RIDA[®]GENE Color Compensation Kit II

Art. No.: PG0002 3 reactions

-20 °C



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1. Intended use

RIDA[®]GENE Color Compensation Kit II is intended for generating a Color Compensation File for duplex and triplex real-time PCR experiments on the LightCycler [®] 1.5 and 2.0. The generated Color Compensation File can be applied to analyze multiplex real-time PCR experiments of RIDA[®]Gene real-time PCR kits on the LightCycler[®] 1.5 and 2.0.

2. Explanation of the test

In a multiplex real-time PCR, the wavelengths of light emitted by the reporter dyes may overlap, causing one channel to pick up signals (crosstalk) from a dye measured by another channel. This crosstalk of fluorescence signal can result in incorrect data unless a correction is made by using a Color Compensation File. Color compensation is used to subtract fluorescence crosstalk from a reporter dye into inappropriate channels outside of its dominant emission channel.

3. Kit components

Kit Code	Reagent	Volume	Lid color
1	Blank	1x 80 µl	white
2	Dye 1	1x 80 µl	green
3	Dye 2	1x 80 µl	yellow
4	Dye 3	1x 80 µl	red

Tab.1: Reagents provided (Reagents provided in the kit are sufficient for 3 Color Compensation experiments)

4. Storage instructions

- Protect RIDA[®]GENE Color Compensation Kit II from light and store at -20 °C.
- RIDA[®]GENE Color Compensation Kit II can be used until the expiration date printed on the label
- -After expiry the quality guarantee is no longer valid.
- Carefully thaw RIDA[®]GENE Color Compensation Kit II reagents before using (e.g. in a refrigerator at 2 - 8 °C).
- During Color Compensation preparation all the reagents should be stored cold in an appropriate way (2 - 8 °C).

5. Additional equipment and materials required

- LightCyler[®] 1.5 or 2.0 (Roche)
- Real-time PCR consumables (LightCycler[®] Capillaries)
- Pipettes (0.5 20 μl, 20 200 μl, 100 1000 μl)
- Filter tips

6. Precautions for users

- This test must only be performed by laboratory personnel trained in molecular biology methods.
- Strictly follow the working instructions.
- When handling samples, wear disposable gloves. After finishing the test, wash your hands.
- Do not smoke, eat or drink in areas where samples or test reagents are being used.
- Do not use the kit after the expiration date.

7. Protocol for creating a Color Compensation File on the LightCycler[®] 1.5

7.1 Preparation of the Color Compensation

For a color compensation experiment it is necessary to pipette 20 μ l of each dye and also of the background (blank) into a LightCycler[®] capillary. Add 1 μ l of Dye 1 to the pre-pipetted 20 μ l of Dye 3 in LightCycler[®] capillary 4 (see Tab.2).

Thaw, mix gently and centrifuge briefly the reagents before use. Keep reagents appropriately cold during working step (2 - 8 °C).

Kit Codo	Descent	Volume per reaction						
KIT CODE	Reagent	Capillary Position 1	CapillaryCapillaryPosition 1Position 2		Capillary Position 4			
1	Blank	20 µl	-	-	-			
2	Dye 1	-	20 µl	-	1 µl			
3	Dye 2	-	-	20 µl	-			
4	Dye 3	-	-	-	20 µl			

Tab.2: Preparation of the Color Compensation

Cap and briefly spin down the LightCycler[®] capillaries after pipetting the reagents.

Note: Do not change the order of the LightCycler[®] capillaries on the LightCycler[®]-Carousel.

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7.2 LightCyler[®] 1.5 Set-up

1. Open the LightCycler[®] 1.5 software. Click the "**Run**" button to open a new "**LighCycler Experiment**".



2. The following window opens.

Roche LightCycler Run 5.32 -	noname.exp							_ _ _ ×
<u>File Edit Tools Options Help</u>								
CCC File Date Use Color Compensation	Choose CCC File	Display F1 V Mode 1 V			User labor Experiment noname.exp			
New Experiment	Cycle Program Data	Analys	is Mode <mark>None</mark> Quantif Melting	ication Curves				*
Save Experiment File	Temperature Targ	ets Imperature (*C) Ibation Time (hrs:mir Temperature T Seconda	:sec) any Target Temp ep Size (°C) Step Delay Acquis	C / s) erature (°C) (cycles) ition Mode	Add Cycle Program Simu 100 - T[°C 90 - 80 -	Persove	Import	Move Up
Edit Samples Real Time Fluorimeter RUN					70 - 60 - 50 - 40 - 30 -			
EXIT					0 1 2 Simulation	3 4 5 Experimenta	6 7 8 al Notes Edi	9 10 t Exp. Notes
Overview 100- T[*C] 80- 60- 40-								
20-1 1	2:00 3:00	4:00	5:00	6:00	1 7:00	8:00	9:00	t(min] 10:00

3. Program the LightCycler[®] according to the real-time PCR profile (see Tab.3). Click the "**Add**" button to program the 4 protocol steps.

