Instructions for Use

Color Compensation Kit for ViroQ Kits

RUO

EN

REF 728258 ViroQ CC LightCycler®

For use on the Roche LightCycler® 480 System

Contents

Version: V6/2020 Issue: 11/2020

BAG Diagnostics GmbH
Amtsgerichtsstr. 1-5
35423 Lich/Germany
Tel.: +49 (0) 6404 / 925-100
Fax: +49 (0) 6404 / 925-460
www.bag-diagnostics.com
info@bag-diagnostics.com

Ordering:
Tel.: +49 (0) 6404 / 925-450
Fax: +49 (0) 6404 / 925-460
order@bag-diagnostics.com

Customer Service:
Tel.: +49 (0) 6404 / 925-125
Fax: +49 (0) 6404 / 925-421
service@bag-diagnostics.com

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 I + II and Cobas z 480. The Color Compensation Set is to be used in combination with the ViroQ real-time PCR diagnostic kits (REF 728250 / 728251 / 728261 / 728262 / 728263 / 728264 / 728267 / 728268 / 728269 / 728270). The ViroQ kits require a color compensation run once a year or after the calibration of the optical parts of the LC® 480 system. 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 ViroQ real-time PCR kits.

For research use only – data not suitable for use in diagnostic purposes.

2. Product description and principle

The Color Compensation kit simultaneously detects five different colors on the LC® 480 System. 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 ViroQ kits. 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 ViroQ 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.

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	
QUASAR 705 calibrator	1 **yellow cap** tube 40 µl	
DNA-amplification-control (DAC)	1 **black cap** tube 140 µl	
ViroQ ENZYME	1 pouch	
ViroQ SOLV	1 uncolored cap tube 400 µl	

Please note: For Cobas z 480 replace Atto 425 with Quasar 705!

4. Additionally required reagents and devices

- Variable pipettes (0,5 – 1000 µl) and pipette tips
- Application spatula for PCR foil
- Molecular grade DNase or Nuclease 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 without cooling. Upon receipt store all reagents in temperature monitored devices at ≤ -20 °C. The expiry date or production date is indicated on the label of each reagent. The reagents ViroQ Enzyme and ViroQ Solvent can be stored at room temperature until expiry date, as long as the enzyme lyophilisate is not solved with the reconstitution buffer. After solving it can be used upon 12 months. Repeated thawing and freezing of reagents (more than twice) should be avoided, as this might affect the performance of the assay. In terms of intermittent use the reagents should be aliquoted.

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® 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. CC for ViroQ)
- Set filter combination as follows:

BAG Diagnostics GmbH
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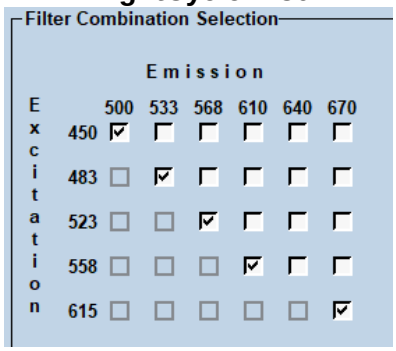
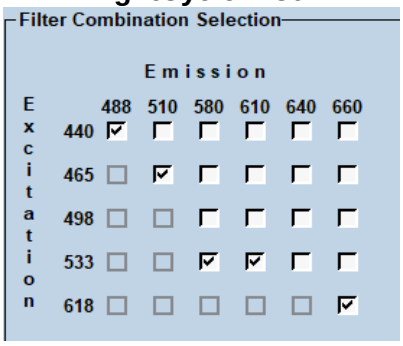
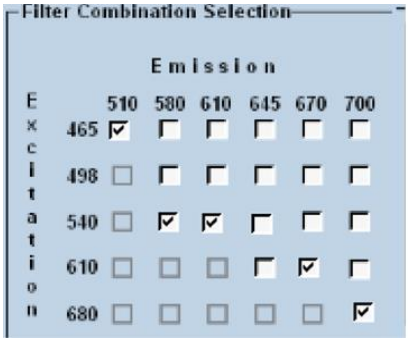
LightCycler 480 I



