RIDA®GENE Color Compensation Kit I

Art. No.: PG0001
3 reactions

-20 °C

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1. Intended use

RIDA®GENE Color Compensation Kit I is intended for generating a Color Compensation File for multiplex real-time PCR experiments on the LightCycler ® 480. The generated Color Compensation File can be applied to analyze multiplex real-time PCR experiments of RIDA®GENE real-time PCR kits on the LightCycler® 480.

2. Explanation of the test

In a multiplex real-time PCR, the wavelengths of light emitted by the reporter dyes may overlap, causing one channel to pick up signals (crosstalk) from a dye measured by another channel. This crosstalk of fluorescence signal can result in incorrect data unless a correction is made by using a Color Compensation File. Color compensation is used to subtract fluorescence crosstalk from a reporter dye into inappropriate channels outside of its dominant emission channel.

3. Kit components

Tab.1: Reagents provided (Reagents provided in the kit are sufficient for 3 Color Compensation experiments)

Kit Code	Reagent	Volume	Lid color
1	Blank	1x 400 µl	white
2	Dye 1	1x 400 µl	green
3	Dye 2	1x 400 µl	yellow
4	Dye 3	1x 400 µl	orange
5	Dye 4	1x 400 µl	red

4. Storage instructions

- Protect RIDA®GENE Color Compensation Kit I from light and store at -20 °C.
- RIDA®GENE Color Compensation Kit I can be used until the expiration date printed on the label. After expiry the quality guarantee is no longer valid.
- Carefully thaw RIDA®GENE Color Compensation Kit I reagents before use (e.g. in a refrigerator at 2 - 8 °C).
- During Color Compensation preparation all the reagents should be stored cold in an appropriate way (2 - 8 °C).

5. Additional equipment and materials

- LightCycler® 480I, LightCycler® 480II or LightCycler® 480z (Roche)
- Real-time PCR consumables (plate, foil)
- Pipettes (0.5 – 20 µl, 20 – 200 µl, 100 – 1000 µl)
- Filter tips

6. Precautions for users

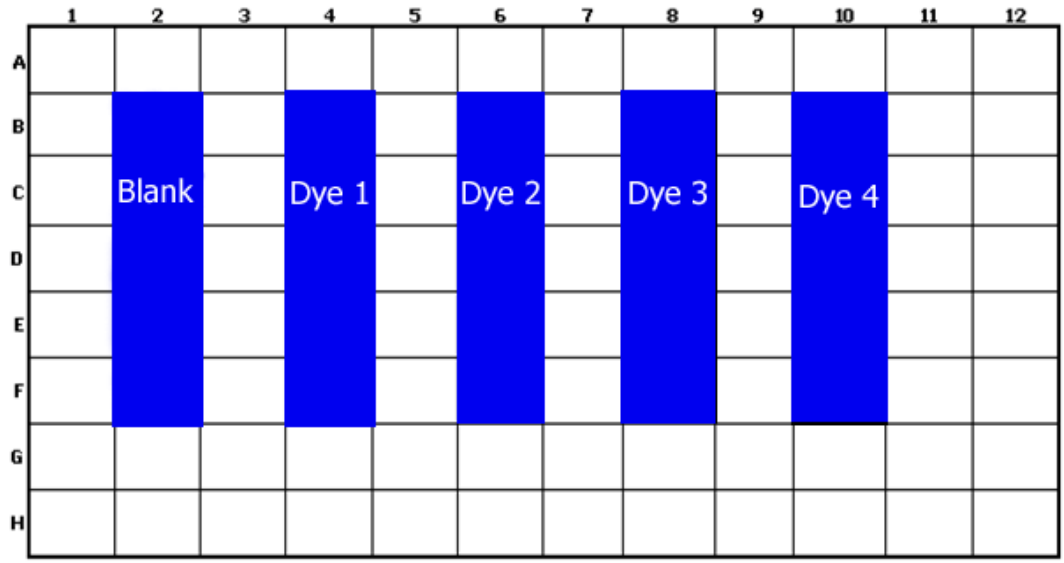
- This test must only be performed by laboratory personnel trained in molecular biology methods.
- Strictly follow the working instructions.
- When handling samples, wear disposable gloves. After finishing the test, wash your hands.
- Do not smoke, eat or drink in areas where samples or test reagents are being used.
- Do not use the kit after the expiration date.

7. Protocol for creating a Color Compensation File on the Light Cycler® 480

7.1 Preparation of the Color Compensation plate

For a color compensation experiment it is necessary to pipette 5 reactions with 20 µl of each dye and also of the background (blank) into a microwell plate (s. Fig.1).

Fig.1: Pipetting scheme for Color Compensation experiment



Thaw, mix gently and centrifuge briefly the reagents before use. Keep reagents appropriately cold during working step (2 - 8 °C).