	Tab.3: LightCy	cler [®] real-time	PCR	profile
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		Temperature targets					
Program	Cycles / Analysis Mode	Target [°C]	Acquisition Mode	Hold [hh:mm:ss]	Ramp rate [°c/s]		
Initial Denat.	1 / none	95	none	00:00:05	20		
Cycling	5 / Quantification	95	none	00:00:05	20		
		60	single	00:00:05	20		
	4 / Oalar	95	none	00:00:05	20		
TM-Analyse	1 / Color Compensation	50	none	00:00:05	20		
	Compensation	70	continuous		0.2 (Acquisitions per $^{\circ}C = 1$)		
Cooling 40	1 / none	40	none	00:00:10	20		

Note: Ensure the correct setting of the number of "**Cycles**" and of the "**Analysis Mode**"



4. The final screen should look like the screenshot below.



5. Place the LightCycler[®]-Carousel with the capillaries in the LightCycler[®] 1.5. Click the "**Run**" button and save the experiment in the proper folder.

The following window will open to program the PCR layout. Enter in the "**Maximum Position**" dialog field the number of capillaries. Click the "**Done**" button to start the experiment.

<u>File E</u> i	LC Carousel		Please edit the san	nple	data for this ru	un.			
#	Sample Name	Type Replicate (of	oncentration Notes	#	Sample Name	Туре	Replicate of	Concentration	Notes
1	Sample 1	Unknown 🖡 0 🖡	0,00E+0	17	Sample 17	Unknow	n 🛊 🛛 💺	0,00E+0	
2	Sample 2	Unknown 🗍 0 🖡	0,00E+0	18	Sample 16	Unknow	n 🛊 🛛 🌲	0,00E+0	
3	Sample 3	Unknown 🗘 0 🕏	0,00E+0	19	Sample 19	Uni now	n 🕴 Ú 🕯	Ú.ÚÚĚ+Ú	
4	Sample 4	Unknown 🕴 0 🛊	0,00E+0	20	Gample 20	Unknow	n 🛊 Օ 💐	0,000 +0	
5	Sampla %	Uni nown 🔹 🖞 🛊	0.008+0	21	Gample 21	Linknow	n 🛊 Օ 💐	0,000+0	
6	Sample 6	Uni nown 🌲 🖞 🕯	Ú.ÚÚŘ.+Ú	22	Sample 22	Linknow	n 🛊 🕠 🐫	0,00E+0	
7	Gample 7	Linknown 🛊 🛛 🗘	0,00£+0	23	Sample 23	Unknow	n 🛊 🌼	0,000+0	j
8	Sanola S	Uni newn 🏮 🖞 🛊	Ú.ÚŒ+Ú	24	Sample 24	Uni now	n 🕴 🖞 🛊	Ú.ÚÚĚ+Ú	
9	Gample 9	Unknown 🗘 🛛 🗘	0,00£+0	25	Sample 25	Unknow	n 🛊 🎊	0,00E+0	
10	Sampla 10	Uni nown 🕴 🗴 🗘	Ú.ÚÚĚ+Ú	26	Semple 24	Uni now	n 🖏 🖞	0.008+0	
11	Cample 11	Linknown 🗘 🕺	0,00£+0	27	Sample 22	Uni now	n 🗘 Ú 🕯	Ú.ÚÚĚ+Ú	
12	Sample 12	Unknown 🛊 🛛 🌲	0,00£+0	28	Sample 28	Unknow	n 🛊 🔿 💐	0,00E+0	
13	Sample 13	Unknown 🗘 🛛 🕯	0,00£+0	29	Sample 29	Unknow	n 🛊 🕠 🗳	0,00E+0	
14	Gaople 14	Unknown 🛊 0 🖡	0,00£+0	30	Sample 30	Uni now	n 🗘 🗘	0.00E+0	
15	Sample 15	Unknown 🗘 0 🗘	0,00£+0	31	Garopie àt	Unknow	n 🛊 🛛 💺	0,00E+0	
16	Sample 16	Unknown 🗘 0 🗘	0,00£+0	32	Sample 32	Uni now	n 🗘 🖞	Ú.ÚÚĚ+Ú	
Se	ek Temperature 30	Maximum Position	4 Done	Ente	r Samples later Clea	ar Sample List	: Defaul	t Sample List	Concentration Units



1. After the experiment ends the following window opens.

2. Click "Select a Program" button to select segment 3 (TM-Analyse) for Color Compensation.

Ensure that the green cursors are placed around the start and end of the melting curve segment. Then select "**Calibration**" from the Color Compensation pull-down menu.



Save the Color Compensation File. The created Color Compensation File can now be applied for LightCycler[®] experiments.

To apply Color Compensation for a multiplex PCR experiment click the "**Select CC Data**" button to select and import the appropriate Color Compensation File. Select the appropriate channel (e.g. F1 or F2) and click the "**Quantification**" button to analyze the experiment.

Note: The Color Compensation File is specific for every LightCycler[®] instrument. A new Color Compensation File has to be created if the LightCyler[®] instrument is changed or after the optical system has been repaired.