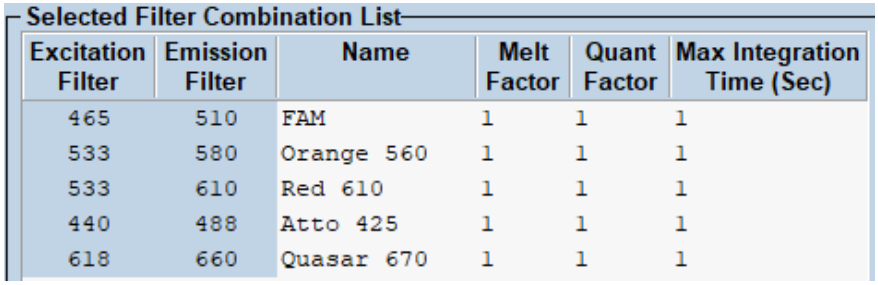
LightCycler 480 II



Cobas z 480



- Change the names in the filter combination list as follows (e.g. **LightCycler480 II**):
  → 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

➔ For LightCycler I and Cobas z 480 as follows:

LightCycler 480 I			Cobas z 480		
Excitation Filter	**Emission Filter**	**Name**	**Excitation Filter**	**Emission Filter**	**Name**
450	500	Atto 425	465	510	FAM
483	533	FAM	540	580	Orange 560
523	568	Orange 560	540	610	Red 610
558	610	Red 610	610	670	Quasar 670
615	670	Quasar 670	680	700	Quasar 705

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. CC for ViroQ)
- **Please note**: Do not assign a ”Test ID’’ or ”CC ID’’

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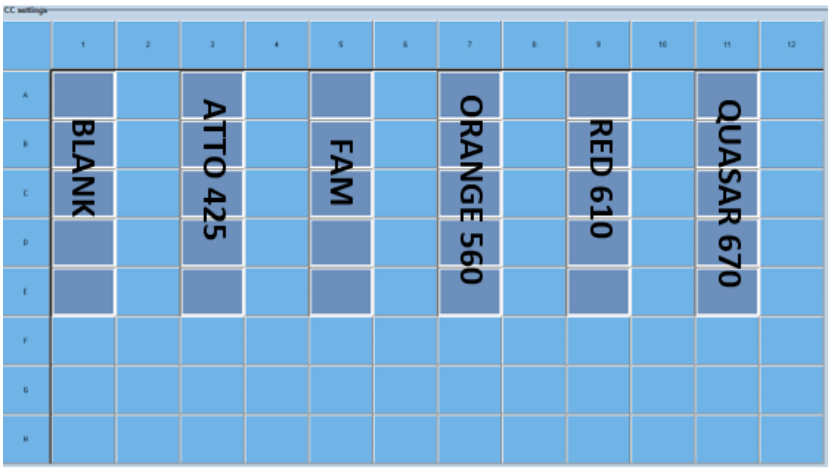
- Click “Customize” and make sure all five filter combinations are active (e.g. 440-488; 465-510; 533-610; 533-580; 618-660) **→ depends on cycler system!** and the “Integration Time Mode” is set to “Dynamic”
- Set the reaction volume to **20 µl**
- Set the PCR program as follows:

Program Name	Cycles	Analysis Mode	Target (°C)	Acquis. Mode	Hold (hh:mm:ss)	Ramp Rate (°C/s)	Acquis. (per °C)
Reverse Transcription	1	None	48	None	00:20:00	2,5	-
Enzyme Activation	1	None	95	None	00:03:00	2,5	-
PCR stage	45	Quantification	95	None	00:00:15	2,5	-
			58	Single	00:00:30	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. CC-ViroQ
- Choose positions for each calibration mix in “CC-ViroQ settings” as follows (the designation will be in the next step)



Please note: For Cobas z 480 replace Atto 425 with Quasar 705!

