

Creating a Color Compensation file for Roche LightCycler 480 vers.I & vers. II

Due to ROX and Cy5 dye emission spectra overlap Roche LightCycler 480 can pick up signals from a dye measured by another channel. This phenomenon is called “crosstalk” or “bleed-over” and can lead to misleading results. To correct for crosstalk Roche recommends to create a so-called Color Compensation file. This permits the LightCycler 480 software to compensate for fluorescence crosstalk. For further details, please refer to the Roche LightCycler 480 Instrument Operator Manual.

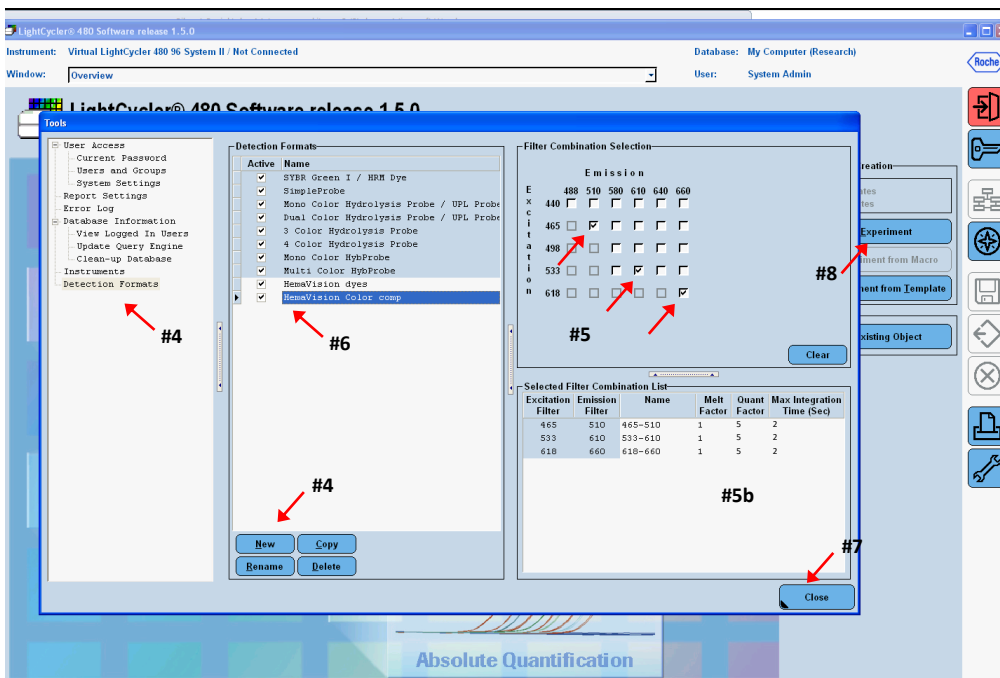
Note: The detection format for the Color Compensation must be identical to the format used when performing a HemaVision 7Q/28Q qPCR. Also a Color Compensation file can only be applied to experiments that were run on the same LightCycler 480 instrument.

Note: The settings given below are recommendations only.

Content: The HemaVision 7Q/28Q Color Compensation kit for Roche LightCycler 480 includes four 8-tube strips with dye. Each strip contains 5 replicates of the same dye, either FAM, ROX or Cy5 dye or no dye in a volume of 20 µL HemaVision 7Q/28Q qPCR mix. The dye content of the strip is marked with a white label on the caps of the 3 empty tubes in each 8-tube strip.

