

RIDA® GENE Color Compensation Kit IV

REF PG0004



R-Biopharm AG, An der neuen Bergstrasse 17, 64297 Darmstadt, Germany

+49 (0) 61 51 81 02-0 / +49 (0) 61 51 81 02-20 / www.r-biopharm.com

1. Intended use

For *in vitro* diagnostic use. The RIDA®GENE Color Compensation Kit IV is used for color calibration of 2-plex and higher RIDA®GENE real-time PCR runs on the LightCycler® 480 II. The RIDA®GENE Color Compensation Kit IV can be used to generate a color compensation file to enable analysis of qualitative and quantitative 2-plex and higher RIDA®GENE real-time PCR tests on the LightCycler® 480 II.

The product is intended for professional use.

2. Summary and explanation of the test

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.

3. Test principle

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

4. Reagents provided

Table 1: Reagents provided (The reagents provided in the kit are sufficient for 3 color compensation runs.)

Kit code	Reagent	Amount		Lid color
1	Blank	1 ×	400 µl	White
2	Dye 1	1 ×	400 µl	Blue
3	Dye 2	1 ×	400 µl	Green
4	Dye 3	1 ×	400 µl	Yellow
5	Dye 4	1 ×	400 µl	Orange
6	Dye 5	1 ×	400 µl	Red

5. Storage instructions

- Protect the RIDA®GENE Color Compensation Kit IV from light and store at -20 °C. If unopened, it can be used until the expiration date printed on the label. After the expiration date, the quality guarantee is no longer valid.
- Before use, carefully thaw the RIDA®GENE Color Compensation Kit IV (e.g., in a refrigerator at 2 – 8 °C).
- During color compensation preparation, all reagents should be appropriately cooled (2 – 8 °C).

6. Reagents required but not provided

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

Table 2: Necessary equipment

Real-time PCR device	
Roche	LightCycler® 480 II

Should you wish to use other real-time PCR devices, please contact R-Biopharm to check compatibility at mdx@r-biopharm.de.

- 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

7. Warnings and precautions for the users

For *in vitro* diagnostic use only.

- This test must be carried out only by trained laboratory personnel. The guidelines for working in medical laboratories must be followed.
- Always adhere strictly to the instructions for use when carrying out this test.
- Do not pipette samples or reagents using your mouth. Avoid contact with broken skin and mucous membranes.
- Wear personal protective equipment (appropriate gloves, lab coat, safety glasses) when handling reagents and samples, and wash hands after completing the test.
- Do not smoke, eat, or drink in areas where samples are handled.
- Ensure that the extraction, PCR preparation, and PCR are carried out in different rooms in order to avoid cross-contaminations.
- Clinical samples must be viewed as potentially infectious and must be disposed of appropriately, like all reagents and materials that come into contact with potentially infectious samples.
- Do not use the kit after the expiration date. Users are responsible for proper disposal of all reagents and materials after use. For disposal, please adhere to national regulations.

For further details, see the safety data sheets (SDSs) at www.r-biopharm.com.

8. Protocol for generating a color compensation file on the LightCycler® 480 II

8.1 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).

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D		BLANK		DYE 1		DYE 2		DYE 3		DYE 4		DYE 5
E												
F												
G												
H												