Tab.2: Preparation of the Color Compensation

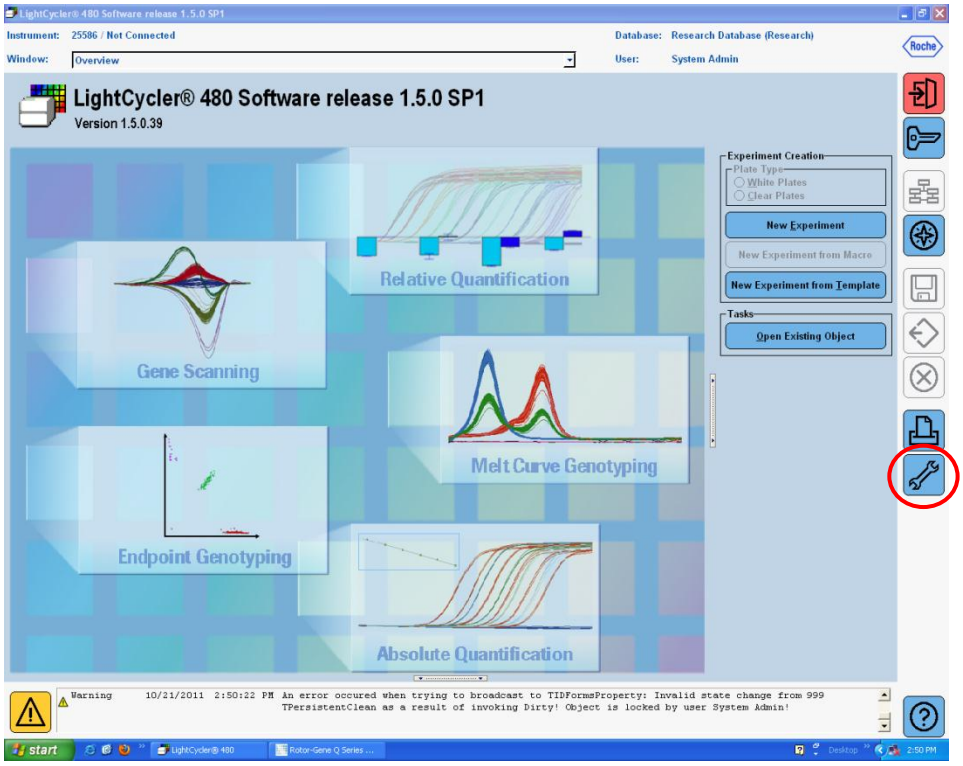
Kit Code	Reagent	Volume per reaction	Pipette 20 µl into the following wells
1	Blank	20 µl	B2, C2, D2, E2, F2
2	Dye 1	20 µl	B4, C4, D4, E4, F4
3	Dye 2	20 µl	B6, C6, D6, E6, F6
4	Dye 3	20 µl	B8, C8, D8, E8, F8
5	Dye 4	20 µl	B10, C10, D10, E10, F10

Cover the microwell plate with an optical sealing foil after pipetting the reagents. Start the real-time PCR according to LightCyler® 480 Set-up.

