



QIAGEN Supplementary Protocol:

LightCycler[®] 480 Software Setup for the QuantiFast[™] SYBR[®] Green RT-PCR Kit

This protocol shows the necessary parameters that need to be entered into the LightCycler 480 software when using the QuantiFast SYBR Green RT-PCR Kit.

IMPORTANT: Please read the *QuantiFast SYBR Green RT-PCR Handbook*, paying careful attention to the safety information, before beginning this procedure. The QuantiFast SYBR Green RT-PCR Kit is intended for research use. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

Procedure

1. Launch the software and set up the programs as described in the next few steps.
2. Select "RT" and set up the parameters for the reverse-transcription step as shown below.

Reaction Format	SYBR Green I	Customize	Block Type	384	Plate ID		Reaction Volume	10
Programs								
Program Name							Cycles	Analysis Mode
▶ RT							1	None
Reactivation							1	None
Cycling							40	Quantification
Melting Curve							1	Melting Curves
Cooling							1	None
Program Temperature Targets								
Target (°C)	Acquisition Mode	Hold (hh:mm:ss)	Ramp Rate (°C/s)	Acquisitions (per °C)	Sec Target (°C)	Step Size (°C)	Step Delay (cycles)	
▶ 50	None	00:10:00	4.8		0	0	0	

3. Select "Reactivation" and set up the parameters for the initial activation step as shown below.

Reaction Format Block Type Plate ID Reaction Volume

Programs		
Program Name	Cycles	Analysis Mode
RT	1	None
▶ Reactivation	1	None
Cycling	40	Quantification
Melting Curve	1	Melting Curves
Cooling	1	None

Reactivation Temperature Targets							
Target (°C)	Acquisition Mode	Hold (hh:mm:ss)	Ramp Rate (°C/s)	Acquisitions (per °C)	Sec Target (°C)	Step Size (°C)	Step Delay (cycles)
▶ 95	None	00:05:00	4.8		0	0	0

4. Select "Cycling" and set up the parameters for PCR cycling as shown below. Be sure to select "Single" for "Acquisition Mode" at the 60°C step.

Reaction Format Block Type Plate ID Reaction Volume

Programs		
Program Name	Cycles	Analysis Mode
RT	1	None
Reactivation	1	None
▶ Cycling	40	Quantification
Melting Curve	1	Melting Curves
Cooling	1	None

Cycling Temperature Targets							
Target (°C)	Acquisition Mode	Hold (hh:mm:ss)	Ramp Rate (°C/s)	Acquisitions (per °C)	Sec Target (°C)	Step Size (°C)	Step Delay (cycles)
▶ 95	None	00:00:10	4.8		0	0	0
60	Single	00:00:30	2.5		0	0	0

5. Select "Melting Curve" and set up the parameters for melting curve analysis as shown below. Be sure to select "Continuous" for "Acquisition Mode" at the 95°C step.

Reaction Format Block Type Plate ID Reaction Volume

Programs		
Program Name	Cycles	Analysis Mode
RT	1	None
Reactivation	1	None
Cycling	40	Quantification
▶ Melting Curve	1	Melting Curves
Cooling	1	None

Melting Curve Temperature Targets							
Target (°C)	Acquisition Mode	Hold (hh:mm:ss)	Ramp Rate (°C/s)	Acquisitions (per °C)	Sec Target (°C)	Step Size (°C)	Step Delay (cycles)
▶ 95	None	00:00:15	4.8				
60	None	00:00:15	2.5				
95	Continuous			5			

6. Select "Cooling" and set up the parameters for cooling as shown below.

Reaction Format Block Type Plate ID Reaction Volume

Programs		
Program Name	Cycles	Analysis Mode
RT	1	None
Reactivation	1	None
Cycling	40	Quantification
Melting Curve	1	Melting Curves
▶ Cooling	1	None

Cooling Temperature Targets							
Target (°C)	Acquisition Mode	Hold (hh:mm:ss)	Ramp Rate (°C/s)	Acquisitions (per °C)	Sec Target (°C)	Step Size (°C)	Step Delay (cycles)
▶ 40	None	00:00:30	2.5		0	0	0

7. Load your PCR plate and start the program.

QIAGEN handbooks can be requested from QIAGEN Technical Service or your local QIAGEN distributor. Selected handbooks can be downloaded from www.qiagen.com/literature/handbooks/default.aspx. Material safety data sheets (MSDS) for any QIAGEN product can be downloaded from www.qiagen.com/ts/msds.asp.

Trademarks: QIAGEN®, QuantiFast™ (QIAGEN Group); LightCycler® (Roche Group); SYBR® (Molecular Probes, Inc.).

Purchase of the QuantiFast SYBR Green RT-PCR Kit is accompanied by a limited, non-transferable immunity from suit to use it with detection by a dsDNA-binding dye as described in U.S. Patents Nos. 5,994,056 and 6,171,785 and corresponding patent claims outside the United States for the purchaser's own internal research. No real-time apparatus or system patent rights or any other patent rights, and no right to use this product for any other purpose are conveyed expressly, by implication or by estoppel.

Purchase of the QuantiFast SYBR Green RT-PCR Kit is accompanied by a limited, non-transferable license under RT and Reverse Transcription-PCR patents owned by Roche Molecular Systems, Inc. and F. Hoffmann-La Roche Ltd to use it for the purchaser's own internal research. No real-time patent rights of any kind, no right under any other patent claims (such as apparatus or system claims), and no right to use this product for any other purpose is hereby granted expressly, by implication or by estoppel.

The melting curve technology is covered by United States Patent No. 5,871,908, and corresponding foreign patents, owned by Evotec Biosystems GmbH and licensed to Roche Diagnostics GmbH. The purchase of this product does not convey to the buyer any right under these patent rights to perform the melting curve technology claimed in those patents. In particular, the purchase of this product does not include nor carry any right or license to use, develop, or otherwise exploit the melting curve technology commercially, and no rights are conveyed to the buyer to use the product or components of the product for any other purposes, including without limitation, provision of services to a third party, generation of commercial databases, research and development, human diagnostics or veterinary diagnostics. Roche Diagnostics GmbH reserves all rights under these patent rights. For information on purchasing a license to the patent rights for uses in conjunction with this product or to use the melting curve technology for other purposes, please contact Roche Diagnostics GmbH, Patent Department, Werk Penzberg, Nonnenwald 2, 82372 Penzberg, Germany.

PCR65 Apr-07 © 2007 QIAGEN, all rights reserved.



Sample & Assay Technologies