



QIAGEN Supplementary Protocol:

LightCycler[®] 2.0 Software Setup for the QuantiFast[™] SYBR[®] Green PCR Kit

This protocol shows the necessary parameters that need to be entered into the LightCycler 2.0 software (version 4.0) when using the QuantiFast SYBR Green PCR Kit.

IMPORTANT: Please read the *QuantiFast SYBR Green PCR Handbook*, paying careful attention to the safety information, before beginning this procedure. The QuantiFast SYBR Green 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 "Reactivation" and set up the parameters for the initial activation step as shown below.

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

Reactivation Temperature Targets						
Target (°C)	Hold (hh:mm:ss)	Ramp Rate (°C/s)	Sec Target (°C)	Step Size (°C)	Step Delay (cycles)	Acquisition Mode
▶ 95	00:05:00	20	0	0	0	None

3. 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.

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

Cycling Temperature Targets							
Target (°C)	Hold (hh:mm:ss)	Ramp Rate (°C/s)	Sec Target (°C)	Step Size (°C)	Step Delay (cycles)	Acquisition Mode	
▶ 95	00:00:10	20	0	0	0	None	
60	00:00:30	20	0	0	0	Single	

4. 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.

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

Melting Curve Temperature Targets							
Target (°C)	Hold (hh:mm:ss)	Ramp Rate (°C/s)	Sec Target (°C)	Step Size (°C)	Step Delay (cycles)	Acquisition Mode	
▶ 95	00:00:15	20	0	0	0	None	
60	00:00:15	20	0	0	0	None	
95	00:00:00	0.1	0	0	0	Continuous	

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

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

Cooling Temperature Targets							
Target (°C)	Hold (hh:mm:ss)	Ramp Rate (°C/s)	Sec Target (°C)	Step Size (°C)	Step Delay (cycles)	Acquisition Mode	
▶ 40	00:00:30	20	0	0	0	None	

6. Load your PCR capillaries and start the program.

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Material safety data sheets (MSDS) for any QIAGEN product can be downloaded from www.qiagen.com/ts/msds.asp.

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