

LightMix® Color Compensation HybProbe

Cat.-No. 40-0318-00

2013: New Version

Color Compensation reagents for:

Roche LightCycler® 1.2 / 1.5 / 2.0 / 480 / 480 II and Cobas® Z480 Instruments.

New version (2013) - This kit replaces the previous Color Compensation kits:

40-0318-00 LightMix® Color Compensation Kit for use of LightMix® typing kits with 530/640 dye combination and LightMix® detection kits with 640/690 dyes combination, and

40-0319-99 Color Compensation for use of the Scrapie LightMix® Kit PRNP 40-0297, and partially **LightCycler® Multicolor Demo Set (Roche, discontinued).**

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1. Set contents, Reagents preparation and Storage

This kit contains seven different dyes. Three new dyes, required for specific kits, are included. Spin down fluorophore tubes for 30 seconds. Dissolve each tube in 75 µl water.

Do not mix standards.

Vials	V130113	Cap color	Labeled with Fluorophore	PCR-grade H ₂ O
1	NEW	Orange	Cyan500	75.0 µl
1		Red	Fluorescein 515	75.0 µl
1		Yellow	SimpleProbe® 530	75.0 µl
1	NEW	Black	LC Red 610	75.0 µl
1		Green	LC Red 640	75.0 µl
1	NEW	Blue	LC Red 670	75.0 µl
1		Violet	LC Red 690	75.0 µl

Lyophilized reagents are stable for at least 6 months from delivery, if stored protected from light at room temperature (18 - 25°C). Dissolved reagents can be stored refrigerated at 4°C for 7 days (in the dark) and long term stored frozen (-20°C). **Expiration date is printed on the kit label; do not use after expiry.**

The final master mix used for the Color Compensation containing PCR-buffer and MgCl₂ can not be stored.

2. Additional Reagents and Materials required:

LightCycler® FastStart DNA Master HybProbe	Cat. No. 03 003 248 001
LightCycler® Capillaries (Glass)	Cat. No. 04 929 292 001
LightCycler® 480 Multiwell Plate 96 (White)	Cat. No. 04 729 692 001
or LightCycler® 480 Multiwell Plate 384 (White)	Cat. No. 04 729 749 001

Note: The use of plastic capillaries (LightCycler® 1.x, / 2) and clear multiwell plates (LightCycler® 480 System) is discouraged. The use of other master mix reagents than FastStart DNA Master HybProbe has not been tested.

3. Introduction

The emission spectra of the fluorophores used with the Roche LightCycler® Instruments overlap, causing fluorescence signals to be recorded in the adjacent channels when used simultaneously. The Color Compensation corrects the signals and enables to read each dye in the corresponding channel. The Color Compensation is influenced by different parameters depending on the dye combination used.

Every instrument has to be calibrated once by running a melting curve analysis with pure dye samples; local laboratory regulations may require to run the calibration regularly. The procedure is described in this manual.

Regardless the present/current use of the LightCycler® we suggest to generate all the color compensation available for your instrument.

The following detection channels are available in the various LightCycler® Instruments:

Channel ►	530 F1	610	640 F2	670	>660	700	705 F3	New Dye
Instrument ▼								LC690
LightCycler® 1.2 / 1.5	•		•				•	• (F3)
LightCycler® 2.0	•	•	•	•			•	• (705)
LightCycler® 480	•	•	•	•				
LightCycler® 480 II	•	•	•		•			• (>660)
LightCycler® Z480	•	•	•			•		• (700)

A) LightMix® kits for Genotyping contain a combination of 530 and 640 probes

As from the table above, channels 530 and 640 are present in all the instruments, making this combination appropriate for duplex kits and suitable for the use with all the instruments. LightMix® kits for genotyping tests contain a combination of 530 and 640 probes.

CC 530-640 for TIB MOLBIOL genotyping kits 530/640
for LightCycler® 1.2 / 1.5 / 2.0 / 480 / 480 II / Cobas® Z480 Instruments.

B) LightMix[®] Kits Detection/Quantification using a combination of 640 / 690 probes

Probes with 530 label are not suitable for detection / quantification tests, thus excluding the 530/640 combination for such kits. Of the remaining channels the LightCycler[®] Instrument family shares only the 640 channel, making the interchangeability of dual-color kits with hybridization probes impossible.

We have introduced the new dye LC690 (with a broad emission range) which can be read in either the 705 or F3 channel of the capillary based LightCycler[®] Instruments or in the >660 channel of the LightCycler[®] 480 II / Z480 Systems. The 670 channel of the LightCycler[®] 480 “first version” is too narrow and can not be used.

