

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



LightCycler[®] PCR QC Kit

 **Version 03**

Content version: November 2012

Kit for Quality Control of the LightCycler[®] 96 System

Cat. No. 06 746 381 001

Kit for 1 test run



-  Store the kit at -15 to -25°C
-  Store vial 3 protected from light!

Table of Contents

1.	What this Product Does	3
	Introduction	3
	Number of Tests	3
	Kit Contents	3
	Storage and Stability	3
	Additional Equipment Required	4
	Assay Time	4
2.	How to Use this Product	5
2.1	Before You Begin	5
2.2	Preparation of PCR Mix	5
2.3	Workflow	6
2.4	Experimental Protocol	7
2.5	Quality Control	7
3.	Results	8
4.	Troubleshooting	9
5.	Supplementary Information	10
5.1	Conventions	10
	Text Conventions	10
	Symbols	10
5.2	Changes to Previous Version	10
5.3	Ordering Information	11
5.4	Disclaimer of License	11
5.5	Trademarks	11
5.6	Regulatory Disclaimer	11

1. What this Product Does

Introduction

The LightCycler® PCR QC Kit contains all reagents for a test run to verify the correct function of a LightCycler® 96 Instrument. The SYBR Green I detection format is used to perform amplification and melting curve analysis. The test is comprised of 96 reactions to determine temperature accuracy across the entire block by calculating T_m values. Potential instrument effects on PCR results using C_q values are also measured. In addition, there is a check for fluorescence intensity by precisely monitoring the initial fluorescence between cycles 1 to 17.

The QC kit uses the FastStart Essential DNA Green Master, a ready-to-use reaction mix for hot start PCR in the SYBR Green I detection format. This master mix includes FastStart Taq Polymerase, a chemically modified thermostable recombinant Taq DNA Polymerase, showing no activity up to +75°C.

This recombinant enzyme is active only at high temperatures at which primers can no longer bind nonspecifically. The enzyme is completely activated by removal of blocking groups in a single pre-incubation step (+95°C, 10 min) before PCR cycling begins. Activation does not require the extra handling steps typical of other hot start techniques.

Number of Tests

The kit is designed for one test run with 96 reactions in a final volume of 10 µl each.

Kit Contents

Vial	Label	Contents/Function	Storage
1	DNA template 15 ng/µl	Human genomic DNA	-15 to -25°C
2	Primer Mix, 10× conc.	β-Globin primer pair mix	-15 to -25°C
3	PCR master, 2× conc.	FastStart Essential DNA Green Master	-15 to -25°C
4	H ₂ O, PCR grade	Water, PCR grade	-15 to -25°C

Storage and Stability

The kit is shipped on dry ice. Store the kit at -15 to -25°C in the dark until the expiration date printed on the label.

 Keep the PCR Master (vial 3) away from light.

What this Product Does

Additional Equipment Required

Equipment	Cat. No.
LightCycler® 480 Multiwell Plate 96, white (with sealing foils)	04 729 692 001
LightCycler® 480 Sealing Foil	04 729 757 001
LightCycler® 96 Instrument	05 815 916 001

- Standard swing-bucket centrifuge containing a rotor for multiwell plates with a suitable adaptor
- Nuclease-free, aerosol-resistant pipette tips
- Pipettes and multichannel pipettes with disposable, positive-displacement tips
- Autoclaved reaction tubes for preparing master mix

Assay Time

Procedure	Time
PCR Setup	20 min thawing reagents and pipetting
LightCycler® 96 QC run	1 h 25 min
Total assay time	1 h 45 min

2. How to Use this Product

2.1 Before You Begin

- Protect the PCR Master mix (vial 3) from intense light.
- Do not touch the surface of the microwell plate. Use the LightCycler® 480 Multiwell Plate 96, white. Always wear gloves when handling these plates.

2.2 Preparation of PCR Mix

Reaction mixture	1 × (μl)	final conc.	115 × (μl)
PCR Master, 2 × (vial 3)	5.0	1 ×	575
Primer Mix, 10 × (vial 2)	1.0	1 ×	115
Water, PCR grade (vial 4)	2.0	-	230
DNA Template (vial 1)	2.0	30 ng	230
Total	10.0	-	1150

