INSTRUCTION MANUAL

Color Compensation CK-19 610-670 Kit Cat.# OL0010006 Not for diagnostic use.

MATERIALS PROVIDED

Materials Provided	Quantity	Color Code
Fluorescein (FL)	120 µl	Green
RED610	120 µl	Red
RED670	120 µl	Purple
BLANK	120 µl	Blue

STORAGE CONDITIONS

The kit is shipped on room temperature. This product should be stored at -20°C, protected from light, and has a shelf-life of at least 6 months when stored under these conditions.

ADDITIONAL MATERIAL REQUIRED

Microcentrifuge tubes (for reaction setup)
PCR plates, including seals
LightCycler® 480II (Roche)
Standard laboratory equipment (Centrifuge, pipettes, gloves, etc.)

WARNINGS & PRECAUTIONS

Before using this kit, carefully read the whole instruction manual. Do not use after expiry date is exceeded. Never heat kit components in order to thaw them. Treat and dispose of waste using proper precautions and in accordance with local, state, and federal regulations. Safety data sheets can be found on the manufacturer's homepage. Visit oncolab.at for further information.

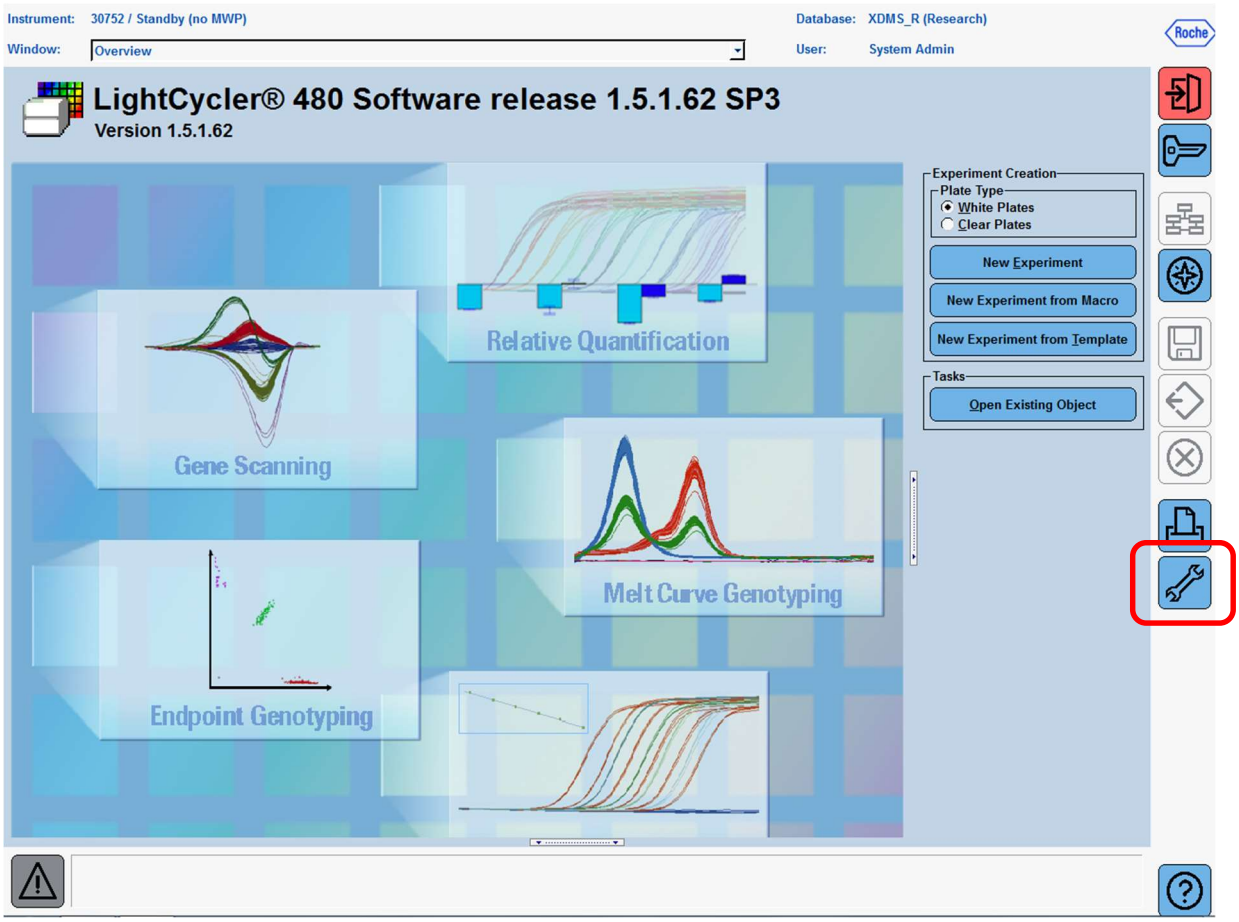
INTENDED USE

The Color Compensation CK-19 610-670 Kit is used to generate a Color Compensation (CC) File on the LightCycler® 480II instrument. This CC File is necessary to compensate for overlapping emission spectra generated in multiplex RT-qPCR experiments. To ensure correct signal detection in each channel, the CC File subtracts fluorescence signals from the inappropriate channel (crosstalk). This Kit is specifically designed to compensate the crosstalk between the emission channels 610 and 670.

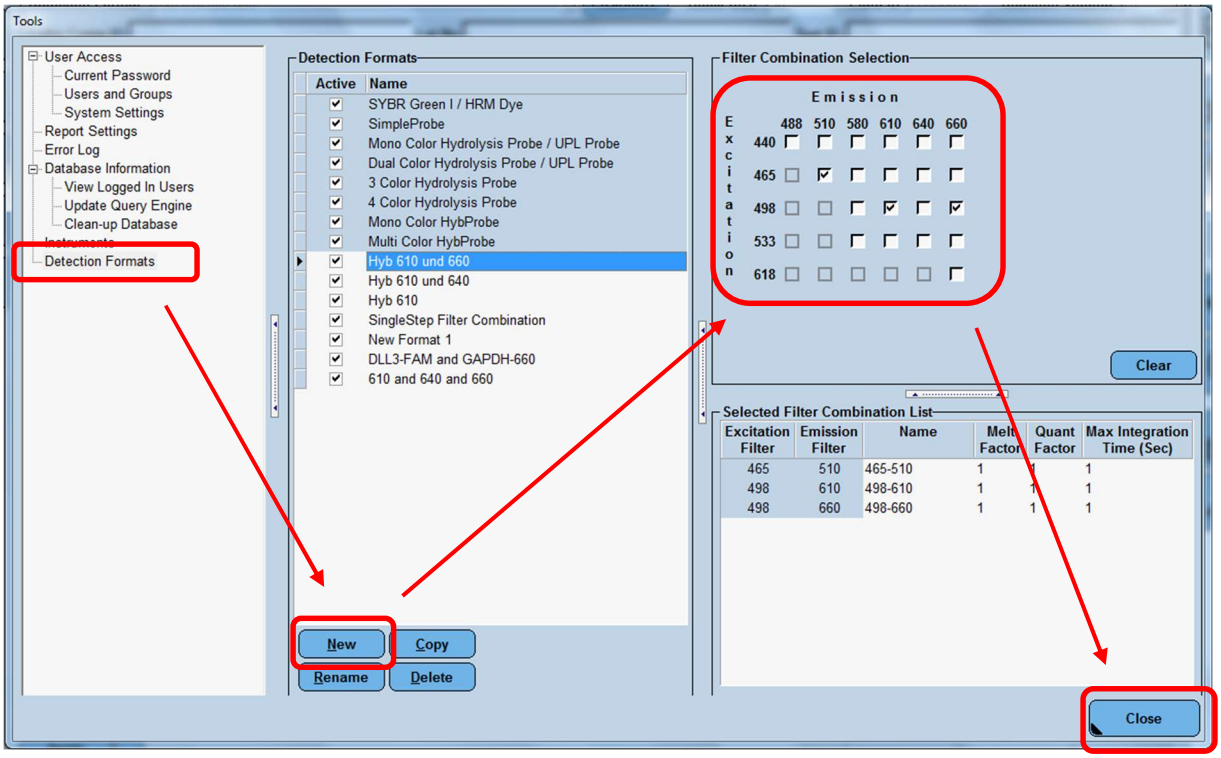
PROGRAM SET-UP

Before generating the CC file the correct program needs to be set up in the LightCycler® 480II software. To do so, follow the step-by-step description below.

