

Color Compensation Set 5

Revision B, November 2024

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

Product No. KIT230011

For 5 calibration runs

Store the kit at -15 to -25 °C



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1. What This Product Does

1.1 Number of Tests

The set is designed for 5 calibration runs.

1.2 Storage and Stability

- Store in the dark 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:

1.3 Kit 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 <ul style="list-style-type: none"> ○ Calibrator 1: FAM ○ Calibrator 2: HEX ○ Calibrator 3: ROX ○ Calibrator 4: Cy5 • To determine the crosstalk of single fluorophores between the LightCycler® channels. • Store in the dark at -15 to -25 °C.
2 red cap	Calibrator 2	
3 purple cap	Calibrator 3	
4 blue cap	Calibrator 4	
5 -	Tube Strips & Caps	<ul style="list-style-type: none"> • Six (6) tube strips and caps

1.4 Additional Equipment and Reagents Required

- LightCycler® 480 System
- LightCycler 480 compatible PCR plate with optical sealing foil **or** If necessary, tube strips and caps (Product No. MLD230010) can be ordered. For tube strips, a special adapter (Product No. MIS230005) is necessary. The PCR strips should be placed in a balanced order. For example, two strips can be placed in columns 1 and 12.
- Standard benchtop microcentrifuge for 2.0 mL reaction tubes and PCR strips
- Pipettes and pipette tips

1.5 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 Hygiena 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.



KIT230018	foodproof® <i>Yersinia enterocolitica</i> and <i>Yersinia pseudotuberculosis</i> Detection Kit
KIT230117	foodproof® <i>Vibrio</i> Detection LyoKit (only LightCycler® 480 II)
KIT230121	foodproof® Spoilage Yeast Detection 1 LyoKit
KIT230124	foodproof® Spoilage Yeast Detection 2 LyoKit
KIT230131	foodproof® <i>Salmonella</i> plus <i>Cronobacter</i> Detection LyoKit
KIT230137	foodproof® <i>Enterobacteriaceae</i> plus <i>Salmonella</i> Detection LyoKit
KIT230140	foodproof® Spoilage Yeast Detection 3 LyoKit
KIT230145	foodproof® <i>Aspergillus</i> Detection LyoKit
KIT230119	microproof® <i>Legionella</i> Quantification LyoKit

2. How to Use This Product

2.1 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.



2.2 Preparation of the Calibration Mixes

Do not touch the upper surface of the PCR plate. 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 the provided tube strips and caps (Product No. MLD230010).
2. Thaw the solutions, mix gently, and, for maximal recovery of contents, briefly spin vials in a microcentrifuge before opening.
3. Pipette the following volumes

3.1 For Analysis of Any PCR Kit Listed Above Except the foodproof *Salmonella* plus *Cronobacter* LyoKit:

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

3.2 For Analysis of the foodproof *Salmonella* plus *Cronobacter* LyoKit:

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	22 µL	-	-	3 µL	-
5	618-660	20 µL	-	-	-	5 µL

4. Seal the plate accurately with an optical sealing foil and centrifuge at 1,500 x *g* for 30 seconds in a swing bucket centrifuge.
or
Seal the tube strips accurately with optical caps and briefly centrifuge.
5. Transfer the PCR plate to the LightCycler
or
Insert the PCR strips into the support frame and load the instrument.
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.



2.3 Analysis

Select 'Color Compensation' from the Analysis menu, click 'Calculate' and then click the 'Save CC Object' button. The stored Color Compensation Object can be used afterward for the analysis of runs conducted with the products mentioned in the "Applicability Statement".

3. Warranty and Disclaimer of Liability

"Limited Warranty" and "Disclaimer of Liability." Hygiena Diagnostics GmbH warrants that this product is free from defects in materials and workmanship through the expiration date printed on the label and only if the following are complied with:

- (1) The product is used according to the guidelines and instructions set forth in the product literature;
- (2) Hygiena Diagnostics GmbH does not warrant its product against any and all defects when: the defect is as a result of material or workmanship not provided by Hygiena Diagnostics GmbH; defects caused by misuse or use contrary to the instructions supplied, or if the product is contaminated by improper storage or handling;
- (3) All warranties of merchantability and fitness for a particular purpose, written, oral, expressed or implied, shall extend only for a period of one year from the date of manufacture. There are no other warranties that extend beyond those described on the face of this warranty;
- (4) Hygiena Diagnostics GmbH does not undertake responsibility to any purchaser of its product for any undertaking, representation or warranty made by any dealers or distributors selling its products beyond those herein expressly expressed unless expressed in writing by an officer of Hygiena Diagnostics GmbH;
- (5) Hygiena Diagnostics GmbH does not assume responsibility for incidental or consequential damages, including, but not limited to responsibility for loss of use of this product, removal or replacement labor, loss of time, inconvenience, expense for telephone calls, shipping expenses, loss or damage to property or loss of revenue, personal injuries or wrongful death;
- (6) Hygiena Diagnostics GmbH reserves the right to replace or allow credit for any modules returned under this warranty.

4. Supplementary Information

4.1 Ordering Information

Hygiena Diagnostics is offering a broad range of reagents and services. For a complete overview and for more information, please visit our website at www.hygiena.com.

4.2 Trademarks

foodproof[®], **microproof**[®], **vetproof**[®], **ShortPrep**[®], **StarPrep**[®], **RoboPrep**[®] and **LyoKit**[®] are registered trademarks of Hygiena Diagnostics GmbH. **Hygiena**[®] is a registered trademark of Hygiena.

Other brand or product names are trademarks of their respective holders.

4.3 Contact and Support

If you have questions or experience problems with this or any other product of Hygiena Diagnostics GmbH, please contact our Technical Support staff (www.hygiena.com/support). 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.



4.4 Reference Number

The reference number and original Hygiena Diagnostics GmbH article number: A 500 15

5. Change Index

Version 1:

First version of the package insert.

Revision A, February 2024:

Rebranding and new layout.

A 500 15 20 -> INS-KIT230011-RevA

Revision B, November 2024:

Edits to product numbers in 1.5.

Additional pipetting scheme in 2.2.



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