Color Compensation Kit IV

The Color Compensation Kit IV is used for color calibration of 2-plex and higher for the LightCycler® 480 II real-time PCR instrument.

For Research Use Only. Not for Use in Diagnostic Procedures.

Catalog Number: 86-CC4HU-03
Size: 3 Color Compensation Runs
Version: 2021-09-09 - ALPCO 1.0

INTENDED USE

The Color Compensation Kit IV is used for color calibration of 2-plex and higher for the LightCycler® 480 II real-time PCR instrument. The Color Compensation Kit IV can be used to generate a color compensation file to enable analysis of qualitative and quantitative 2-plex and higher real-time PCR tests on the LightCycler® 480 II. For Research Use Only. Not for use in diagnostic procedures.

TEST SUMMARY

In a real-time PCR, the emitted fluorescent signal of a fluorescent reporter dye can overlay an adjacent color channel, thus generating a signal (crosstalk) in this channel. Crosstalk from fluorescent signals can cause incorrect results unless a correction is carried out by a color compensation file. A color compensation file can compensate for crosstalk between the color channels.

REAGENTS PROVIDED

Reagents provided (The reagents provided in the kit are sufficient for 3 color compensation runs.)

Table 1. Reagents Provided

Kit Code	Reagent	Amount		Lid Color
1	Blank	1x	400 uL	White
2	Dye 1	1x	400 uL	Blue
3	Dye 2	1x	400 uL	Green
4	Dye 3	1x	400 uL	Yellow
5	Dye 4	1x	400 uL	Orange
6	Dye 5	1x	400 uL	Red

STORAGE INSTRUCTIONS

- Protect all reagents from light and store at -20°C. All reagents can be used until the expiration date. After expiry the quality guarantee is no longer valid.
- Carefully thaw reagents before use (e.g., in a refrigerator at 2 - 8°C).
- During color compensation preparation, all reagents should be stored cold (2 - 8°C).

MATERIALS REQUIRED BUT NOT PROVIDED

The Color Compensation Kit IV is appropriate for use with the following real-time PCR device:

Table 2. Necessary Equipment

Real-time PCR Instrument	
Roche	LightCycler 480 II

Note: Only use 0.1 ml tubes on the Rotor-Gene Q (QIAGEN).

- Real-time PCR consumables (microtiter plate, optical foil)
- Centrifuge with rotor for reaction vials or plates
- Pipettes (0.5 to 20 µl, 20 to 200 µl, 100 to 1,000 µl)
- Pipette tips with filters
- Powder-free disposable gloves

WARNINGS and PRECAUTIONS

- Good laboratory practices must be used and the instructions for carrying out the test must be strictly followed.
- Do not mix reagents from kits with different lot numbers.
- Do not pipet samples or reagents by mouth. Avoid contact with bruised skin or mucosal membranes.
- During handling of reagents or samples, wear appropriate safety clothing (appropriate gloves, lab coat, safety goggles) and wash your hands after finishing the test procedure.
- Do not smoke, eat, or drink in areas where samples or reagents are being used.
- Extraction, PCR preparation and the PCR run should be separated in different rooms to avoid cross-contaminations.
- Samples must be treated as potentially infectious as well as all reagents and materials exposed to the samples and must be handled according to safety regulations.
- Do not use the kit after the expiration date.
- All reagents and materials used must be disposed properly after use. Please refer to the relevant regulations for disposal.

PROTOCOL FOR GENERATING COLOR COMPENSATION FILE

Color compensation preparation

For a color compensation run, pipette five reactions with 20 µl of each dye including the background (blank) into a microtiter plate (s. Fig.1).