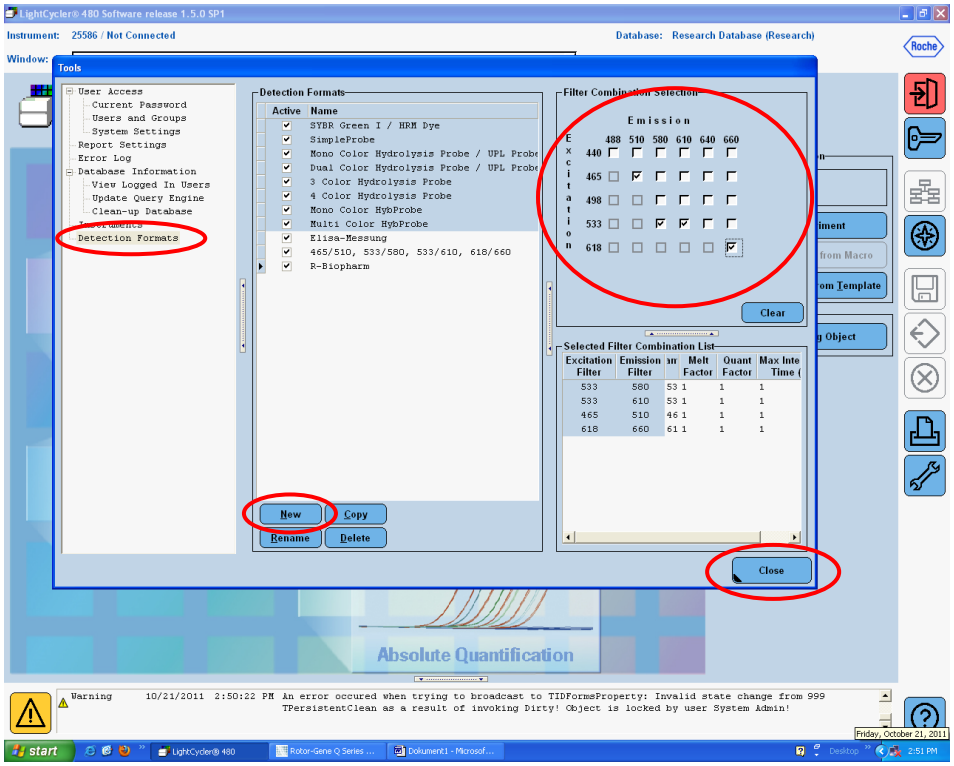
7.2 LightCyler® 480Set-up

Note: Login as administrator to set up the detection format in the LightCyler® 480 software.

1. After opening the LightCycler® 480 software, it is necessary to click the "**Tools**" icon to program the detection format.



2. The following window opens. Select “**Detection formats**” in the Tools window. Click the “**New**” button and set-up the detection format (see Tab.3). Save the detection format as “**R-Biopharm**”. Click the “**Close**” button to exit the Tools window.

