cycles)
95 None 00:00:10 4.4
40 None 00:00:30 2.2
70 Continuous 0.03 6
Overview
Temperature (°C)
Estimated Time (h:mm:ss)
Apply Template
End Program
+ 10 Cycles
Start Run

Sample setting

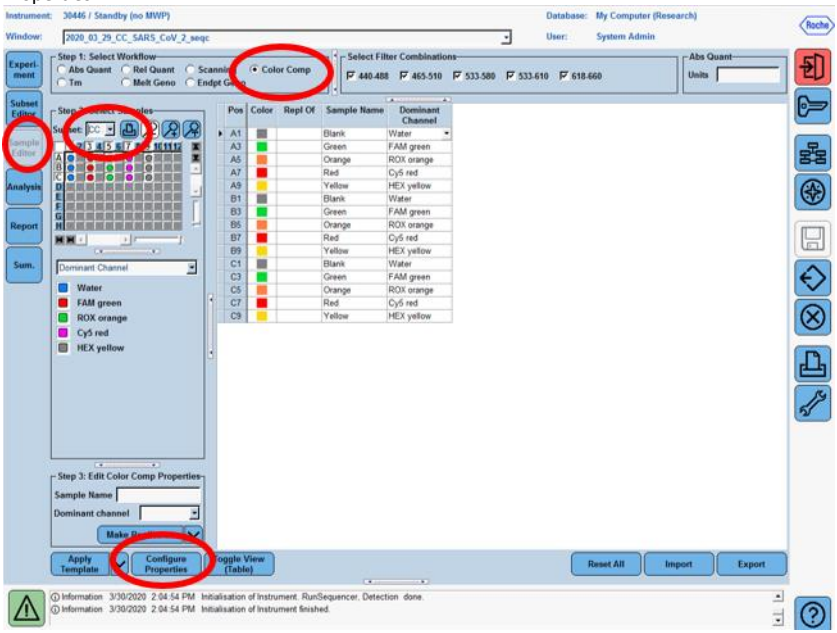
Subset Editor:

For optimal measurement and calibration of the LightCycler® 480 II it is necessary to create a subset for each well which is included in the Color Compensation experiment. Choose 'Subset Editor', click on '+' and add the name of the new subset e.g. MP2-CC. Press 'Strg' (Ctrl) and select the wells included in the experiment and click 'Apply'.

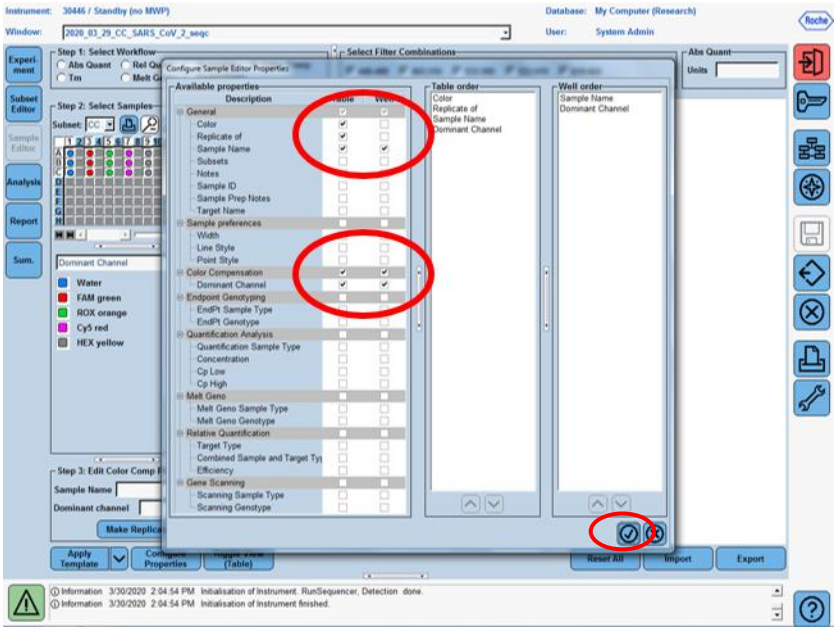


Sample Editor:

Choose the ‘Sample Editor’, tick ‘Color Comp’ and choose the created subset. Select ‘Configure Properties’.



Click ‘Color’, ‘Replicate of’, ‘Sample Name’, ‘Color Compensation’ and ‘Dominant Channel’. Click ‘ok’.



Insert ‘Sample Name’ and ‘Dominant Channel’ into ‘Step 3: Edit Color Comp Properties’ according to Table 7. Coloring of the respective rows can be edited in ‘Color’.