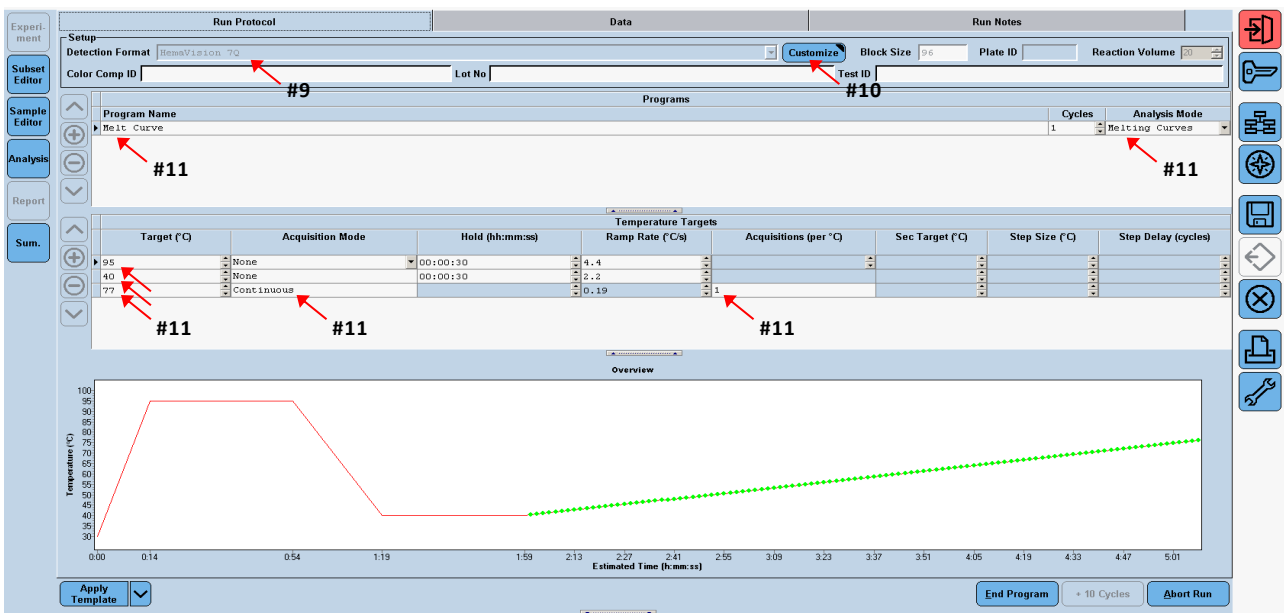
Creating a Color Compensation file:

1. Turn on the LightCycler480 and let it warm-up for at least 10 min.
2. Prior to use, spin the 8-tube strips quickly to collect the dye solution at the bottom of the tubes.
3. In the software window, select the Tools Icon (the wrench symbol).