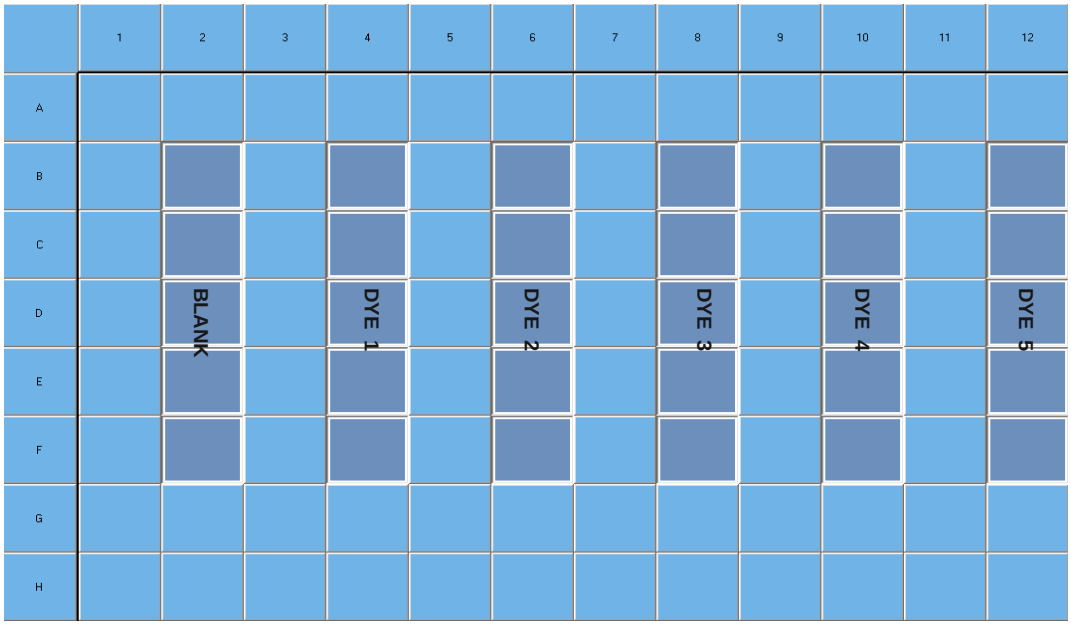


Fig. 1: Pipetting scheme for color compensation on the LightCycler® 480 II.

Thaw, mix, and briefly centrifuge the reagents before use.
Always cool reagents appropriately during the work steps (2 °C to 8 °C).

Table 3: Preparation of color compensation LightCycler® 480 II

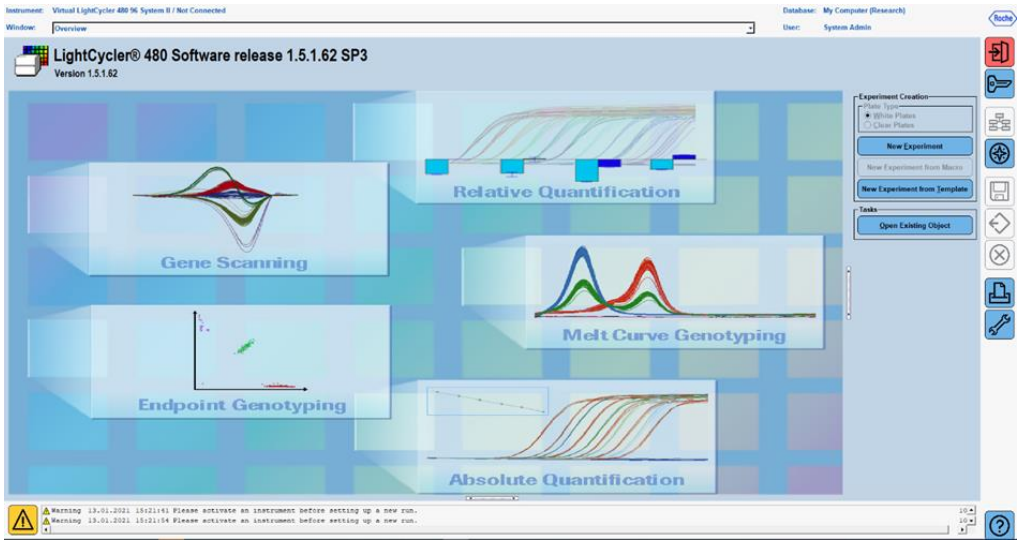
Kit code	Reagent	Quantity per reaction	Pipette 20 µl each in the following wells
1	Blank	20 µl	B2, C2, D2, E2, F2
2	Dye 1	20 µl	B4, C4, D4, E4, F4
3	Dye 2	20 µl	B6, C6, D6, E6, F6
4	Dye 3	20 µl	B8, C8, D8, E8, F8
5	Dye 4	20 µl	B10, C10, D10, E10, F10
6	Dye 5	20 µl	B12, C12, D12, E12, F12