Most LightMix[®] detection/quantification kits contain 640 and 690 probes.

CC 640-690 for TIB MOLBIOL detection / quantification kits 640/690 for LightCycler[®] 1.2 / 1.5 / 2.0 / 480 II / Z480 Cobas[®] Instruments.

The 670 channel of the LightCycler[®] 480 Instrument first version cannot read dye LC690. Kits, which exploit LC690 probes for an optional IC, may be used with these instruments by omitting the IC from the test.

C) MultiColor HybProbe assays performed on the LightCycler[®] 2.0 Instrument

The LightCycler[®] 2.0 Instrument has four different dye channels 610, 640, 670, and 705. The use of different labeled hybridization probes requires a specific multicolor compensation.

CC MultiColor (similar to the discontinued Roche Multicolor Demo Set) for TIB MOLBIOL kits like LightMix[®] kit 40-0356-16 JAK2 exon 12, for LightCycler[®] 2.0 Instruments.

D) Scrapie PRNP Genotyping on the LightCycler[®] 480 Instruments

The LightMix[®] Kit 40-0297 PRNP uses Cyan500, fluorescein and hybridization probes.

CC PRNP for TIB MOLBIOL LightMix[®] Kit PRNP 40-0297 for LightCycler[®] 480 / 480 II Instruments.

4. Protocol for LightCycler® 480, 480 II and Cobas® Z480 Instruments


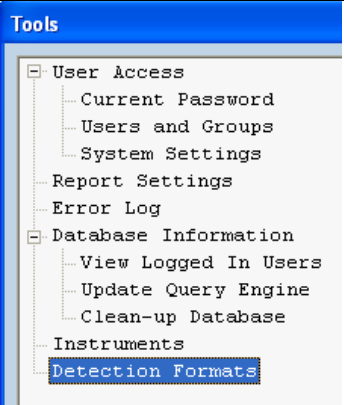

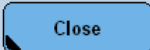
4.1 Introduction

To obtain the best performance of the LightMix® kits, several specific detection formats can be generated:

- 1) TIB Molbiol 530-640 Detection format.
- 2) TIB Molbiol 515-640-690 Detection format.
- 3) TIB Molbiol Multicolor Detection format.
- 4) TIB Molbiol PRNP Detection format.

The Multicolor and Scrapie detection formats are used for the generation of all the CC files; generation of all detection format combinations will allow an easier programming of LightMix® kits.

4.2.1 Opening steps

Open Tools	
Select Detection Format	
Select New	
Create all the detection formats	See chapter 4.2.2
Close window	

4.2.2 Definition of the Detection Format

	LightCycler® 480	LightCycler® 480 II	Cobas® Z480																																																																																																																																																								
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4.3 Programming

The programming procedure is independent from the CC file to be generated. Start programming the instrument before preparing the solutions.

Detection Format		MultiColor		
Program Name	Melting			Cooling
Analysis Mode	Color Compensation			None
Cycles	1			1
Segment	1	2	3	1
Target [°C]	95	40	85	40
Acquisition Mode	None	None	Continu.	None
Hold [hh:mm:ss]	00:00:30	00:02:00		00:00:30
Ramp Rate [°C/s] 96	4.4	1.5	0.14	1.5
Ramp Rate [°C/s] 384	4.6	2.0	0.14	2.0
Acquisitions [per °C]	---	---	1	---
Sec Target [°C]	0	0	0	0
Step Size [°C]	0	0	0	0
Step Delay (cycles)	0	0	0	0

Save protocol for future use. See the LightCycler® specific operator’s manual for details.

4.4 Subset Editor

Using the subset editor, generate **My Samples** and all the subsets relevant to your instrument as depicted below to restrict the use of signals only on the wells containing the dye standards.

Instrument ▶	All	All	480	480II / Z480	480 / 480II
Subset ▼	SP-640	FL-640-690	MultiColor	MultiColor	PRNP
My Samples	SP-640	FL-640-690	MultiColor	MultiColor	PRNP
	1 2 3	1 2 3	1 2 3	1 2 3	1 2 3
A					
B					
C					
D					
E					
F					
G					
H					

4.5 Sample Editor

For a better overview restrict to subset: “My Samples” only.

Set each row as replicate.

Select the first column of subset: “My Samples” only

Set the dominant channel as described.

Note: with the detection format set to “MultiColor” some Dominant Channel are not selectable.

Use “Water” for non relevant channels.

Example: Set B1=Cyan500 to “Water”.