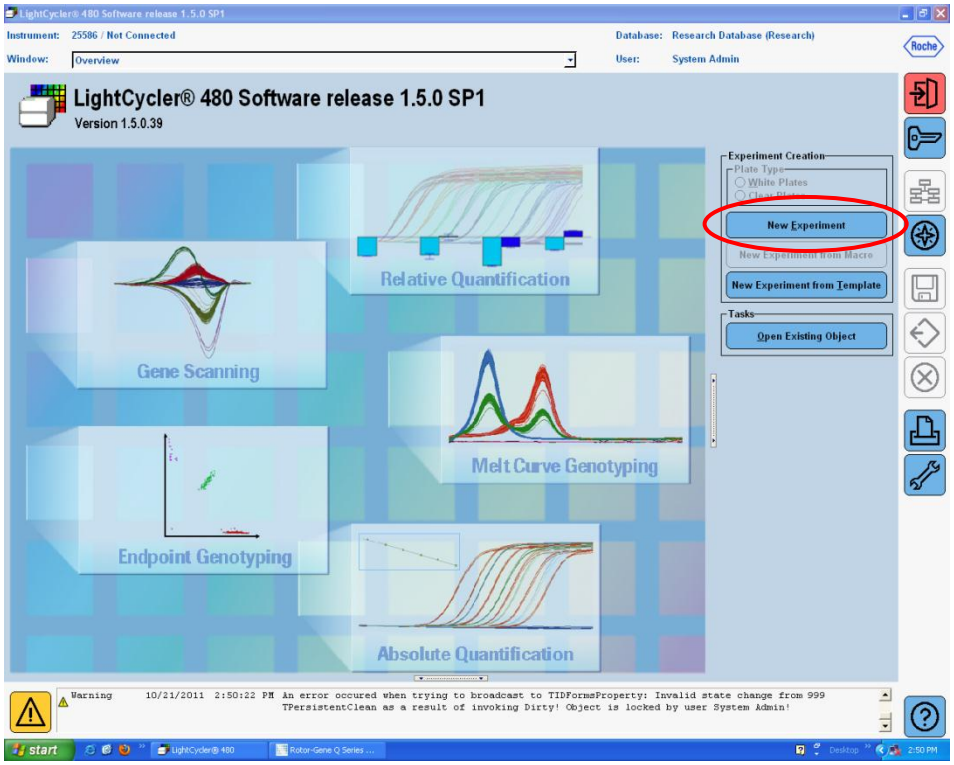


Tab.3: Detection Channels Set-up for LightCycler® 480

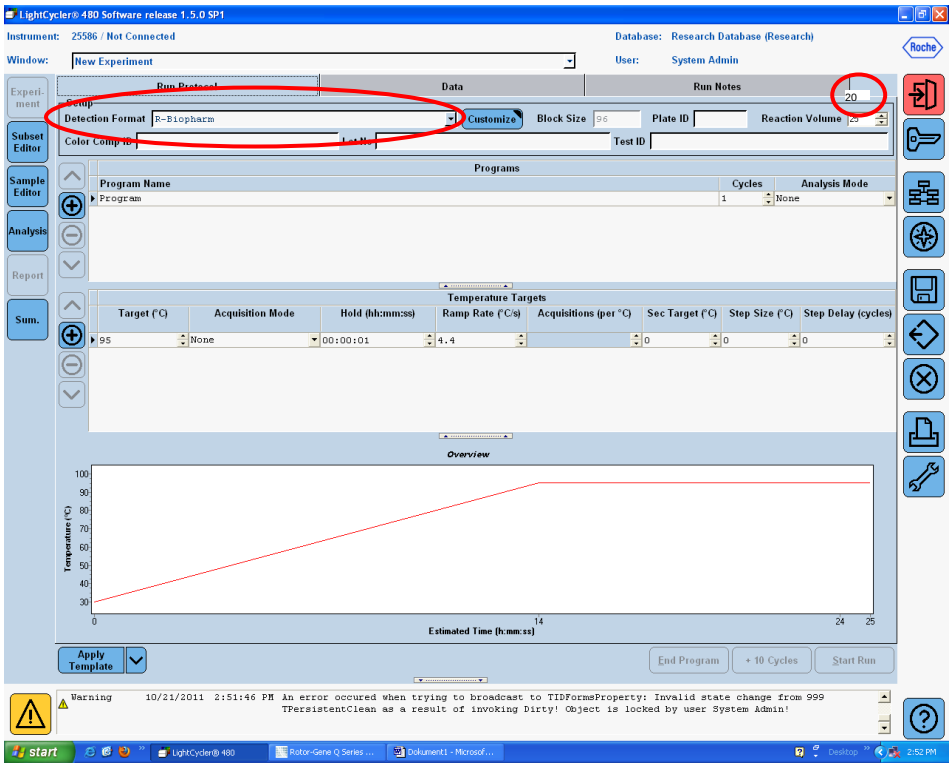
Filter Combination		
LightCycler® 480I	**LightCycler® 480II**	**LightCycler® 480z**
450 / 500	465 / 510	465 / 510
523 / 568	533 / 580	540 / 580
558 / 610	533 / 610	540 / 610
615 / 670	618 / 660	610 / 670

Note: Set the value for Quant Factor, Melt Factor as well as the Integration Time to 1.

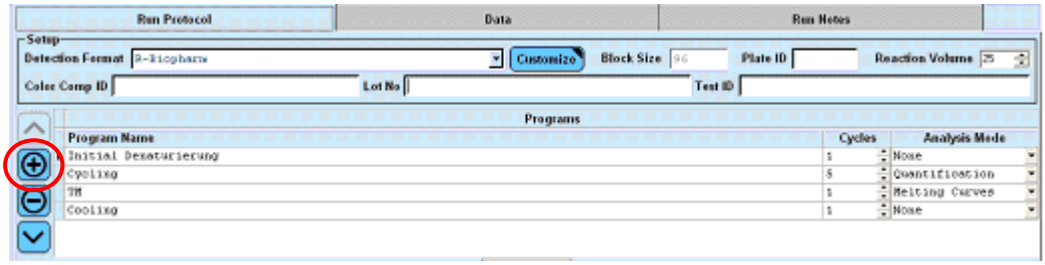
3. After programming the detection format, click the „**New Experiment**“ button.



4. The following window will open. Select detection format “**R-Biopharm**” and enter a reaction volume of 20 µl.



5. Program the LightCycler® 480 according to the real-time PCR profile (see Tab.4). Click the "**Plus**" button to program the four protocol steps.

