

RIDA® GENE Color Compensation Kit IV

REF PG0004



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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). 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, ready for use
2	Dye 1	1 ×	400 µL	blue, ready for use
3	Dye 2	1 ×	400 µL	green, ready for use
4	Dye 3	1 ×	400 µL	yellow, ready for use
5	Dye 4	1 ×	400 µL	orange, ready for use
6	Dye 5	1 ×	400 µL	red, ready for use

5. Storage instructions

- Please follow the handling guidelines in Table 2 and store the kit directly after use according to the information specified.
- All reagents must be stored away from light at -16 °C to -28 °C and, if unopened, can be used until the expiration date printed on the label. After the expiration date, the quality guarantee is no longer valid.
- All reagents should be carefully thawed prior to use (e.g., in a refrigerator at 2 - 8 °C).
- Repeated freezing and thawing up to 3 times does not affect the test properties.
- Cool all reagents appropriately during PCR preparation (2 - 8 °C).

Table 2: Storage conditions and information

	Storage temperature	Maximum storage time
unopened	-16 °C to -28 °C	Can be used until the printed expiration date
opened	-16 °C to -28 °C	3 thaw-freeze cycles

6. Reagents required but not provided

6.1 Reagents

None.

6.2 Laboratory equipment

The following equipment is needed to carry out the RIDA®GENE Color Compensation Kit IV test:

Equipment
Real-time PCR instrument: LightCycler® 480 II (Roche)
Real-time PCR consumables (plates (low profile, white wells, clear frame), reaction vials, films)
Centrifuge with rotor for plates / reaction vials
Vortexer
Pipettes (0.5 - 20 µL, 20 - 200 µL, 100 - 1000 µL)
Pipette tips with filters
Powder-free disposable gloves

For questions please contact R-Biopharm AG at pcr@r-biopharm.de.

7. Warnings and precautions for the users

For *in vitro* diagnostic use only.

This test must be carried out only by qualified laboratory personnel. The guidelines for working in medical laboratories must be followed.

Always adhere strictly to the operating manual 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.

Separate rooms, special clothing, and instruments for extraction, PCR preparation, and PCR must be used to prevent cross-contamination and false-positive results.

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 the proper disposal of all reagents and materials after use. For disposal, please adhere to national regulations.

Further details on the Safety Data Sheet (SDS) can be found under the item number at <https://clinical.r-biopharm.com/search/>.

For users in the European Union: Report all serious adverse events associated with the product to R-Biopharm AG and the appropriate national authorities.

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

8.1 Color compensation preparation

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

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

Table 3: Preparation of color compensation for the LightCycler® 480 II

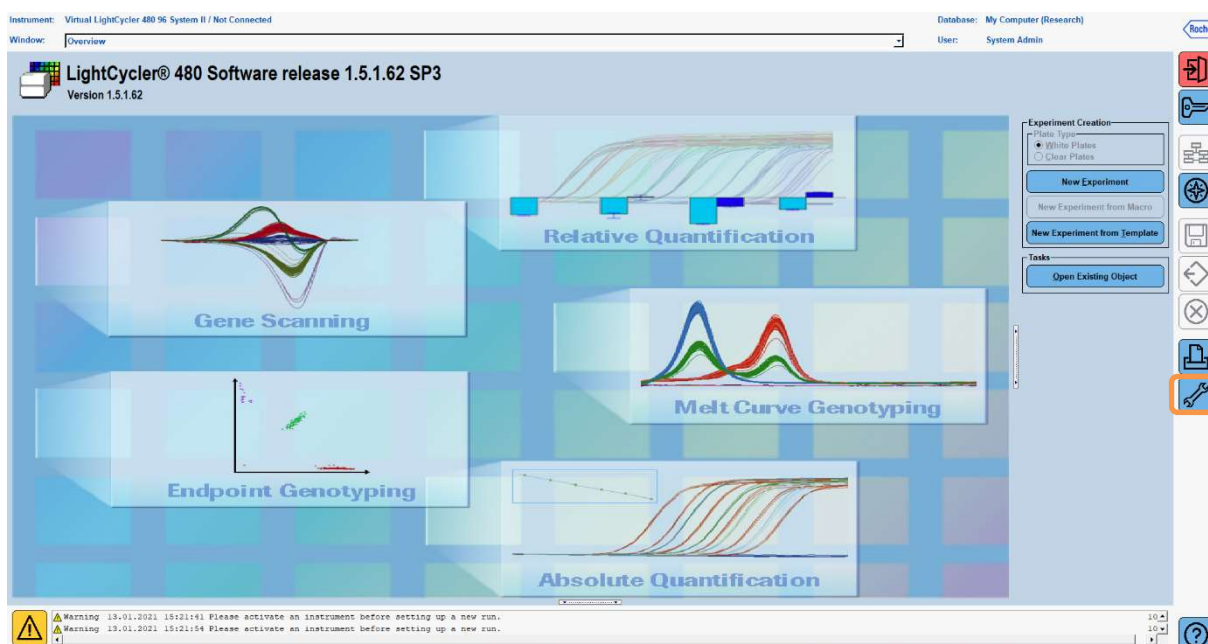
Kit code	Reagent	Quantity per reaction	Pipette 20 µL each in the following wells
1	Blank	20 µL	B2, C2, D2, E2, F2
2	Dye 1	20 µL	B4, C4, D4, E4, F4
3	Dye 2	20 µL	B6, C6, D6, E6, F6
4	Dye 3	20 µL	B8, C8, D8, E8, F8
5	Dye 4	20 µL	B10, C10, D10, E10, F10
6	Dye 5	20 µL	B12, C12, D12, E12, F12

