

Ordering No. 57-0010

Thermal Cycler Color Compensation Kit

Instructions for Use

Reagents for 10 calibrations

Manufacturer:

Minerva Biolabs GmbH, Koepenicker Strasse 325, 12555 Berlin, Germany

FOR PROFESSIONAL USE ONLY!

Symbols



Lot no.



Order No.



Expiry date



Store at



Contains reagents for 25, 100 or 250 tests



Manufacturer



GENERAL NOTES

The Color Compensation reagents are designed for the generation of color compensation objects on the LightCycler®480 system, type I and II, to allow proper interpretation of multiplex experiments. Subsequently, the generated file may be used for analyzing runs with our multiplex PCR-kit Onar®MRSa.

As real-time cyclers may vary in their fluorescence detection sensitivity, color compensation experiments must be performed on each specific multiplex-PCR instrument.

In the case of overcompensation or remaining crosstalk it is recommended to adjust the Calibration dye concentration used for performing the color compensation experiment to result in an equal level of (absolute) fluorescence signal.

EXPLANATION OF THE TEST

To generate a color compensation object, the labeled Calibration dyes are used in an initial color compensation run. The Calibration dyes contain labeled oligonucleotides for the determination of crosstalk corresponding to each individual dye being used in the multiplex assay, and a blank reaction buffer without dye for the determination of background fluorescence of the buffer.

REAGENTS

Each kit contains reagents for 10 calibrations (25 µl each). The expiry date of the unopened package is marked on the package label. The kit components are stored until use at room temperature. Please handle solutions protected from light to avoid photobleaching. The lot specific quality control certificate (Certificate of Analysis) can be downloaded from our website (www.minerva-biolabs.com).

Kit Component	Quantity	Cap Color
Calibration Dye 1	1 x lyophilized	Green
Calibration Dye 2	1 x lyophilized	Yellow
Calibration Dye 3	1 x lyophilized	Orange
Rehydration Buffer	1 x 0.8 ml	Black

NEEDED, BUT NOT INCLUDED IN THE KIT

The kit contains the reagents for the calibration. General industrial supplies and reagents, usually available in PCR laboratories are not included:

- LightCycler® 480 Instrument I or II (Roche Diagnostics)
- PCR plates and sealing compatible with the LightCycler® 480 system
- Microcentrifuge for 2 ml standard tubes
- Pipettes and pipette tips
- Recommended: centrifuge for multiwell plates

PRECAUTIONS

For in vitro diagnostic use. This kit should be used only by trained persons. This kit does not contain hazardous substances and may be disposed of according to local regulations.

CALIBRATION PROCEDURE

All reagents and samples must be equilibrated to +2 to +8 °C prior use.

1. Rehydration of the reagents

After reconstitution, the reagents should be stored at below -18 °C. Repeated freezing and thawing should be avoided and reagents stored in aliquots if necessary.

1.	Calibration Dye 1 Calibration Dye 2 Calibration Dye 3	green cap yellow cap orange cap	Spin for 5 sec at maximum speed of the mini centrifuge
2.	Calibration Dye 1 Calibration Dye 2 Calibration Dye 3	green cap yellow cap orange cap	Add 260 µl rehydration buffer (black cap)
3.	Calibration Dye 1 Calibration Dye 2 Calibration Dye 3	green cap yellow cap orange cap	Incubate 5 min at room temperature
4.	Calibration Dye 1 Calibration Dye 2 Calibration Dye 3	green cap yellow cap orange cap	Vortex DNA briefly and spin for 5 sec

2. Loading the test tubes

Preparation of the reagents and loading of the PCR tubes should not take more than 45 minutes to avoid a reduction in the fluorescent signal. Each of these color compensation reactions should be run in replicates of 4.

1. For each Calibration dye dispense 25 µl into each of 4 wells in an optical PCR reaction plate
Dispense 25 µl of the Rehydration Buffer for a blank control
2. Seal the plate with an optical sealing foil
3. Centrifuge at 1500 x g for 30 s
4. Transfer the plate in the qPCR instrument
5. Start the Color Compensation run/program
6. Open the "Sample Editor", click the "Color Comb" tab and indicate the dominant channel for each color compensation sample. For the blank samples containing no Calibration dye choose "water" as the dominant channel

4. Starting the reaction

1. Load the cycler, check each PCR tube and the cycler lid for tight fit
2. Program the qPCR cycler or check stored temperature profiles. Please refer to the LightCycler® Instrument Operator's Manual for detailed device handling and programming. Programs for additional cyclers might be available on request.
3. Start the program and data reading.