1. Open the LightCycler® software and select the tools menu:

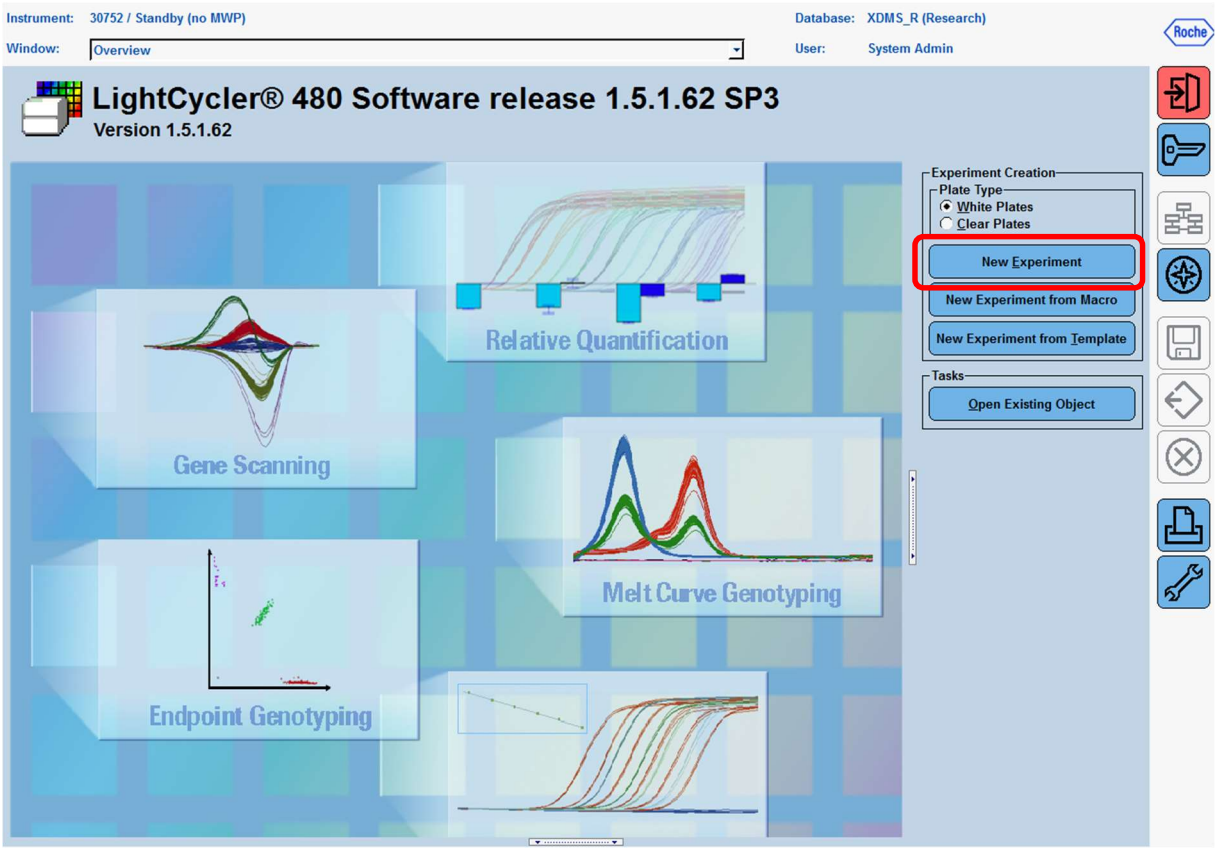


2. Select 'Detection Formats' in the menu tree on the left side. Add a detection format by clicking 'New' and set up the filter combinations as listed below. Name the newly created detection format and close the menu.