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 (see the following figure).



- The following window opens. In the Tools window, select “**Detection Formats**”. Click the “**New**” button to create a new detection format (s. Tab. 4) and save it as “**RIDA®GENE**” (see the following figure).

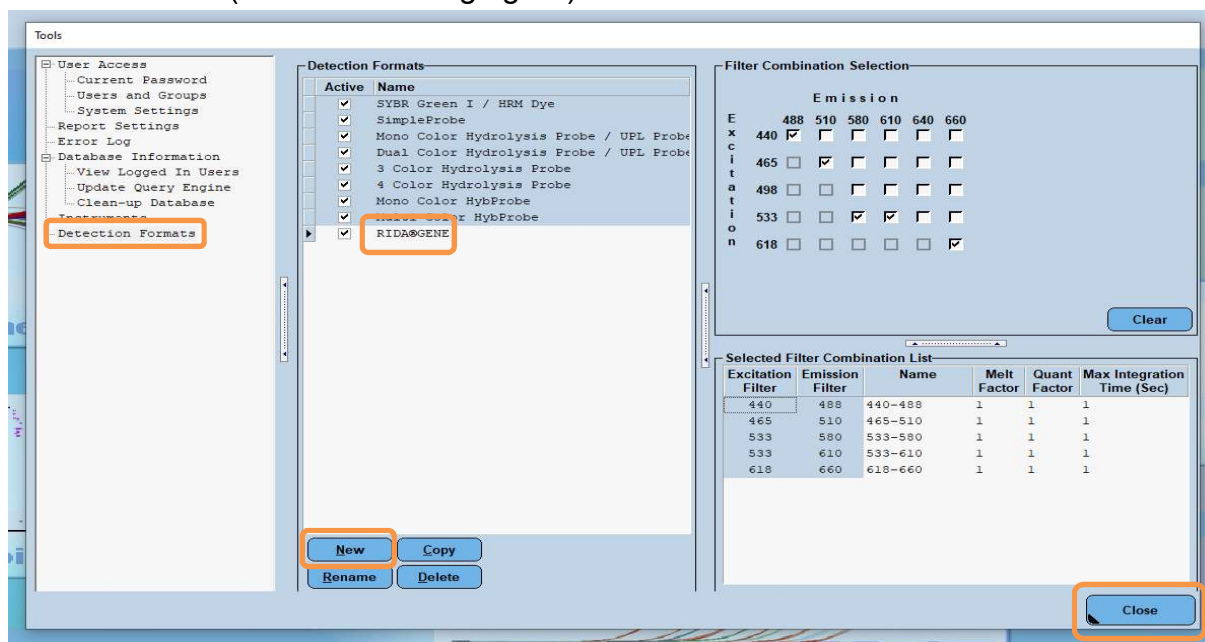


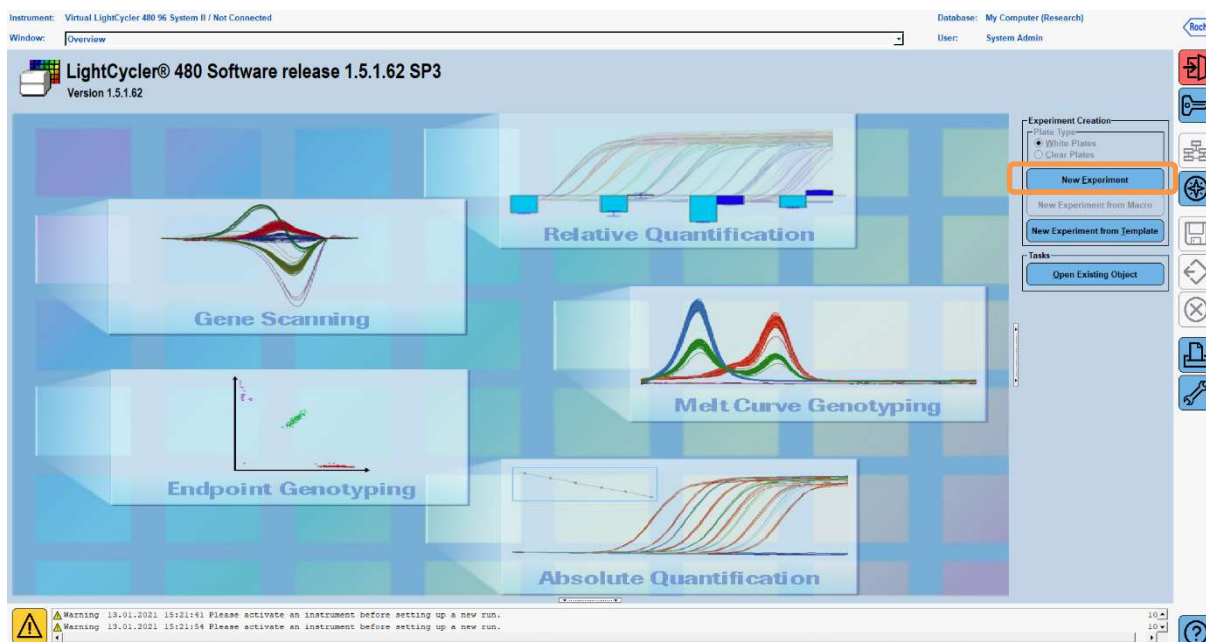
Table 4: Detection channel set-up for the LightCycler® 480 II

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

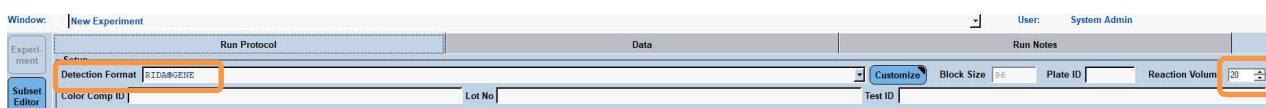
Note: Set the value for Quant Factor, Melt Factor and Integration Time to 1 (default).

Click the “**Close**” button to exit the Tools window.

3. After programming the detection format, click the “**New Experiment**” button (see the following figure).



4. Select the “**RIDA®GENE**” detection format and enter a reaction volume of 20 µL (default) (see the following figure).