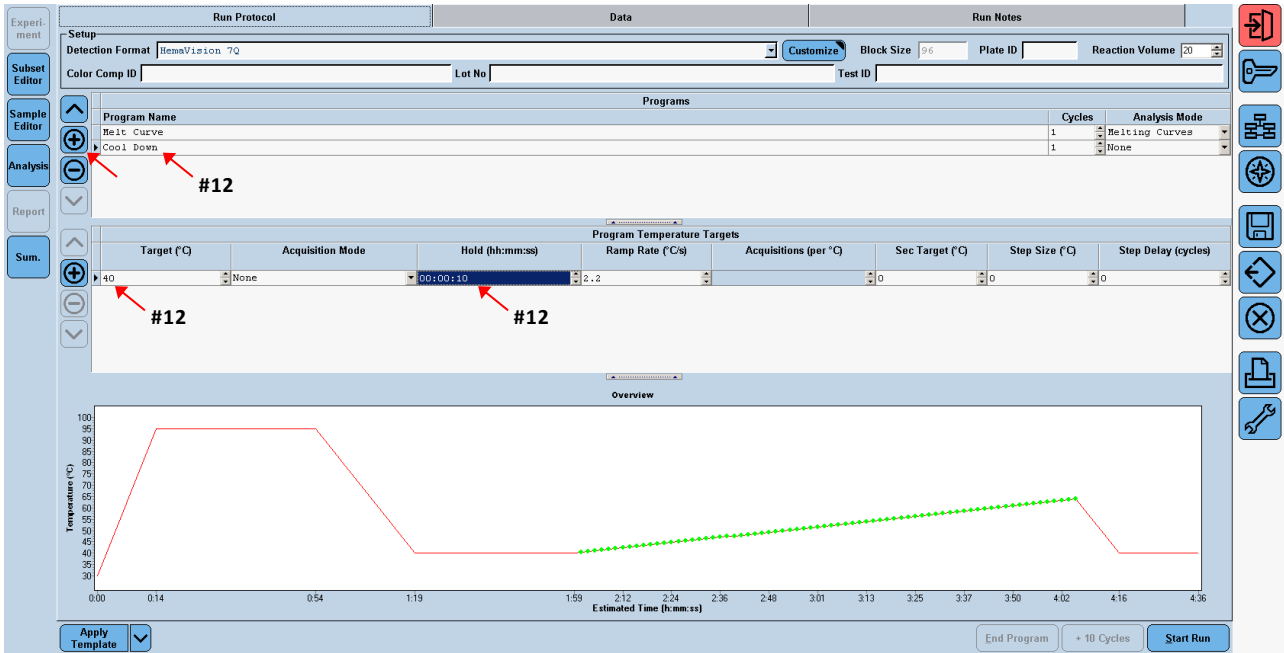


4. Select Detection Format, and then choose “New”.

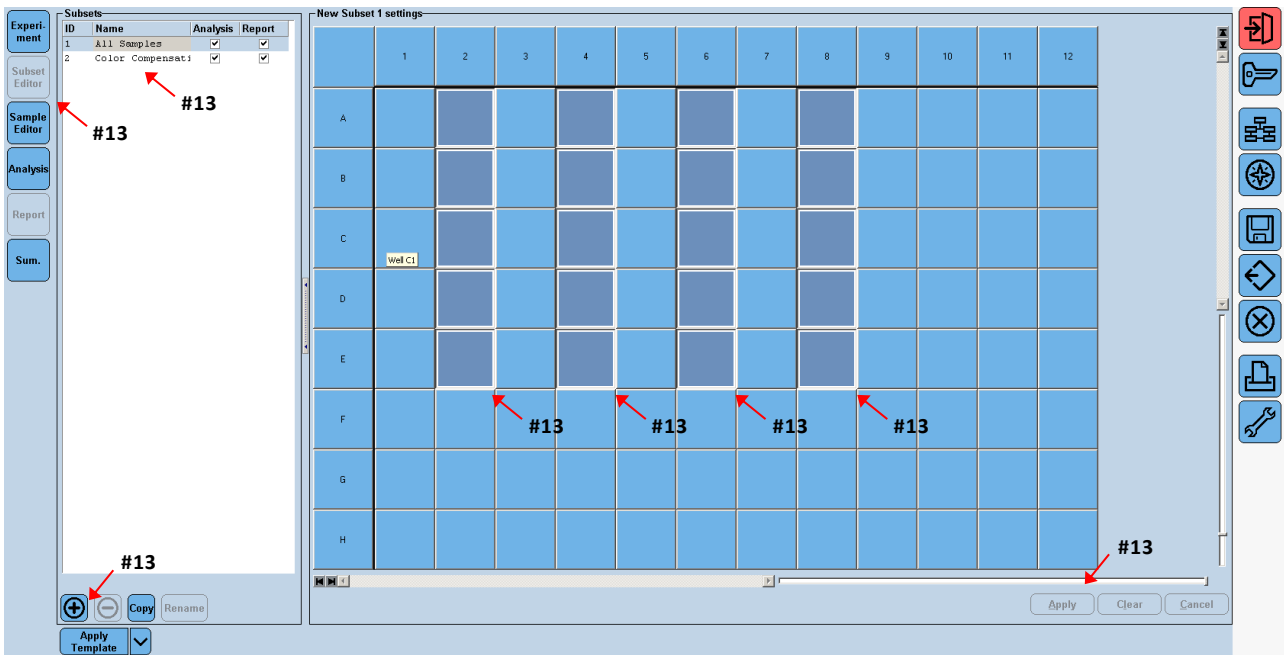
5. Select the excitation/emission filter combinations 465/510 nm for FAM, 533/610 nm for ROX and 618/660 nm for Cy5 as shown above.
NOTE: The instrument settings of melt factor, quant factor and max. integration time should be the same as when running HemaVision 7Q/28Q qPCR. The recommended settings are melt factor (1), quant factor (5), max. integration time (2).
6. Type in a name that describes the calibration series, for instance “HemaVision 7Q”.
7. Click “Close”.
8. From the Software “Overview” window, choose “New experiment”.
9. Under “Setup, Detection Format” choose the name of the filter combination you created in step 5 - 6.
10. Click the “Customize” tab to ensure the right filter combinations have been chosen.
11. Set up a Melt Curve program as shown below:



