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EliGene® 4-channel Color Compensation Kit

REF 90080-CC

Kit components:

1 × 50 µl FAM Sample
1 × 50 µl HEX Sample
1 × 50 µl Texas Red Sample
1 × 50 µl Cy5 Sample
1 × 50 µl Blank Sample

Storage and shelf life:

All components of the kit must be transported and stored at -20 °C. Kit and remaining MasterMixes must be stored at -20 °C in a dark.

Intended use

EliGene® 4-channel Color Compensation Kit is intended for color compensation of the fluorescence overlaps between excitation and emission channels for FAM, HEX, Texas Red and Cy5 on the LightCycler 480 II instrument. The color compensation file generated with the kit can be used in all EliGene® kits on a particular LightCycler 480 II instrument without restrictions. Color compensation file is unique for the particular LightCycler 480 II instrument; therefore, the color compensation must be performed on each instrument, it is not advised to transfer color compensation files among multiple instruments.

Principle of the method

In multiplex qPCR, the wavelengths of light emitted by the reporter dyes may overlap, which is manifested by the fluorescence signal presence in adjacent channels. Presence of non-specific fluorescent signal can result in the data misinterpretation and provision of the incorrect results. Color compensation is therefore required to specify the fluorescence spectrum of the particular fluorescent dye in its dominant channel and reduce the overlap to adjacent channels.

Equipment required

- Automatic pipettes 1-1000 µl and sterile tips with filter DNA-, RNA- free, DNase-, RNase- free (we recommended plastic with CE certificate for diagnostic purposes).
- Please, use white plates only intended for LightCycler® 480 II. The usage of natural plates can lead to decreased sensitivity of the kit. Do not reuse plates; the contamination of your laboratory could occur during the manipulation with plates. **The utilization of non-original plastic can lead to difficulties with the fluorescence readout and determination of the threshold. We cannot guarantee a correct interpretation of the results when non-original or disapproved plastics are used.**
- Sterile stand DNA-, RNA- free, DNase-, RNase- free.
- Equipment for qPCR – the kit is designed for qPCR instrument LightCycler® 480 (Roche)
- Lab safety gloves. Please work in appropriate biohazard boxes.

Reagent preparation

- To avoid contamination, keep all tubes closed and follow the instructions.

- All reagents must be completely thawed before the usage, briefly mixed on vortex, and shortly spun.

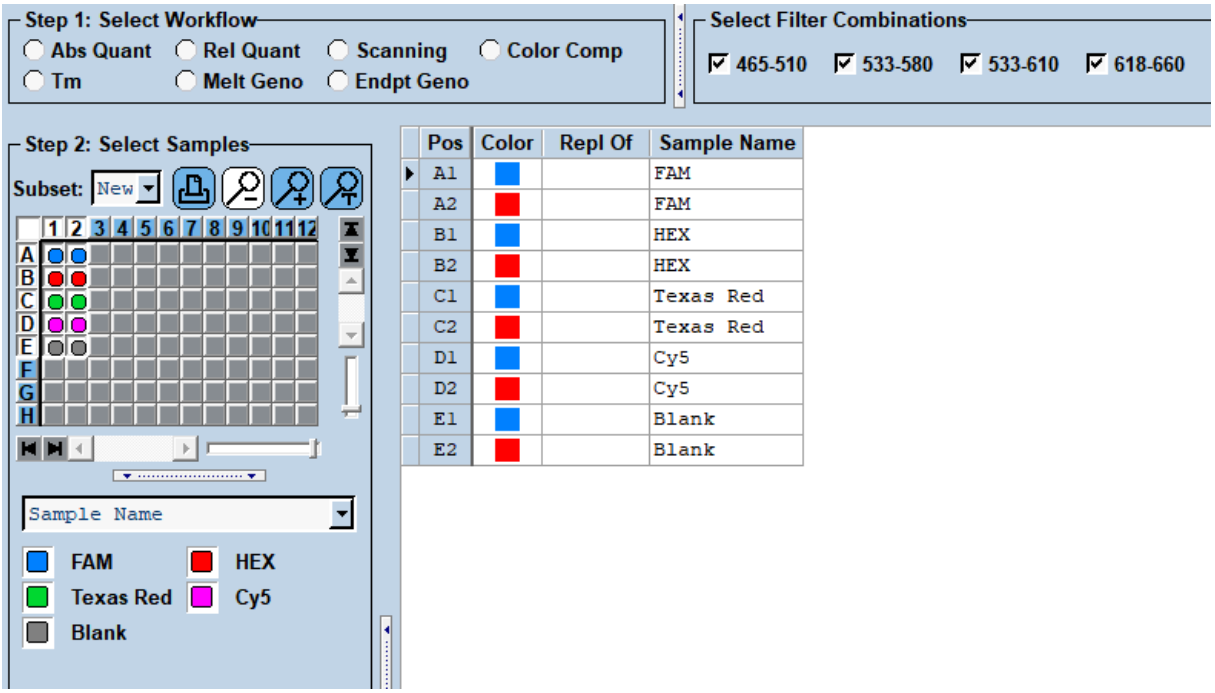
WARNING: The contamination in laboratory space is possible. Use a separate pipette for manipulation with mastermixes and samples!