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

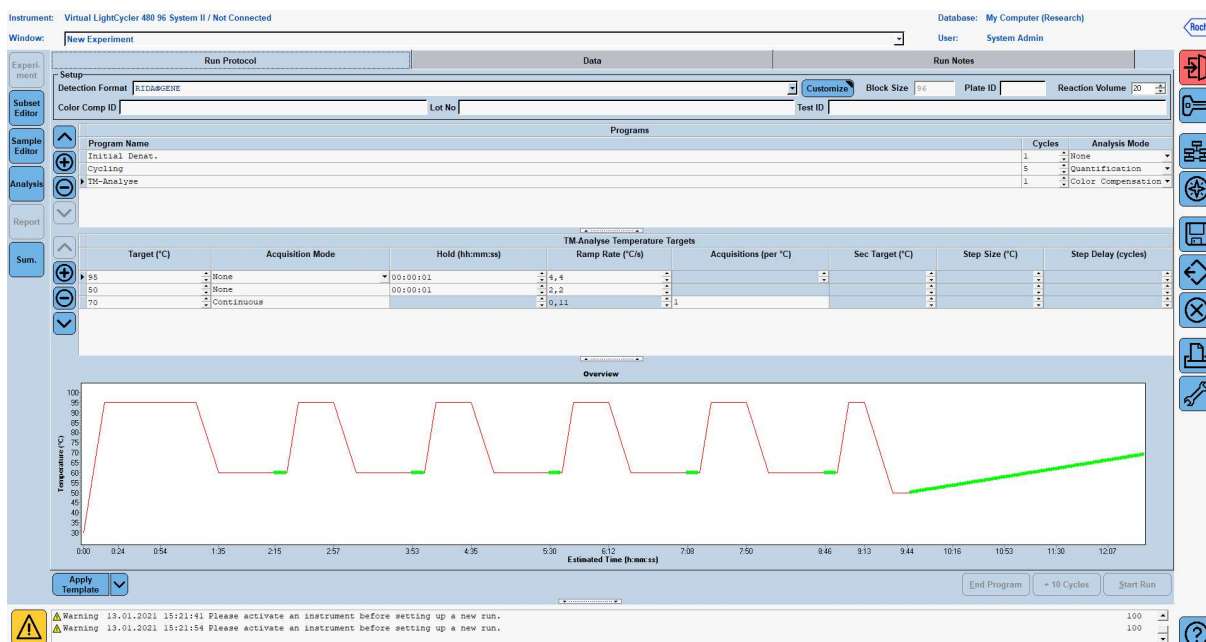
Table 5: Thermal profile

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*

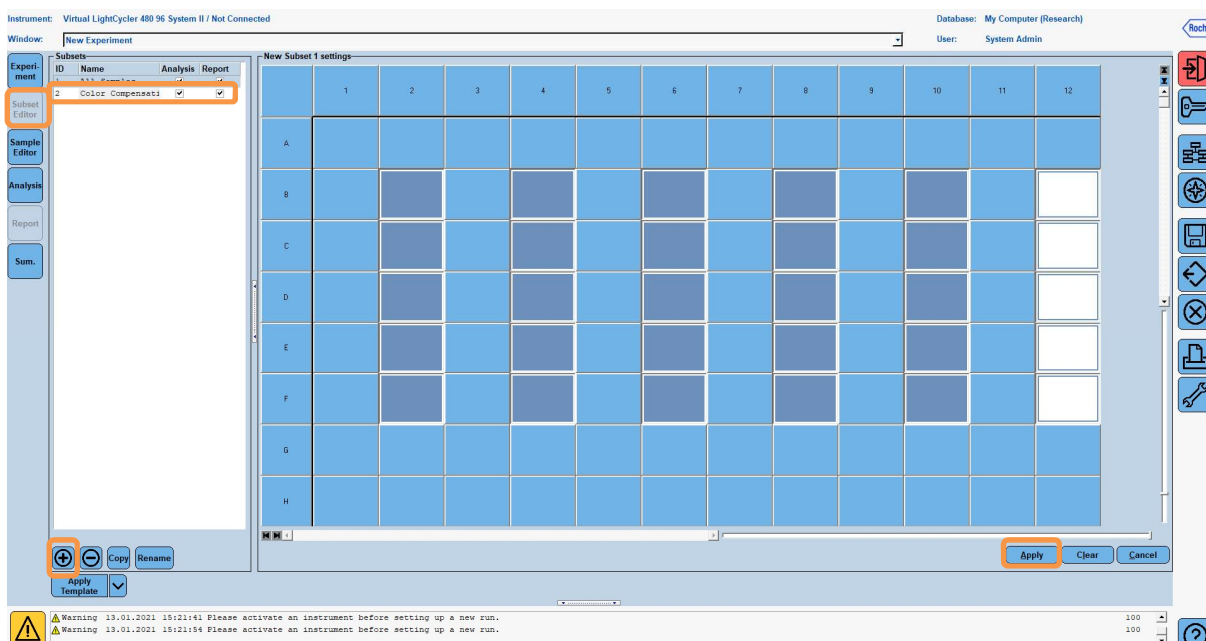
Note: Ensure that the number of “**Cycles**” and “**Analysis Mode**” is correct.

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

6. After programming is complete, the experiment should look as follows (see the following figure).



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 (see the following figure).



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) (see the following figure).