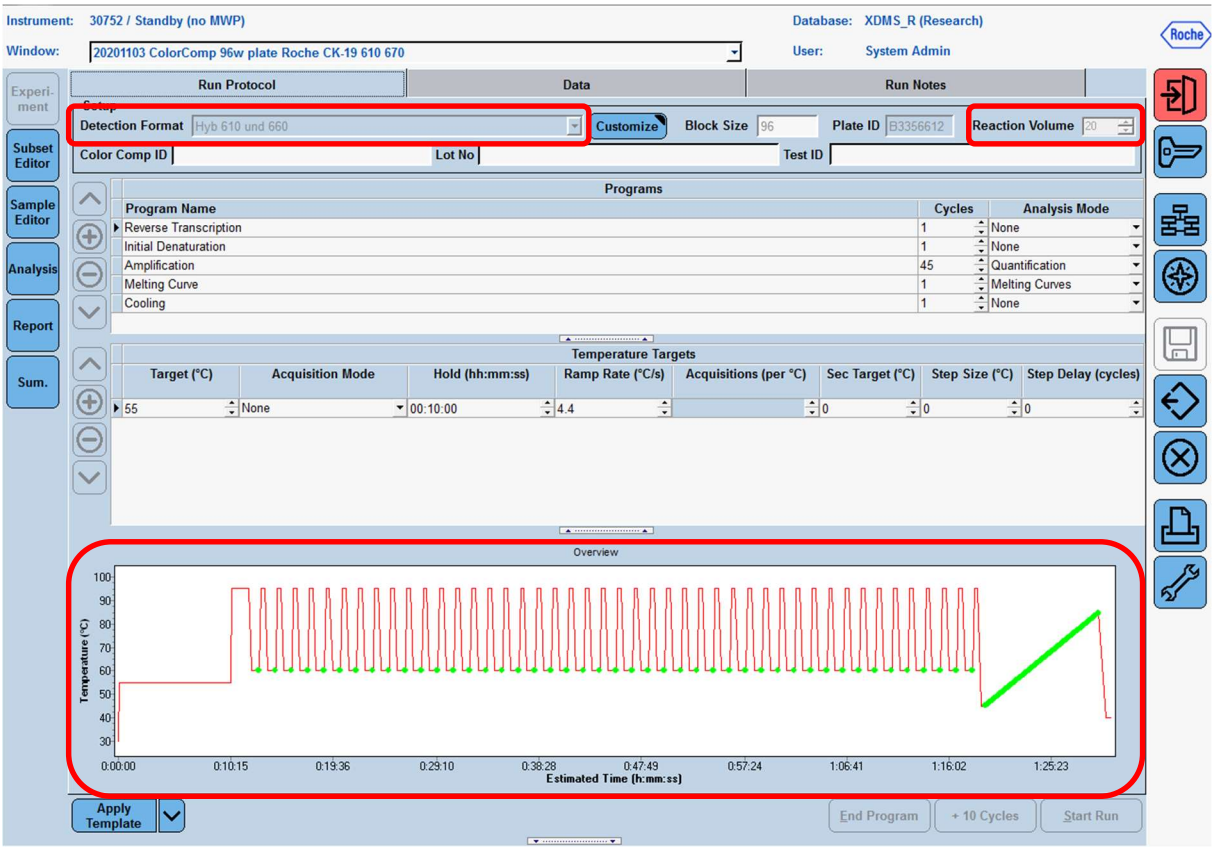


Filter Combination	Excitation Filter	Emission Filter
1	465 nm	510 nm
2	498 nm	610 nm
3	498 nm	660 nm

3. The new detection format can now be applied to the experiment set-up. To program the settings, select 'New experiment' in the software home screen.