Preparation of the qPCR plate

1. Each tube with the specific fluorescent dye sample (FAM, HEX, Texas Red, Cy5 and Blank) must be completely thawed at the room temperature and spun briefly in a centrifuge. Do not freeze samples again.

2. Add 20 µl of each sample and Blank on the plate in duplicate. Be careful when pipetting the sample to avoid cross-contamination of the samples.

Example of the plate layout with samples in duplicates



Insert the plate into the LightCycler 480 II instrument and continue with LightCycler 480 II instrument setup.

LightCycler 480 II Setup

Creation of the detection profile:

Open "Toolbox" in the "Main menu" (icon with a wrench), select "Detection formats". Select "New" detection format and assign it a name according to your choice. In the excitation and emission spectra matrix on the top right corner, click on boxes with the following combinations:

Excitation Filter	Emission Filter	Name	Melt Factor	Quant Factor	Max Integration Time
465	510	FAM	1	10	2
533	580	HEX	1	10	2
533	610	Texas Red	1	10	2
618	660	Cy5	1	10	2

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LightCycler 480 II instrument setup

Select “New Experiment” from the “Overview” window.

In option Detection format, choose the detection profile you have created

Set up the following temperature profile:

Step 1 - Analysis mode "None", 1 Cycle

95°C	2 min	Ramp rate (4.4°C/s)	Acquisition mode “None”

Step 2 - Analysis mode "Quantification", 45 Cycles

95°C	5 s	Ramp rate (4.4°C/s)	Acquisition mode “None”
55°C	15 s	Ramp rate (2.2°C/s)	Acquisition mode “Single”
67°C	15 s	Ramp rate (4.4°C/s)	Acquisition mode “None”

