For general laboratory use.

LightCycler® PCR QC Kit

Version: 08

Content Version: November 2023

Kit for Quality Control of the LightCycler® PRO or LightCycler® 96 Systems. The kit is designed for one test run with 48 or 96 reactions in a final volume of 10 µL each.

Cat. No. 06 746 381 001	1 kit for 1 test run Up to 96 reactions with 10 µL final volume each

Store the kit at −15 to −25°C.

1. General Information

1.1. Contents

Vial / bottle	Label	Function / description	Content
1	LightCycler® PCR QC Kit, DNA Template, 15 ng/µL	Human genomic DNA	1 vial, 250 µL
2	LightCycler® PCR QC Kit, Primer Mix, 10x conc.	β-Globin primer pair mix	1 vial, 118 µL
3	LightCycler® PCR QC Kit, PCR Master, 2x conc.	FastStart Essential DNA Green Master	1 vial, 1,000 µL
4	LightCycler® PCR QC Kit, Water, PCR Grade	To adjust final reaction volume.	1 vial, 1,000 µL

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	Label	Storage
1	DNA Template, 15 ng/µL	Store at −15 to −25°C.
2	Primer Mix, 10x conc.	
3	PCR Master, 2x conc.	Store at −15 to −25°C. ⚠ ***Keep protected from light.***
4	Water, PCR Grade	Store at −15 to −25°C.

1.3. Additional Equipment and Reagent required

Standard laboratory equipment

- Nuclease-free pipette tips
- 1.5 mL RNase-free microcentrifuge tubes to prepare master mixes and dilutions
- To minimize risk of RNase contamination, autoclave all vessels

For quality control

- Real-Time PCR systems such as the LightCycler® PRO or LightCycler® 96 Systems*
- LightCycler® 480 Multiwell Plate 96, white*
- LightCycler® 480 Multiwell Plate 384, white*
- LightCycler® 480 Multiwell Plate 96, white, 4 bar codes*
- LightCycler® 480 Multiwell Plate 384, white, 4 bar codes*
- Sealing Foil Applicator*
- LightCycler® 480 Sealing Foil*
- Centrifuge with swinging-bucket rotor

1.4. Application

The LightCycler® PCR QC Kit contains all reagents for a test run to verify the correct function of a LightCycler® PRO or LightCycler® 96 System.

- The SYBR Green I detection format is used to perform amplification and melting curve analysis. The test is comprised of 48 or 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 LightCycler® PCR 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 minutes) before PCR cycling begins. Activation does not require the extra handling steps typical of other hot start techniques.

1.5. Preparation Time

Typical Run Time

Procedure	Time
PCR Setup	20 minutes thawing reagents and pipetting
LightCycler® PRO and LightCycler® 96 QC run	1 hour, 25 minutes
Total assay time	**1 hour, 45 minutes**

2. How to Use this Product

2.1. Protocols

LightCycler® PRO and LightCycler® 96 System protocols

The following procedure is optimized for use with the corresponding LightCycler® System you are using.

⚠ ***Program the LightCycler® Instrument before preparing the reaction mixes.***
i *For details on how to program the experimental protocol, see the LightCycler® PRO System User Assistance or LightCycler® 96 System Operator's Guide.*

Protocol for use with the LightCycler® PRO Instrument

Setup of the qPCR reaction for the LightCycler® PRO Instrument

Follow the procedure below to prepare one 10 µL standard reaction.
i *Always wear gloves during handling.*

1. Thaw the 4 reagent vials from the LightCycler® PCR QC Kit.

2. Mix each vial sufficiently by vortexing for a few seconds; spin down reaction mix briefly.

3. To a separate 1.5 mL reaction tube, add the components listed below:

Reagent	Volume [µL] 1 reaction	Final conc.	Volume [µL] 58 reactions 96-well plates	Volume [µL] 115 reactions 384-well plates
PCR Master, 2x conc. (Vial 3)	5.0	1x	290	575
Primer Mix, 10x conc. (Vial 2)	1.0	1x	58	115
Water, PCR Grade (Vial 4)	2.0	–	116	230
DNA Template (Vial 1)	2.0	30 ng	116	230
Total volume	**10.0**	**–**	**580**	**1,150**

4 When using multichannel pipettes, prepare the reaction mixture with sufficient extra volume. For example, set up enough volume for 58 or 115 reactions as indicated in the table. Dispense 10 µL of this mixture into **48 wells** of a 96-well plate or into **96 wells** of a 384-well plate. See the pipetting schemes below:

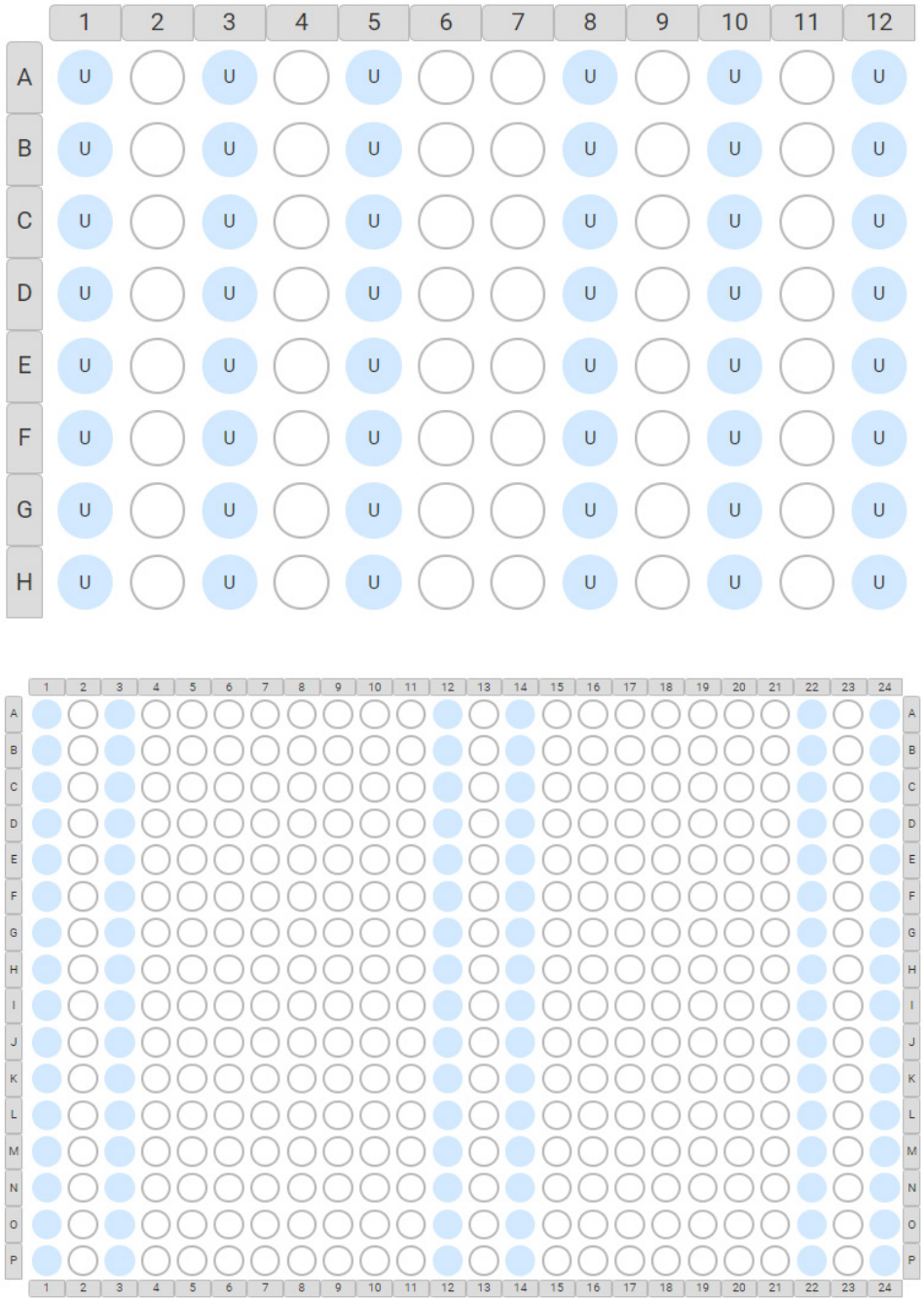

Fig. 1: Pipetting schemes for the LightCycler® PRO Instrument.

– Ensure that the rims of the wells are free of any liquid.

5 Seal the multiwell plate with a LightCycler® 480 Sealing Foil.

⚠ ***Make sure that the peripheral wells are completely sealed.***

6 Place the multiwell plate into the centrifuge with a swinging-bucket rotor and suitable adapter, and balance it with a suitable counterweight, such as another multiwell plate.
– Centrifuge at 1,500 × *g* for 2 minutes.

7 Make sure that there are no air bubbles visible in the wells of the multiwell plate.

8 Log into the LightCycler® PRO Instrument by using the touch screen.

9 If the QC Kit function is turned **off**, an **Administrator** has to enable this function, otherwise continue with Step 10.

