

# Color Compensation Set 1

Version 2, March 2012

Solutions for the generation of color compensation objects for the LightCycler® System.

Order No. A 500 08

For 5 calibration runs.

Store in dark at -15 to -25 °C

## 1. What this Product Does

### Number of Tests

The set is designed for 5 calibration runs.

### Storage and Stability

- Store at -15 to -25 °C through the expiration date printed on the label.
- Keep away from light!
- Once opened, store the components as described in the following table:

### Contents

Vial / Cap Color	Label	Contents / Function / Storage
0 white cap	Blank	<ul style="list-style-type: none"> <li>• 550 µl PCR buffer</li> <li>• To determine the background fluorescence of PCR buffer.</li> <li>• Store at -15 to -25 °C.</li> </ul>
1 yellow cap	Calibrator 1	<ul style="list-style-type: none"> <li>• 25 µl labeled probe</li> <li>• To determine the crosstalk of single fluorophores between the LightCycler® channels.</li> <li>• Store in dark at -15 to -25 °C.</li> </ul>
2 red cap	Calibrator 2	
3 purple cap	Calibrator 3	
4 blue cap	Calibrator 4	

### Additional Equipment and Reagents Required

- LightCycler® 2.0 Carousel-Based System<sup>1</sup> **OR** LightCycler® 480 System<sup>1</sup>
- LightCycler® 20 µl – Capillaries<sup>1</sup> **OR** white LightCycler® 480 compatible PCR plate with optical sealing foil<sup>1</sup>
- Standard benchtop microcentrifuge containing a rotor for 2.0 ml reaction tubes.

- LC Carousel Centrifuge 2.0<sup>1</sup> for use with the LightCycler® 2.0 Sample Carousel (optional) **OR** Standard swing bucket centrifuge containing a rotor for multi-well plates  
The LightCycler® Carousel-Based System provides adapters that allow LightCycler® Capillaries to be centrifuged in a standard microcentrifuge rotor.
- Pipettes and pipette tips

<sup>1</sup> Available from Roche Diagnostics

### Applicability Statement

The Color Compensation Set 1 is intended for the generation of color compensation (CC) objects for the LightCycler® System. The generated CC object can subsequently be used to analyze multicolor experiments of the following assays from BIOTECON Diagnostics:

R 300 13 **foodproof** *E. sakazakii* Detection Kit, Hybridization Probes (LC 2.0)

R 310 15 **foodproof** *Enterobacteriaceae* plus *E. sakazakii* Detection Kit, Hybridization Probes (LC 2.0, 480)

As color compensation is instrument-specific, it is necessary to generate a CC object for every LightCycler® Instrument. A new object has to be created after the optical system has been repaired.

## 2. How to Use this Product

### LightCycler® System Protocol

The following tables show the setup for the LightCycler® 2.0 Carousel-Based System and the LightCycler® 480 System (please note the different ramp rates). Program the LightCycler® before preparing the calibration mixes. Please refer to the LightCycler® Instrument Operator's Manual for details on how to program the experimental protocol.

	Temperature Gradient			Cooling
<b>Programs/Cycle Program Data</b>	<b>Value</b>			<b>Value</b>
Cycles	1			1
Analysis Mode	Color Compensation or Melting Curve			None
<b>Temperature Targets</b>	<b>Segment 1</b>	<b>Segment 2</b>	<b>Segment 3</b>	<b>Segment 1</b>
Target [°C]	95	40	95	40
Hold [hh:mm:ss]	00:00:01	00:00:10	00:00:00	00:00:30
Ramp Rate [°C/s] LightCycler® 2.0	20	20	0.2	20
Ramp Rate [°C/s] LightCycler® 480	4.4	2.2	0.19*	2.2
Acquisition Mode	None	None	Cont	None

\*Set acquisitions/°C to 1.

Sec Target, Step Size, Step Delay: 0

**LightCycler® 2.0 Carousel-Based System**

Parameter	Setting
Default channel	530
Seek Temperature	30 °C
Max. Seek Pos.	5
Instrument Type	6 Ch.
Capillary Size	20 µl

**LightCycler® 480 System**

Parameter	Setting
Detection Format	Multi Color HybProbe 465-510 498-610 498-640 498-660
Block Size	96
Reaction Volume	20 µl

On a LightCycler® 480 System the Color Compensation reactions can be run in parallel to experimental samples, e.g. of your *Enterobacteriaceae* plus *E. sakazakii* Detection System assay. In this case, add the temperature gradient after the amplification step of the experimental protocol.