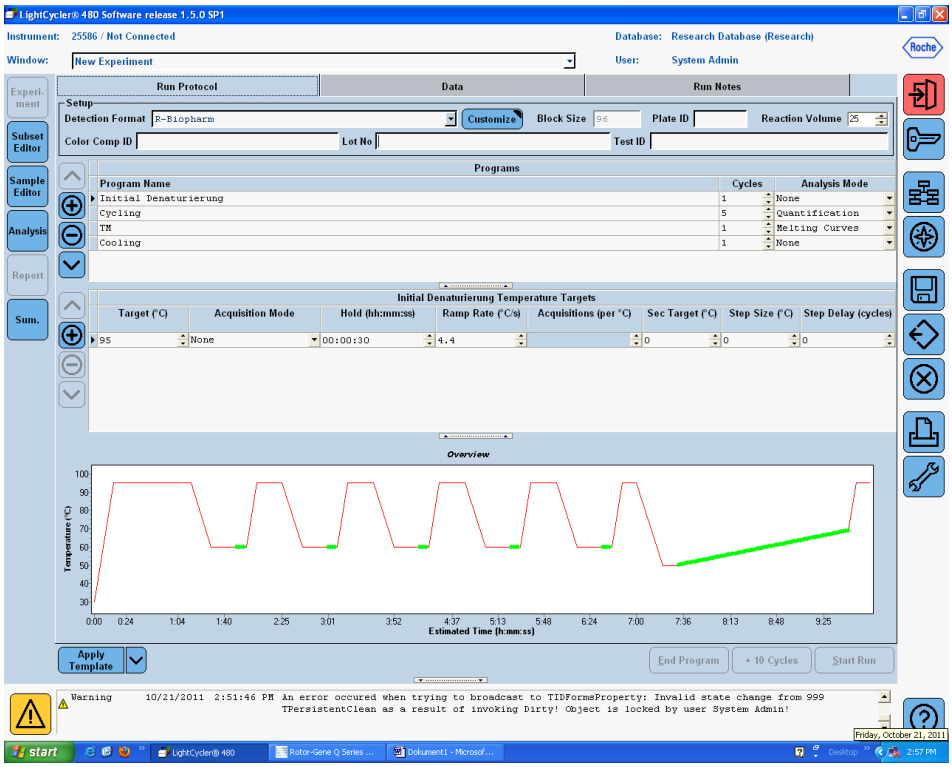


Tab.4: LightCycler® 480 real-time PCR profile

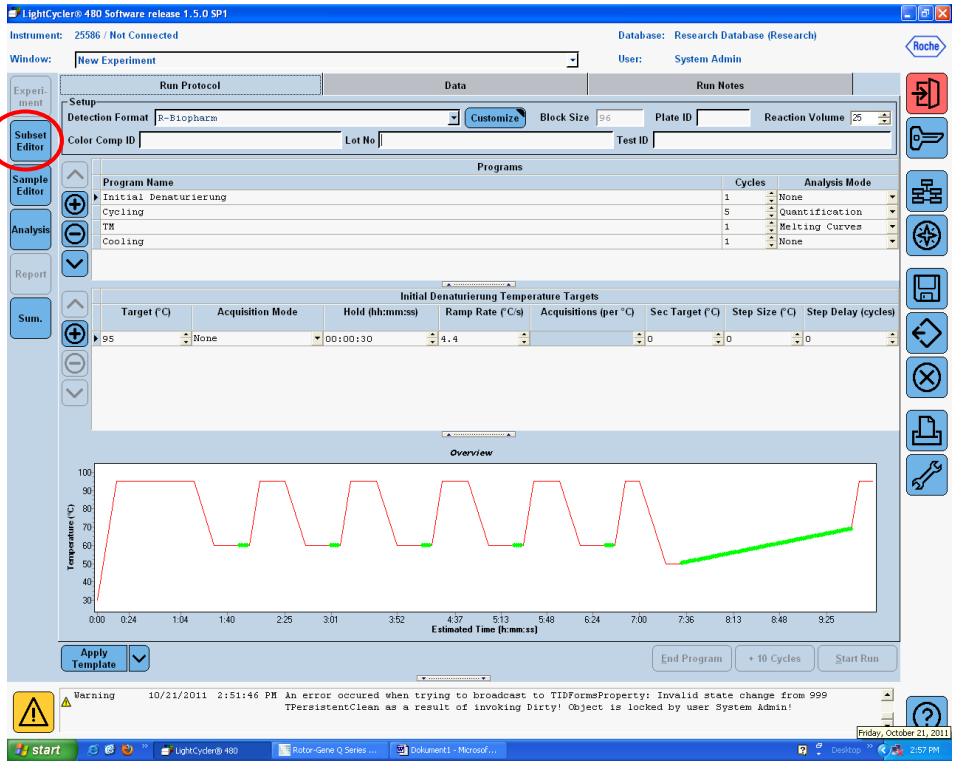
		Temperature targets			
Program	Cycles / Analysis Mode	Target [°C]	Acquisition Mode	Hold [hh:mm:ss]	Ramp rate [°c/s]
Initial Denat.	1 / none	95	none	00:00:30	4.4
Cycling	5 / Quantification	95	none	00:00:15	4.4
		60	single	00:00:30	2.2
TM-Analyse	1 / Color Compensation	95	none	00:00:01	4.4
		50	none	00:00:30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

Note: Ensure the correct setting of the number of "**Analysis Mode**" and of the "**Cycles**".