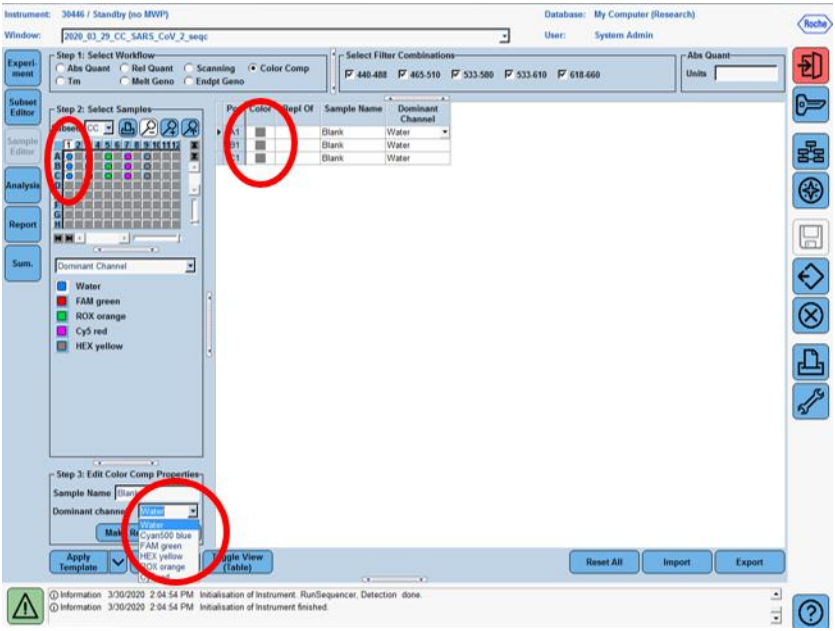
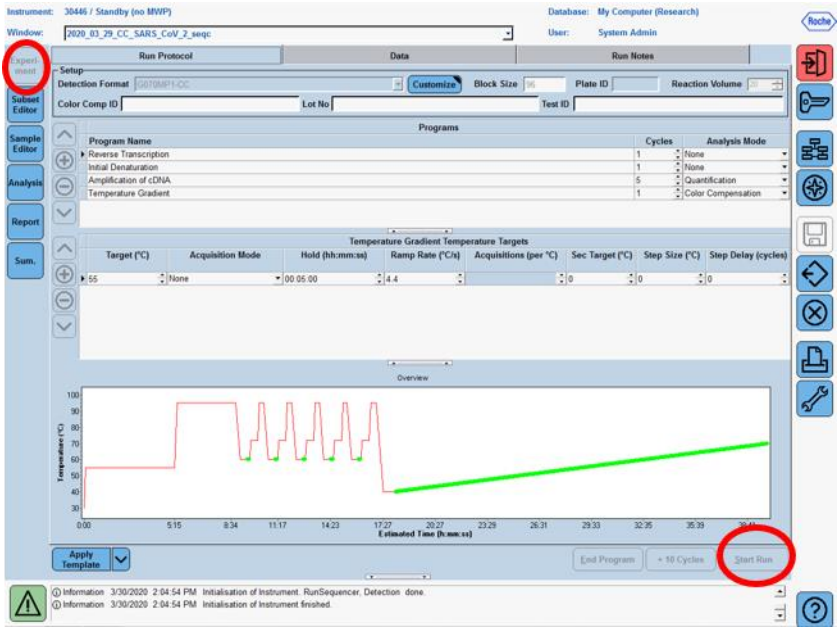


Table 7: Dominant channels for Color Compensation

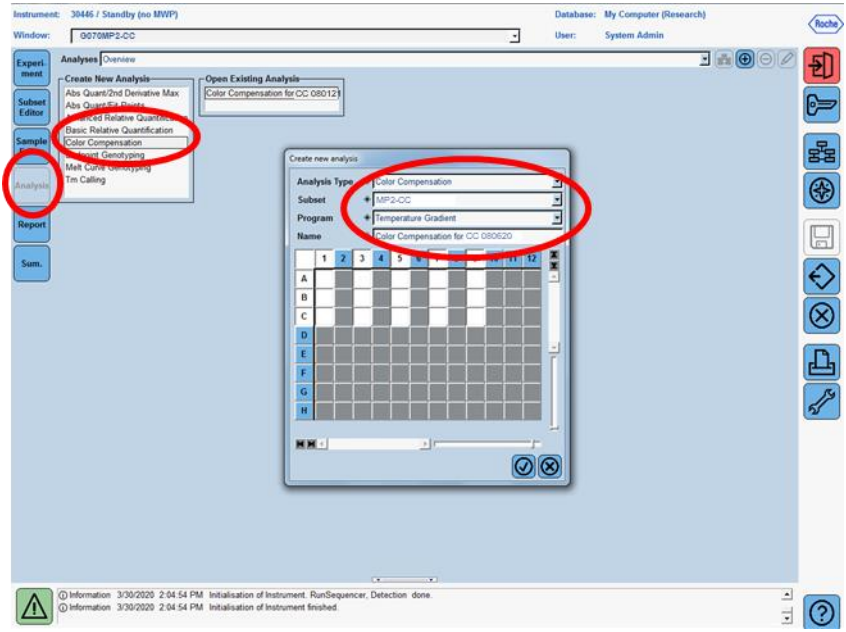
Row	Reagent	LightCycler® 480 II
1	Blank	Water
3	Green	465 / 510
5	Orange	533 / 610
7	Red	618 / 660
9	Yellow	533 / 580