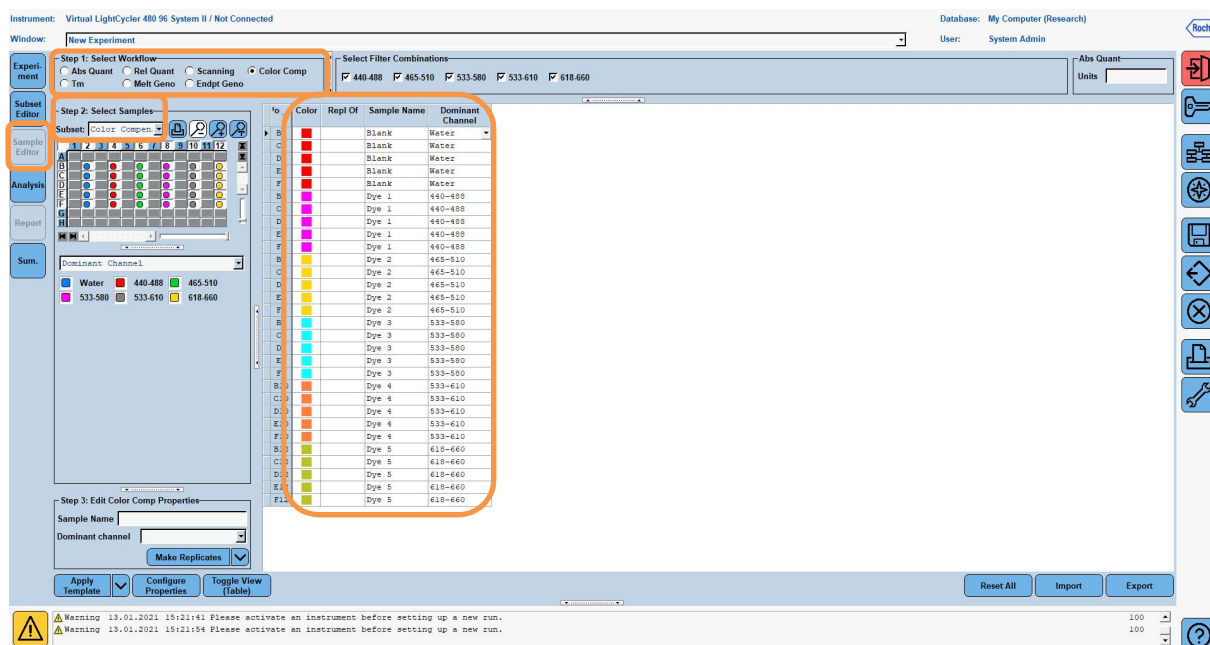
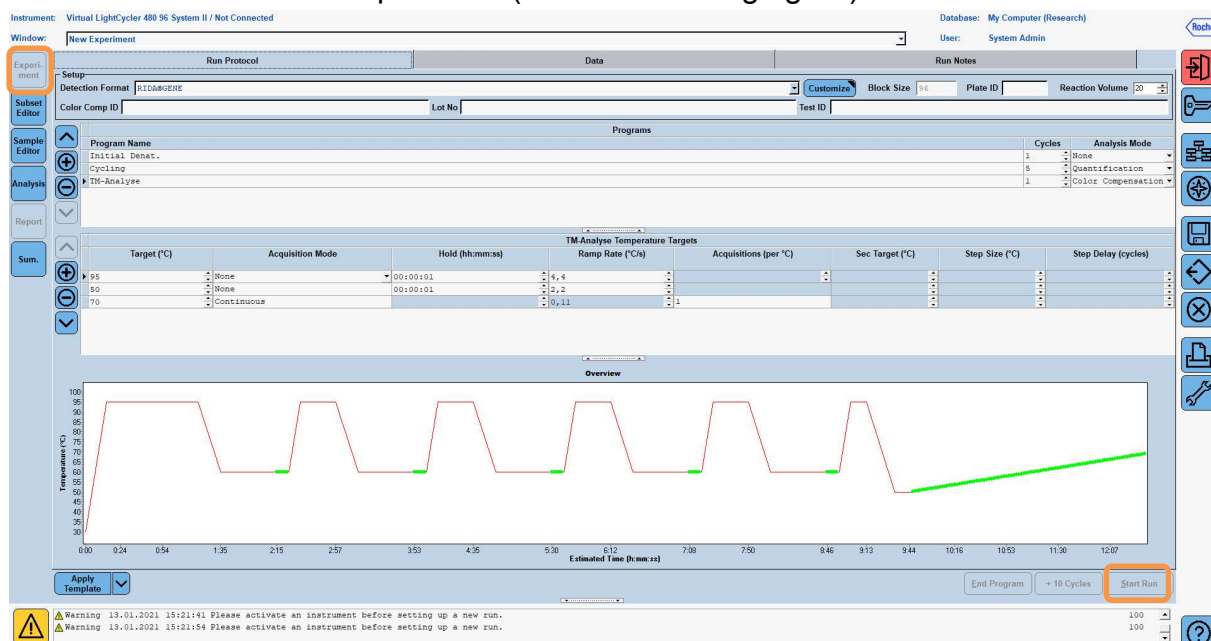


Table 6: Dominant Channel settings for the reagents (LightCycler® 480 II)