Pos	Sample Name	Dominant Channel
A1	H2O	Water
B1	Cyan500	Cyan500
C1	Fluorescein 515	Fuor 515
D1	SimpleProbe® 530	Fuor 515
E1	LC Red 610	LC Red 610
F1	LC Red 640	LC Red 640
G1	LC Red 670	LC Red 670
H1	LC Red 690	LC Red 690

4.6 Reagents Preparation

The plate based instruments require a two melting curve performed on the same plate. The second melting curve, specific for PRNP, can be omitted by skipping all specific steps. See the LightCycler® operator's manual about the relevant details for the generation of a CC file

Prepare Master Mix containing:

Instruments ►	1 reaction	All	LC 480 PRNP include	LC480 II PRNP include
		18 (+1) reactions	21 (+1) reactions	24 (+1) reactions
Water, PCR-grade	11.8 µl	224.2 µl	259.6 µl	295.0 µl
FastStart DNA Master 10 X	2.0 µl	38.0 µl	44.0 µl	50.0 µl
MgCl ₂ solution 25 mM	1.2 µl	22.8 µl	26.4 µl	30.0 µl
Final volume	15.0 µl	285.0 µl	330.0 µl	375.0 µl

One excess reaction has been included

Mix gently and spin down

4.7 Plate preparation

Transfer 15 µl of the reaction mix into each well and add fluorophore as indicated:
Optional PRNP specific wells are highlighted.

Instruments ►	Dominant Channel	LC480		LC480 II		Z480	
		Position	Volumes	Position	Volumes	Position	Volumes
H ₂ O	Water	A1,A2,A3	5.0 µl	A1,A2,A3	5.0 µl	A1,A2,A3	5.0 µl
Cyan500	Cyan500	B1,B2,B3	5.0 µl	B1,B2,B3	5.0 µl		
Fluorescein 515	Flous	C1,C2,C3	5.0 µl	C1,C2,C3	5.0 µl	C1,C2,C3	5.0 µl
SimpleProbe 530	Flous	D1,D2,D3	5.0 µl	D1,D2,D3	5.0 µl	D1,D2,D3	5.0 µl
LC Red 610	Red 610	E1,E2,E3	5.0 µl	E1,E2,E3	5.0 µl	E1,E2,E3	5.0 µl
LC Red 640	Red 640	F1,F2,F3	5.0 µl	F1,F2,F3	5.0 µl	F1,F2,F3	5.0 µl
LC Red 670	Red 670	G1,G2,G3	5.0 µl	G1,G2,G3	5.0 µl		
LC Red 690	Red 690			H1,H2,H3	5.0 µl	H1,H2,H3	5.0 µl

Centrifuge plate before loading on the LightCycler® to eliminate bubbles.

4.8 Multicolor Run

Start run.

Save run as: **Universal CC YYYY-MM-DD** (e.g., “Universal CC 2013-01-13”).

Save plate for a second PRNP specific run (see 4.9)

At the end of run:

Create a new analysis

Analysis Type “Color Compensation”

Subset “**Multicolor**”

Calculate Save CC object as: **CC MultiColor YYYY-MM-DD**

Create a new analysis

Analysis Type “Color Compensation”

Subset “**SP-640**”

Calculate Save CC object as: **CC 530-640 YYYY-MM-DD**

LightCycler® 480 II users generate additional CC files

Create a new analysis

Analysis Type “Color Compensation”

Subset “**CC FL-640-690**”

Calculate Save CC object as: **CC 640-690 YYYY-MM-DD**

4.9 PRNP Run

Reload program saved in **4.3 Programming**;

Modify detection format

Detection Format

PRNP

Select Dominant Channel as described in **4.5 Sample Editor**

Dominant Channel Cyan500 must be selectable.

Use “Water” for non relevant channels.

Example: Set H1=LC Red 690 to “Water”.

Start run.

Save run as: **PRNP CC YYYY-MM-DD** (e.g., “PRNP CC 2013-01-13”).

At the end of run:

Create a new analysis

Analysis Type “Color Compensation”

Subset “**PRNP**”

Calculate Save CC object as: **CC PRNP YYYY-MM-DD**

5. Protocol for LightCycler® 1.x /2.0 Instruments

5.1 Programming

The programming procedure is independent from the CC file to be generated. Start programming the instrument before preparing the solutions.

Program for LC1.X / LC2.0				
Program Name	Melting			Cooling
Analysis Mode	Color Compensation			None
Cycles	1			1
Segment	1	2	3	1
Target [°C]	95	40	85	40
Hold [hh:mm:ss]	00:00:20	00:00:20	00:00:00	00:00:30
Ramp Rate [°C/s]	20	20	0.2	20
Sec Target [°C]	0	0	0	0
Step Size [°C]	0	0	0	0
Step Delay (cycles)	0	0	0	0
Acquisition Mode	None	None	Continu.	None

Save protocol for future use. See the LightCycler® specific operator's manual for details.