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

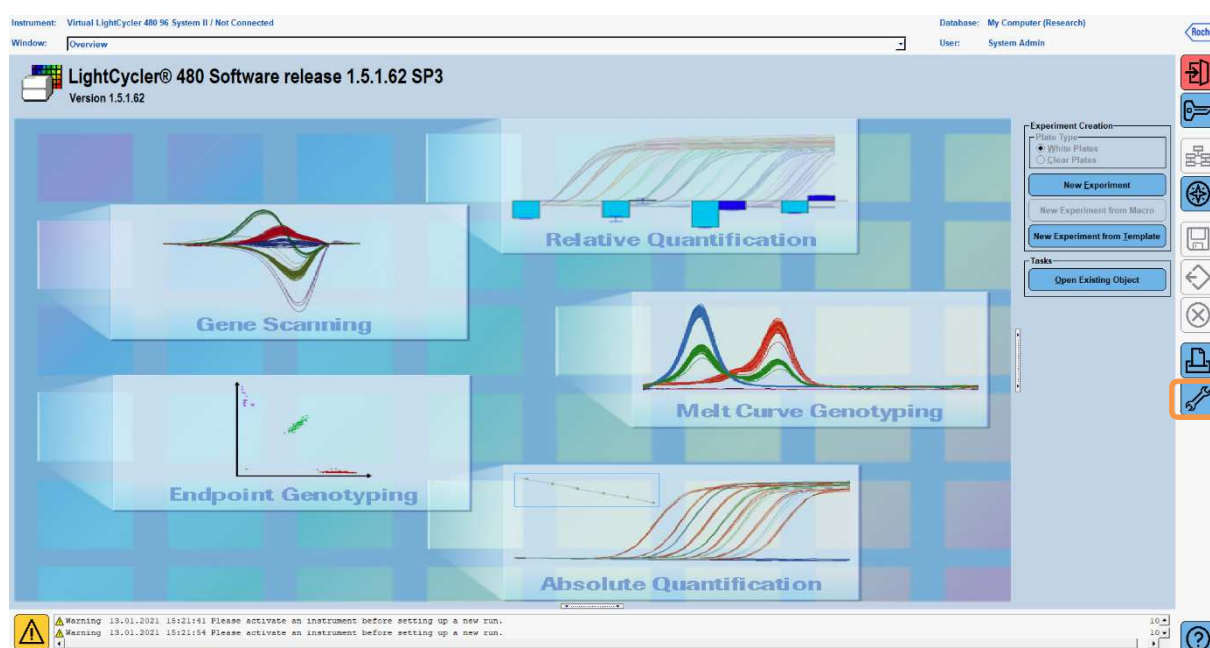
Kit code	Reagent	Quantity per reaction	Pipette 20 µl each in the following wells
1	Blank	20 µl	B2, C2, D2, E2, F2
2	Dye 1	20 µl	B4, C4, D4, E4, F4
3	Dye 2	20 µl	B6, C6, D6, E6, F6
4	Dye 3	20 µl	B8, C8, D8, E8, F8
5	Dye 4	20 µl	B10, C10, D10, E10, F10
6	Dye 5	20 µl	B12, C12, D12, E12, F12

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.

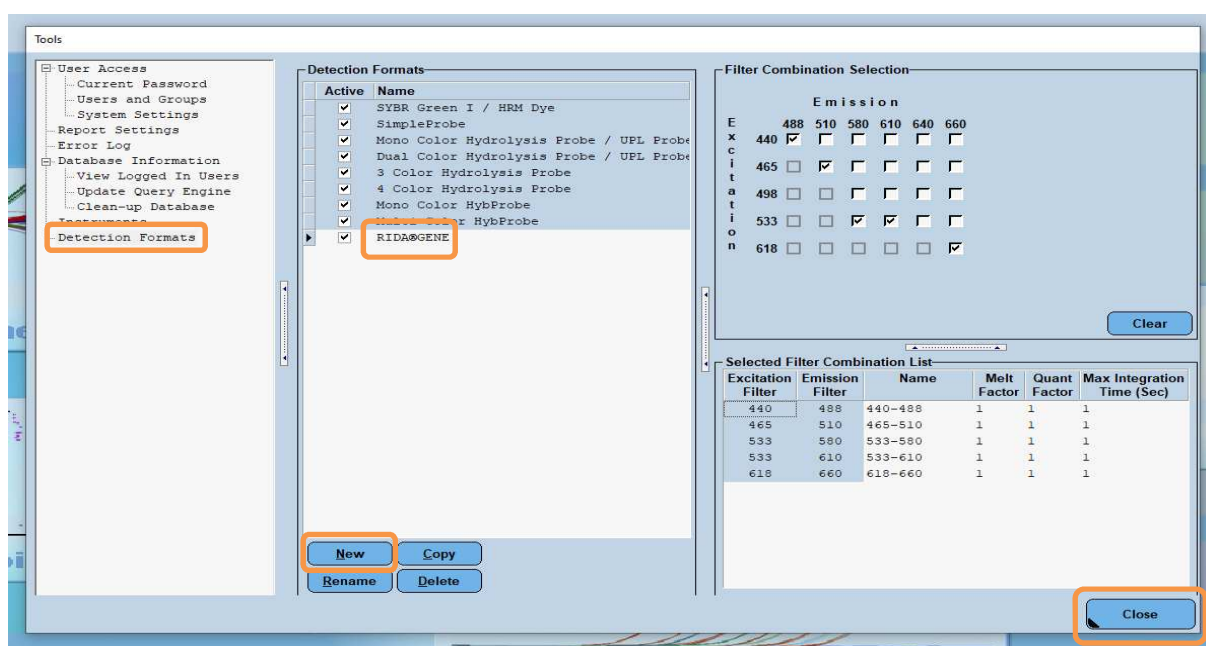
8.2 PCR instrument set-up

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.