8. Protocol for creating a Color Compensation File on the LightCycler[®] 2.0

8.1. Preparation of the Color Compensation

For a color compensation experiment it is necessary to pipette 20 µl of each dye and also of the background (blank) into a LightCycler[®] capillary. (see Tab.4).

Thaw, mix gently and centrifuge briefly the reagent before using. Keep reagents appropriately cold during working step (2 - 8 °C).

Kit Code	Reagent	Volume per reaction	Capillary position
1	Blank	20 µl	1
2	Dye 1	20 µl	2
3	Dye 2	20 µl	3
4	Dye 3	20 µl	4

Tab 4 [.]	Preparation	of the (Color Com	pensation
т upт.	ricparation			pensation

Cap and briefly spin down the LightCycler[®] capillaries after pipetting the reagents.

Note: Do not change the order of the LightCycler[®] capillaries on the LightCycler[®] Carousel.

8.2 LightCyler[®] 2.0 Set-up

1. Open the LightCycler[®] 2.0 software. Click the "**File**" menu, and select "**New**" to open the "**Create New Object**" dialog box. Double-click the "**LightCycler Experiment**" icon to open a new LightCycler experiment file.

ntCycler Software (bu	ild 4.1.1.21)							_ 8 ×
File Edit View Tools Wind	low Help							
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				Experiment	Instrument			
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			Query	User	Folder			
						1		
					ок	Cancel		

2. The following window opens.



3. Program the the LightCycler[®] according to the Set-up (see Tab. 5) and the protocol steps of the real-time PCR profile (see Tab. 6)

Parameter	Setting
Default Channel	530
Seek Temperature	70 °C
Max. Seek Pos.	4
Instrument Type	6 Ch.
Capillary Size	20µI

Tab.5: LightCycler[®] Set-up

Tab.6: LightCycler[®] real-time PCR profile

		Temperature targets					
Program	Cycles / Analysis Mode	Target [°C]	Acquisition Mode	Hold [hh:mm:ss]	Ramp rate [°c/s]		
Initial Denat.	1 / none	95	none	00:00:05	20		
Cualing	5 / Quantification	95	none	00:00:05	20		
Cycling		60	single	00:00:05	20		
	1 / Color	95	none	00:00:05	20		
TM-Analyse	Compensation	50	none	00:00:05	20		
	Compensation	70	continuous		0.2 (Acquisitions per $^{\circ}C = 1$)		
Cooling 40	1 / none	40	none	00:00:10	20		

Note: Ensure the correct setting of the number of "**Cycles**" and of the "**Analysis Mode**"

<i> </i> LightCycle	Software (build 4.1.1.21) - [New Experiment]	
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] [] [] <u>N</u> ew	la Run 🙋 Analysis 🔗 🛛 pen 🔊 Save 🙆 Beport 🛛 🖓 Template 🍪 Run Macro	
	Run	
172	Start Run End Program + 10 Cycles Color Comp (Off) - LC_15448: 6 Ch. on CDM2 (LC_15448) Options -	
	Programs on me Deta Display Run Notes	
Summary		Programs
	Default Channel: 530 🚺 🛆 Program Name	Cycles Analysis Mode
Bun	Seek Temperature: 70 Program	None
		5 Quantification
	Max. Seek Pos. 4 I Melt	1 Color Compensation
Samples	Instrument Type: 6 Ch. 💌 🚽 Cooling	1 🕄 None
	Capillary Size: 20 µl 👻	



4. The final screen should look like the screenshot below.

5. Click the "**Samples**" icon on the left-hand side of the window. Then click the "**Analysis Type**" menu, and select "**Color Compensation**".



6. Define in the "**Dominant Channel**" dialog fields the dominant channel for the selected dyes for each position (see Tab.7). Select for the reagent "**Blank**" the dominant channel "**Water**". It is not necessary to specify a **"Sample Name**".

Reagent	Detection Channel
Blank	Water
Dye1	530
Dye2	560
Dye3	705



Place the LightCycler[®]-Carousel with the capillaries in the LightCycler[®] 2.0. Click the "**Run**" icon and save the experiment in the proper folder.

8.3 Evaluation and creation of a Color Compensation File

After the experiment ends, click the "Analysis" button to open the "Create New Analysis" dialog box. Under "Other Methods", select "Color Compensation". Then click "OK"

In the "**Color Compensation**" window which appears, click the "**Save CC Object**" button to save the Color Compensation File in the "**CCC**" folder. The created Color Compensation File can now be applied for LightCycler[®] experiments.

To apply Color Compensation for a multiplex PCR experiment open the experiment and click the "**Analysis**" button. Click the "**Color Compensation (Off)**" button, and select the appropriate Color Compensation File. The "**Color Compensation (Off)**" button switches to "**Color Compensation (On)**" to confirm that color compensation is active. The multiplex real-time PCR experiment can now be analyzed.

Note: The Color Compensation File is specific for every LightCycler[®] instrument. A new Color Compensation File has to be created if the LightCyler[®] instrument is changed or after the optical system has been repaired.