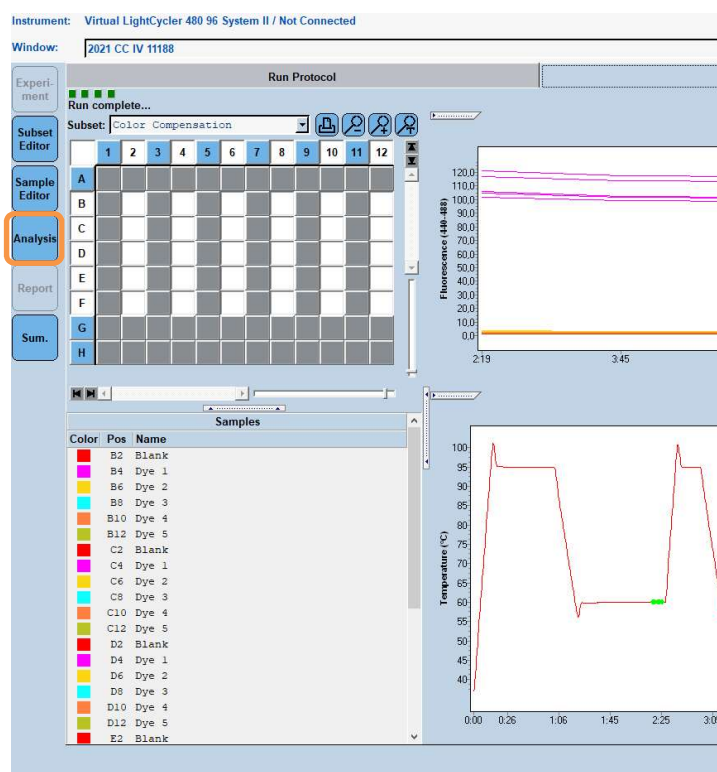
Reagent	Dominant Channel
Blank	Water
Dye 1	440 / 488
Dye 2	465 / 510
Dye 3	533 / 580
Dye 4	533 / 610
Dye 5	618 / 660

9. Place the plate with the prepared reactions into the device. Click **“Experiment”** and then **“Start Run”** to start the experiment (see the following figure).