	Temperature settings			Cooling
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	65	40
Hold hh:mm:ss	00:00:00	00:00:30		00:00:30
Ramp Rate °C/s	4.8	2.5	0.14	2.2
Acquisition Mode	None	None	None	None

5. Result reading with LightCycler®

After the Color Compensation run the data must be converted into a CC object and saved separately. Select "Color Compensation" from the Analysis Menu, click "Calculate" and then click "Save CC Object". The stored Color Compensation Object can be used for the analysis of runs performed with the Onar®MRSa Detection Kit.

Component	Cap Color	dominant channel (Excitation – Detection)	
		Setting LC 480 I	Setting LC 480 II
Rehydration Buffer for the determination of background fluorescence	black cap	"water"	"water"
Calibration Dye 1 (for "mecA channel") for the determination of crosstalk of the dedicated fluorophore	green cap	483 – 533 nm	465 – 510 nm
Calibration Dye 2 (for "internal control channel") for the determination of crosstalk of the dedicated fluorophore	yellow cap	523 – 568 nm	533 – 580 nm
Calibration Dye 3 (for "femA channel") for the determination of crosstalk of the dedicated fluorophore	orange cap	558 – 610 nm	533 – 610 nm

NOTES ON THE PROCEDURE

1. This leaflet must be widely understood for a successful use of the Thermal Cycler Color Compensation kit. The reagents supplied should not be mixed with reagents from different lots and used as an integral unit. The reagents of the kit should not be used beyond its shelf life.
2. Any deviation from the method can affect the results.

APPENDIX

Limited Product Warranty

This warranty limits our liability for replacement of this product. No warranties of any kind, express or implied, including, without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided. Minerva Biolabs shall have no liability for any direct, indirect, consequential, or incidental damages arising out of the use, the results of use, or the inability to use this product.

Trademarks

LightCycler is a registered trademark of a member of the Roche Group. FAM™ and ROX™ are trademarks of Applied Biosystems Corporation or its subsidiaries in the US and certain other countries. Onar is registered trademarks of Minerva Biolabs GmbH.

Related Products

MB Taq DNA Polymerase

53-0050/-0100/-0200/-0250	MB Taq DNA Polymerase (5 U/μl)	50/100/200/250 units
53-1050/-1100/-1200/-1250	MB Taq DNA Polymerase (1 U/μl)	50/100/200/250 units

Clinical Diagnostic Kits for qPCR

20-2025/-2100/-2250	Onar®Mp <i>Mycoplasma pneumoniae</i>	25/100/250 tests
21-2025/-2100/-2250	Onar®Ls <i>Legionella species</i>	25/100/250 tests
21-3025/-3100/-3250	Onar®Lp <i>Legionella pneumophila</i>	25/100/250 tests
22-2025/-2100/-2250	Onar®MRSa Methicillin-resistant <i>S. aureus</i>	25/100/250 tests
23-2025/-2100	Onar®Syphilis <i>Treponema pallidum</i>	25/100 tests
24-2025/-2100/-2250	Onar®Ct <i>Chlamydia trachomatis</i>	25/100/250 tests
25-2025/-2100/-2250	Onar®Pertussis <i>Bordetella pertussis/parapertussis</i>	25/100/250 tests

Quantification Standards, 100 μl each, 1x10⁶ genomes/μl

52-0101	<i>Legionella pneumophila</i> DNA Standard
52-0119	<i>Mycoplasma pneumoniae</i> DNA Standard

Genomic DNA Extracts, 100 μl each, +/- 10 ng / 100 μl

51-0566	<i>Streptococcus pneumoniae</i> , DSMZ 20566
51-5571	<i>Bordetella pertussis</i> , DSMZ 5571
51-3415	<i>Bordetella parapertussis</i> , DSMZ 13415
51-0177	<i>Ureaplasma urealyticum</i> , NC 10177
51-0111	<i>Mycoplasma hominis</i> , NC 010111
51-0112	<i>Mycoplasma orale</i> , NC 010112
51-0117	<i>Mycoplasma fermentans</i> PG19, NC 010117
51-0119	<i>Mycoplasma pneumoniae</i> , NC 010119
51-0129	<i>Mycoplasma arginini</i> , NC 010129
51-0162	<i>Mycoplasma arthritidis</i> , NC 010162
51-0195	<i>Mycoplasma genitalium</i> , NC 010195
51-1746	<i>Mycoplasma penetrans</i> , NC 11746

DNA Remover™

15-2025	DNA Decontamination Reagent, spray bottle	250 ml
15-2200	DNA Decontamination Reagent, refill bottles	4 x 500 ml

Extraktion Kit

56-1100	MB DNA Extraction Kit	100 extractions
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Last technical revision: November 2010