**Preparation of the Calibration Mixes**

Do not touch the surface of the capillaries or the upper surface of the PCR plate. Always wear gloves when handling the PCR vessels.

- Place 5 LightCycler® Capillaries in centrifuge adapters or in a LightCycler® Sample Carousel in a LC Carousel Centrifuge Bucket **OR**  
 Prepare a white LightCycler® 480 compatible PCR plate that you normally use for your LightCycler® 480 hybridization probe assays.
- Thaw the solutions, mix gently, and, for maximal recovery of contents, briefly spin vials in a microcentrifuge before opening.
- Pipet the following volumes:

Capillary Well	Dominant Channel LC 2.0 / 480	Tube 0 Blank	Tube 1 Calibrator 1	Tube 2 Calibrator 2	Tube 3 Calibrator 3	Tube 4 Calibrator 4
1	Water / Water	20 µl	-	-	-	-
2	530 / Fluos	15 µl	5 µl	-	-	-
3	610 / Red 610	15 µl	-	5 µl	-	-
4	640 / Red 640	15 µl	-	-	5 µl	-
5	670 / Cy 5(.5)	15 µl	-	-	-	5 µl

Do not change the order of the capillaries on the LightCycler® 2.0 Carousel-Based System.

4. Place the adapters (containing the capillaries) in a standard benchtop microcentrifuge. (Place the centrifuge adapters in a balanced arrangement within the centrifuge.) Centrifuge at 700 x g for 5 s (3,000 rpm in a standard benchtop microcentrifuge). Alternatively, use the LC Carousel Centrifuge for spinning the capillaries.

**OR**

Seal the plate accurately with an optical sealing foil.  
Place the plate in a swing bucket centrifuge and centrifuge at 1,500 x g for 30 s.

5. Transfer the capillaries or the PCR plate to the LightCycler®.
6. Cycle the samples as described above.
7. In the Sample Editor, define the dominant channel for each position (LightCycler® 2.0: Analysis Type Color Comp; LightCycler® 480: Workflow Color Comp) as indicated in the table above.

**Analysis**

Select “Color Compensation” from the Analysis menu, click “Calculate” (LightCycler® 480 only) and then click the “Save CC Object” button. The stored Color Compensation Object can afterwards be used for the analysis of runs conducted with the following products:

- R 300 13 **foodproof** *E. sakazakii* Detection Kit, Hybridization Probes (LC 2.0)
- R 310 15 **foodproof** *Enterobacteriaceae* plus *E. sakazakii* Detection Kit, Hybridization Probes (LC 2.0, 480)

**3. Troubleshooting**

The Color Compensation Set 1 is designed to generate suitable color compensation objects for a broad range of LightCycler® Instruments. However, due to variations of the optical systems of different instruments it is sometimes advisable to adjust the volumes of the calibrators for an optimal result. The following table refers to the analysis of **foodproof** *Enterobacteriaceae* plus *E. sakazakii* Detection System runs:

Observation	Possible Reason	Recommendation
<u>Channel 640</u> Negative control samples show a slight increase at cycles > 28.	Signal of the Internal Control not fully compensated.	Generate a new color compensation object with a larger volume of Calibrator 2 (vial 2, red cap). <i>E.g.</i> , pipet 10 µl Blank (vial 0, white cap) and add 10 µl Calibrator 2.
<u>Channel 660/670</u> <i>Enterobacteriaceae</i> -positive samples show a <b>slight</b> increase at cycles similar to their respective Cp-value in channel 640.	Signal of channel 640 not fully compensated.	Generate a new color compensation object with a larger volume of Calibrator 3 (vial 3, purple cap). <i>E.g.</i> , pipet 10 µl Blank (vial 0, white cap) and add 10 µl Calibrator 3.

## 4. Supplementary Information

### Ordering Information

BIOTECON Diagnostics is offering a broad range of reagents and services. For a complete overview and for more information, please visit our website at [www.bc-diagnostics.com](http://www.bc-diagnostics.com).

### Trademarks

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### Contact and Support

If you have questions or experience problems with this or any other product of BIOTECON Diagnostics, please contact our Technical Support staff (for details see [www.bc-diagnostics.com](http://www.bc-diagnostics.com)). Our scientists commit themselves to providing rapid and effective help. We also want you to contact us if you have suggestions for enhancing our product performance or using our products in new or specialized ways. Such customer information has repeatedly proven invaluable to us and the worldwide research community.

## 5. Change Index

### Version 1:

First version of the package insert.

### Version 2:

Page 2: Ramp Rate [°C/s] LightCycler® 480 = **0.19 (Set acquisitions/°C to 1.)**

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