12. Introduce a “Cool Down” step by pressing “+”, write Cool Down and set “Target” to 40 for 10 seconds.

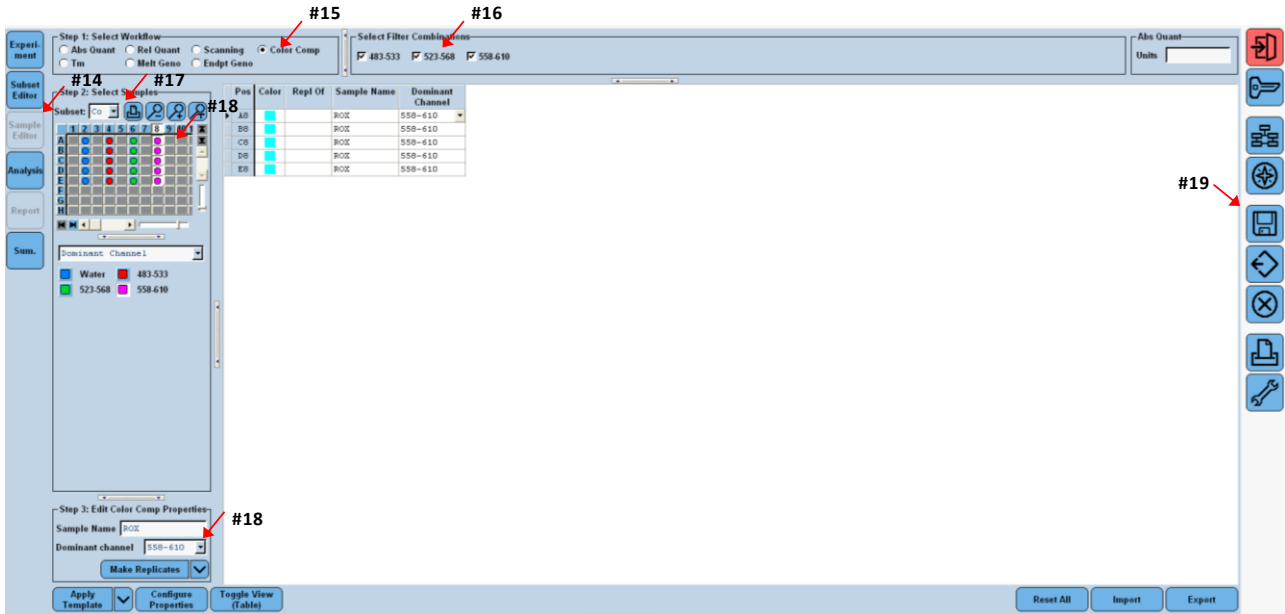


- In the "Subset Editor" on the left hand column, choose a new subset by pressing the "+" tab in the lower left corner. Name the subset "Color Compensation" and mark 4 rows with each 5 wells. Hold down the control button on your keyboard to select multiple rows. Once done click "Apply".

