Instructions for Use

BAG RT-PCR Universal Color Compensation Kit

RUO

EN

REF 728259 RT CC Universal LightCycler®

For use on the Roche Light Cycler®480 System II

Contents

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1. Application

The 5-color compensation set is used to create an application-specific color compensation object (or file) on the Light Cycler®480 system II. The 5-Color Compensation Set is to be used in combination with BAG Diagnostics real-time PCR kits: FastQ®, ERY Q® and HISTO TYPE Rainbow. The kits require a color compensation run once a year or after repair, maintenance in combination with the calibration of the optical parts of the LC®480 system II. Once the application-specific color compensation object has been performed and the data file created, it is used to analyze all the data generated with the mentioned real-time PCR kits.

For research use only

2. Product description and principle

The Color Compensation kit simultaneously detects five different colors on the LC®480 System II. Due to the overlap of the emission spectra of organic dyes, crosstalk emission between detector channels can occur. This phenomenon is described as the overspill of one dye into the next detector channel which may result in the misinterpretation of the data. To correct for cross-talk emission between detector channels, color compensation can be applied when analyzing the data.

The dye calibrators used in the color compensation set are identical to the dyes used in the PCR kits: FastQ®, ERY Q® and HISTO TYPE Rainbow. During a color compensation run, the LC®480 instrument measures the fluorescence of each dye calibrator in all the channels and generates an instrument-specific color compensation file or object. When analyzing the PCR kits: FastQ®, ERY Q® and HISTO TYPE Rainbow, the software uses this color compensation file/object data to reassign the fluorescence in each detector channel to the appropriate dye. As a result, only one dye signal is detected in each channel.

3. Kit contents for color compensation

Components	Description	Storage conditions
ATTO 425 calibrator	1 **orange cap** tube 40 µl	≤ -20°C
FAM calibrator	1 **green cap** tube 40 µl	
ORANGE 560 calibrator	1 **blue cap** tube 40 µl	
RED 610 calibrator	1 **red cap** tube 40 µl	
QUASAR 670 calibrator	1 **purple cap** tube 40 µl	
DNA-amplification-control (DAC)	1 **black cap** tube 80 µl	
Plex Mix	1 clear cap tube 230 µl	

4. Additionally required reagents and devices

- Variable pipettes (0,5 – 1000 µl) and pipette tips
- Application spatula for PCR foil
- Molecular grade DNase free water
- Centrifuge (e.g. PlateFuge – MicroCentrifuge Benchmark Scientific)
- PCR Plate (e.g. Roche Multiwell Plate 96, white, Product No. 04729692001)
- PCR Foil (e.g. Optically clear adhesive film, Product No. 4ti-0560)

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5. Storage and stability

The kits are shipped on blue ice. Upon receipt store all reagents in temperature monitored devices at ≤ -20 °C. The manufacturer date is indicated on the label of each reagent. The freeze-thaw cycle testing for the Plex Mix has shown that up to 6 cycles have no detrimental effects on the performance of the kit.

6. Test procedure

6.1 Safety conditions and special remarks

Molecular genetic techniques are particularly sensitive and should be performed by well trained personnel experienced in molecular genetic techniques.

Special safety conditions must be observed to avoid contamination and thus false reactions:

- Wear gloves during work (powder-free, if possible).
- Use new tips with each pipetting step (with integrated filter).
- Use separate working areas for pre-amplification (DNA isolation and PCR set up) and post-amplification (detection). Preferably, use two separate rooms.
- Use devices and other materials only at the respective places and do not exchange them.

