

Color Compensation Set 4

Version 1, March 2012

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

Order No. A 500 11

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"> • 550 µl PCR buffer • To determine the background fluorescence of PCR buffer. • Store at -15 to -25°C.
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. • Store in dark at -15 to -25°C.
2 red cap	Calibrator 2	
3 purple cap	Calibrator 3	
4 blue cap	Calibrator 4	
5 green cap	Calibrator 5	

Additional Equipment and Reagents Required

- LightCycler® 2.0 Carousel-Based System¹
- LightCycler® 20 µl - Capillaries¹
- Standard benchtop microcentrifuge containing a rotor for 2.0 ml reaction tubes.

- LC Carousel Centrifuge 2.0¹ for use with the LightCycler® 2.0 Sample Carousel (optional)
The LightCycler® Carousel-Based System provides adapters that allow LightCycler® Capillaries to be centrifuged in a standard microcentrifuge rotor.
- Pipettes and pipette tips

¹ Available from Roche Diagnostics

Applicability Statement

The Color Compensation Set 4 is intended for the generation of color compensation (CC) objects for the LightCycler® 2.0 System. The generated CC object can subsequently be used to analyze multicolor experiments of the Hygiene Screening System (*Staphylococcus*, *Micrococcus*, *Corynebacterium*), Hybridization Probes (LC 2.0) from BIOTECON Diagnostics (Cat. No. R 300 31).

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. 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	40	95	40
Hold [hh:mm:ss]	00:00:00	00:00:10	00:00:00	00:00:30
Ramp Rate [°C/s]	20	20	0.2	20
Acquisition Mode	None	None	Cont	None

Sec Target, Step Size, Step Delay: 0

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

Preparation of the Calibration Mixes

Do not touch the surface of the capillaries. Always wear gloves when handling the capillaries.

1. Place 6 LightCycler® Capillaries in centrifuge adapters or in a LightCycler® Sample Carousel in a LC Carousel Centrifuge Bucket.
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:

Capillary	Dominant Channel	Tube 0 Blank	Tube 1 Calibrator 1	Tube 2 Calibrator 2	Tube 3 Calibrator 3	Tube 4 Calibrator 4	Tube 5 Calibrator 5
1	Water	20 µl	-	-	-	-	-
2	530	15 µl	5 µl	-	-	-	-
3	610	15 µl	-	5 µl	-	-	-
4	640	15 µl	-	-	5 µl	-	-
5	670	15 µl	-	-	-	5 µl	-
6	705	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.
5. Transfer the capillaries to the LightCycler®.
6. Cycle the samples as described above.
7. In the Sample Editor, define the dominant channel for each position (Analysis Type Color Comp) as indicated in the table above.

Analysis

Select “Color Compensation” from the Analysis menu and click the “Save CC Object” button. The stored Color Compensation Object can afterwards be used for the analysis of runs conducted with the following product:

R 300 31 Hygiene Screening System (*Staphylococcus, Micrococcus, Corynebacterium*), Hybridization Probes (LC 2.0)

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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