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Not for use in diagnostic procedures.



LightCycler[®] 480 QC Kit

 **Version: 01**

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Kit for quality control of the LightCycler[®] 480 System and the **cobas**[®] 4800 User Defined Workflow (UDF).

Cat. No. 04 697 804 001 1 kit

for 1 test run on a 96-well block, with 20 µl final volume each
for 2 test runs on a 384-well block, with 10 µl final volume each

Store the kit at –15 to –25°C.

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1. General Information

1.1. Contents

Vial / bottle	Cap	Label	Content
1	colorless	LightCycler® 480 QC Kit, Blank	1 vial
2	green	LightCycler® 480 QC Kit, Melting Mix	1 vial
4	purple	LightCycler® 480 QC Kit, Detection Mix	1 vial
5	red	LightCycler® 480 QC Kit, Reaction Mix	1 vial

i The Vial 3 (Fluor Standard) available in previous versions of the kit is no longer needed for the **gCAT Check Report Tool** software and was removed from the kit.

1.2. Storage and Stability

Storage Conditions (Product)

The kit is shipped on dry ice.

When stored at –15 to –25°C, the kit is stable through the expiry date printed on the label.

Vial / bottle	Cap	Label	Storage
1	colorless	Blank	Store at –15 to –25°C.
2	green	Melting Mix	Store at –15 to –25°C.
4	purple	Detection Mix	⚠ Keep protected from light.
5	red	Reaction Mix	

1.3. Additional Equipment and Reagent required

Standard laboratory equipment

- LightCycler® 480 Instrument, 96- or 384-well, or **cobas z** 480 Analyzer operated with the **cobas**® 4800 UDF (User Defined Workflow) Software*
- LightCycler® 480 Multiwell Plate 96 or 384, white with LightCycler® 480 Sealing Foil, or AD-plate 0.3 mL (multiwell) and Sealing Film*
- Standard swinging-bucket centrifuge containing a rotor for multiwell plates with suitable adaptors
- Nuclease-free, aerosol-resistant pipette tips
- Pipettes
- Autoclaved 1.5 ml reaction tubes
- QC Macro depending on instrument version and block type

1.4. Application

The LightCycler® 480 QC Kit contains all reagents for a test run to verify the correct function of a LightCycler® 480 Instrument, or **cobas z** 480 Analyzer operated with the User Defined Workflow (UDF).

- The test comprises a Melting Mix for precise determination of temperature accuracy and several replicates of a PCR composition for analysis of instrument effects on PCR results.
- The automated evaluation of raw data is done by your Roche representative using a dedicated software.
- The analysis results in a PASS/FAIL decision provided as a signed QC certificate. In case of FAIL, the detailed report contains hints for further investigation of a potential instrument malfunction.

1.5. Preparation Time

Procedure	Time [min]
PCR Setup	20
QC run	70
Total preparation time	90

2. How to Use this Product

2.1. Before you Begin

General Considerations

Precautions

- Keep the Melting Mix (Vial 2), Detection Mix (Vial 4), and Reaction Mix (Vial 5) protected from light.
- All reagents must be completely thawed and mixed well before use, for example, using a vortex for 5 seconds, or by inverting the tube 15 times.
- Do not touch the surface of the microwell plate. Use the LightCycler® 480 Multiwell Plate 96, or 384, white, or the **cobas**® 4800 System AD-plate 0.3 mL and Sealing Film (use only specified consumables to ensure valid test results).
- Always wear gloves when handling the PCR mixes and plates.
- Use only swinging-bucket centrifuges. Other types of centrifuges may lead to failure of the QC test.

2.2. Protocols

Experimental overview

To prevent input errors, the Roche pre-defined protocol files for each instrument (QC macro .ixo) must be used. A zipped file containing the QC Macros will be provided by your Roche representative. When one LightCycler® 480 Instrument is operated with a 96-well and a 384-well block, you must run two separate tests for the two block types.