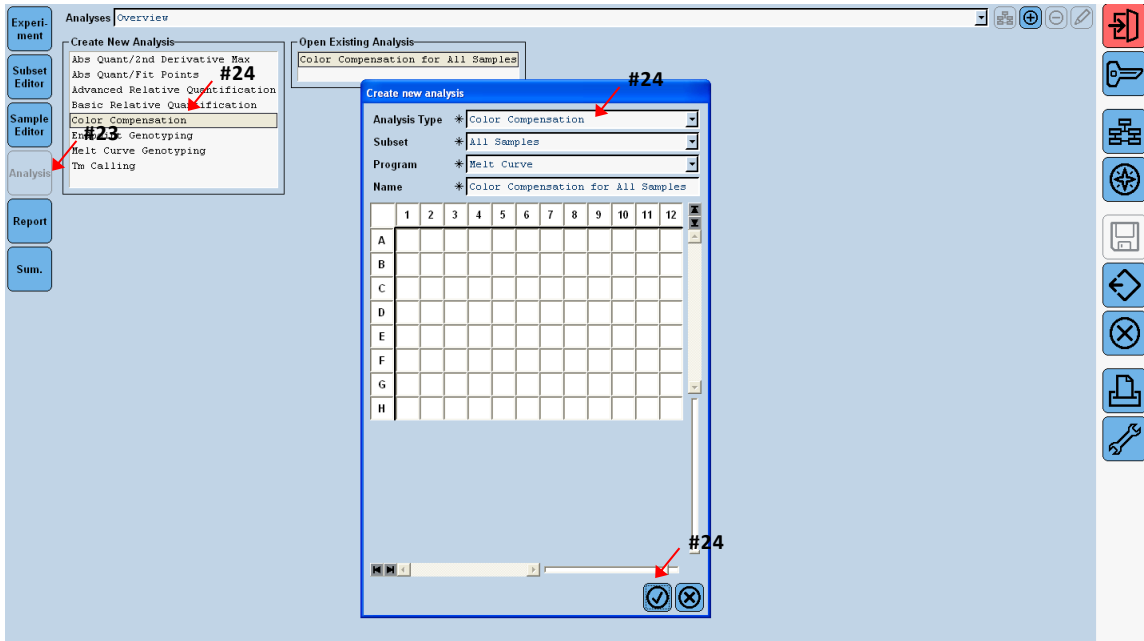


- Click the "Sample Editor" in the left hand column.
- Under "Step 1: Select Workflow" click "Color Comp"
- Under "Select Filter Combinations" check that the correct filter combinations have been chosen.
- Under "Step 2: Select Samples" select the sample subset created in step 12.

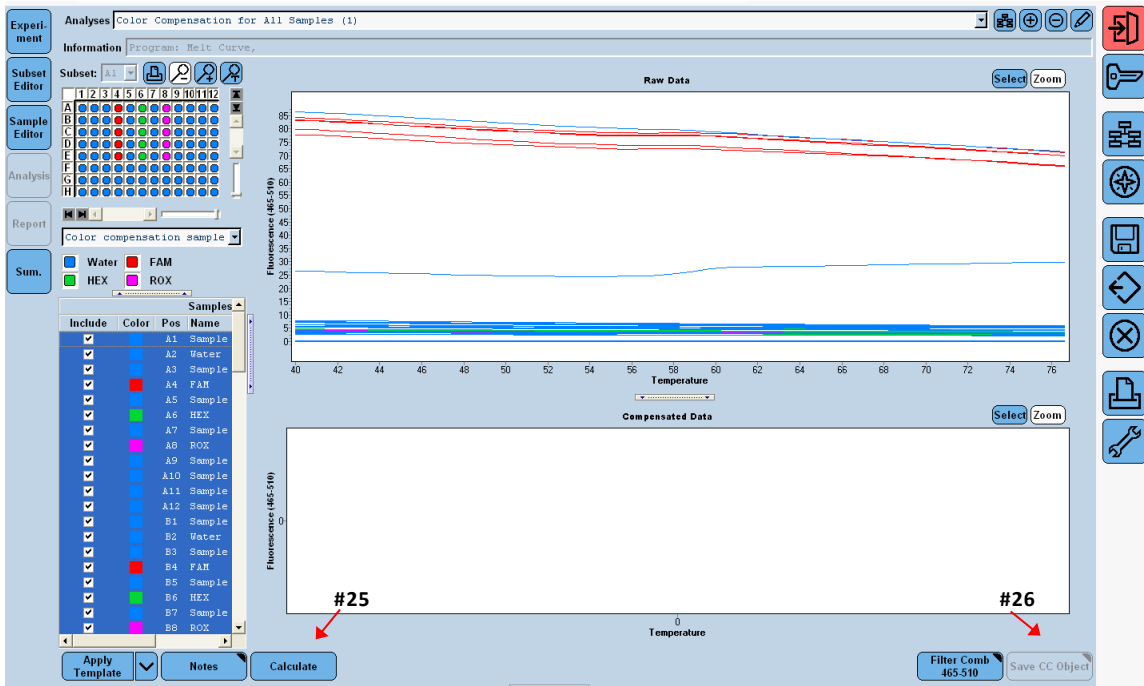
- Under “Step 3: Edit Color Comp Properties”, use the drop down menu to select the dominant channel for each of the 5 dye strips. In the strip without dye, choose water as dominant channel (see figure below).



