

# Color Compensation Set 3

**Version 2, March 2012**

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

**Order No. A 500 10**

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 <ul style="list-style-type: none"> <li>• Calibrator 1: FAM</li> <li>• Calibrator 2: HEX</li> <li>• Calibrator 3: ROX</li> <li>• Calibrator 4: Cy5</li> </ul> </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® 480 System
- LightCycler® 480 compatible PCR plate with optical sealing foil **or**
- Tube strips (Order No. Z 100 25) and caps (Order No. Z 100 23). For tube strips, a special adapter (Order No. Z 100 24) is necessary. The PCR strips should be placed in a balanced order. For example two strips can be placed in column 1 and 12.
- Standard benchtop microcentrifuge containing a rotor for 2.0 ml reaction tubes.
- Standard swing bucket centrifuge containing a rotor for multi-well plates
- Pipettes and pipette tips

## Applicability Statement

The Color Compensation Set 3 is intended for the generation of color compensation (CC) objects for the LightCycler® 480 System. The generated CC object can subsequently be used to analyze multicolor experiments of the following assays from BIOTECON Diagnostics:

- R 302 04 **foodproof**® *Brucella* Detection Kit
- R 302 05 **foodproof**® *Campylobacter* Quantification Kit
- R 302 15.1 **foodproof**® *Enterobacteriaceae* plus *Cronobacter* Detection Kit
- R 302 17 **foodproof**® GMO Screening Kit (35S, NOS, bar, FMV)
- R 302 28 **foodproof**® *Alicyclobacillus* Detection Kit
- R 302 37 **foodproof**® Hepatitis A Virus Detection Kit
- R 302 38.1 **foodproof**® Norovirus Detection Kit (GI, GII)
- R 302 50 **foodproof**® Norovirus (GI, GII) plus Hepatitis A Virus Detection Kit
  
- R 602 02-1 **foodproof**® Beer Screening 1 LyoKit
- R 602 06-1 **foodproof**® Beer Screening 2 LyoKit
- R 602 11-1 **foodproof**® STEC Screening LyoKit
- R 602 12-1 **foodproof**® STEC Identification LyoKit
- R 602 17-1 **foodproof**® GMO Screening 1 LyoKit
- R 602 18-1 **foodproof**® GMO Screening 2 LyoKit
- R 602 24-1 **foodproof**® GMO Soya Identification 1 LyoKit
- R 602 25-1 **foodproof**® GMO Soya Identification 2 LyoKit
- R 602 36-1 **foodproof**® GMO Soya Identification 3 LyoKit
- R 602 34-1 **foodproof**® *Salmonella* Enteritidis & Typhimurium Detection LyoKit
- R 602 40-1 **foodproof**® *Clostridium botulinum* Detection LyoKit
- R 602 44-1 **foodproof**® *Vibrio* Detection LyoKit (only LightCycler® 480 I)
- R 602 51-1 **foodproof**® *Listeria* plus *L. monocytogenes* Detection LyoKit
- R 602 57-1 **foodproof**® *Salmonella* plus *Cronobacter* Detection LyoKit
- R 602 59-1 **foodproof**® *Salmonella* Genus plus Enteritidis & Typhimurium Detection LyoKit
  
- F 302 41 **foodproof**® *Clostridium* Control Detection Kit
  
- F 602 46-1 **foodproof**® GMO Maize Identification 1 LyoKit (DAS-40278-9, LY038, VCO-01981-5)
- F 602 58-1 **foodproof**® Plant Taxon Screening LyoKit (Soya, Corn, Canola)
  
- V 900 01 **vetproof**® SE Vaccine Detection 1 Kit
- V 900 03 **vetproof**® STM Vaccine Detection 1 Kit

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® 480 System. 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	37	95	40
Hold [hh:mm:ss]	00:00:01	00:00:10		00:00:30
Ramp Rate [°C/s]	4.4	2.2		2.2
Acquisitions/°C			1	

Sec Target, Step Size, Step Delay: 0

Parameter	Setting	Name	Melt Factor	Quant Factor	Max Integration Time (Sec)
Detection Format	465-510	FAM	1	10	2
	533-580	HEX	1	10	2
	533-610	ROX	1	10	2
	618-660	Cy5	1	10	2
Block Size	96				
Reaction Volume	25 µl				

On the LightCycler® 480 System the Color Compensation reactions can be run in parallel to experimental samples, e.g. of your *Campylobacter* Quantification Kit 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 upper surface of PCR plates. Always wear gloves when handling the PCR vessels.

1. Prepare a LightCycler® 480 compatible PCR plate that you normally use for your LightCycler® 480 hydrolysis probe assays. For our LyoKits, use tube strips (Order No. Z 100 25) and caps (Order No. Z 100 23), matching BIOTECON Diagnostics’ LyoKit consumables.
2. Thaw the solutions, mix gently, and, for maximal recovery of contents, briefly spin vials in a microcentrifuge before opening.
3. Pipet the following volumes:

Well	Dominant Channel	Tube 0 Blank	Tube 1 Calibrator 1	Tube 2 Calibrator 2	Tube 3 Calibrator 3	Tube 4 Calibrator 4
1	Water	25 µl	-	-	-	-
2	465-510	20 µl	5 µl	-	-	-
3	533-580	20 µl	-	5 µl	-	-
4	533-610	20 µl	-	-	5 µl	-
5	618-660	20 µl	-	-	-	5 µl

4. 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 PCR plate to the LightCycler®.
6. Cycle the samples as described above.
7. In the Sample Editor, define the dominant channel for each position (Workflow Color Comp) as indicated in the table above.

**Analysis**

Select “Color Compensation” from the Analysis menu, click “Calculate” 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 products mentioned in “Applicability Statement”.

### 3. 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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