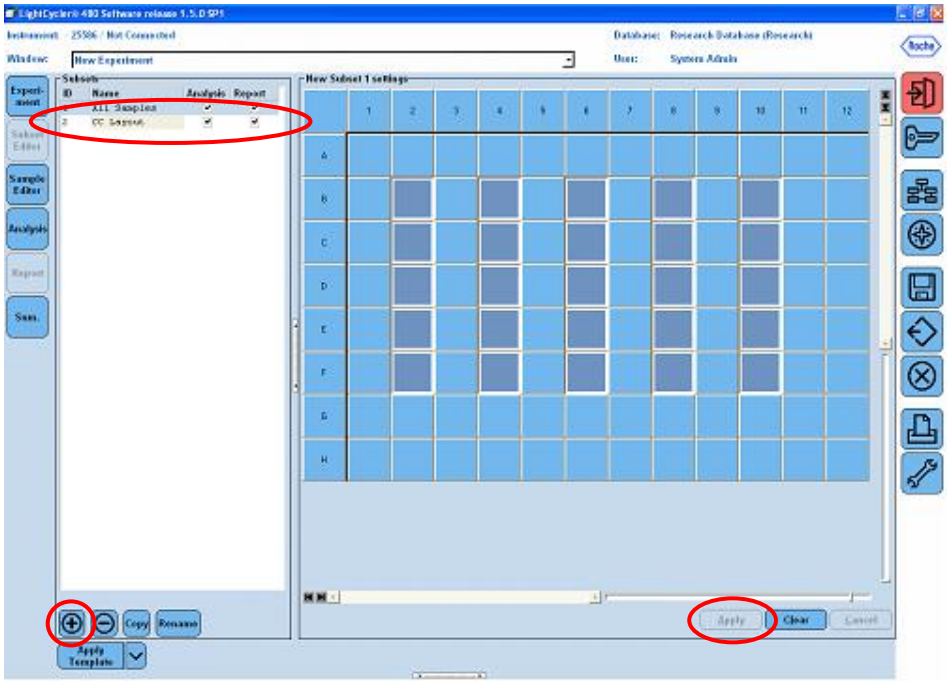
6. After completion of the programming the LightCycler® experiment should look like the picture below.



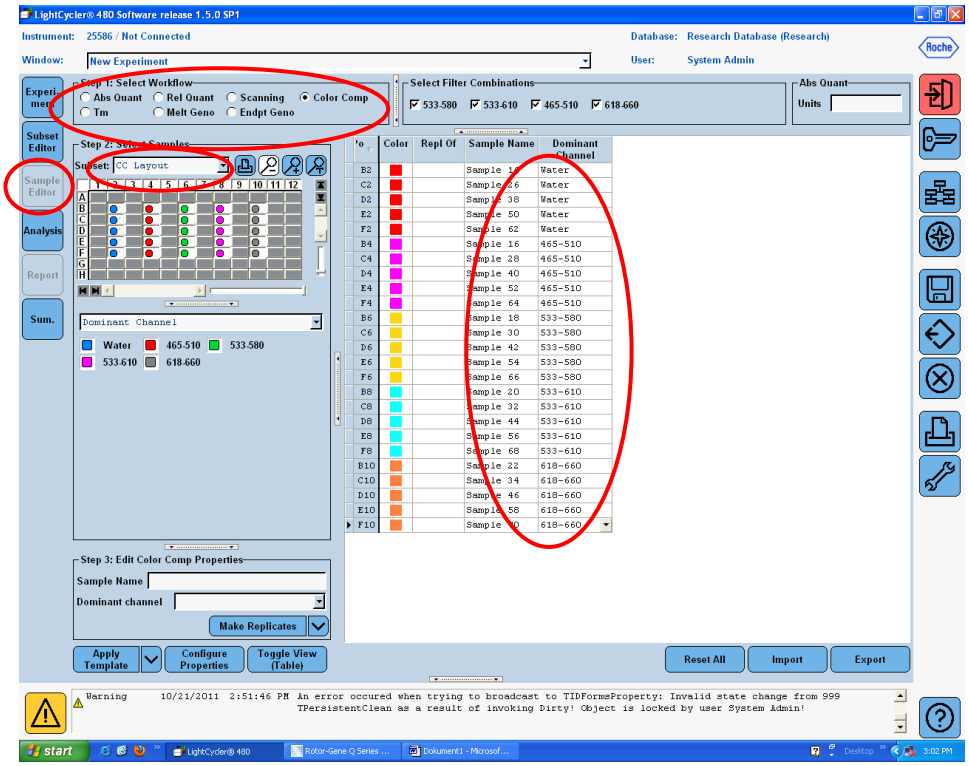
7. To select the right PCR layout click the **„Subset Editor“** button.



8. Click on the “**Plus**” icon to create a new subset and enter a unique name for the layout (e.g. CC Layout). Press the Strg-key as well as the left mouse button simultaneously and mark all wells of the microwell plate with reagents. Click on the **“Apply”** button to finish the subset. The final screen should look like the screenshot below.