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

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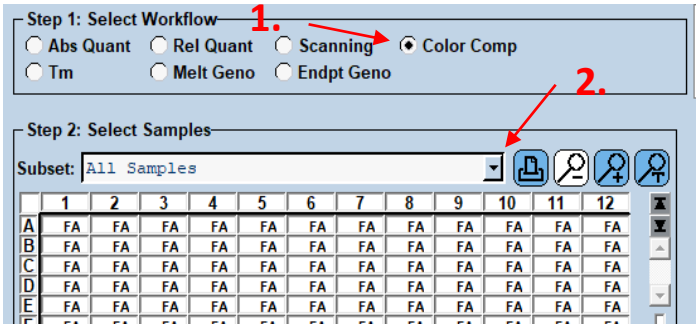
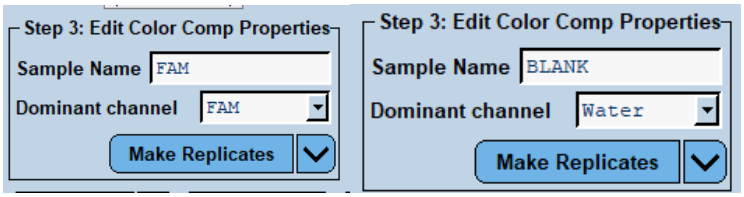
6.6 Set up the “Sample Editor”

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



- In “Step 3: Edit Color Comp Properties” set Sample Name (e.g. BLANK, FAM, ORANGE 560 etc.) 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 (e.g. 440-488; 465-510; 533-610; 533-580; 618-660) **→ depends on cycler system!**

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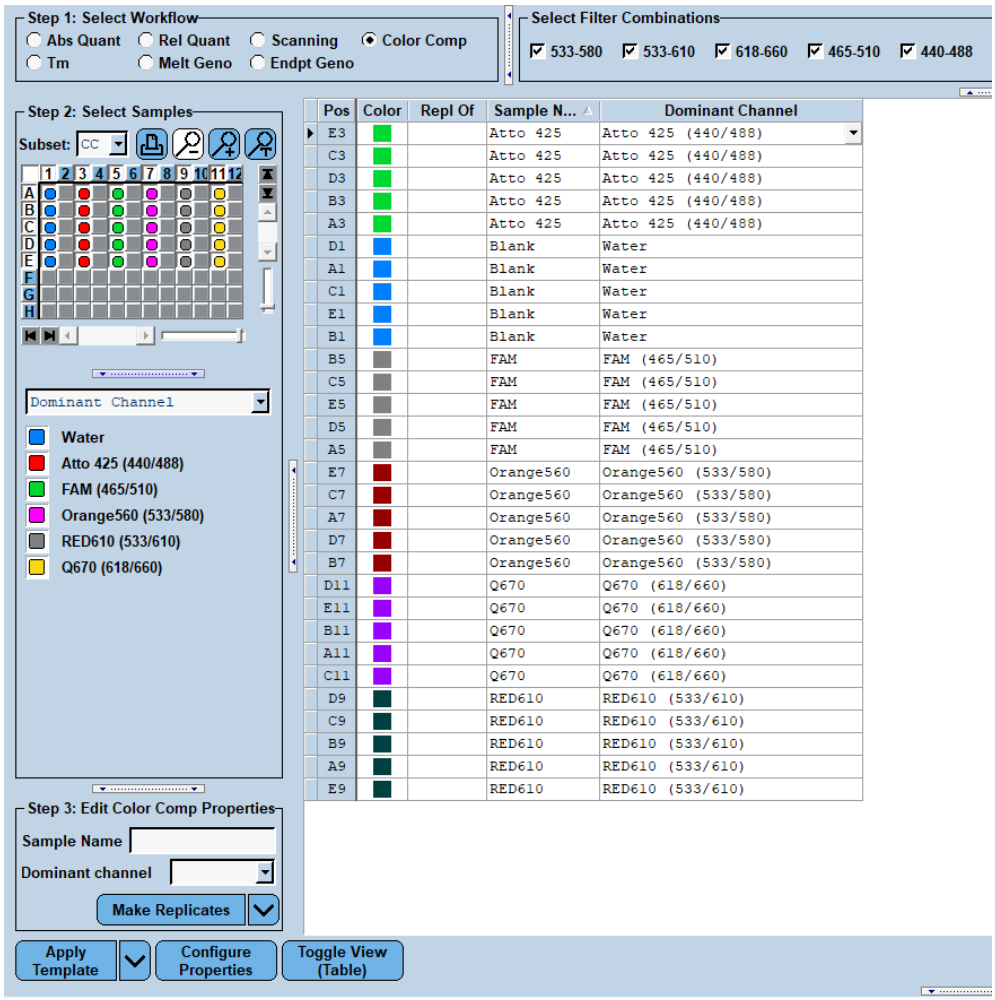