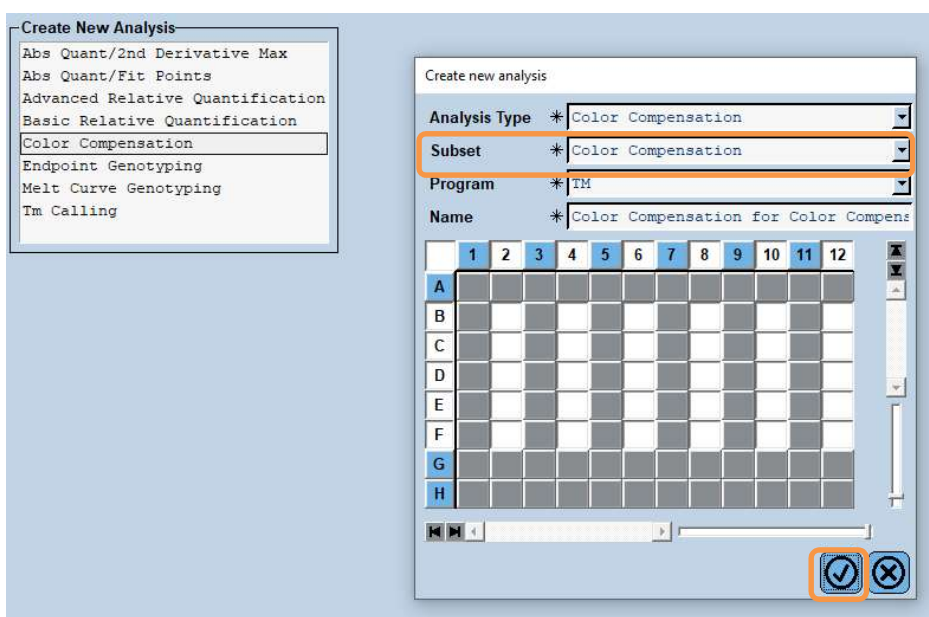


8.3 Evaluation and creation of a color compensation file

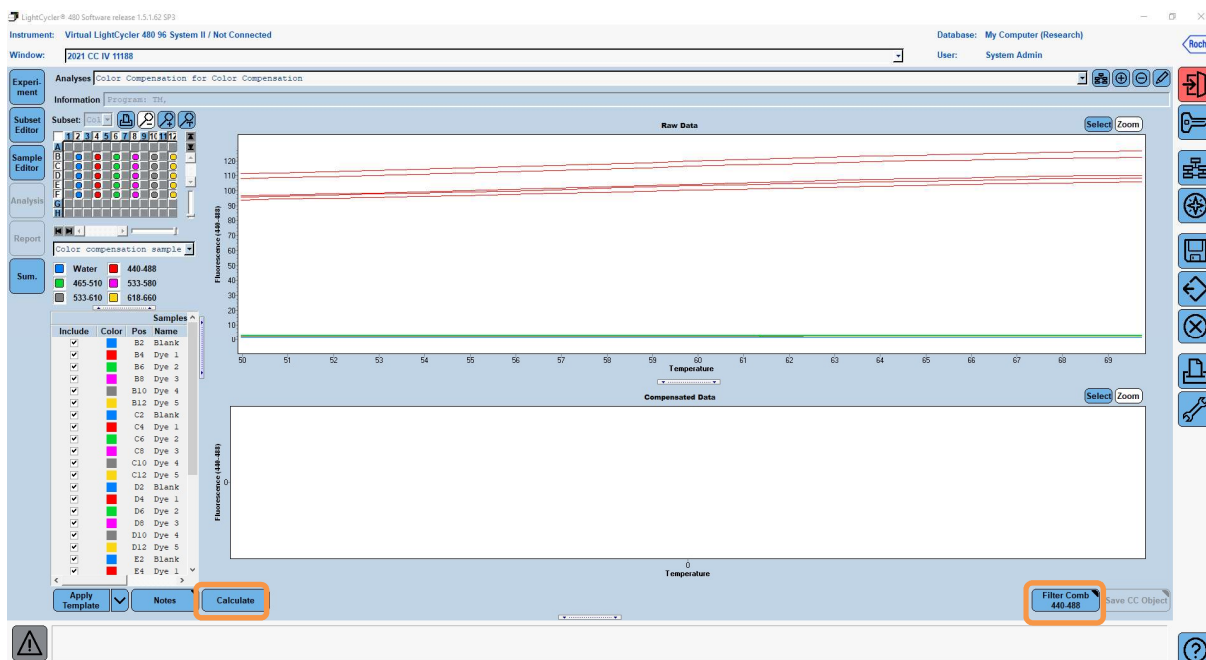
1. After completing the LightCycler® experiment, click the **“Analysis”** button (see the following figure).