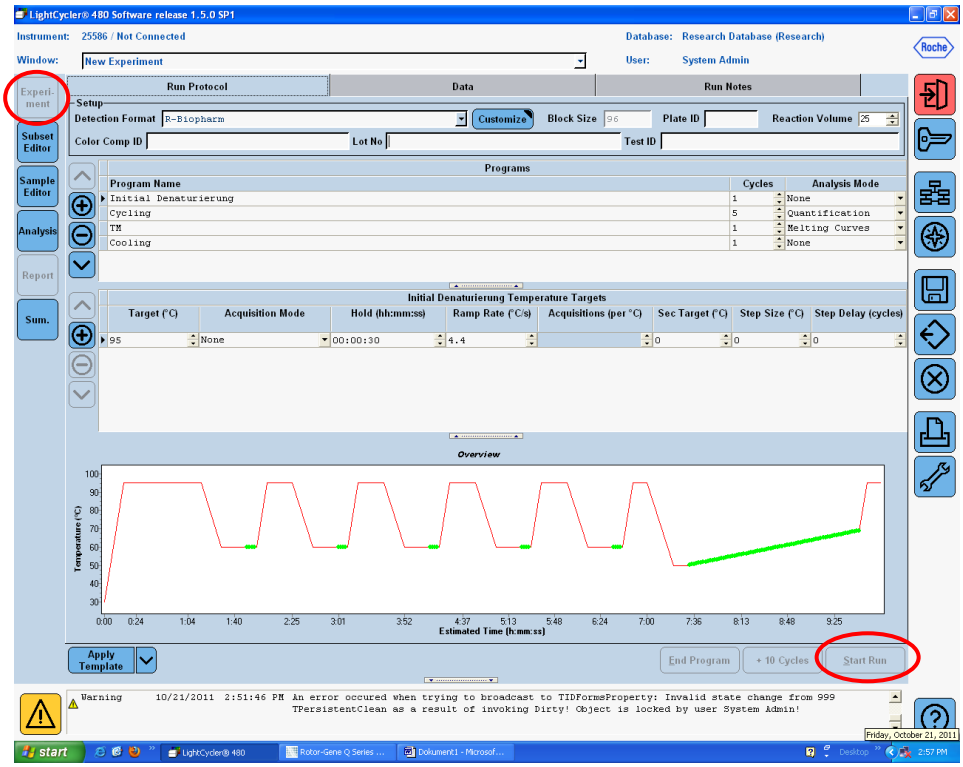
9. Click the **„Sample Editor“** button. Select **„Color Comp“** from the Step 1: “**Select Workflow”** field. Then select the created subset (CC Layout) in the Step 2: **Select Samples** field. To finish the layout select in the **„Dominant Channel“** dialog field for the reagents (Dye 1, Dye 2, Dye 3, Dye 4) the corresponding dominant channel (see Tab.5). Select for the samples with the reagent “Blank” the dominant channel “**Water**”. It is not necessary to enter a “Sample Name”.



Tab. 5: Dominant Channel setting for reagents

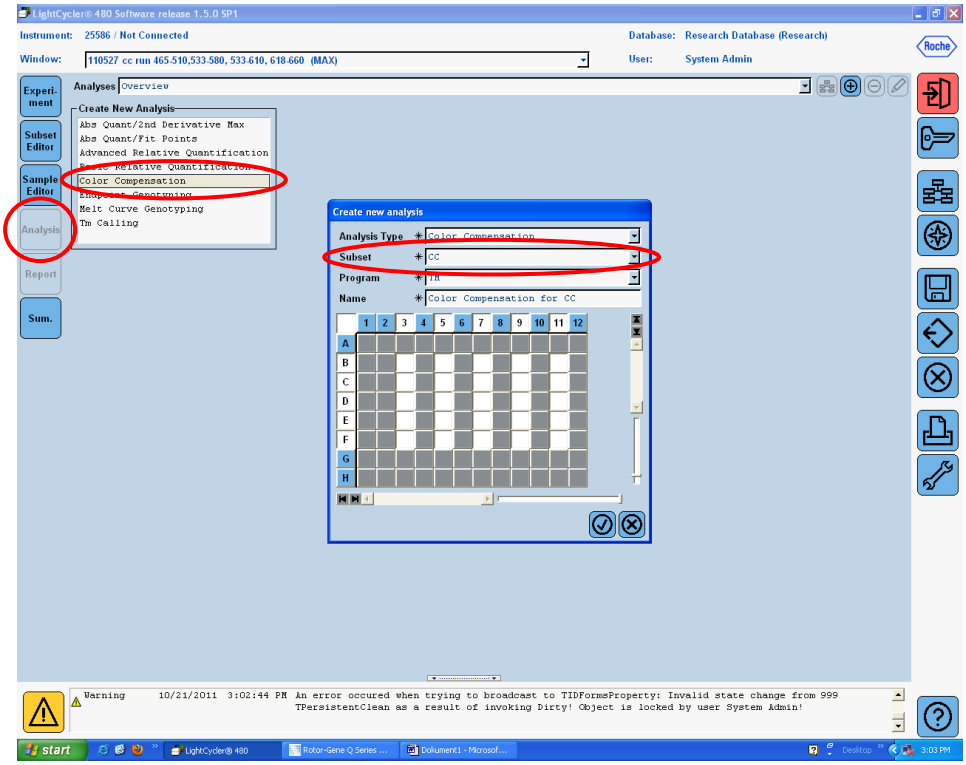
	Dominant Channel		
Reagent	**LightCycler® 480I**	**LightCycler® 480II**	**LightCycler® 480z**
Blank	Water	Water	Water
Dye 1	450 / 500	465 / 510	465 / 510
Dye 2	523 / 568	533 / 580	540 / 580
Dye 3	558 / 610	533 / 610	540 / 610
Dye 4	615 / 670	618 / 660	610 / 670