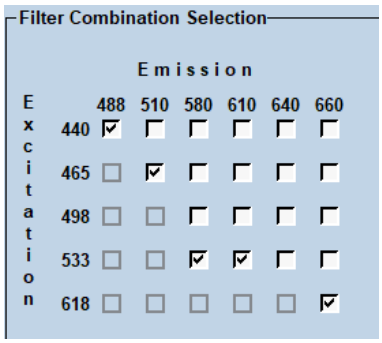
6.2 Before starting color compensation

- A CC object can only be applied to experiments that were run on the same Light Cycler® 480 Instrument it was created on.
- Instead of running a separate color compensation experiment, you can also run the color compensation reactions in parallel to your experimental samples. In this case, apply the appropriate experimental PCR protocol, but always add a temperature gradient or melting curves program.
- For further information, refer to the LC®480 Instruments Operator's Manual, Software version 1.5, section Advanced Software Functionalities, Color Compensation Analysis.

6.3 Set up a color compensation experiment

A new detection Format must be set when using the color compensation for the first time. If the format has already been generated, continue to step 1.1.

- Go to tools -> 
- Select "Detection Formats and click on "New"
- Name your detection formats (e.g. BAG CC universal)
- Set filter combination as follows:



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- Change the names in the filter combination list as follows:
  -> The Melt/Quant Factors and the Max. Integration Time should be set as default.

Selected Filter Combination List

Excitation Filter	Emission Filter	Name	Melt Factor	Quant Factor	Max Integration Time (Sec)
465	510	FAM	1	1	1
533	580	Orange 560	1	1	1
533	610	Red 610	1	1	1
440	488	Atto 425	1	1	1
618	660	Quasar 670	1	1	1

Close the “Detection Formats”.

6.4 Set up a color compensation run protocol.

- Go to “Overview” window -> 
- Click on “ New Experiment”
- In “Experiment”/ ”Setup” select your CC experiment in “detection format” (e.g. BAG CC universal)
- **Please note**: Do not assign a ”Test ID’’ or ”CC ID’’
- Click “Customize” and make sure all five filter combinations are active (440-488; 465-510; 533-610; 533-580; 618-660) and the “Integration Time Mode” is set to “Dynamic”

- Set the reaction volume to 10 µl
- Set the PCR program as follows:

Program Name	Cycles	Analysis Mode	Target (°C)	Acquis. Mode	Hold (hh:mm:ss)	Ramp Rate (°C/s)	Acquis. (per °C)
Initial activation	1	None	96	None	00:02:00	2,5	-
Amplification	13	None	98	None	00:00:05	2,5	-
			68	None	00:00:25	2,2	-
Amplification	37	Quantification	98	None	00:00:05	2,5	-
			68	Single	00:00:25	2,2	-
Color Compensation	1	Color Compensation	50	None	00:00:01	2,2	-
			75	Continuous	-	0,03	5
Cooling	1	None	37	None	00:00:30	2,2	-

6.5 Set up the “Subset Editor”

- Click on “Subset Editor” on the left side 

- Create a new “ID” in “Subsets” with  and rename it to e.g. BAG CC universal
- Choose positions for each calibration mix in “BAG CC universal settings” as follows (The designation will be in the next step)

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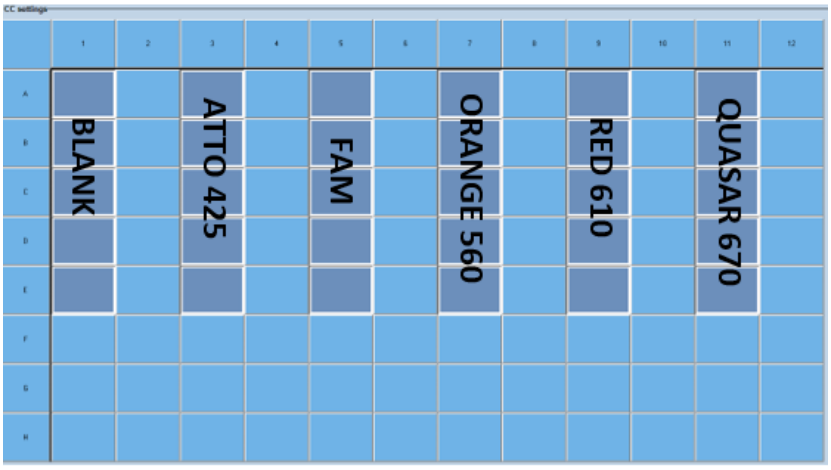
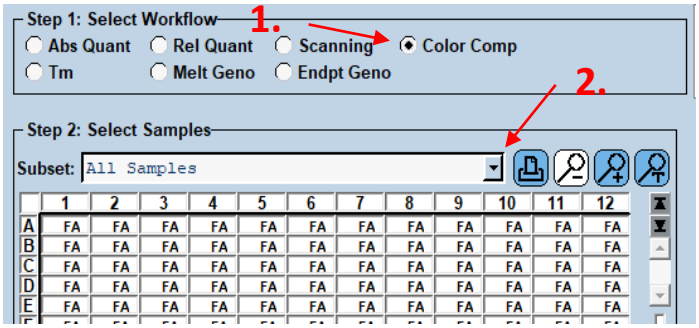