After pipetting the reagents, seal the microtiter plate with optical foil and centrifuge if possible. Start the real-time PCR according to the device settings.

PCR instrument set-up

Note: Log into the software as the administrator to set up the detection format.

1. After opening the software, click the “Tools" icon to program the detection format.



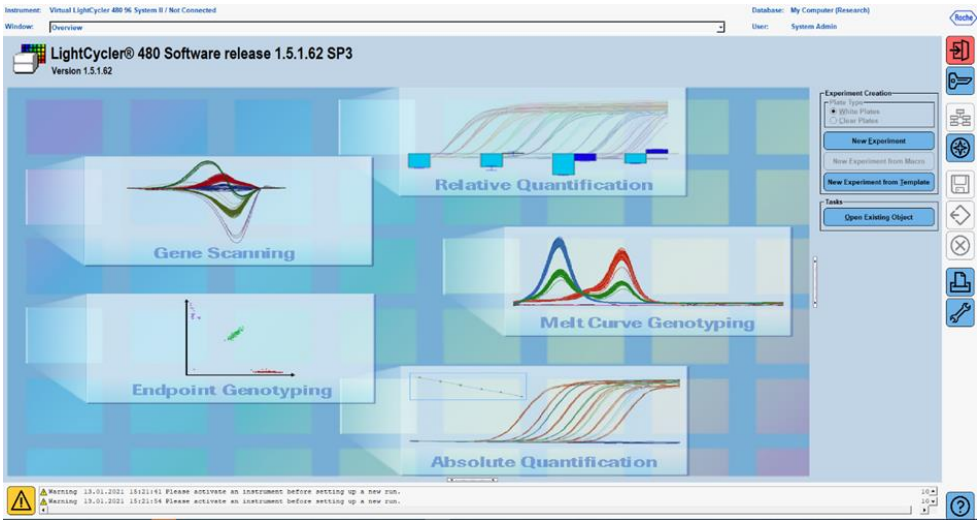
2. The following window opens. In the Tools window, select “Detection Formats”. Click the “New” button to create a new detection format (s. Tab. 5). Click the “Close” button to exit the Tools window.

Tab.4: Detection channel set-up for the LightCycler® 480 II

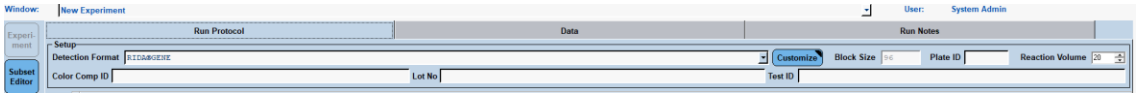
Filter combination
440 / 488
465 / 510
533 / 580
533 / 610
618 / 660