For details on how to select the settings to enable this function, see the LightCycler® PRO System User Assistance.

10 In the **"Overview"** menu, load the multiwell plate into the LightCycler® PRO Instrument with the **"Open loading drawer"** button. When loaded, close the drawer with the **"Close loading drawer"** button.

11 Turn on the **"QC kit run"** feature by pressing the toggle next to it and confirming the next pop-up window.

12 Once the QC kit run has been confirmed:
– **Start the run** with the respective button.

LightCycler® PRO experimental protocol

The PCR runs do not have to be programmed by the user. The run profiles **LC PCR QC Kit 96** *or* **LC PCR QC Kit 384** *are pre-installed on the LightCycler® PRO Instrument and will automatically be assigned to the run when activating the* **"QC kit run"** *function.*

Setup					
Thermal cycler type			**Reaction volume [µL]**		
96 (384)			10		
Detection format					
Select in the menu for the Detection format the intercalating dye **SYBR Green I** in your assays.					
Programs					
Program name	**Cycles**				
Pre-incubation	1				
Amplification	45				
Melting	1				
Cooling	1				
Temperature targets					
	Target [°C]	**Acquisition mode**	**Duration [s]**	**Ramp rate [°C/s]**	**Reading**
Pre-incubation	95	None	600	4.4 (4.8)	–
3-step amplification	95	None	10	4.4 (4.8)	–
	55	None	10	2.2 (2.4)	–
	72	Single	10	4.4 (4.8)	–
Melting	95	None	5	4.4 (4.8)	–
	72	None	300	2.2 (2.4)	–
	97	Continuous	1	0.05 (0.05)	20
Cooling (automatically added)	40	None	30	2.2 (2.4)	–

QC Kit run evaluation

1. Insert a USB flash drive into the front of the instrument or use the SFTP connection to allow for the export of the QC Kit run data.

2. In the user interface of the LightCycler® PRO Instrument, select the **“Results”** menu.
   – Select your run of interest by checking the respective box and press the **“Export”** button. Depending on whether there is a USB flash drive inserted into the front of the instrument or if there is a SFTP connection set up, you can choose between **“SFTP”** and **“USB”** as an exporting location. Once the location of interest is chosen, confirm with the **“Export”** button.

3. Start up the LightCycler® PRO Development Software on your computer.

4. Insert the USB flash drive with the run data in your computer or set up a connection to the SFTP in the LightCycler® PRO Development Software.

5. Go to **“Projects”** menu and select the **“QCKitProject”** project.

6. Click the **“Import”** button in the **“Run overview”** and select the import location of your run data and confirm with **“Proceed”**.

7. Select the experiment file of interest and confirm by pressing **“Open”**.

8. Locate your run in the **“Run Overview”** and press the **“>”** icon on the far right to view the run data.

9. Proceed to the **“Results”>”Target results”** tab and press **“Calculate results”**.
   – If necessary, exclude up to 3 wells by switching the **“Include/Exclude”** toggle bar of the respective wells.

10. Switch to the **“Overall results”** tab and **Create QC kit report** by adding the Material and Lot number and the Expiry date of the LightCycler® PCR QC Kit used.

11. Chose the location where the report should be saved.

Protocol for use with the LightCycler® 96 Instrument

Setup of the qPCR reaction for the LightCycler® 96 Instrument

Follow the procedure below to prepare one 10 µL standard reaction.
Always wear gloves during handling.

1. Thaw the 4 reagent vials from the LightCycler® PCR QC Kit.

2. Mix each vial sufficiently by vortexing for a few seconds; spin down reaction mix briefly.

3. To a separate 1.5 mL reaction tube, add the components listed below.

Reagent	Volume [µL] 1 reaction	Final conc.	Volume [µL] 115 reactions
PCR Master, 2x conc. (Vial 3)	5.0	1x	575
Primer Mix, 10x conc. (Vial 2)	1.0	1x	115
Water, PCR Grade (Vial 4)	2.0	–	230
DNA Template (Vial 1)	2.0	30 ng	230
Total volume	**10.0**	**–**	**1,150**

4. When using multichannel pipettes, prepare the reaction mixture with sufficient extra volume. For example, set up enough volume for 115 reactions as indicated in the table. 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.

5. Seal the multiwell plate with a LightCycler® 480 Sealing Foil.

   ⚠ ***Make sure that the peripheral wells are completely sealed.***

6. Place the multiwell plate into the centrifuge with a swinging-bucket rotor and suitable adapter, and balance it with a suitable counterweight, such as another multiwell plate.
   – Centrifuge at 1,500 × *g* for 2 minutes.