Select ‘Experiment’. Open tray of the LightCycler® 480 II instrument. Insert centrifuged plate and close tray. Start the run on the ‘start run’ button and save the experiment.

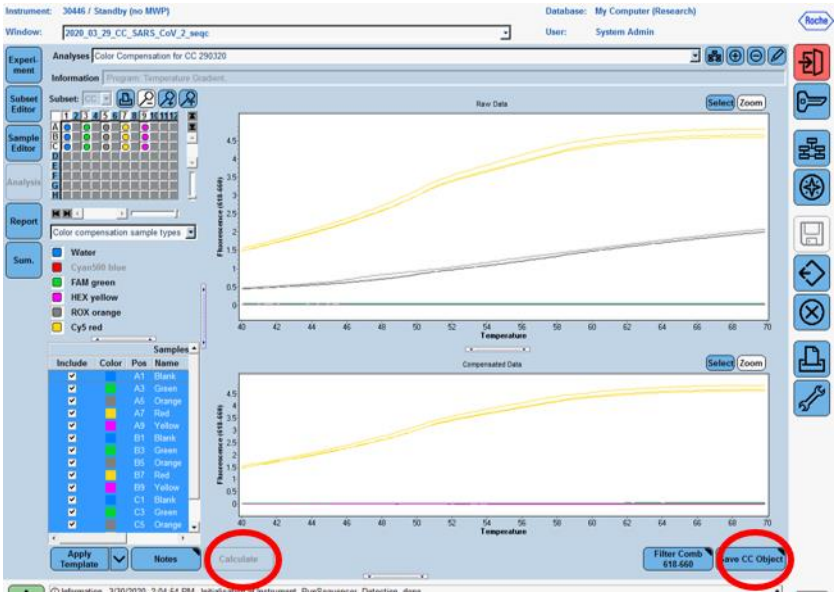


7.5 Creation of the Color Compensation File

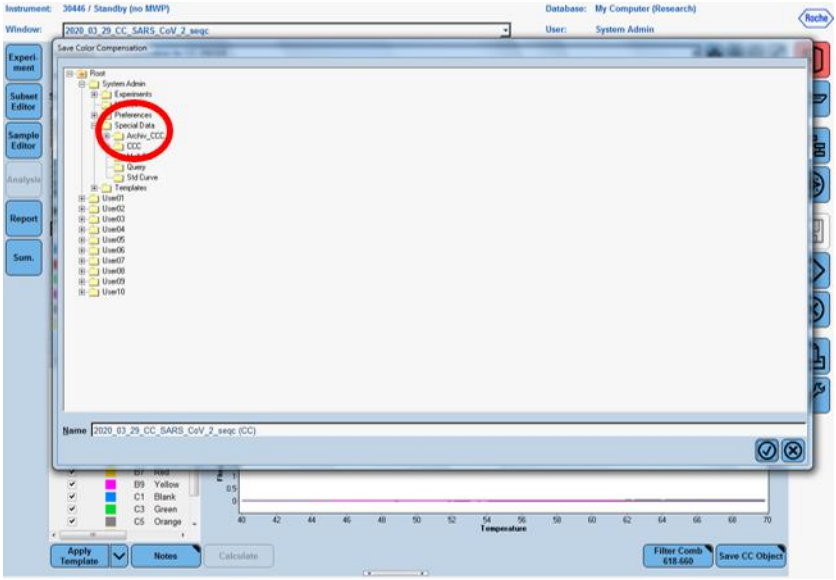
After the run has finished click on ‘Analysis’ on the module bar and choose the digital box ‘Create New Analysis’ the field ‘Color Compensation’. Choose and confirm your subset (e.g. MP2-CC) in the new window. Click ‘ok’.



Click on ‘Calculate’ to perform the Color Compensation analysis. Confirm the analysis with ‘Save CC Object’ in the folder ‘Special Data’ - ‘CCC’. The Color Compensation file can now be used in other LightCycler® 480 II experiments, the creation of the file is finished.