Software version	Instrument	Block type	Macro name
LightCycler® 480 Software v1.5.1 and higher	LightCycler® 480 I	96	Macro_I_v1_5_QC1_96.ix0
		384	Macro_I_v1_5_QC1_384.ix0
	LightCycler® 480 II	96	Macro_II_v1_5_QC1_96.ix0
		384	Macro_II_v1_5_QC1_384.ix0
cobas ® 4800 UDF Software v2.1	cobas ® z 480	96	Macro_z480_UDF_QC1.ix0

- 1 Unzip the folder and select the macro corresponding to the instrument version and block type in use.
- 2 Import the appropriate macro.ix0 file into the LightCycler® 480 Software, or into the **cobas**® 4800 UDF Software and save it in the “Macros” folder of the database.

Preparation of reagent

- 1 Thaw the 4 reagent vials from the LightCycler® 480 QC Kit and mix them well, for example, using a vortex for 5 seconds.
- 2 Centrifuge the vials for 30 seconds at $1,500 \times g$.
- 3 To a separate 1.5 ml reaction tube, add the components listed below.

i The required volume of the PCR Mix solution depends on the block type (96-well or 384-well block).

Reagent	Vial cap color	Volumes for 96-well block [µl]	Volumes for 384-well block [µl]
Blank (Vial 1)	colorless	688	344
Detection Mix (Vial 4)	purple	86	43
Reaction Mix (Vial 5)	red	86	43
Total Volume	–	860 µl	430 µl

- 4 Mix the PCR Mix solution gently by tipping the tube 15 times.
- 5 Centrifuge the tube for 10 seconds at $2,500 \times g$.
- 6 Pipette the Melting Mix (Vial 2), and the freshly prepared PCR Mix solution into the respective plate wells indicated in the pipetting schemes in Figures 1 and 2.

– Dispense 20 µl using 96-well plates.

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Melting Mix

PCR Mix

Fig. 1: Pipetting scheme for a 96-well plate. Positions are indicated for Melting Mix (Vial 2) in green, and PCR Mix solution in red, respectively.

2. How to Use this Product

– Dispense 10 µl using 384-well plates.

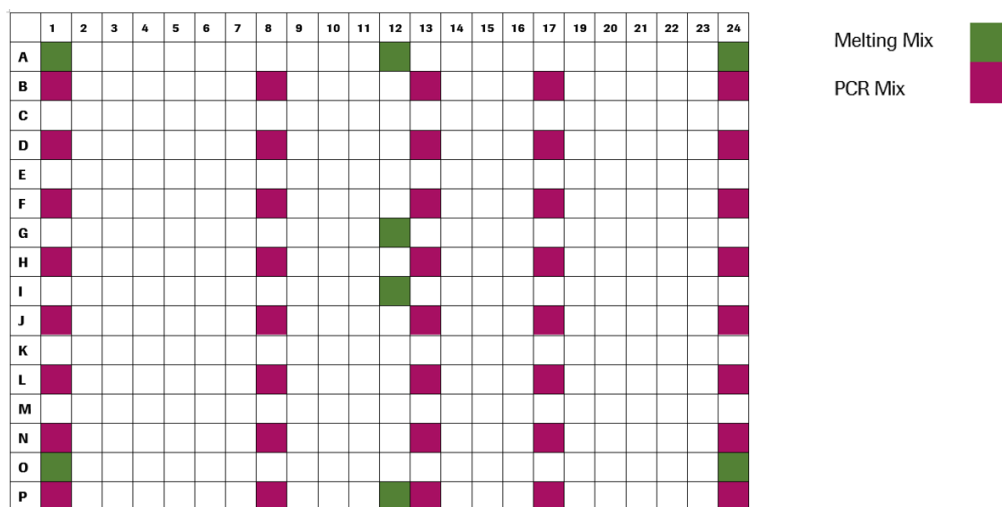


Fig. 2: Pipetting scheme for the LightCycler® 480 Multiwell Plate 384. Positions are indicated for Melting Mix (Vial 2) in green, and PCR Mix solution in red, respectively.