- The following window opens. In the Tools window, select “**Detection Formats**”. Click the “**New**” button to create a new detection format (s. Tab. 5) and save it as “**RIDA®GENE**”. Click the “**Close**” button to exit the Tools window.

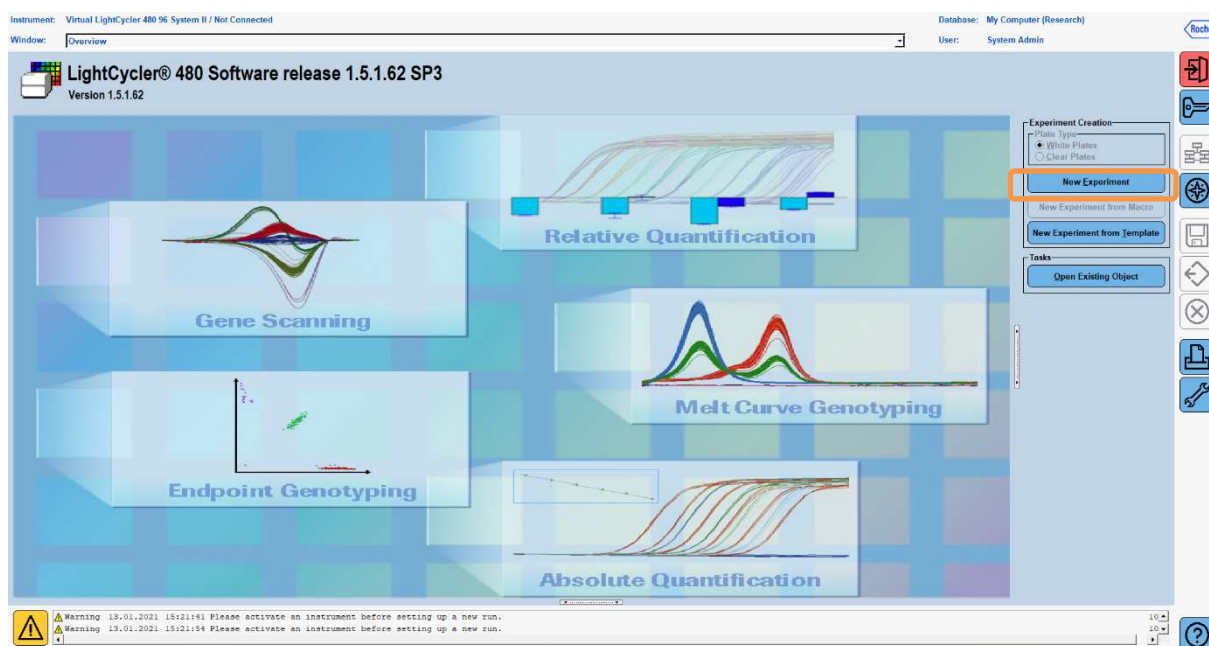


Tab.4: Detection channel set-up for the LightCycler® 480 II

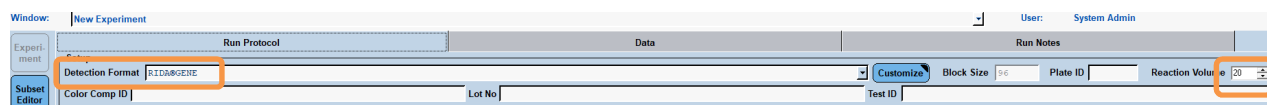
Filter combination
440 / 488
465 / 510
533 / 580
533 / 610
618 / 660

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 “**RIDA®GENE**” detection format and enter a reaction volume of 20 µl (default).



