

Color Compensation Set 5

Version 1, February 2016

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

Order No. A 500 15

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 in dark at –15 to –25 °C through the expiration date printed on the label.
- Keep away from light!

Contents

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

Additional Equipment and Reagents Required

- LightCycler® 480 System
- LyoKit compatible consumables¹
 - Optical white cap strip, 120 pcs, Order No. Z 100 26
 - EU 0.2 ml Thin-wall 8-tube strip, white 120 pcs, Order No. Z 100 25
- Standard benchtop microcentrifuge for 2.0 ml reaction tubes and PCR strips
- Pipettes and pipette tips

¹ Available from BIOTECON Diagnostics

Applicability Statement

The Color Compensation Set 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. 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 or exchanged.

- R 602 44-1 **foodproof®** *Vibrio* Detection LyoKit
- R 602 47-1 **foodproof®** Spoilage Yeast Detection 1 LyoKit
- R 602 48-1 **foodproof®** Spoilage Yeast Detection 2 LyoKit
- R 602 57-1 **foodproof®** *Salmonella* plus *Cronobacter* Detection LyoKit
- R 602 67-1 **foodproof®** *Enterobacteriaceae* plus *Salmonella* Detection LyoKit
- R 602 72-1 **foodproof®** *Aspergillus* Detection LyoKit
- F 302 53 **foodproof®** *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* Detection Kit
- R 602 45-1 **microproof®** *Legionella* Quantification LyoKit

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
Programs/Cycle Program Data	Value			Value
Cycles	1			1
Analysis Mode	Color Compensation or Melting Curve			None
Temperature Targets	Segment 1	Segment 2	Segment 3	Segment 1
Target [°C]	95	37	95	40
Acquisition Mode	None	None	Cont	None
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. with the **foodproof®** *Vibrio* Detection LyoKit assay.

Preparation of the Calibration Mixes

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

1. Set up the Color Compensation reactions with tube strips (Order No. Z 100 25) and caps (Order No. Z 100 26) 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 strips accurately and centrifuge at 1,500 x g for 30 s.
5. Insert the PCR strips into a support frame matching LightCycler® plates and load to the instrument.
6. Cycle the samples as described above.
7. In the Subset Editor, create a new subset for the Color Compensation reactions
8. 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.

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). 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.

4. Change Index

Version 1:

First version of the package insert.

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