- 7 Seal the multiwell plate with the respective sealing foil.
 - ⚠ **Make sure that the peripheral wells are completely sealed.**
- 8 Place the multiwell plate in a standard swinging-bucket centrifuge that contains a rotor for multiwell plates with suitable adaptors, and balance it with a suitable counterweight, such as another multiwell plate.
 - Centrifuge for 2 minutes at 1,500 × g.
- 9 Load the correctly sealed multiwell plate into the instrument.
- 10 Start the PCR run, by clicking on **New Experiment from Macro** on the Overview page, and by selecting the corresponding QC Macro.
 - Save the experiment under the name QC.dd-mmm-yyyy.serial number, for example, QC.12-APR-2017.6372.
 - Enter the 29 digit lot-specific number from the label on the LightCycler® 480 QC Kit container, in the **Run Notes** section of the experiment.

QC test evaluation

The automated analysis of the raw data of a QC test run is performed by your Roche representative using a dedicated software.

- 1 Open the LightCycler® 480 Software, or the **cobas**® 4800 UDF Software on the Control Unit.

- 2 Search in the Experiment folder for the QC test and export the experiment as .ixo file.

- 3 Send this QC run.ixi file (zipped) via mail to your Roche representative.
 - Make sure, the 29-digit lot-specific number on the label of the LightCycler® 480 QC Kit container is included, as this number is needed to analyze the generated data.
 An example is shown below:



- 4 Your Roche representative will perform the QC run analysis and provide the signed QC Certificate when the system performance check passes all criteria settings. In case of failure, a QC report and further information or instructions will be sent.

3. Troubleshooting

Observation	Recommendation
QC run abort.	Prepare a plate with fresh reagents.

4. Additional Information on this Product









4.1. Quality Control

The LightCycler® 480 QC Kit is function tested using the LightCycler® 480 Instrument.

5. Supplementary Information

5.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
 Information Note: Additional information about the current topic or procedure.	
 Important Note: Information critical to the success of the current procedure or use of the product.	
   etc.	Stages in a process that usually occur in the order listed.
   etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

5.2. Changes to previous version

First version.

5.3. Ordering Information

Roche offers a large selection of reagents and systems for life science research. For a full overview of related products and manuals, please visit and bookmark our homepage lifescience.roche.com.

Product	Pack Size	Cat. No.
Accessories general (hardware)		
LightCycler® 480 Block Kit 384 Silver	1 block kit	05 015 197 001
LightCycler® 480 Block Kit 96 Silver	1 block kit	05 015 219 001
Consumables		
LightCycler® 480 Multiwell Plate 96, white	5 x 10 plates	04 729 692 001
LightCycler® 480 Multiwell Plate 384, white	5 x 10 plates	04 729 749 001
LightCycler® 480 Sealing Foil	50 foils	04 729 757 001
Instruments		
LightCycler® 480 Instrument II	1 instrument	05 015 278 001
	1 instrument	05 015 243 001
cobas z 480 analyzer		05 200 881 001
		05 200 881 692

5.4. Trademarks

LIGHTCYCLER, COBAS Z and COBAS are trademarks of Roche.
All other product names and trademarks are the property of their respective owners.

5.5. License Disclaimer

For additional documentation such as certificates and safety data sheets, please visit:
documentation.roche.com.

5.6. Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

5.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

5.8. Contact and Support

If you have questions or experience problems with this or any Roche product for Life Science, please contact our Technical Support staff. Our scientists are committed to providing rapid and effective help.
Please also contact us if you have suggestions for enhancing Roche product performance or using our products in new or specialized ways. Such customer information has repeatedly proven invaluable to the research community worldwide.

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support** Site.

Visit documentation.roche.com, to download or request copies of the following Materials:

- Instructions for Use
- Safety Data Sheets
- Certificates of Analysis
- Information Material

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