Instruction for Use

Color Compensation Kit for HISTO TYPE B*27 Q

Electronic instructions for use see www.bag-healthcare.com

RUO

REF XXXXXX CC-HT-B*27 Q

For use on the Roche Light Cycler®480 System II

Contents

Intended use

The 3-Color Compensation Set is used to create an application-specific color compensation object (or file) on the Light Cycler®480 system II. The 3-Color Compensation Set is to be used in combination with the HISTO TYPE B*27 Q real-time PCR diagnostic kit (Ref 728200). The HISTO TYPE B*27 Q kit requires a color compensation run once a year after 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 HISTO TYPE B*27 Q real-time PCR diagnostic test.

Product description and principle

The HISTO TYPE B*27 Q real-time PCR diagnostic kit (Ref 728200) simultaneously detects three 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 HISTO TYPE B*27 Q diagnostic kit. 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 HISTO TYPE B*27 Q experimental data, 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.

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Kit contents for color compensation

Components	Description	Storage conditions
FAM calibrator	1 **green cap** tube 30 µl	≤ -20°C
Yakima Yellow calibrator	1 **yellow cap** tube 30 µl	
Texas Red calibrator	1 **red cap** tube 30 µl	
DNA-amplification-control (DAC)	1 **blue cap** tube 30 µl	
Q Mastermix	1 **violet cap** tube 230 µl	
IFU		

Storage and stability

The kits are shipped at 2...8°C. Upon receipt store all reagents in temperature monitored devices at ≤ -20 °C. The expiry date is indicated on the label of each reagent. The expiry date indicated on the outer label refers to the reagent with the shortest stability contained in the kit. The freeze-thaw cycle testing has shown that up to 15 cycles has no detrimental effects on the quality of the kit.

Test procedure

- **Safety conditions and special remarks**

Molecular genetic techniques are particularly sensitive and should be performed by well trained personnel experienced in molecular genetic techniques. The results of these tests must not be used as sole basis for clinical decisions.

Special safety conditions must be observed in order 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.

- **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.

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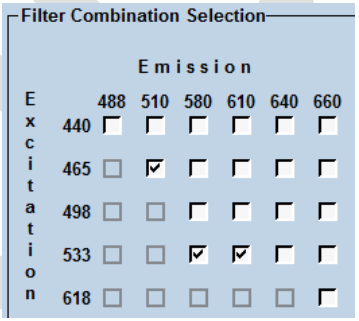
- ♦ For further information, refer to the LC®480 Instruments Operator's Manual, Software version 1.5, section Advanced Software Functionalities, Color Compensation Analysis.

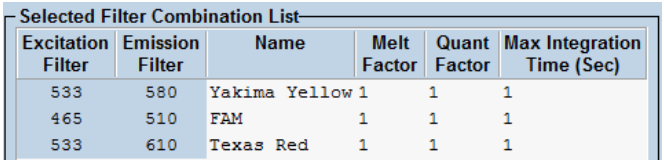
1. Set up a color compensation experiment

A new detection Format has to 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. CC-HT-B*27Q)
- Set filter combination as follows:



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



- Close the “Detection Formats”.

1.1 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. CC-HT-B*27Q)
- Click “Customize” and make sure all three filter combinations are active (465-510; 533-580; 533-610) 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	00:10:00	4,4	-
Amplification	40	Quantification	96	None	00:00:20	4,4	-
			64	Single	00:00:40	2,2	-
Color Compensation	1	Color Compensation	50	None	00:00:01	2,2	-
			75	Continuous	-	0,04	5
Cooling	1	None	37	None	00:00:30	2,2	-

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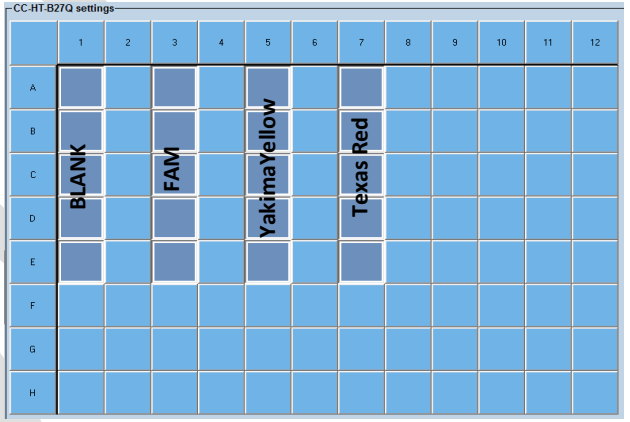
1.2 Set up the “Subset Editor”