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

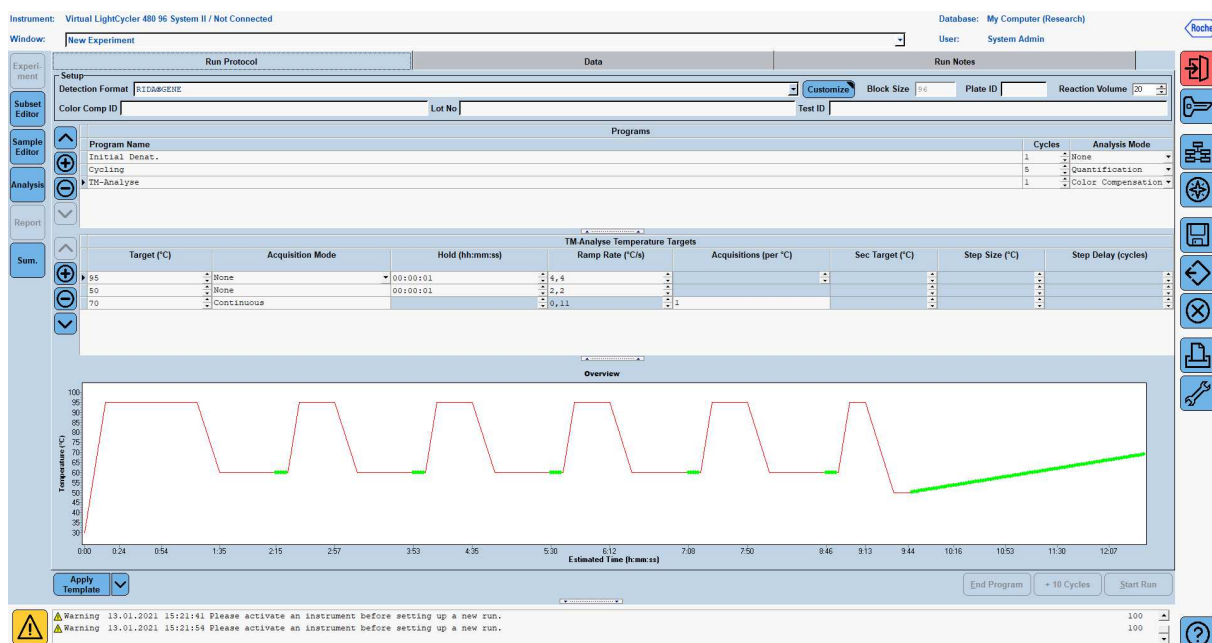
Table 5: Thermal profile

Program	Cycles / Analysis Mode	Temperature targets			
		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*

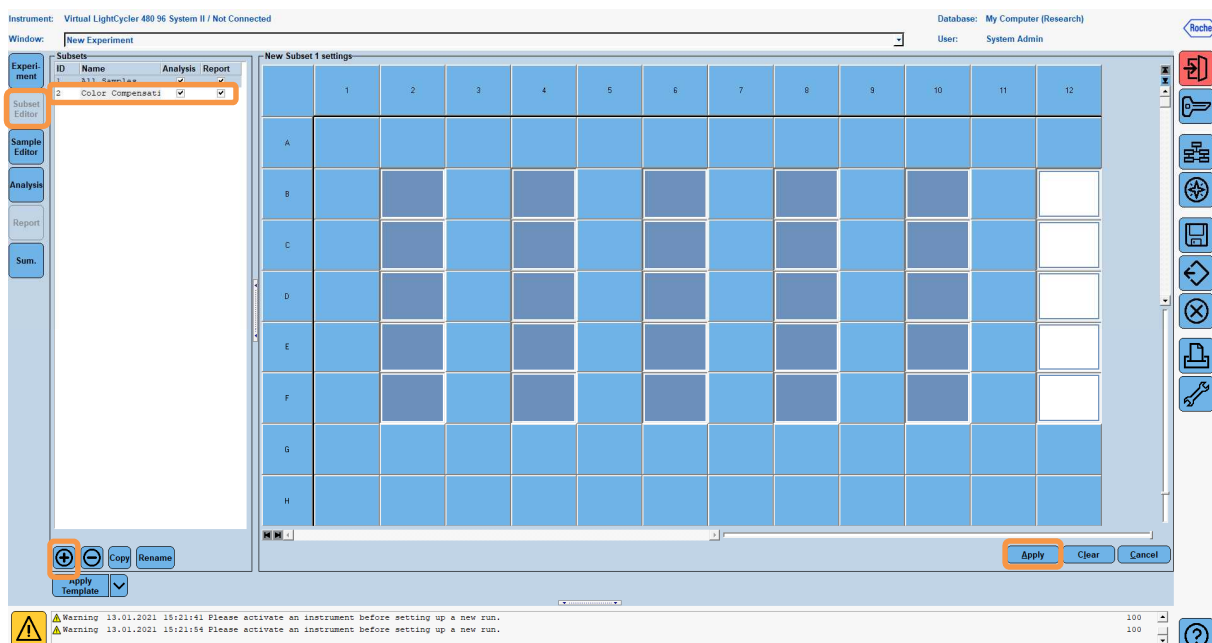
Note: Ensure that the number of “**Cycles**” and “**Analysis Mode**” is correct.

* 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).