10. Place the PCR plate with the prepared reactions into the LightCyler® 480. Click the “**Experiment**” icon and start the experiment by clicking the "**Start Run**" button in the experiment window.

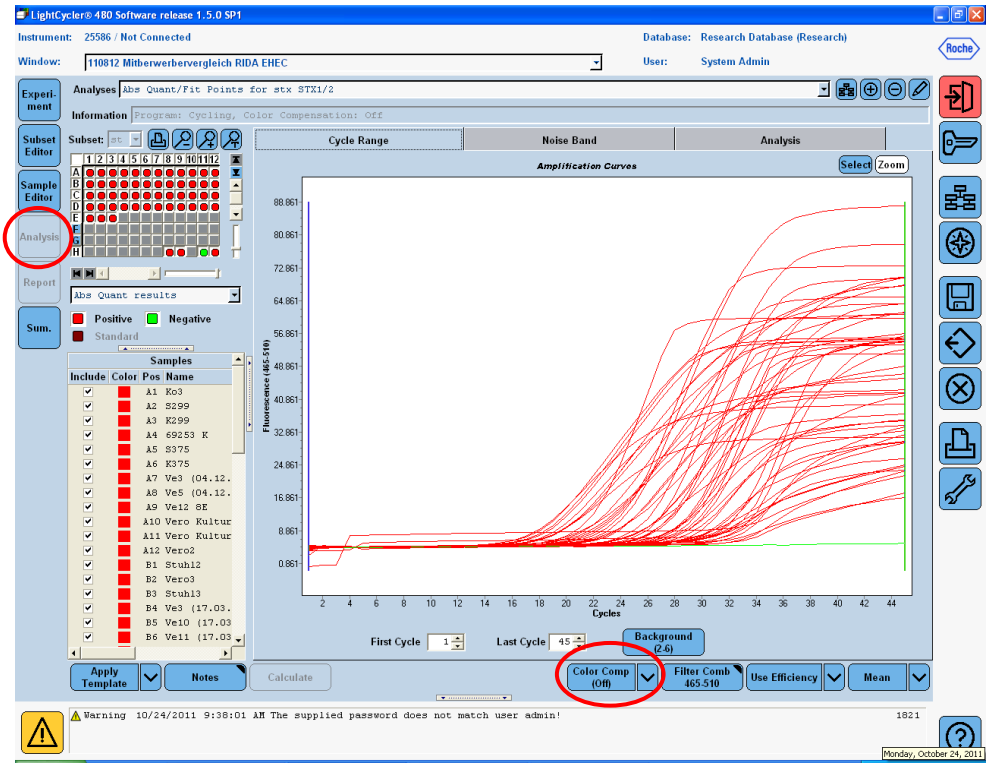


7.3 Evaluation and creation of a Color Compensation File

After the experiment ends click the **“Analysis”** button to open the **“Create New Analysis”** dialog box and select **“Color Compensation”**. Select in the appearing dialog box the appropriate subset (e.g. CC Layout) and confirm your selection. Click the button **“Calculate”** in the analysis window to perform color compensation analysis. Click the **“Save CC Object”** button to save the Color Compensation file as “**R-Biopharm**” in the “**CCC”** folder. The created Color Compensation File can now be applied for other LightCycler® 480 experiments.



To apply Color Compensation open the multiplex assay and click the **“Analysis”** button to select the appropriate filter combination. Select **“in Database”** from the Color Compensation drop-down menu and choose the stored Color Compensation file you want to apply to the experiment. Click the “**Color Compensation (Off)**” button, and select the appropriate Color Compensation File. The “**Color Compensation (Off)**” button switches to “**Color Compensation (On)**” to confirm that Color Compensation is active. The multiplex real-time PCR experiment can now be analyzed.



Note: The Color Compensation File is specific for every LightCycler®480 instrument. A new Color Compensation File has to be created if the LightCyler®480 instrument is changed or after the optical system has been repaired.