5.2 Reagents Preparation

The capillary based instruments require a three melting curve performed using different set of dyes. LightCycler® 1.x detects only 3 channels: the third run must be omitted.

See the LightCycler® operator's manual about the relevant details for the generation of a CC file

Prepare Master Mix containing:

Instruments ►	Components ▼		LC 1.x	LC 2.0
		1 reaction	5 (+1) reactions	7 (+1) reactions
	Water, PCR-grade	11.8 µl	70.8 µl	94.4 µl
	FastStart DNA Master 10 X	2.0 µl	12.0 µl	16.0 µl
	MgCl ₂ solution 25 mM	1.2 µl	7.2 µl	9.6 µl
	Final volume	15.0 µl	90.0 µl	120.0 µl

One excess reaction has been included

Mix gently and spin down

5.3 Sample Editor for LightCycler® 1.x using Software Version 3.5

P.	Name
1	H2O
2	FL
3	640
4	690

Designate "Sample Name" as described.
Note that sample order for software 3.5 is mandatory.

5.4 Sample Editor for LightCycler® 1.5 / 2.0 using Software Version 4.05 or 4.1

Pos	Sample Name	Dominant Channel
▶ 1	H2O	Water
2	FL	530
3	LC640	640
4	LC690	705
5	LC610	610
6	LC670	670

Designate "Sample Name", select Color Compensation from the Analysis Type menu and select "Dominant Channel" as described

5.5 CC Runs

The capillary instruments require running 2 (LightCycler® 1.x) or 3 melting curves.

First run Color Compensation 530-640.

Transfer 15 µl each of the reaction mix into four LightCycler® capillaries and add to capillary:

Rotor position	Fluorophore	Dominant Channel	Volumes
1	H ₂ O	Water	5.0 µl
2	SimpleProbe 530	530	5.0 µl
3	LC Red 640	640	5.0 µl
4	LC Red 690	705	5.0 µl

Start run. Save as: **CC 530-640**

NOTE: See chapter 5.6 or 5.7

Second run Color Compensation 640-690.

Leave capillaries 1, 3 and 4 in the rotor and replace capillary 2 containing probe 530 by a capillary containing 15 µl of mix and 5 µl of probe 515 (as listed in the table below):

Rotor position	Fluorophore	Dominant Channel	Volumes
1	H ₂ O	Water	5.0 µl
2	Fluorescein 515	530	5.0 µl
3	LC Red 640	640	5.0 µl
4	LC Red 690	705	5.0 µl

Start run. Save as: **CC 640-690**

NOTE: See chapter 5.6 or 5.7

Third run Color Compensation Multicolor: LightCycler® 2.0 only.

Leave capillaries from 1 to 4 in the rotor and add capillary in position 5 and 6 containing 15 µl of mix and 5 µl of the probes as listed in the table below:

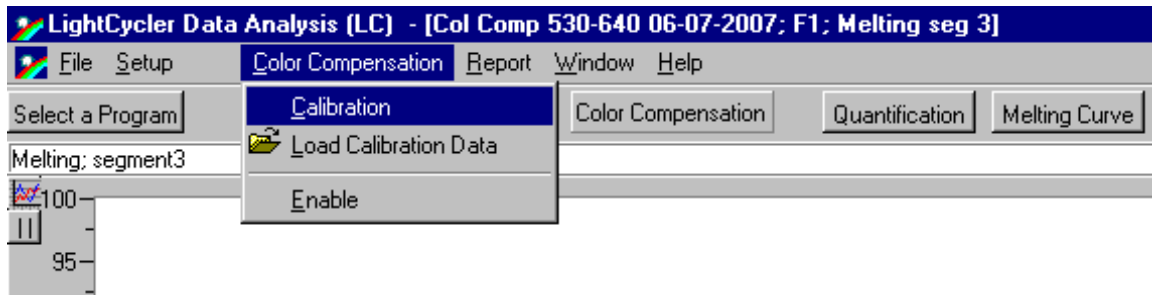
Rotor position	Fluorophore	Dominant Channel	Volumes
1	H ₂ O	Water	5.0 µl
2	Fluorescein 515	530	5.0 µl
3	LC Red 640	640	5.0 µl
4	LC Red 690	705	5.0 µl
5	LC Red 610	610	5.0 µl
6	LC Red 670	670	5.0 µl

