



## QIAGEN Supplementary Protocol:

### LightCycler® 2.0 Software Setup for the QuantiFast™ SYBR® Green RT-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 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.

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

  

RT Temperature Targets						
Target (°C)	Hold (hh:mm:ss)	Ramp Rate (°C/s)	Sec Target (°C)	Step Size (°C)	Step Delay (cycles)	Acquisition Mode
▶ 50	00:10:00	20	0	0	0	None

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

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)	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

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.

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)	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

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.

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)	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

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

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

**7. Load your PCR capillaries and start the program.**

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