***Note**:* Set the value for Quant Factor, Melt Factor and Integration Time to 1 (default).

3. After programming the detection format, click the “New Experiment” button.



4. Select the saved detection format and enter a reaction volume of 20 µl (default).



5. Program the thermal profile (s. Tab. 5).

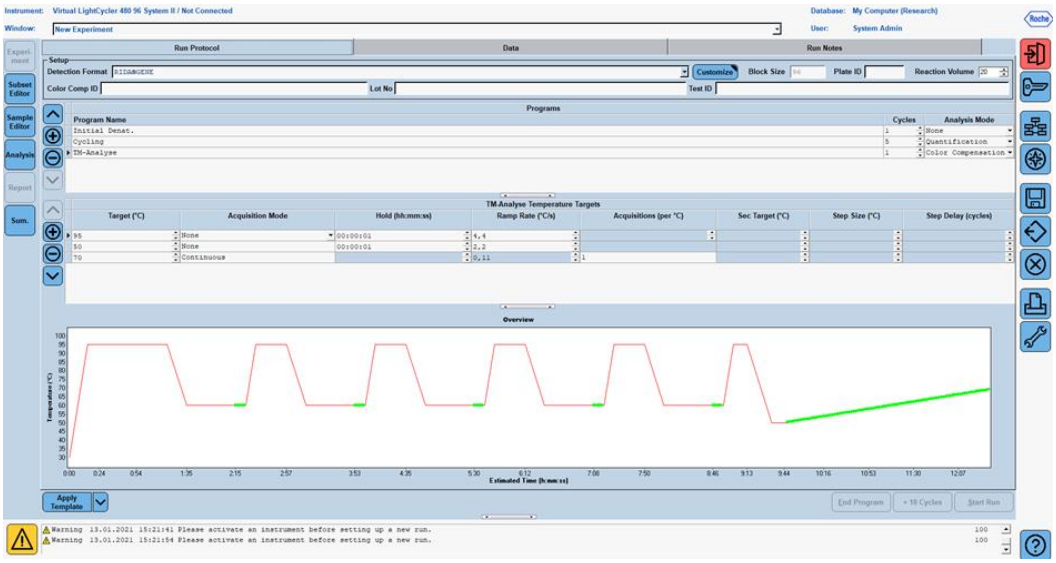
Table 5: Thermal profile

		Temperature targets			
Program	**Cycles / Analysis Mode**	**Target [°C]**	**Acquisition Mode**	**Hold [hh:mm:ss]**	**Ramp rate [°c/s]**
Initial Denat.	1 / none	95	none	00:00:30	4.4
Cycling	5 / Quantification	95	none	00:00:15	4.4
		60	single	00:00:30	2.2
TM Analysis	1 / Color Compensation	95	none	00:00:01	4.4
		50	none	00:00:30	2.2
		70	continuous		Acquisitions (per °C) = 1 0.14*

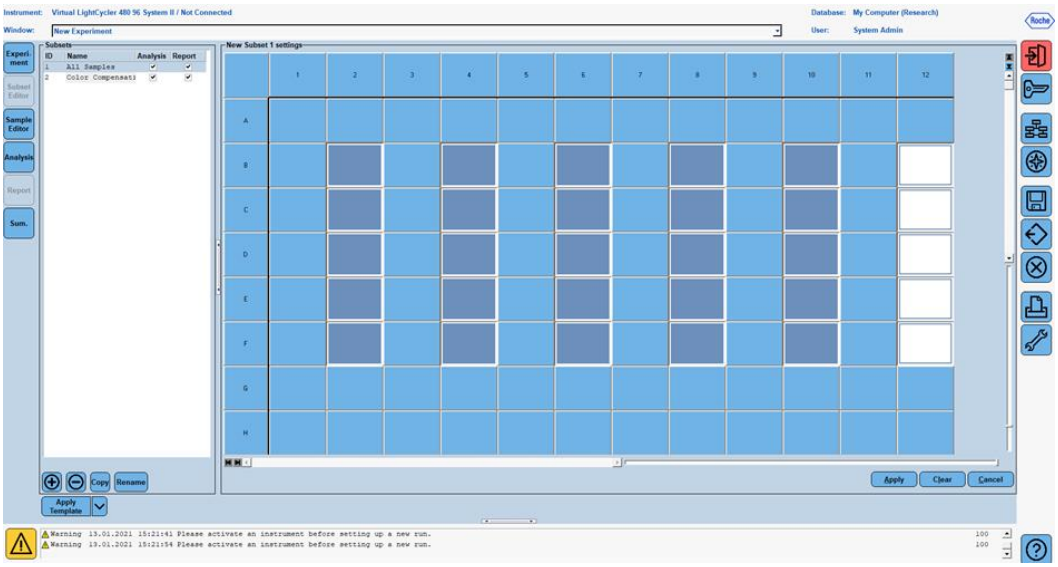
Note: Ensure that the number of “Cycles” and “Analysis Mode” is correct.