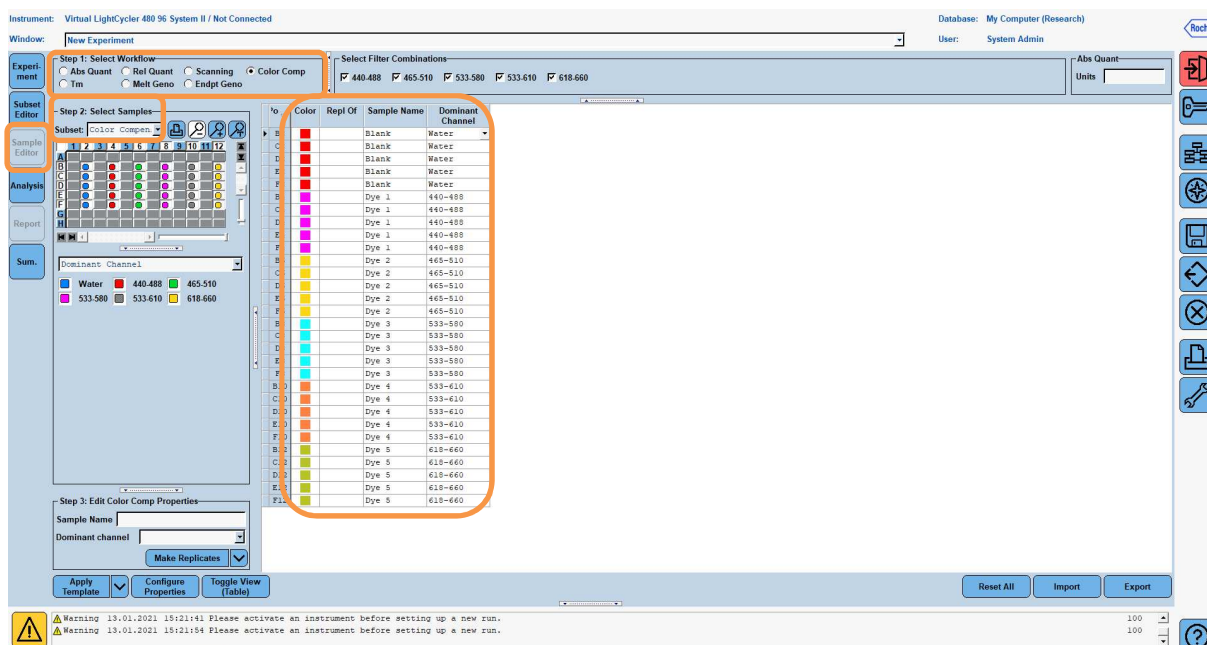
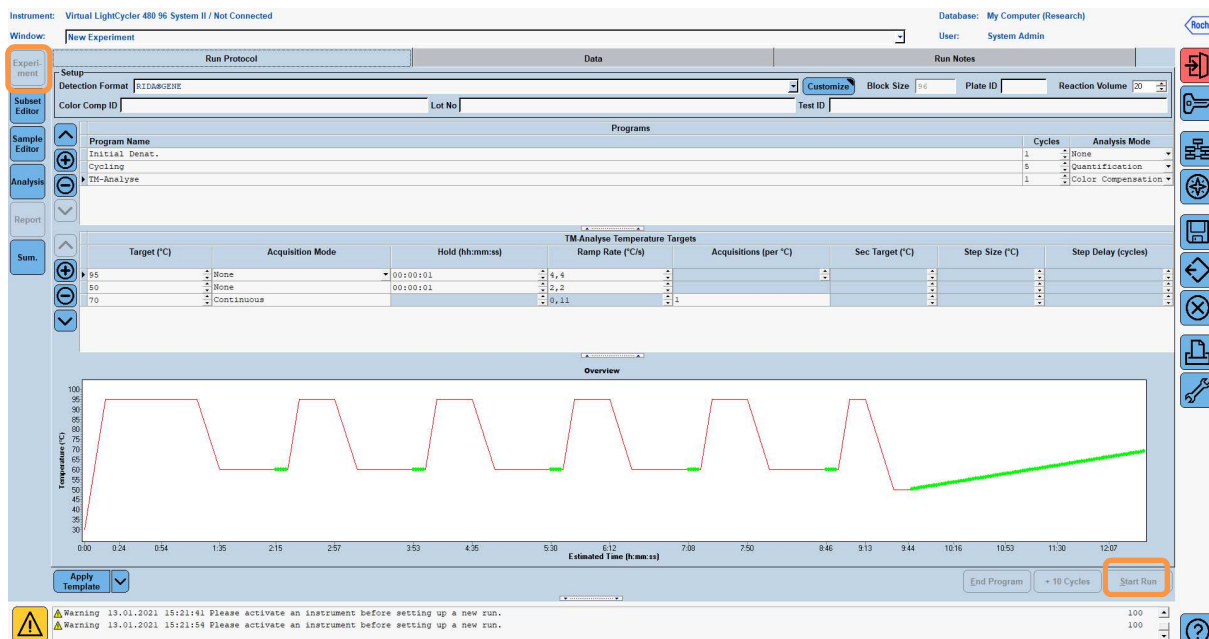