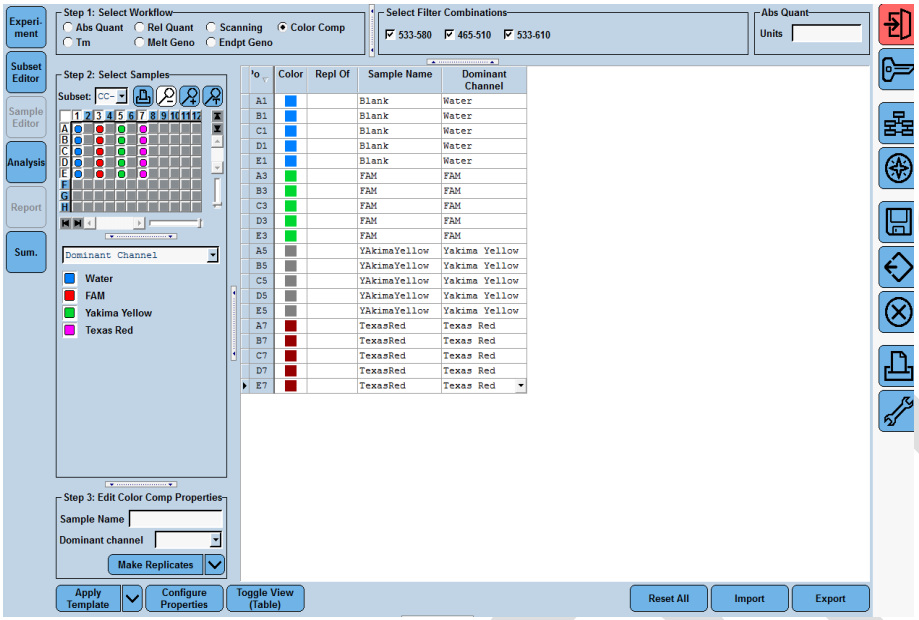
- Click on “ Subset Editor”
- Create a new “ID” in “Subsets” with  and rename it to CC-HT-B*27Q
- Choose positions for each calibration mix in “CC-HT-B*27Q settings” as follows



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

1.3 Set up the “Sample Editor”

- In “Step1: Select Workflow” select “Color Comp”
- In “Step2: Select Samples” select in “Subset” the name CC-HT-B*27Q
- Make sure all three filter combinations in “Select Filter Combinations” are active (465-510; 533-580; 533-610)
- Set the sample names and dominant channels for each position and calibration mix as follows. For blank the dominant channel is water.



1.4 Prepare the reaction mix

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

	Blank (µl)	FAM (µl)	YY (µl)	Texas Red (µL)
Calibrator mix	0	10	10	10
Q Mastermix	10	10	10	10
DAC	0	5	5	5
Water	40	25	25	25
Total reaction volume	50	50	50	50

- Pipette 10 µl of each reaction mix as shown in figure chapter 1.2.

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- After preparing and sealing the 96-well plate spin down the plate and set into the LC 480.
- Click “Start Run” in “Experiment”

2. Data analysis

- After run is completed go to “Analysis”
- Select “Color Compensation” from “Analyses”
- Click “Save CC Object”
- Choose a folder and name the CC object “CC-HT-B*27Q”
- Click  to save the CC object.

The stored color compensation object should be used for the analysis of runs performed on the following product: HISTO TYPE B*27 Q kit (Ref 728200). If available please use pre-typed samples to ensure the CC object is valid.

- After performing the HISTO TYPE B*27 Q kit go to “Analysis” and choose the color comp object (CC-HT-B*27Q) from “In Database”.
- Click “Calculate” for each “Filter Comb” to get Cp results.
- Save your experimental data.

EXPLANATION OF SYMBOLS USED ON THE LABELS

	Sufficient for n tests
	Storage temperature / Lower limit of temperature
	Use by
	Consult instructions for use
	Manufacturer
IFU	Instructions for use
RUO	For research use only
LOT	Batch code
CAL | FAM	FAM calibrator
CAL | Yakima Yellow	VIC calibrator
CAL | Texas Red	Texas Red calibrator
DAC	DNA-amplification-control
Q Mastermix	PCR mastermix
REF	Catalogue number

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Technical assistance

http://service.bag-healthcare.com or phone +49 (0)6404-925-125

Instructions for use in other languages see http://www.bag-healthcare.com