** The ramp rate can vary slightly depending on the detector format selected.*

6. After programming is complete, the experiment should look as follows**.**



7. To program the layout of the microtiter plate, switch into the “Subset Editor**”**. Click the “Plus” icon to create a new subset and enter a name for the layout (e.g., Color Compensation). Press and hold the Ctrl key and the left mouse button and mark all wells containing reagents in the microtiter plate (see Fig. 1 and 2). Click the “Apply**”** button to finish the subset. The screen should appear as follows.



8. Switch into the “Sample Editor”. From Step 1: “Select Workflow”, choose “Color Comp”. In Step 2: “Select Samples”, choose the previously set subset (Color Compensation). To finish the layout, select the corresponding dominant channel for each reagent (Blank, Dye 1, Dye 2, Dye 3, Dye 4, Dye 5) in the “Dominant Channel” field (s. Tab. 6). Please select “Water” for the reactions with the color background (Blank).

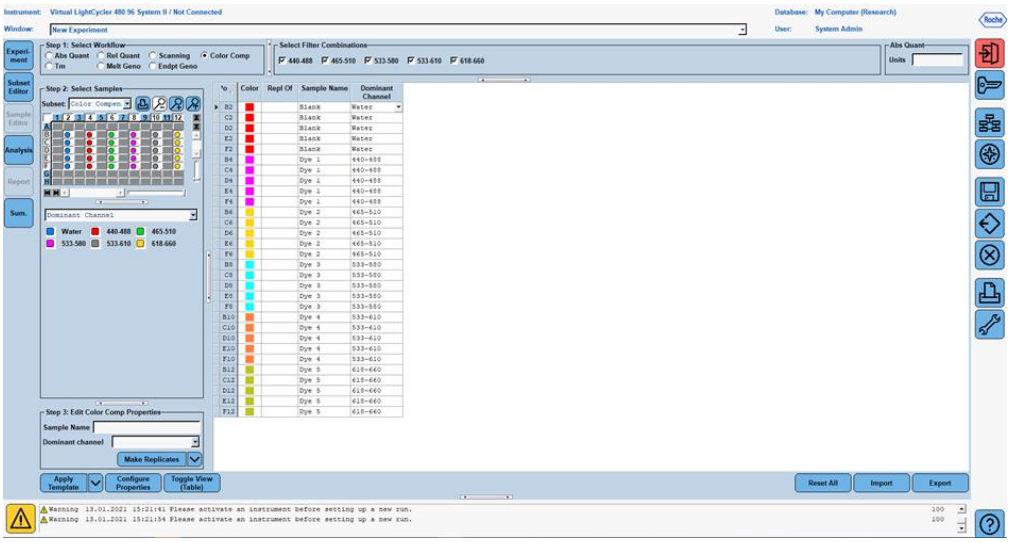
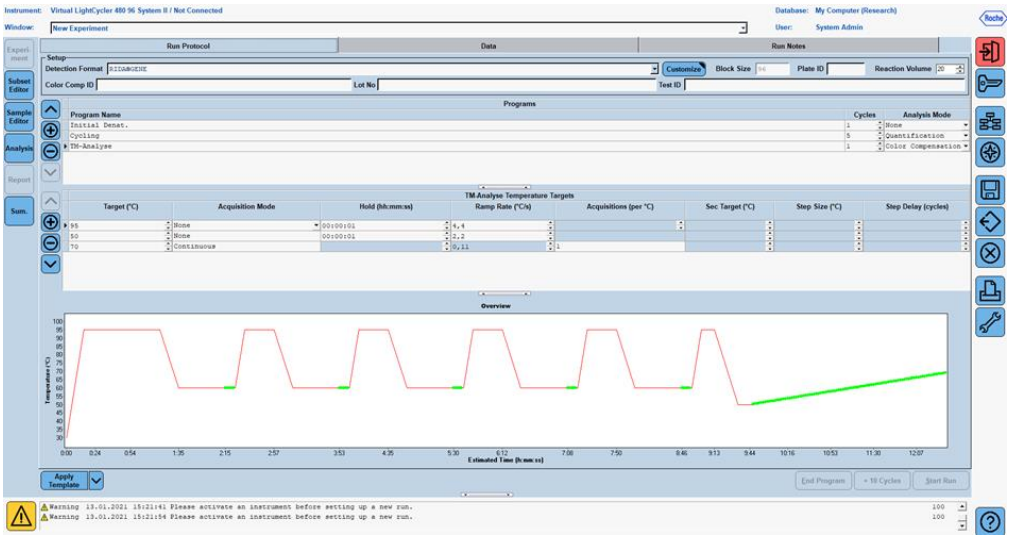