- 1 Mix sufficiently by vortexing for a few seconds and spin down reaction mix briefly.
- 2 When using multichannel pipettes, prepare the reaction mixture with sufficient extra volume. For example, set up enough volume for 115 reactions as indicated above. Dispense 10 μl of this mixture into each of the wells of the LightCycler® 480 Multiwell Plate 96, white. Ensure that the rims of the wells are free of any liquid.
- 3 Seal the Multiwell Plate with a LightCycler® 480 Sealing Foil, making certain that the edge wells are completely sealed.
- 4 Place the Multiwell Plate in a standard swing-bucket centrifuge containing a rotor for multiwell plates using suitable adaptors. Balance it with a suitable counterweight, *e.g.*, another LightCycler® 480 Multiwell Plate 96, white and centrifuge for 2 min at 1,500 × *g*.
- 5 Make sure that there are no air bubbles visible in the wells of the multiwell plate.
- 6 Load the multiwell plate into the LightCycler® 96 Instrument. Before starting the PCR run, type in the Plate Id of the 96 well plate under field "**Plate ID**" using the LightCycler® 96 Software. Use the template "**ExperimentTemplate_QC-Test**" from the LightCycler 96 Application software or downloaded from the LightCycler 96 USB stick.
- 7 Perform the PCR run, including melting curve analysis using the workflow below.

2.3 Workflow

- Open LightCycler® 96 Application Software on your PC.
- In the Startup wizard on the Tab "**Quick Start**", choose the option "**Create new Experiment from Roche Template**" or alternatively click on the menu "**File**" > "**New**" > "**Create new Experiment from Roche Template**".
- In the file **open dialog** select "**ExperimentTemplate_QC-Test**" and open it.
- By doing this, a new experiment, including run and analysis settings, is generated.
- Save this new experiment at a place of your choice and close it.

There are 2 options

a) Using the USB stick

- 1 Insert the USB stick into one of the USB interfaces of your computer and create a folder named "**experiments**" on the first level of the USB stick.
- 2 Save the new experiment created above in this folder with a new name and close it.
- 3 Insert the USB stick into the LightCycler® 96 Instrument.
- 4 Select the experiment in the file list of the Overview tab of the LightCycler® 96 Instrument Software.
- 5 Click the "**Synchronize**" button on the touch screen.
- 6 Load the multiwell plate and start the run.

b) Using the Instrument Manager of the LightCycler® 96 Application Software on your PC:

- 1 In the "**Tools**" menu, choose "**Instrument Manager**" to open the Instrument Manager window.
- 2 Select the appropriate LightCycler® 96 Instrument from the "**Instruments**" table.
- 3 Click on the tab "**Send/Receive Experiments**". Now you find, on the left hand side of the screen, a file explorer for navigating to the directory of the experiment on your PC, and on the right hand side, the file list for the instrument you are using.
- 4 Select the folder on the left hand side, where you previously saved the new experiment created above.
- 5 All files of the selected directory are listed in the middle of the "**Send/Receive Experiments**" tab. Select your new experiment.
- 6 Click on the ">" button, and your file will be loaded onto the instrument.
- 7 Select the experiment in the file list of the "**Overview**" tab on the touch screen of the instrument.
- 8 Load the multiwell plate into the instrument, and start the run.

2.4 Experimental Protocol

Please do not change the protocol from the template.

Program Name		Cycles		
Preincubation		1		
Amplification		45		
Melting		1		
Temperature Targets				
Target [°C]	Acquisition Mode	Hold	Ramp Rate [°C/s]	Acquisitions [per °C]
Preincubation				
95	None	600 s	4.4	–
Amplification				
95	None	10 s	4.4	–
55	None	10 s	2.2	–
72	Single	10 s	4.4	–
Melting				
95	None	5 s	4.4	–
72	None	300 s	2.2	–
97	continuous	1 s	0.03	20

Deliver the run file together with the following information to your Roche representative:

- **Lot Number** of LightCycler® PCR QC Kit used
- **Lot Number** of LightCycler® 96 Multiwell Plate used
- **Lot Number** of the LightCycler® Sealing foil used
- **Positions** which should be excluded from analysis (maximum of 3): These well positions could be where you know that something has occurred that could be a problem and has nothing to do with the instrument, *e.g.*, pipetting errors.
- **QC run file**

Your Roche representative may also provide you with further information or instructions.

2.5 Quality Control

The LightCycler® PCR QC Kit is function tested using the LightCycler® 96 Instrument.

3. Results

The following amplification curves were obtained using the LightCycler[®] PCR QC Kit. The intensity in relative fluorescence units versus cycle number is displayed (see Figure 1).

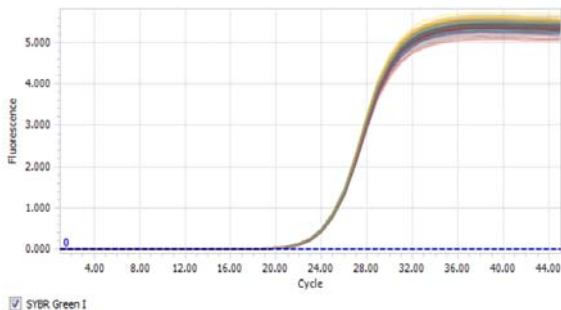


Figure 1: Ninety-six replicates of β -globin primers with human genomic DNA as template from the LightCycler[®] PCR QC Kit.