Table 6: Dominant Channel settings for the reagents (LightCycler® 480 II)

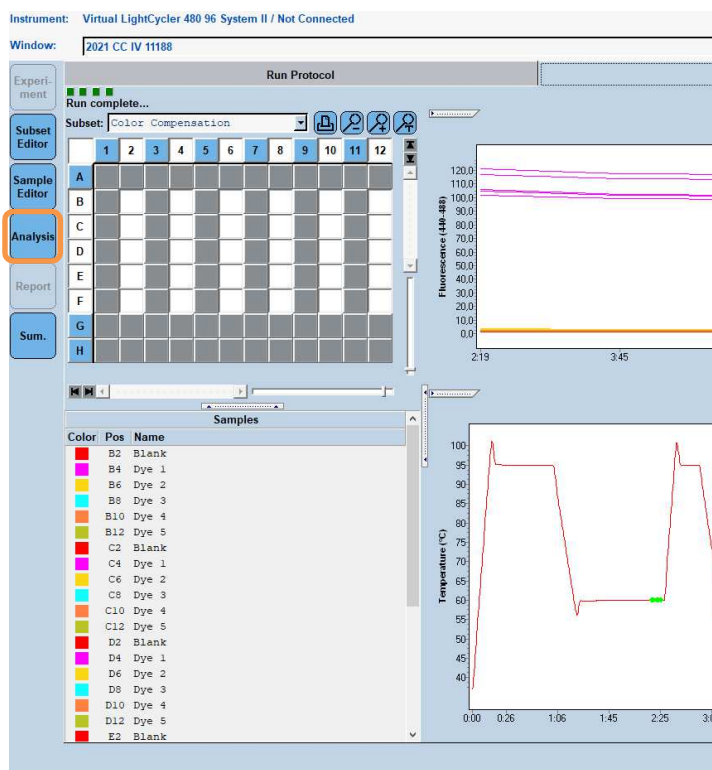
Reagent	Dominant Channel
Blank	Water
Dye 1	440 / 488
Dye 2	465 / 510
Dye 3	533 / 580
Dye 4	533 / 610
Dye 5	618 / 660