Table 6: Dominant Channel settings for the reagents (LightCycler® 480 II)

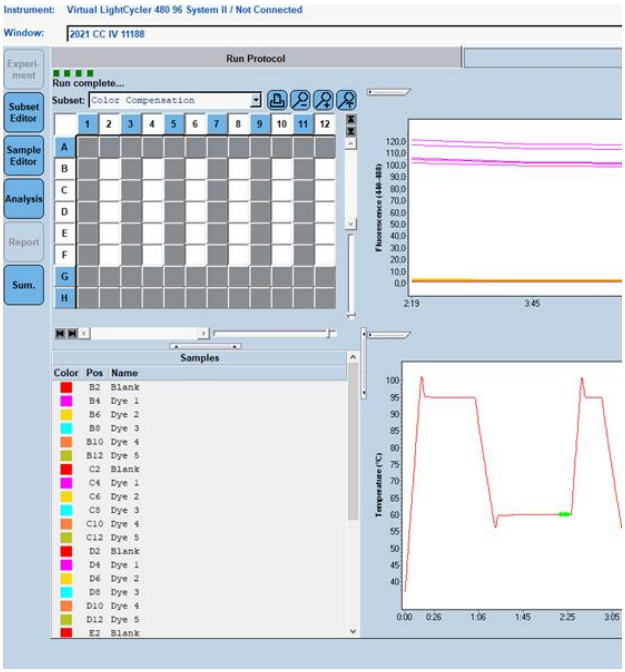
Reagent	Dominant Channel
Blank	Water
Dye 1	440 / 488
Dye 2	465 / 510
Dye 3	533 / 580
Dye 4	533 / 610
Dye 5	618 / 660

9. Place the plate with the prepared reactions into the device. Click “Experiment” and then “Start Run” to start the experiment.