- In the column to the right, click the floppy disk icon to save the experiment. In the folder “Experiments” save the experiment under an appropriate name.
- Place the calibration dye strips in the adapter plate. Make sure that the orientation is the same as you previously have indicated in the “Sample Editor”. The 5 dye containing tubes of each strip are not covered by the white label. Well A1 must be located in the top left corner and the cut corner in the lower right of the plate.
- Insert the adapter plate and calibration dyes in the machine.
- In the “Experiment” window press “Start”.
- When the experiment has finished click “Analysis” in the left hand column.



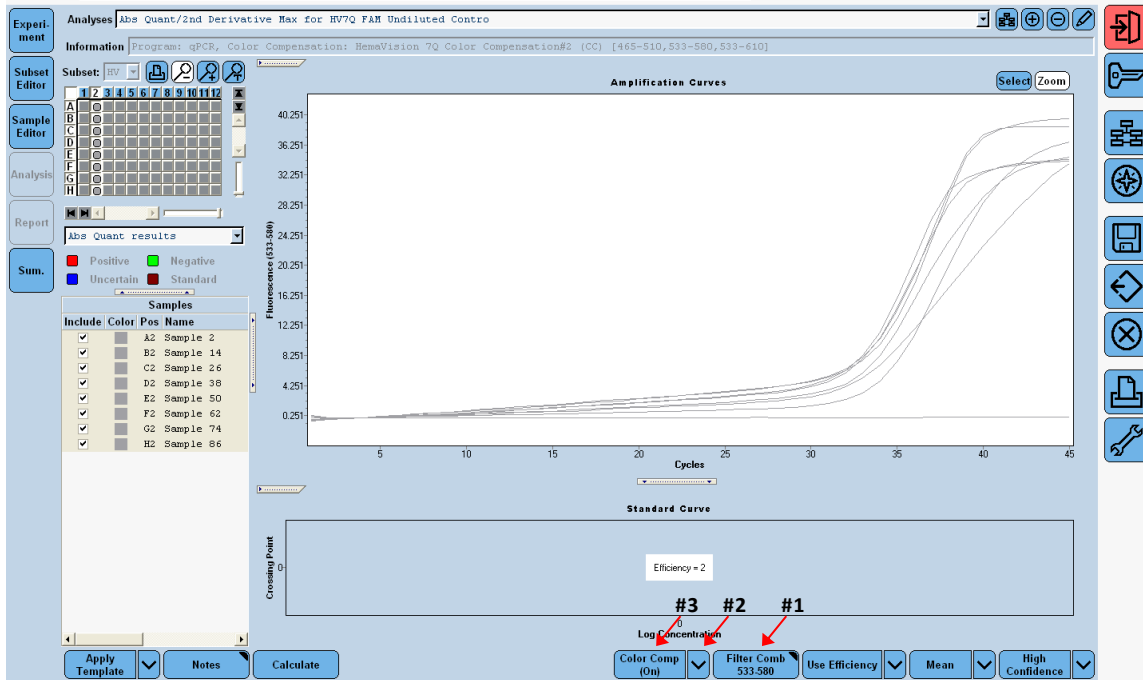
24. In “Create New Analysis” click “Color Compensation” and click the OK symbol.
25. Click “Calculate” to perform the analysis.



26. Click “Save CC Object” to create a Color Compensation file. By default the file is saved under “Special Data” and “CCC”.
27. You can now apply the Color Compensation data to new experiments or re-analyse previous experiments. If the lamp of the Roche LightCycler 480 apparatus has been changed a new Color Compensation file is required. The Color Compensation kit can be re-used a limited number of times. Store the kit dark at -20⁰ C.

Applying Color Compensation

1. Select the filter combination you want to display and compensate by using the “Filter Comb” button.



2. Click the arrow-down button “Color Comp” and select “In Use” or “In Database” to find the Color Compensation file you have created and click “OK”.
3. The amplification curves are now changed using the compensated data. Note that the “Color Comp” button now is labeled “(On)”.

In case of insufficient Color Compensation leading to either overcompensation or remaining crosstalk, please refer refer to the LightCycler 480 Instrument Operator Manual.