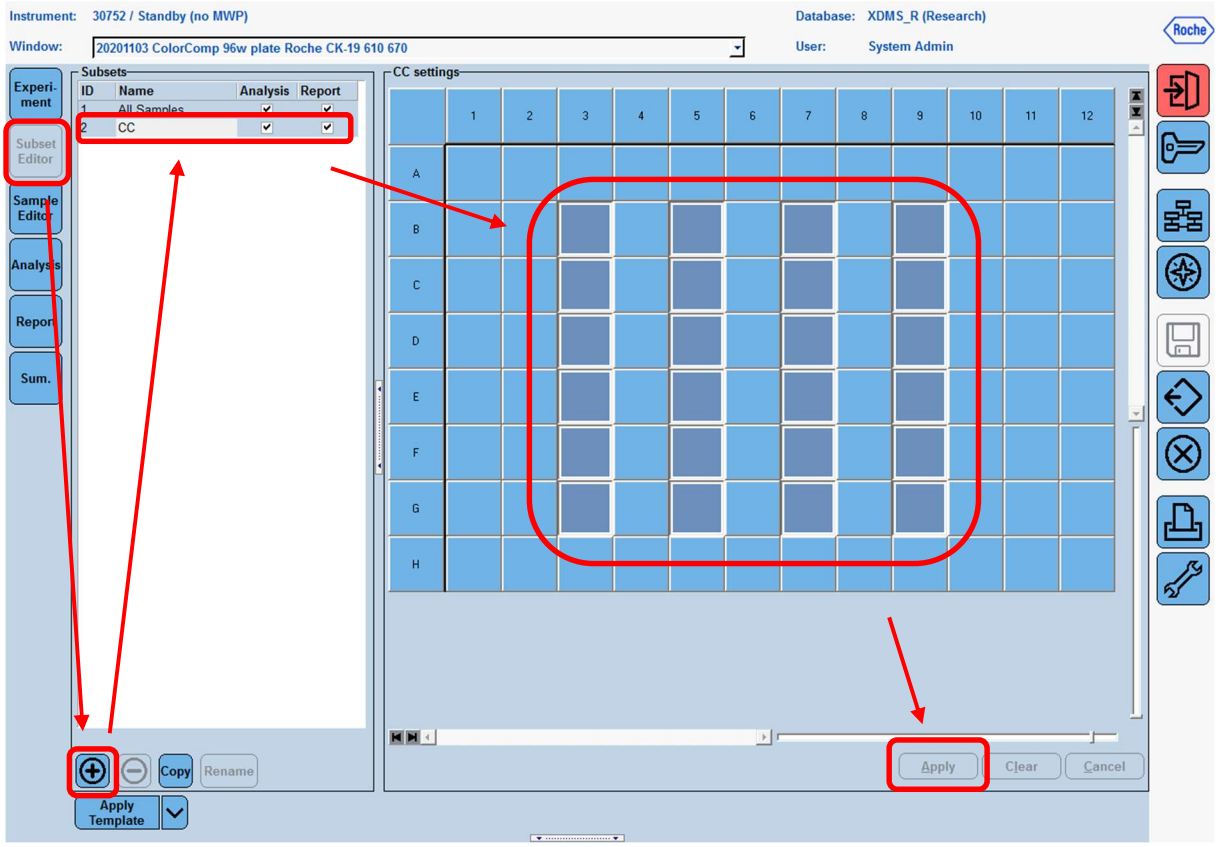
4. Select the previously created detection format and set the reaction volume to 20 µl. Choose the individual steps by adding different programs to the experiment set-up according to the list below. Check the overview to verify the correct set-up.