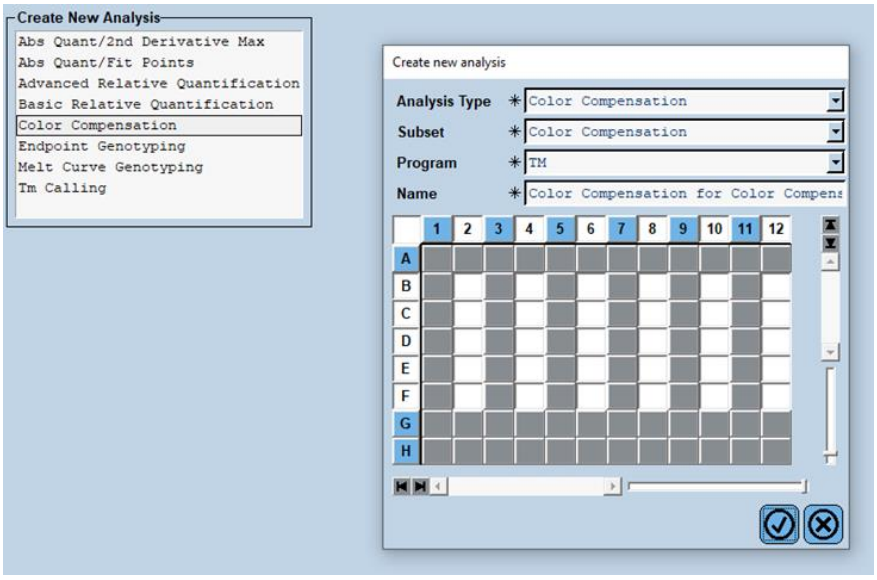


Evaluation and creation of color compensation file

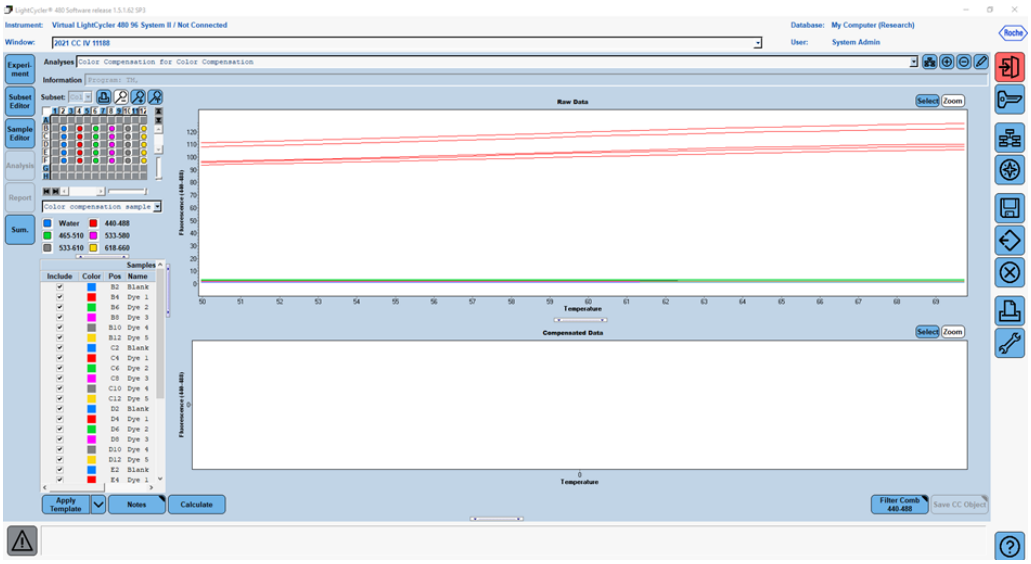
1. After completing the LightCycler® experiment, click the “Analysis” button.