7. Make sure that there are no air bubbles visible in the wells of the multiwell plate.

8. 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.

9. Perform the PCR run, including melting curve analysis using the following Workflow.

Workflow

1. Open the LightCycler® 96 Application Software on your PC.

2. 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"**.

3. In the file **open dialog**, select **"ExperimentTemplate_QC-Test"** and open the file.
   – By doing this, a new experiment, including run and analysis settings, is generated.

4. Save this new experiment at a location of your choice and close the file.

5. Continue with one of the 2 options described below, see **Using the USB stick** or **Using the Instrument Manager of the LightCycler® 96 Application Software on your PC**.

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 the file.

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.

Using the Instrument Manager of the LightCycler® 96 Application Software on your computer

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"**. You will see 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 **">"** icon, 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.

LightCycler® 96 experimental protocol

⚠ ***Do not change the protocol from the template.***

Run editor				
Detection format			Reaction volume [µL]	
SYBR Green I			10	
Programs				
	Temp. [°C]	Ramp [°C/s]	Duration [s]	Acquisition mode
Pre-incubation	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	0.05	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, for example, pipetting errors.
- **QC run file**

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

3. Results

Results on the LightCycler® PRO Instrument

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. 2).

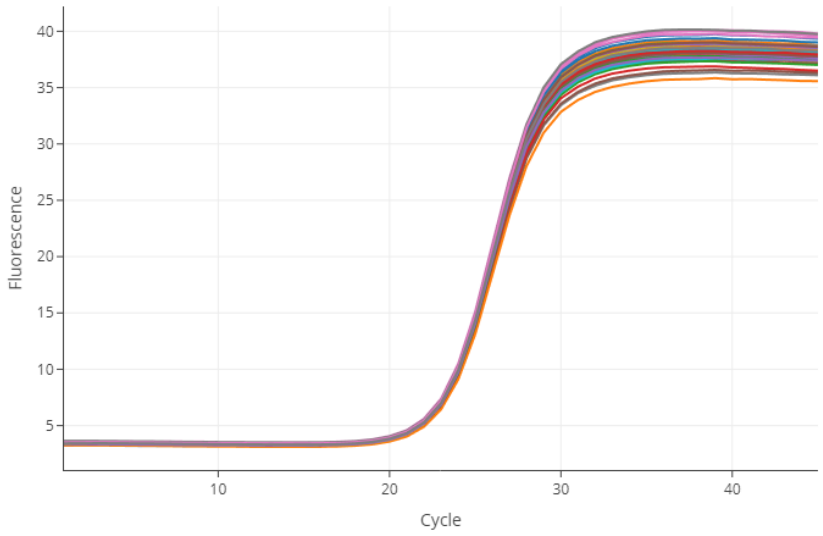

Fig. 2: Forty-eight replicates of ß-globin primers with human genomic DNA as template from the LightCycler® PCR QC Kit.

Specificity of the amplified PCR product was assessed by performing a melting curve analysis on the LightCycler® PRO Instrument. By calculating the melting point (Tm) for 48 wells on the 96-well plate, temperature accuracy is determined for the thermal cycler (see Fig. 3).

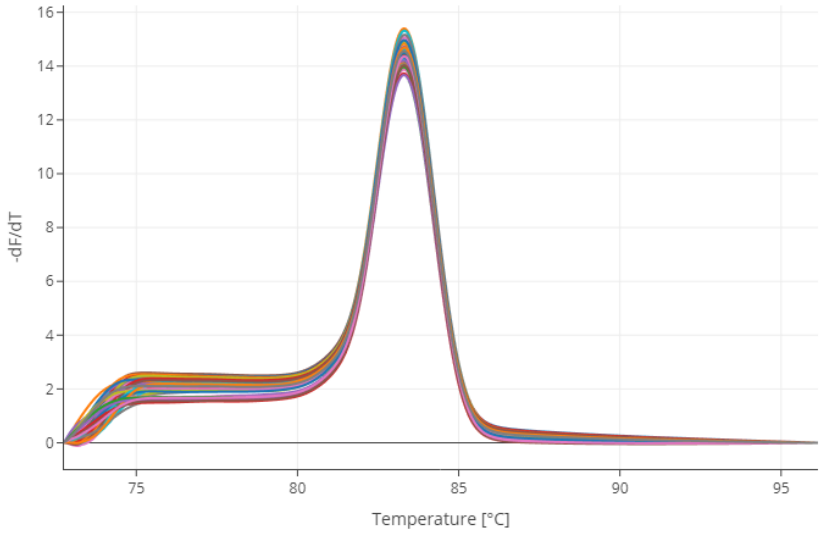

Fig. 3: Melting curve analysis of samples amplified using the LightCycler® PCR QC Kit.