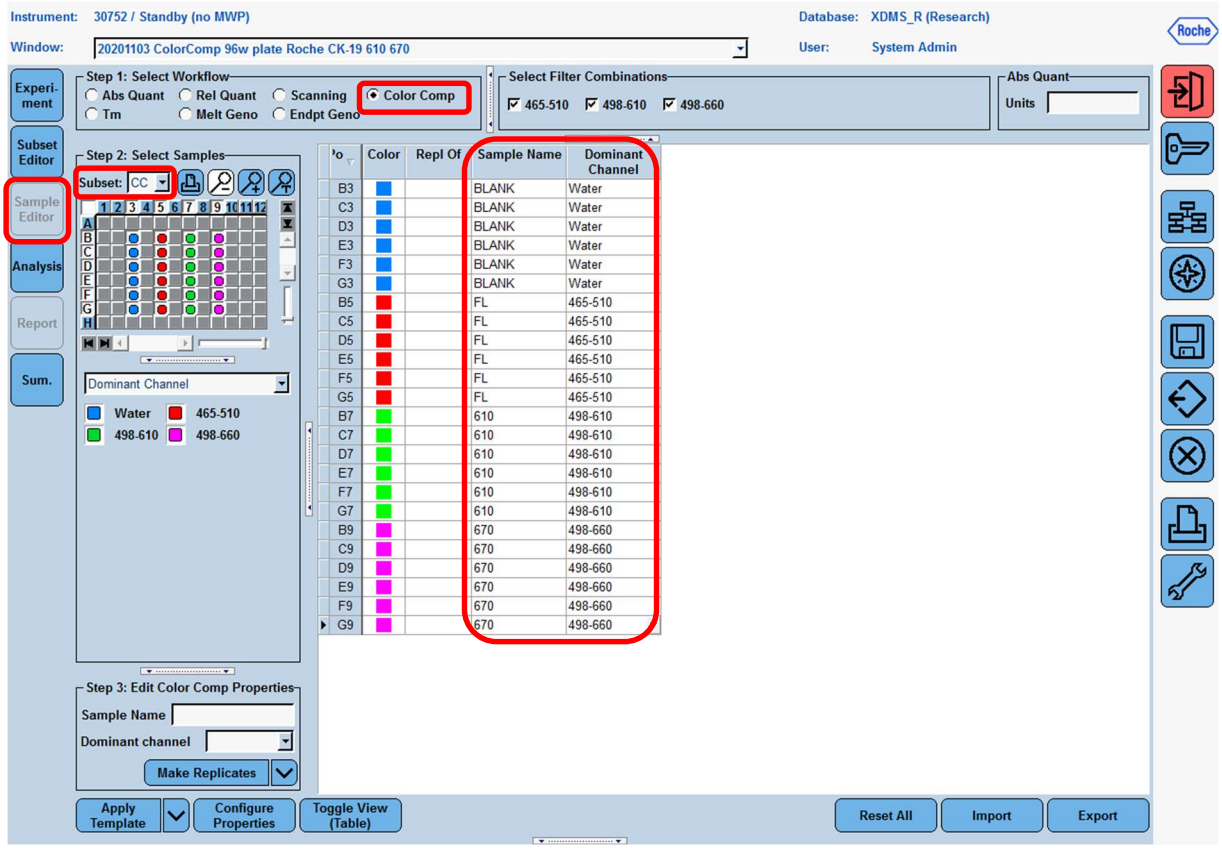
Program Name	Cycles	Analysis Mode
RT-step	1	None
Denaturation	1	None
Cycling	50	Quantification
Melting curve	1	Color Compensation
Cooling	1	None

Program Name	Target Temperature	Acquisition Mode	Hold [hh:mm:ss]	Ramp Rate [°C/s]	Acquisitions [s]
RT-step	55 °C	None	00:10:00	4.4	None
Denaturation	95 °C	None	00:01:00	4.4	None
Cycling	95 °C	None	00:00:10	4.4	None
	63 °C	Single	00:00:30	2.2	None
Melting curve	95 °C	None	00:00:05	4.4	None
	48 °C	None	00:00:10	2.2	None
	85 °C	Continuous	None	0.06	3
Cooling	40 °C	None	00:00:05	2.2	None

5. Next go to the 'Subset Editor' and create a new subset by clicking on the '+' icon in the lower left corner. Name the new subset and select the wells indicated below by holding the Strg-key and the left mouse button. Click apply.