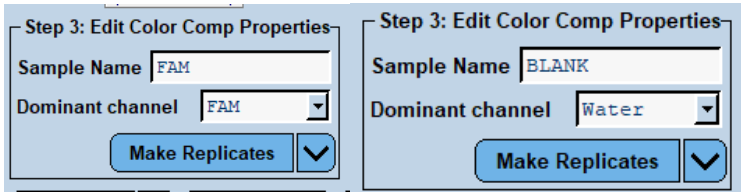
- Click on “Apply” and go to “Sample Editor”

6.6 Set up the “Sample Editor”

- In “Step 1: Select Workflow” select “Color Comp”
- In “Step 2: Select Samples” choose in “Subset” the name BAG CC universal



- In “Step 3: Edit Color Comp Properties” set Sample Name (BLANK, ATTO 425 FAM, ORANGE 560, RED 610 and QUASAR 670) and select Dominant channel for each Blank / Dye and press “Enter”. For BLANK the dominant channel is water.



- Make sure all five filter combinations in “Select Filter Combinations” are active (440-488; 465-510; 533-610; 533-580; 618-660)

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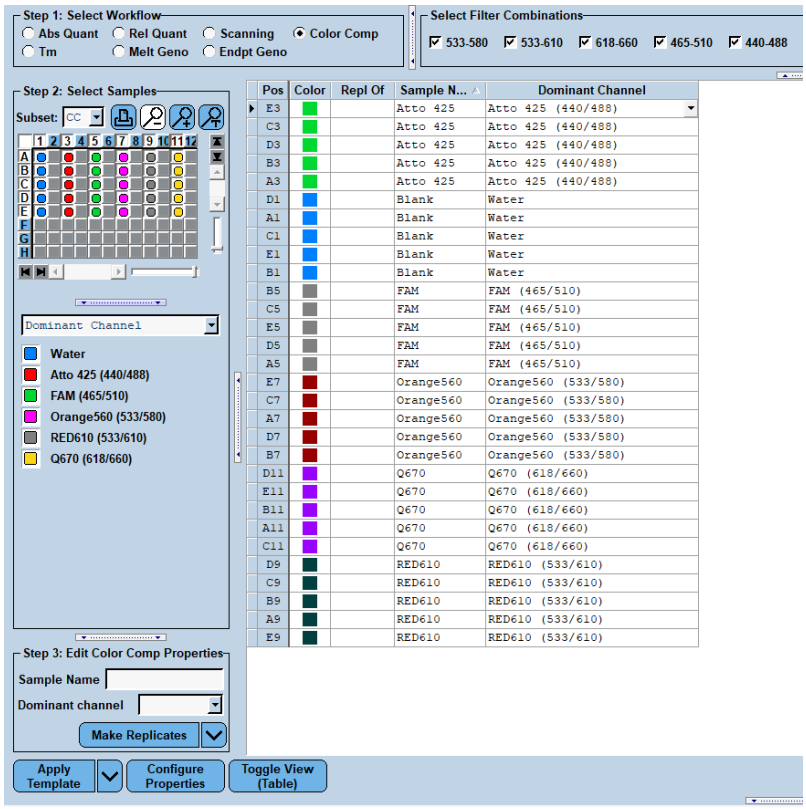

6.7 Prepare the reaction mix

Prepare reaction mixes: one for blank and one for each calibrator dye. (see table below).