Start run. Save as: **CC MultiColor**

NOTE: See chapter 5.6 or 5.7

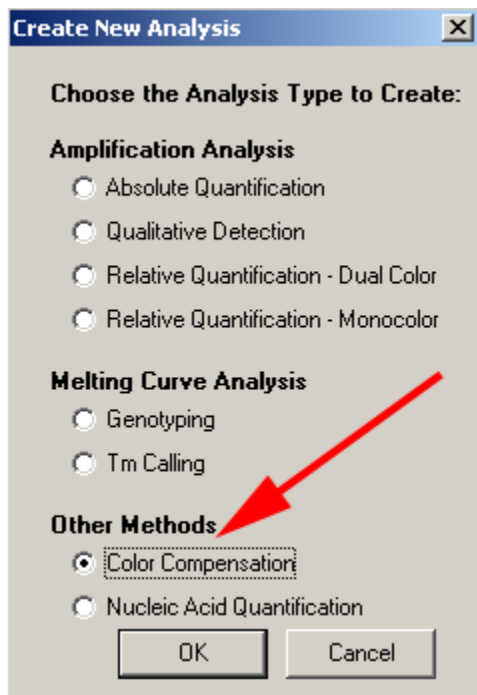
5.6 Saving CC for LightCycler® 1.x using Software Version 3.5

After each run, in the opening page of the “LightCycler Data Analysis” software, select the pull down menu “Color Compensation”. The “Save as” command is called “**Calibration**”.

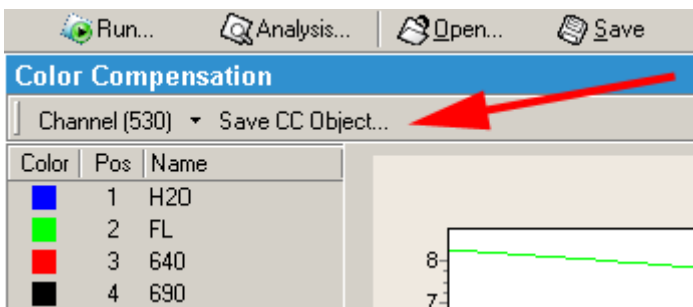


Name the files CC 530-640 or CC 640-690 as described above.

5.7 Saving CC for LightCycler® 1.5 / 2.0 using Software Version 4.05 or 4.1



At the end of the run create a New Analysis selecting “Color Compensation”



Click on “Save CC Object”
Name the files: YYYY-MM-DD followed by CC 530-640, CC 640-690 or CC Multicolor as described above.

7. Material Safety Data Sheet (MSDS)

Product is not hazardous, not toxic, not IATA restricted. Product is not from human, animal or plant origin. Product contains synthetic oligonucleotide primers and probes. For laboratory use only.

According to OSHA 29CFR1910.1200, Commonwealth of Australia [NOHSC:1005, 1008 (1999)] and the European Union Directives 67/548/EC and 1999/45/EC any products which not contain more than 1% of a component classified as hazardous or classified as carcinogenic do not require a Material Safety Data Sheet.

8. Version History

Notes in red mark events require to change procedures
Blue : Improvements, change in composition

V070117	Original Manual ColorCompensation 530-640
V090924	Release version 530-640-690 Introduction of the LC690 dye.
V110104	Editorial Changes
V120908	Calibration of the Z480 Instrument (open channel software) ColorCompensation CE-IVD kits (IVD-use), naming of CC files, Editorial Changes
V130113	New version with 7 instead of 4 different reference dyes to run more versatile Color Compensation files, including a specific CC for the LightMix [®] Kit 40-0297 (replaces Color Compensation Kit 40-0319) and universal Color Compensation files for multicolor hybridization probe based applications using LightCycler [®] 2.0 and 480 Instruments (substituting the Multicolor Demo Set) as required to run LightMix [®] Kit 40-0356. Chapters have been rearranged, editorial changes.
V130412	Clarification for generation of MultiColor Compensation files LightCycler [®] 2.0 and 480 systems. Chapters rearranged, editorial changes.
V131018	Correction of "Final volume" in 4.6 (LC480 II) and editorial changes. Definition of detection format for LightCycler [®] 480 first version. Clarification on Sample Editor and Mix Volumes for capillary instruments.

For technical questions and assistance please contact design@tib-molbiol.de

Roche SAP order n° 05997704001

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Color Compensation can be a prerequisite to run CE-IVD marked LightMix[®] kits (see Instruction For Use). This kit is no CE-IVD device.

Produced by: TIB MOLBIOL Syntheselabor GmbH, Eresburgstrasse 22-23, 12103 Berlin, Germany, www.tib-molbiol.com