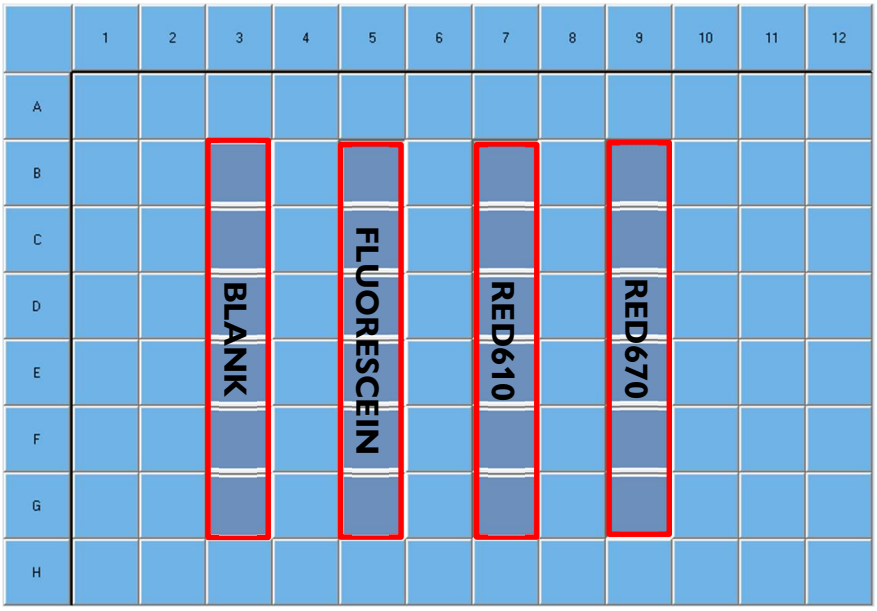


6. Next go to the 'Sample Editor'. At 'Step 1: Workflow' select 'Color Comp'. At 'step 2: Select Samples' select your previously created subset. Optionally, you can name your samples at 'Step 3'. Finally, select the correct 'Dominant Channel' for all your samples (select 'Water' for BLANK; '465-510' for Fluorescein; '498-610' for RED610 and '498-660' for RED670).