	Blank (µl)	Atto (µl)	FAM (µl)	Orange (µl)	Red (µl)	Quasar (µL)
Calibrator mix (e.g. CAL FAM)	0	12	12	12	12	12
Plex Mix	12	12	12	12	12	12
DAC	0	6	6	6	6	6
Molecular grade water	48	30	30	30	30	30
Total reaction volume	60	60	60	60	60	60
Stripe	1	3	5	7	9	11

Pipette 10 µl of each mix into each well as shown in figure chapter 1.2.

- After preparing and sealing the 96-well plate spin down the plate and set into the LC 480.
  Please note: The sloping corner must be at the bottom right
- Choose ”Experiment’’ and click on ”Save’’  to select a folder for the run
- Click “Start Run” at the bottom right

7 Data analysis

- After run is completed go to “Analysis”
  Select “Color Compensation” from “Analysis”

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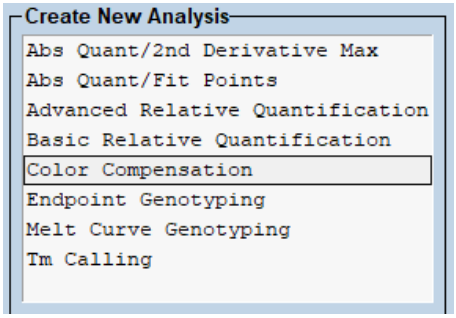
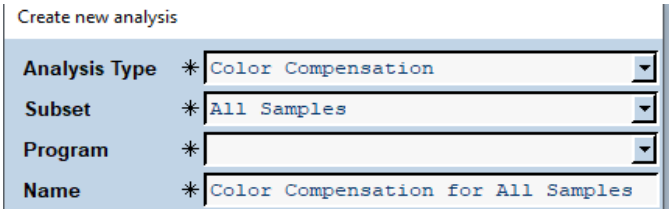
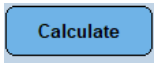


- Open ”Subset’’ and choose the correct run (e.g. CC-FastQ)

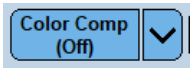


- Click 
- Then click on ”Calculate” 

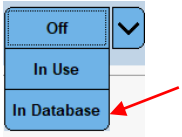
- Click “Save CC Object”
- Choose a folder and name the CC object e.g. “BAG CC universal -Date”
- Click  to save the CC object.

The stored color compensation object should be used for the analysis of runs performed on the following product line: FastQ®, ERY Q® and HISTO TYPE Rainbow kits. If available please use pre-typed samples to ensure the CC object is valid.

- After performing the FastQ® kit go to “Analysis” and click on ”Color Comp (Off)’’



- Click “In Database’’ to choose the correct color comp object (e.g. BAG CC universal Date)



- Choose the “Filter Comb” (1.) and click on “Calculate” (2.) for each to get Cp results.

- Save your experimental data.

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8 Explanation of symbols used on the labels

	Storage temperature / Lower limit of temperature
	Use by
	Consult instructions for use
	Manufacturing Date
	Manufacturer
eIFU | VXX/XXXX	Electronic Instructions for use
RUO	For research use only
LOT	Batch code
CAL | A425	Atto 425 calibrator
CAL | FAM	FAM calibrator
CAL | O560	Orange 560 calibrator
CAL | R610	Red 610 calibrator
CAL | Q670	Quasar 670 calibrator
DAC	DNA-amplification-control
Plex Mix	PCR mastermix
REF	Catalogue number

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