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

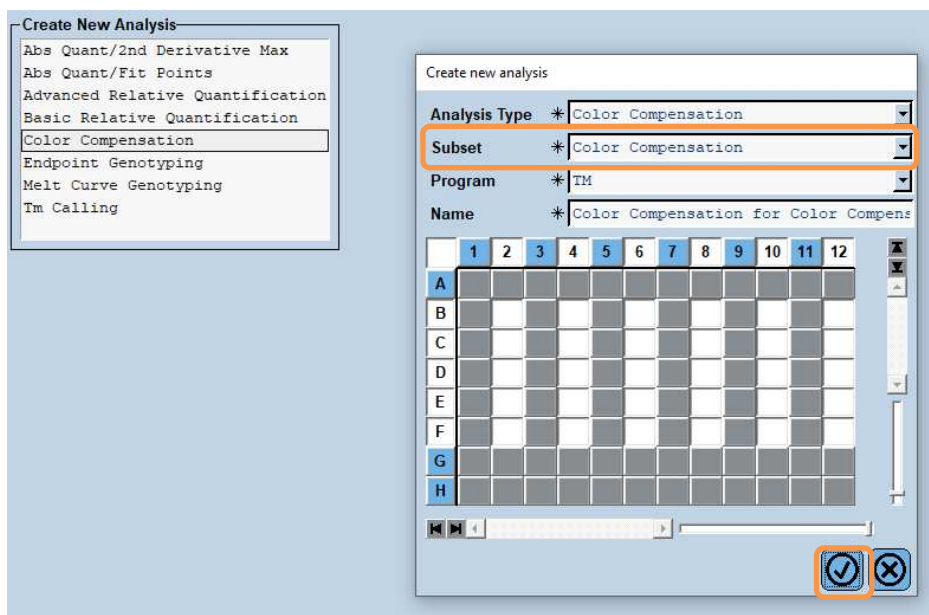


8.3 Evaluation and creation of a color compensation file

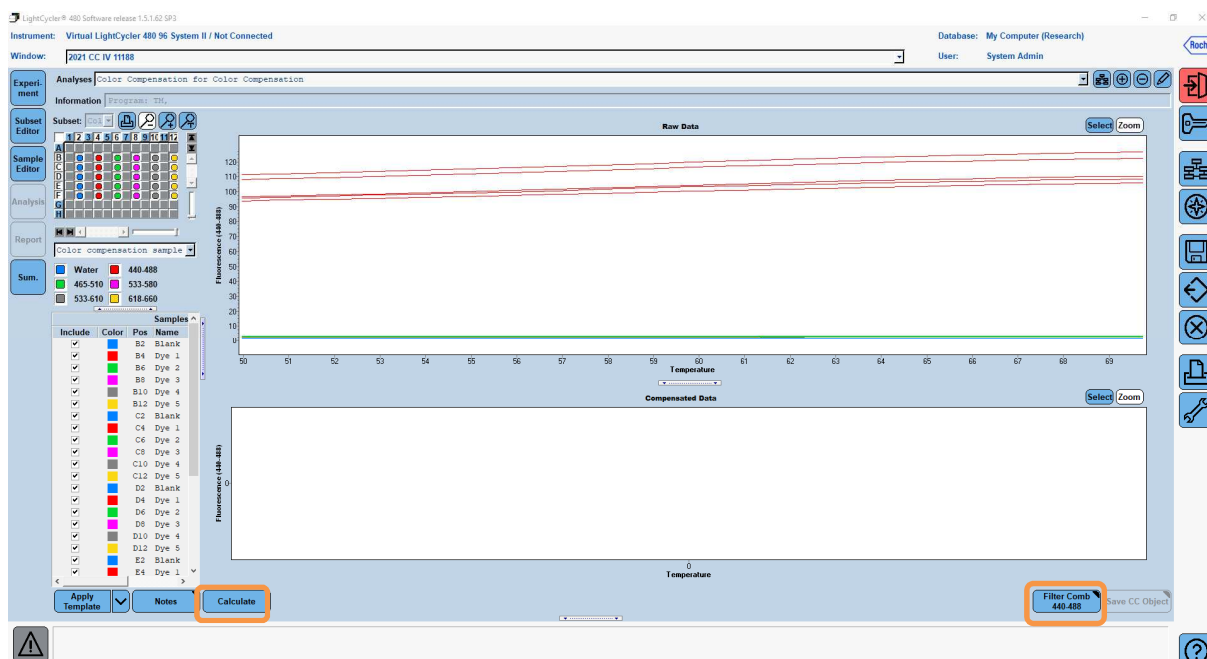
- After completing the LightCycler® experiment, click the **“Analysis”** button.



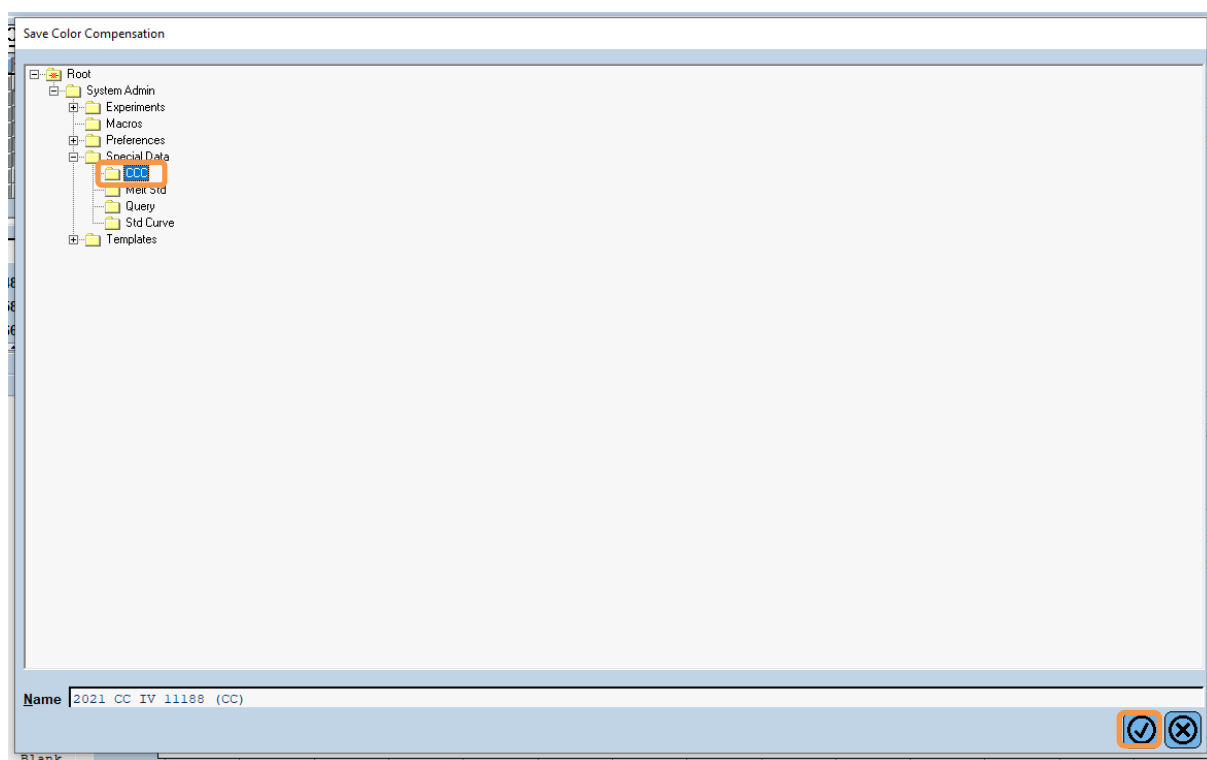
2. In the **“Create New Analysis”** dialog box, go to **“Color Compensation”**. Select and confirm the appropriate subset (e.g., Color Compensation) in the dialog box that opens.