Specificity of the amplified PCR product was assessed by performing a melting curve analysis on the LightCycler[®] 96 Instrument. By calculating the melting point (T_m) for each well on the 96-well plate, temperature accuracy is determined for the entire thermal block covering 96 wells.

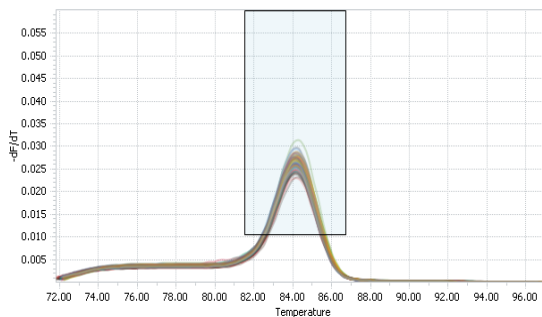


Figure 2: Melting curve analysis of samples amplified using the LightCycler[®] PCR QC Kit.

4. Troubleshooting

	Possible Cause	Recommendation
No amplification detectable	There are pipetting errors or reagents have been omitted.	Email the LightCycler® run data files, if appropriate, to your Roche representative.
Artefacts in melting and/or amplification curves	Sealing foil is not correctly applied to the plate.	Repeat the run.
	Detection dye has bleached.	Make sure the PCR master mix is kept away from light.

5. Supplementary Information



5.1 Conventions

Text Conventions To make information consistent and understandable, the following text conventions are used in this document:

Text Convention	Use
Numbered instructions labeled 1 , 2 etc.	Steps in a procedure that must be performed in the order listed.
Asterisk *	Denotes a product available from Roche Applied Science.

Symbols

Symbols are used in this document to highlight important information:

Symbol	Description
	Information Note: Additional information about the current topic or procedure.
	Important Note: Information critical to the success of the procedure or use of the product.

5.2 Changes to Previous Version

- correction of typing errors in Experimental Protocol

5.3 Ordering Information

Roche Applied Science provides a large selection of reagents and systems for life science research. For a complete overview of related products and manuals, visit and bookmark our home page, www.roche-applied-science.com.

Product	Pack Size	Cat. No.
LightCycler® 480 Multiwell Plate 96, white with sealing foils	5 × 10 plates with sealing foils	04 729 692 001
LightCycler® 480 Sealing Foil	50 foils	04 729 757 001
LightCycler® 96 Instrument	1 instrument plus accessories	05 815 916 001

5.4 Disclaimer of License

Use of this product is covered by US patent claims and corresponding patent claims outside the US. The purchase of this product includes a limited, non-transferable immunity from suit under the foregoing patent claims for using only this amount of product for the purchaser's own internal research. No right under any other patent claim and no right to perform commercial services of any kind, including without limitation reporting the results of purchaser's activities for a fee or other commercial consideration, is conveyed expressly, by implication, or by estoppel. This product is for research use only. Diagnostic uses require a separate license from Roche. Further information on purchasing licenses may be obtained by contacting the Director of Licensing, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, USA.

The technology used for the LightCycler® System is licensed from Idaho Technology Inc., Salt Lake City, UT, USA.

SYBR Green I is manufactured by Molecular Probes, Inc., and is provided under license from Molecular Probes, Inc., for direct research use for PCR, where the dye is present during the PCR.

5.5 Trademarks

LIGHTCYCLER and FASTSTART are trademarks of Roche.

All other product names and trademarks are the property of their respective owners.

5.6 Regulatory Disclaimer

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

Contact and Support

If you have questions or experience problems with this or any Roche Applied Science (RAS) product, please contact our Technical Support staff. Our scientists commit themselves to providing rapid and effective help.

We also want you to contact us if you have suggestions for enhancing RAS product performance or using our products in new or specialized ways. Such customer information has repeatedly proven invaluable to RAS and the worldwide research community.

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

www.roche-applied-science.com/support

To call, write, fax, or email us, visit the Roche Applied Science home page, www.roche-applied-science.com, and select your home country.

Country-specific contact information will be displayed.

On the Roche Applied Science home page select **Printed Materials** to find:

- in-depth Technical Manuals
- Lab FAQs: Protocols and references for life science research
- our quarterly Biochemica Newsletter
- Material Safety Data Sheets
- Pack Inserts and Product Instructions

or to request hard copies of printed materials.