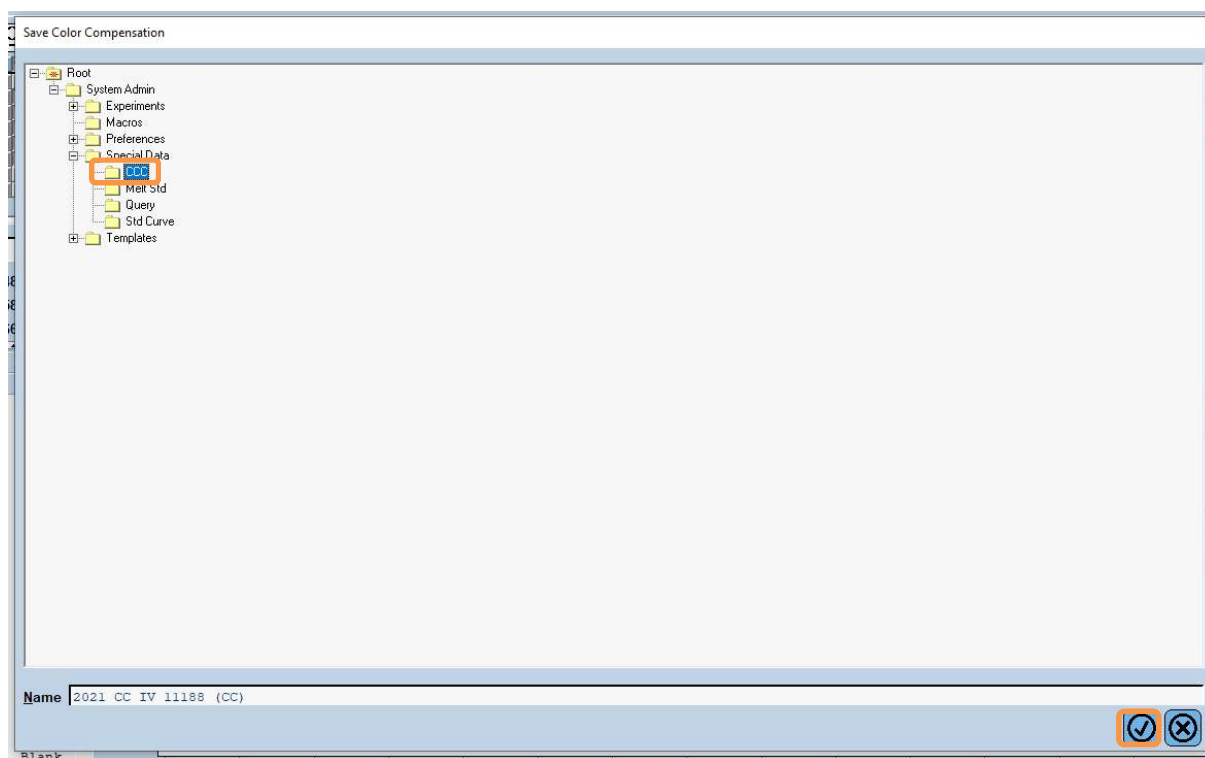
2. In the **“Create New Analysis”** dialog box, go to **“Color Compensation”**. Select and confirm the appropriate subset (e.g., Color Compensation) in the dialog box that opens (see the following figure).