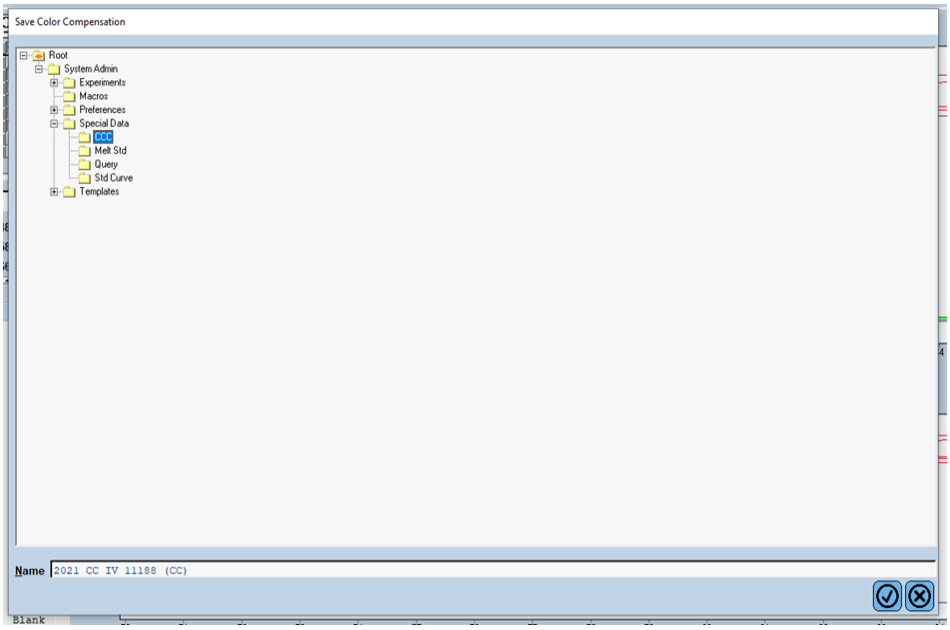
2. In the **“**Create New Analysis” dialog box, go to “Color Compensation”. Select and confirm the appropriate subset (e.g., Color Compensation) in the dialog box that opens.



3. The analysis opens; click “Calculate” and then “Save CC Object”.



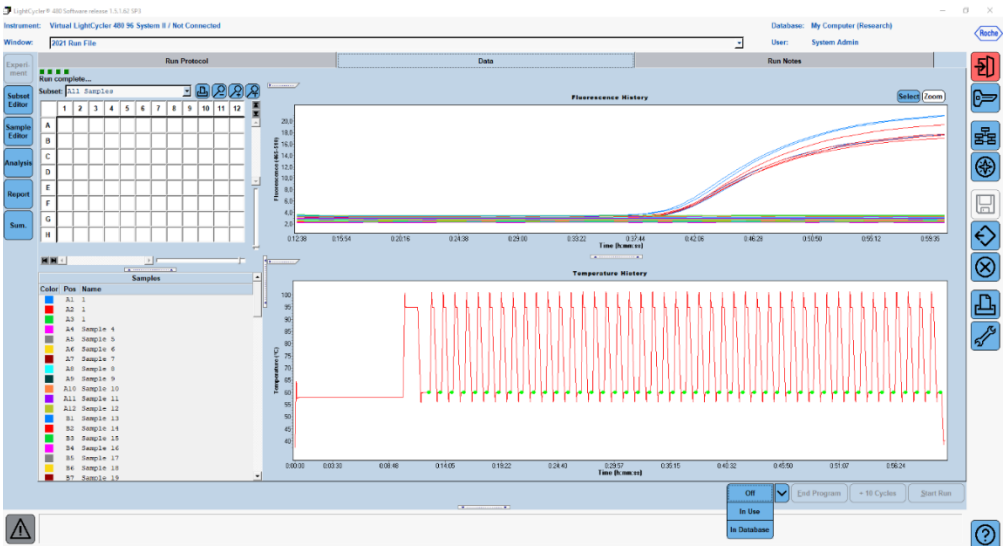
4. Save the color compensation file in the **“**CCC**”** folder.



This file is then available for other LightCycler® 480 II experiments. Generating the color compensation file is now complete

Use of the color compensation file

To use the color compensation file, open the given real-time PCR experiment and load the desired color compensation under “Experiment” “Data”. In the “Color Comp (Off)” drop-down menu, select “in Database” and then the saved color compensation file.



When the color compensation is selected, the “Color Comp (Off)” button changes to “Color Comp (On)”. The selected color compensation is automatically applied to all filters of the analysis. The real-time PCR run can now be analyzed as usual.

Note: The color compensation file is specific for every LightCycler® 480 II. A new color compensation file is needed if the device is exchanged or the optical unit is repaired.