PREPARATION OF THE COLOR COMPENSATION PLATE

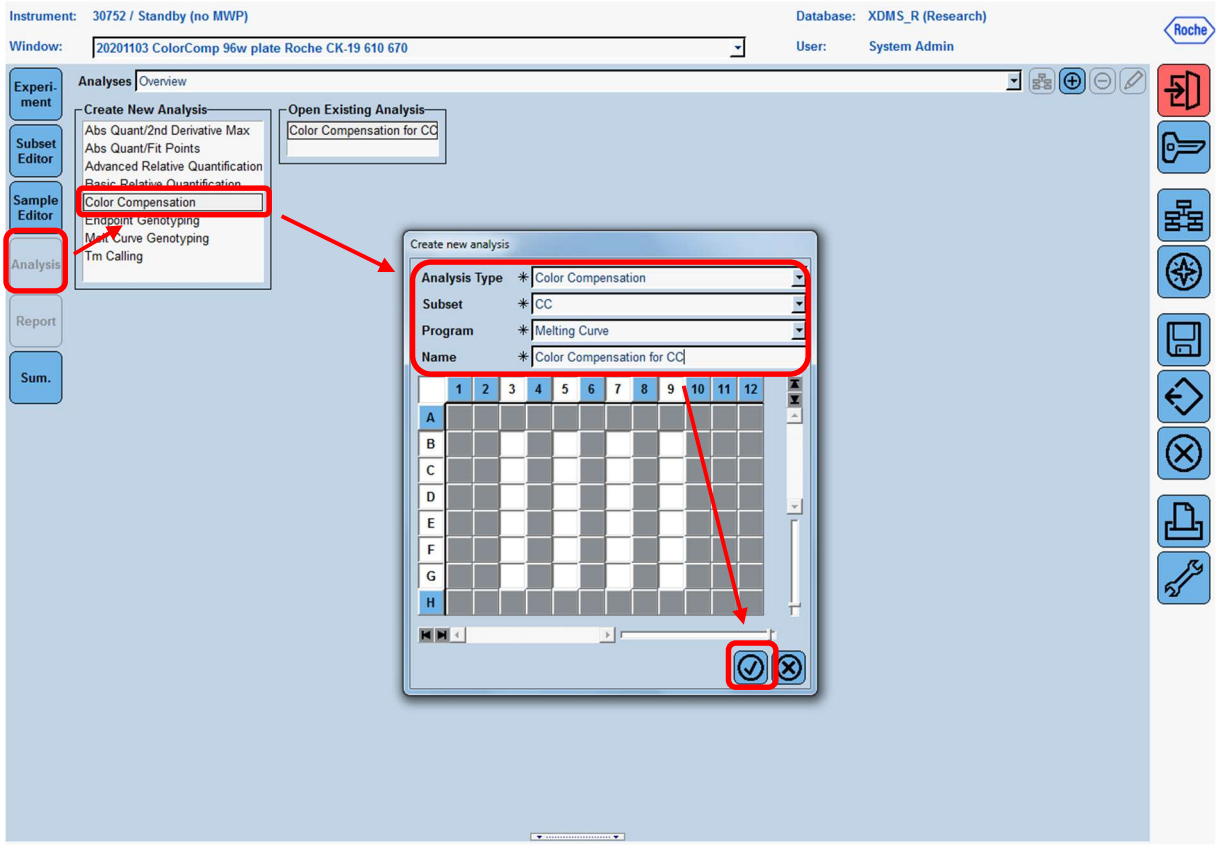
1. Thaw the tubes on room temperature and protected from light. **Never heat the tubes.**
2. Pipette 20 µl of the different dyes into the indicated wells of a PCR plate indicated below.



3. **Seal the plate with a foil.**

STARTING THE RUN AND CC FILE GENERATION

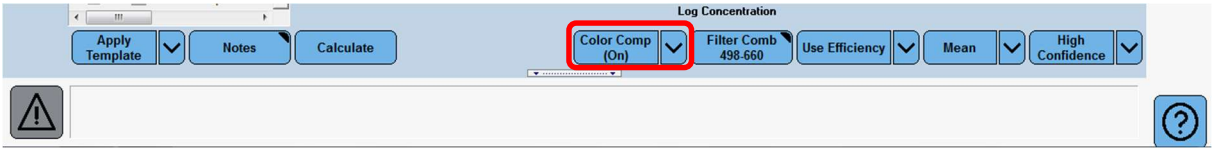
1. Put the plate into the LightCycler® 480II and press the start button in the 'Experiment' menu.
2. Once the run has finished go to the 'Analysis' menu. Under 'Create New Analysis' choose Color Compensation. In the newly opened window, select the correct subset, choose the 'Program' Melting Curve and confirm.



3. Press calculate and subsequently save the CC File in the 'CCC' Folder by clicking on 'Save CC Object'.

USING THE CC FILE

To apply the CC File to an experiment, go to the 'Analysis' menu, klick on the 'Color Comp' dropdown menu and select 'in Database'. There you can select your previously created CC File. After you confirmed your selection the 'Color Comp (Off)' button automatically switches to 'Color Comp (On)'.



DISPOSAL

Dispose of in accordance with local regulations. Dispose of waste in accordance with environmental legislation.

TROUBLESHOOTING

Problem	Possible Cause	Solution
CC File does not compensate adequately	Expiry date exceeded	• Only use Kits before they have expired
	Reduced reagent stability due to heating	• Never heat tubes to speed up the thawing process
	Wrong channel selected	• Re-check the manual for proper channel selection
	Wrong cycling protocol	• Re-check the manual for the correct cycling steps
	LightCycler® 480II instrument was changed	• A new CC File needs to be generated for each individual instrument
	Optical system was repaired	• A new CC File needs to be generated after the optical system of the instrument was repaired

DISCLAIMER

This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

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