- The analysis opens; click **“Calculate”** and then **“Save CC Object”** (see the following figure).



- Save the color compensation file as **“RIDA®GENE CCIV”** in the **“CCC”** folder (see the following figure).



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 (s. Fig.2).

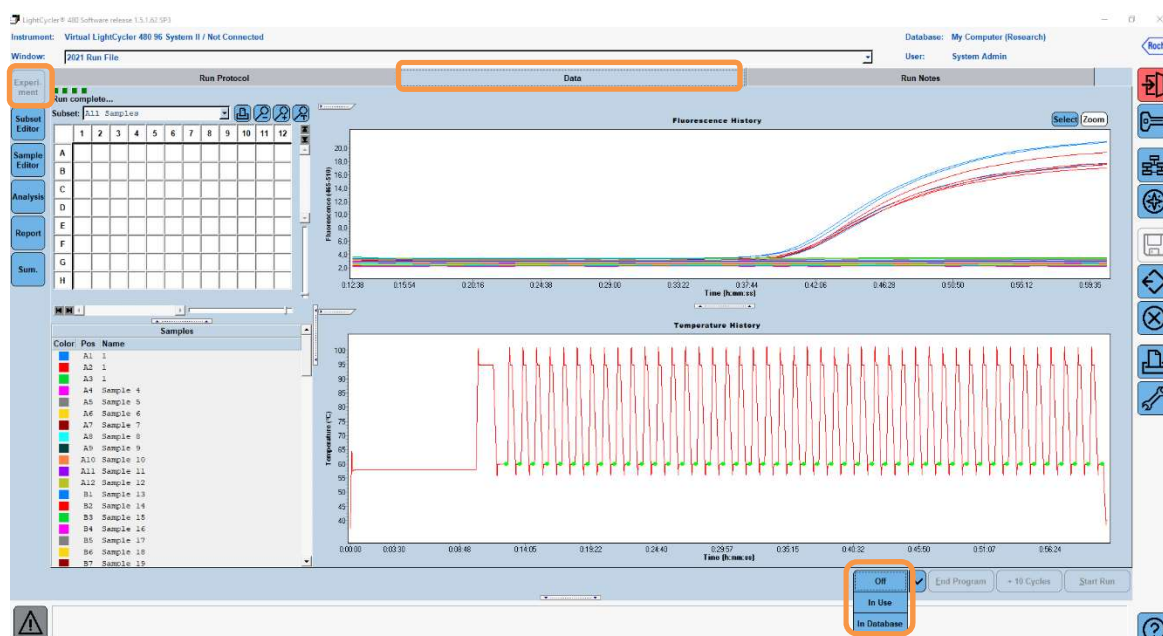


Figure 2: Use of the Color Compensation

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


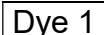

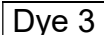
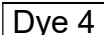
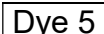
Version number	Section and designation
2021-09-09	Previous version
2022-02-03	General revision: 4. Reagents provided 5. Storage instructions 6. Reagents required but not provided 7. Warnings and precautions for the users

10. Explanation of symbols

General symbols

	For <i>in vitro</i> diagnostic use
	Observe operating manual
	Batch number
	Use before
	Storage temperature
	Item number
	Number of tests
	Date of manufacture
	Manufacturer

Test-specific symbols

	Blank
	Dye 1
	Dye 2
	Dye 3
	Dye 4
	Dye 5