Check all Detection channels, by clicking on ‘Filter Comb’. The ‘Raw Data’ shows a baseline (blank), a channel specific curve and in the ROX-channel and the Cy5 channel a crosstalk curve. The ‘Compensated Data’ (lower picture) must show only the channel specific curve.



7.6 Verification of the received Color Compensation File

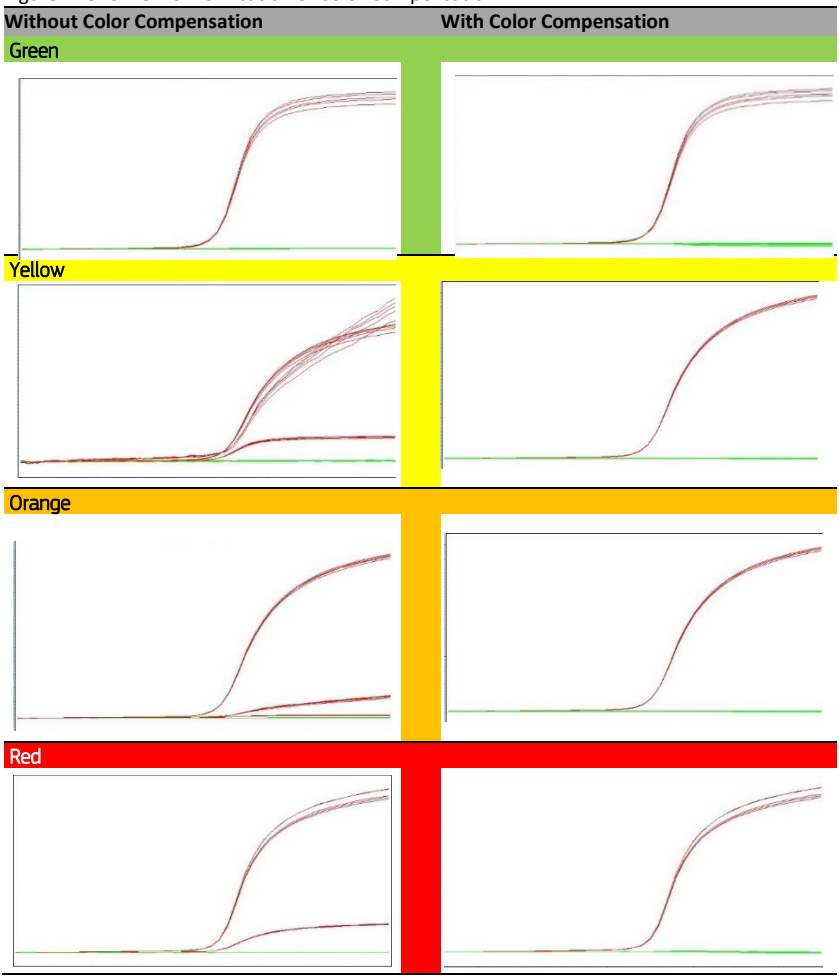
Open a runfile of the respective real time (RT-) multiplex PCR and take a look on the Positive Control. After addition of the Color Compensation, the crosstalk between the channels should be minimized and a difference as shown in Figure 1 should be visible.

8 Installation on multiple LightCycler® 480 II instruments

The Color Compensation Kit SARS-CoV-2 seqc can be used on multiple LightCycler® 480 II instruments.

Therfore use the same plate, that was already used for a Color Compensation Experiment and place it in the next LightCycler® 480 II. Then perform the program again as shown in Table 6 and follow the manual step by step.

Figure 1: Overview of verification of Color Compensation.



8.1 The Use of the Color Compensation File

To apply the Color Compensation open virellaSARS-CoV-2 PCR assay and click the 'Analysis' button to select the appropriate filter combination. Click the Color Compensation dropdown menu and select 'in Database'. Choose the stored Color Compensation File you want to apply to the assay. The 'Color Compensation (off)' button switches to 'Color Compensation (on)'. This confirms that the Color Compensation is active and the multiplex assay can now be analyzed.

9 Abbreviations and Symbols

BLANK MIX	Blank Mix
GREEN MIX	Green Mix
ORANGE MIX	Orange Mix
RED MIX	Red Mix
YELLOW MIX	Yellow Mix
BLANK CONTROL	Blank Control
CONTROL +	Positive Control

REF	Catalog number
Σ	Contains sufficient for <n> test
- 18°C	Upper limit of temperature
	Manufacturer
	Use by YYYY-MM-DD
LOT	Batch code
CONT	Content
	Consult instructions for use
DNA	Deoxyribonucleid Acid
PCR	Polymerase Chain Reaction
RNA	Ribonucleic Acid