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

Print Protocol | Print Changes to Previous Version | Print

LightCycler® PCR QC Kit

Version: 04
Content version: October 2016

Kit for Quality Control of the LightCycler® 96 System. The kit is designed for one test run with 96 reactions in a final volume of 10 µl each.

Cat. No. 06 746 381 001 1 kit
96 reactions with 10 µl final volume each

Store the kit at −15 to −25°C until the expiration date printed on the label.

1. General Information

1.1. Contents

Vial	Label	Content
1	LightCycler® PCR QC Kit, DNA template 15 ng/µl	Human genomic DNA
2	LightCycler® PCR QC Kit, Primer Mix, 10x conc.	ß-Globin primer pair mix
3	LightCycler® PCR QC Kit, PCR Master, 2x conc.	FastStart Essential DNA Green Master
4	LightCycler® PCR QC Kit, H^2O, PCR Grade	Water, PCR grade

1.2. Storage and Stability

Storage Conditions (Product)

The kit is shipped on dry ice.

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

1.3. Additional Equipment and Reagents 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

1.4. Application

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 Tm values. Potential instrument effects on PCR results using Cq 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 mastermix 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.

1.5. Preparation 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

General Considerations

- 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. Protocols

Preparation of PCR Mix

Reaction mixture	1× (µl)	final conc.	115× (µl)
PCR Master, 2× (vial 3)	5.0	1x	575
Primer Mix, 10× (vial 2)	1.0	1x	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.

Workflow

- Open LightCycler® 96 Application Software on your PC.
- In the Startup wizard on the Tab "**Quick Start"**, choose the option **"Create newExperiment 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 location of your choice and close it.

Continue with one of the 2 options described below:

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:

7. In the "Tools" menu, choose "Instrument Manager" to open the Instrument Manager window.

8. Select the appropriate LightCycler® 96 Instrument from the "Instruments" table.

9. 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.

10. Select the folder on the left hand side, where you previously saved the new experiment created above.

11. All files of the selected directory are listed in the middle of the "Send/Receive Experiments" tab. Select your new experiment.

12. Click on the ">" button, and your file will be loaded onto the instrument.

13. Select the experiment in the file list of the "Overview" tab on the touch screen of the instrument.

14. Load the multiwell plate into the instrument, and start the run.

Experimental Protocol

Please do not change the protocol from the template.

Run Editor				
Detection Format			Reaction Volume (µl)	
SYBR Green I			20	
Programs				
	Temp. [°C]	Ramp [°C/s]	Duration [s]	Acquisition Mode
Preincubation	95	4.4	600	None
3-Step Amplification	No.of Cycles: 45			
	95	4.4	10	None
	55	2.2	10	None
	72	4.4	10	Single
Melting	95	4.4	5	None
	72	2.2	300	None
	97	0.03	1	Continuous; 20 Readings / °C

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.

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 Fig. 1).

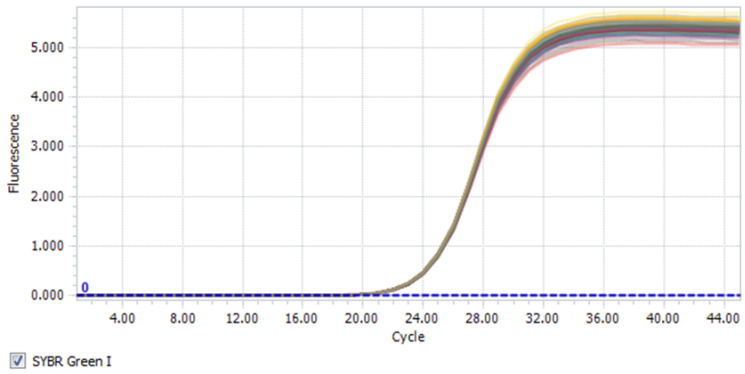

Fig. 1: Ninety-six replicates of ß-globin primers with human genomic DNA as template from the LightCycler® PCR QC Kit.

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

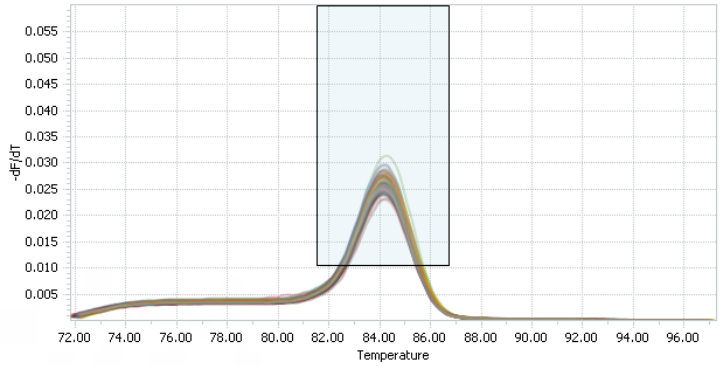

Fig. 2: Melting curve analysis of samples amplified using the LightCycler® PCR QC Kit.

4. Troubleshooting

Observation	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.
Artefacts in melting and/or amplification curves	Detection dye has bleached.	Make sure the PCR master mix is kept away from light.

5. Additional Information on this Product

5.1. Quality Control

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

6. Supplementary Information

6.1. 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.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

6.2. Changes to previous version

Layout changes.
Editorial changes.

6.3. Ordering Information

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

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

6.4. Trademarks

FASTSTART and LIGHTCYCLER are trademarks of Roche.
SYBR is a trademark of Thermo Fisher Scientific Inc..
All third party product names and trademarks are the property of their respective owners.

6.5. License Disclaimer

For patent license limitations for individual products please refer to: http://technical-support.roche.com.

6.6. Regulatory Disclaimer

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

6.7. Safety Data Sheet

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

6.8. 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 **lifescience.roche.com**, to download or request copies of the following **Materials**:
- Instructions for Use
- Safety Data Sheets
- Certificates of Analysis
- Information Material

To call, write, fax, or email us, visit **lifescience.roche.com** and select your home country to display country-specific contact information.

1016.06781721001 ©

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim
Germany