



QIAGEN Supplementary Protocol:

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

This protocol shows the necessary parameters that need to be entered into the LightCycler 480 software 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.

Reaction Format	SYBR Green I	Customize	Block Type	384	Plate ID		Reaction Volume	10
Programs								
Program Name	Cycles	Analysis Mode						
Reactivation	1	None						
Cycling	40	Quantification						
Melting Curve	1	Melting Curves						
Cooling	1	None						
RT 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	

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.

Reaction Format Block Type Plate ID Reaction Volume

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)	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

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.

Reaction Format Block Type Plate ID Reaction Volume

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)	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			

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

Reaction Format	SYBR Green I	Block Type	384	Plate ID		Reaction Volume	10
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)	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

6. Load your PCR plate 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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