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



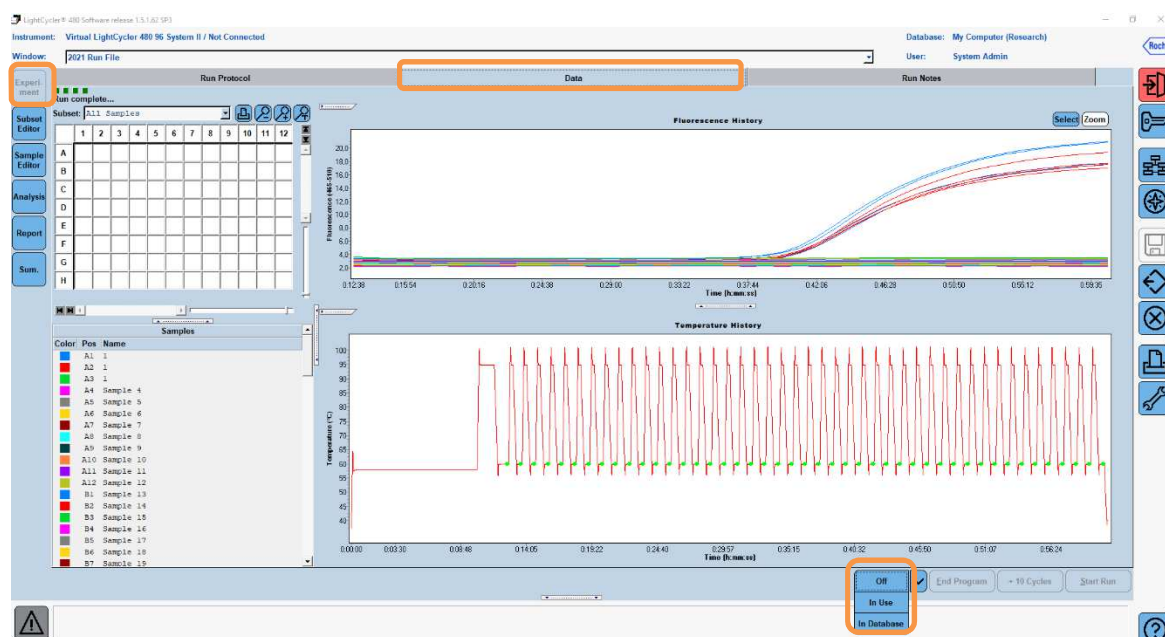
4. Save the color compensation file as “**RIDA®GENE CCIV**” in the “**CCC**” folder.



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

8.4 Use of the color compensation file

To use the color compensation file, open the given RIDA®GENE 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 RIDA®GENE 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.

9. Version history

Version number	Section and designation
2021-03-16	Previous version
2021-09-09	Adaptation / Correction Title page

10. Explanation of symbols

General symbols

	For in vitro diagnostic use
	Consult instructions for use
	Lot number
	Expiry
	Store at
	Article number
	Number of tests
	Date of manufacture
	Manufacturer

Testspecific symbols

Blank

Dye 1

Dye 2

Dye 3

Dye 4

Dye 5