Results on the LightCycler® 96 Instrument

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. 4).

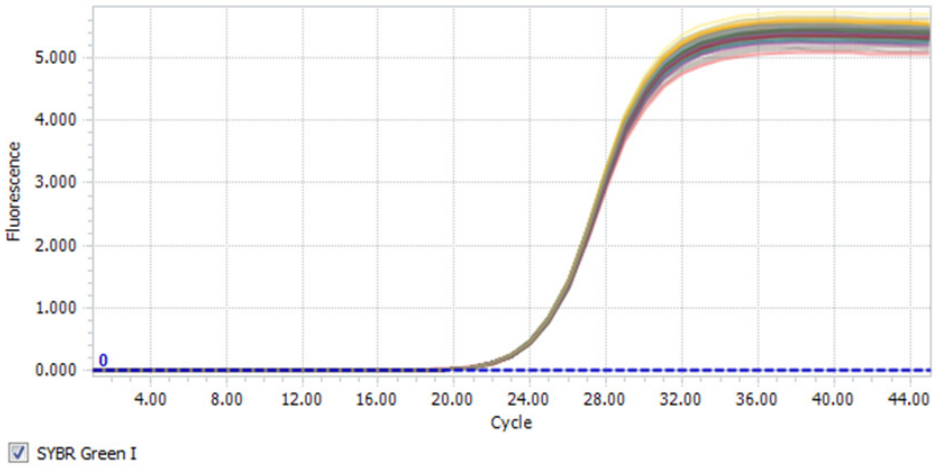

Fig. 4: Ninety-six replicates of ß-globin primers with human genomic DNA as template from the LightCycler® PCR QC Kit.

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 cycler covering 96 wells (see Fig. 5).

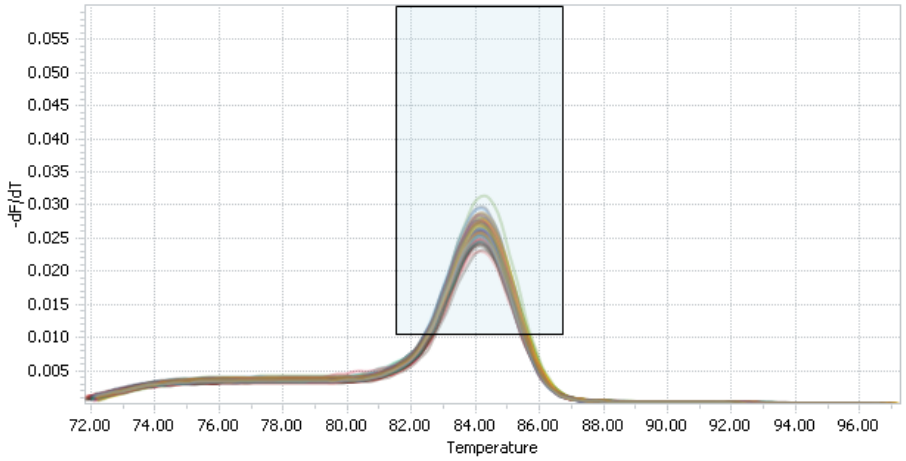

Fig. 5: Melting curve analysis of samples amplified using the LightCycler® PCR QC Kit.

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.
Artifacts in melting and/or amplification curves.	Sealing Foil is not correctly applied to the plate.	Repeat the run.
	Detection dye has bleached.	⚠ ***Keep PCR master mix away from light.***

5. Additional Information on this Product

5.1. Quality Control

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

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

Editorial changes.
Information about the LightCycler® PRO System has been added.
List of additional reagents and equipment has been updated.

6.3. 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)		
Thermal Cycler Assembly 96	1 piece	09 742 565 001
Thermal Cycler Assembly 384	1 piece	09 742 581 001
Sealing Foil Applicator	1 piece	10 018 607 001
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
LightCycler® 480 Multiwell Plate 384, white	5 x 10 plates	04 729 749 001
LightCycler® 480 Multiwell Plate 384	5 x 10 plates without sealing foils	05 217 555 001
LightCycler® 480 Multiwell Plate 96	5 x 10 plates without sealing foils	05 220 319 001
Instruments		
LightCycler® 96 Instrument	1 instrument	05 815 916 001
LightCycler® PRO Instrument	1 instrument, 96-well version	09 541 713 001
	1 instrument, 384-well version	09 582 487 001

6.4. Trademarks

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

6.5. License Disclaimer

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

6.6. Safety Data Sheet

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

6.7. 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

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

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