6.7 Prepare the reaction mix

Please note: The enzyme mix ViroQ Enzyme has to be dissolved prior use with 400 µl ViroQ Solvent by pipetting up and down.

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

	Blank (µl)	Atto 425 or Quasar 705 (µl)	FAM (µl)	Orange 560 (µl)	Red 610 (µl)	Quasar 670 (µL)
Calibrator mix (e.g. CAL FAM)	0	12	12	12	12	12
ViroQ Enzyme	24	24	24	24	24	24
DAC	0	12	12	12	12	12
Molecular grade water	96	72	72	72	72	72
Total reaction volume	120	120	120	120	120	120
Stripe/Row	**1**	**3**	**5**	**7**	**9**	**11**

Please note: For Cobas z 480 replace Atto 425 with Quasar 705!

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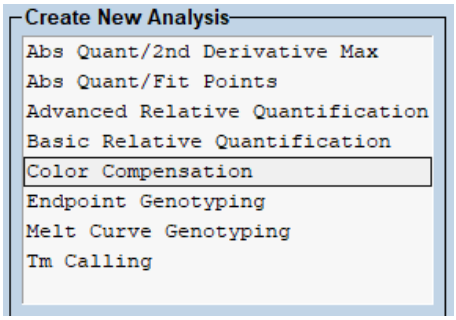
Pipette 20 µl of each mix into each well as shown in figure chapter 1.2.

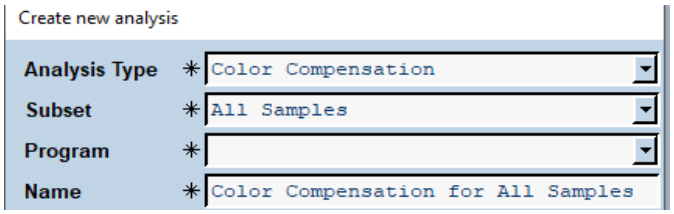
- After preparing and sealing the 96-well plate spin down the plate and set into the cycler.
  Please note: The sloping corner must be at the bottom right
- Choose ”Experiment’’ and click on ”Save’’  to select a folder for the run
- Click “Start Run” at the bottom right

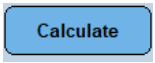
7. Data analysis

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



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

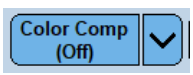


- Click 
- Then click on ”Calculate” 

- Click “Save CC Object”
- Choose a folder and name the CC object e.g. “CC-ViroQ-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: ViroQ kits. If available, please use pre-typed samples to ensure the CC object is valid.

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



- Click ”In Database’’ to choose the correct color comp object (e.g. CC-ViroQ Date)

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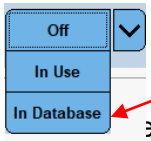

- e “Filter Comb” (1.) and click on “Calculate” (2.) for each to get Cp results.

- Save your experimental data.

8. Explanation of symbols used on the labels

	Storage temperature / Lower limit of temperature
	Use by
	Consult instructions for use
	Manufacturer
	Manufacturing Date
eIFU | VXX/XXXX	Electronic instructions for use Version of the actual instruction 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
CAL | Q705	Quasar 705 calibrator
DAC	DNA-amplification-control
ViroQ | ENZYME	Enzyme mix for ViroQ products
ViroQ | SOLV	Solvent for ViroQ enzyme mix
REF	Catalogue number
	**Warning** H302: Harmful if swallowed. H412: Harmful to aquatic life with long lasting effects.
	**Health hazard** H371: May harm the central nervous system. Route of exposure: Oral

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