Step 3 - Analysis mode "Color Compensation", 1 Cycle

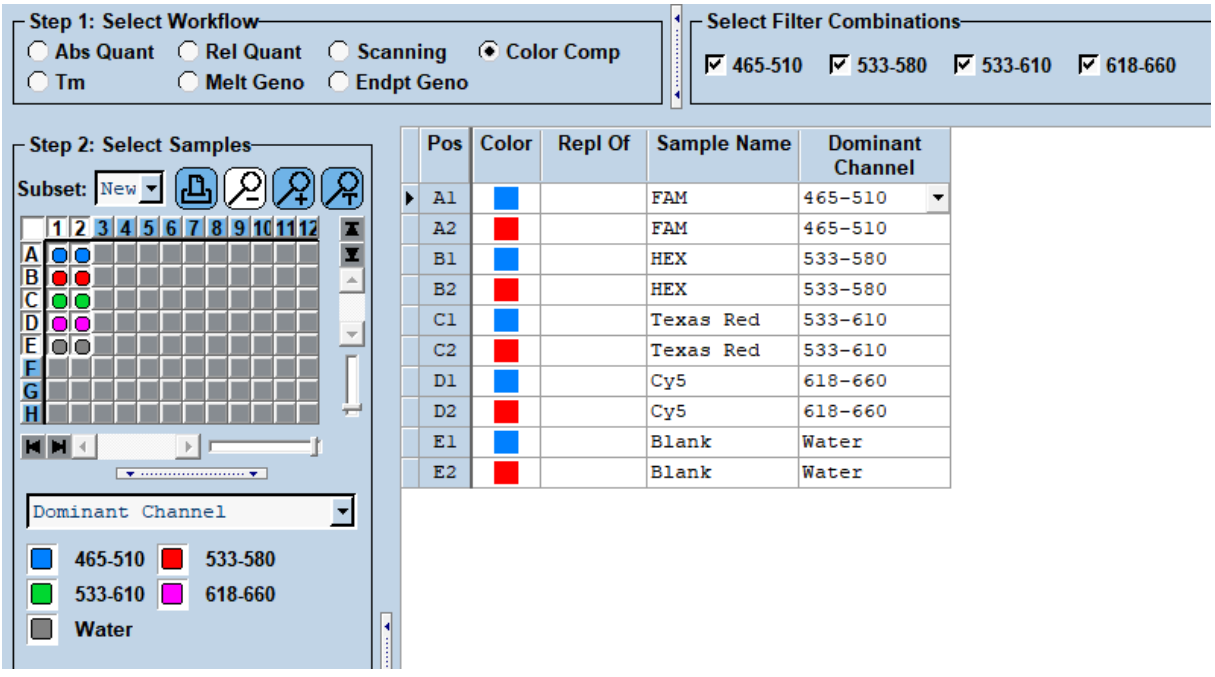
95°C	1 s	Ramp rate (4.4°C/s)	Acquisition mode “None”
50°C	30s	Ramp rate (2.2°C/s)	Acquisition mode “None”
95°C	-	5 Acquisitions per °C	Acquisition mode “Continuous”
40°C	30s	Ramp rate (2.2°C/s)	Acquisition mode “None”

In the “Subset Editor” tab, create a subset comprising wells with samples.

In the “Sample Editor” tab, in the “Step 1: Select Workflow” select “Color Comp” option.

Assign the excitation and emission filters to the well with relevant dye according to the table above.

Example of the plate with the filled parameters



Pos	Color	Repl Of	Sample Name	Dominant Channel
A1			FAM	465-510
A2			FAM	465-510
B1			HEX	533-580
B2			HEX	533-580
C1			Texas Red	533-610
C2			Texas Red	533-610
D1			Cy5	618-660
D2			Cy5	618-660
E1			Blank	Water
E2			Blank	Water

Return to the “Experiment” tab, press “Start Run” and save the experiment.

Creation of the Color Compensation File

After the experiment ends, click “Analysis” tab and select “Color Compensation” in the “Create New Analysis” window.
Select “Subset” that include well with the samples for the Color Compensation and “Program” that contains Color Compensation data (Step 3 in the Temperature profile).
Click the button “Calculate”. The Color Compensation File is created.
Click “Save CC Object” and save the file to the CCC folder.

Application of the Color Compensation File

To apply the Color Compensation File, click “Color Comp” button in the “Analysis” tab in particular run. Select “In Database” and select the saved Color Compensation File from the table to apply it for fluorescence correction in the selected channel. Please note, that Color Compensation File must be selected for each subsequent analysis. The option “In Use” can be used in this case.

Warnings and precautions specific to components of the kit

The tubes containing samples are disposable and therefore must be used only once.

The mixes carry the following safety warnings (P):

P280 Wear protective gloves/protective clothing/eye protection/face protection.
P281 Use personal protective equipment as required.

Manufacturer

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