



QIAGEN Supplementary Protocol:

LightCycler[®] 1.x Software Setup for the QuantiFast[™] SYBR[®] Green RT-PCR Kit

This protocol shows the necessary parameters that need to be entered into the LightCycler 1.x software (version 3.5) 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 cycle program data as described in the next few steps.
2. Select "None" and set up the parameters for the reverse-transcription step as shown below.

Cycle Program Data

Analysis Mode: None

Cycles: 1

Quantification
Melting Curves

Temperature Targets

Target Temperature (°C): 50

Incubation Time (hrs:min:sec): 10:00

Temperature Transition Rate (°C/s): 20.00

Secondary Target Temperature (°C): 0

Step Size (°C): 0.0

Step Delay (cycles): 0

Acquisition Mode: NONE

Ins Del

3. Select "None" and set up the parameters for the initial activation step as shown below.

Cycle Program Data

Analysis Mode: None

Cycles: 1

Temperature Targets

Target Temperature (°C): 95

Incubation Time (hrs:min:sec): 5:00

Temperature Transition Rate (°C/s): 20.00

Secondary Target Temperature (°C): 0

Step Size (°C): 0.0

Step Delay (cycles): 0

Acquisition Mode: NONE

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

Cycle Program Data

Analysis Mode: Quantification

Cycles: 40

Temperature Targets

Target Temperature (°C): 95

Incubation Time (hrs:min:sec): 10

Temperature Transition Rate (°C/s): 20.00

Secondary Target Temperature (°C): 0

Step Size (°C): 0.0

Step Delay (cycles): 0

Acquisition Mode: NONE

Target Temperature (°C): 60

Incubation Time (hrs:min:sec): 30

Temperature Transition Rate (°C/s): 20.00

Secondary Target Temperature (°C): 0

Step Size (°C): 0.0

Step Delay (cycles): 0

Acquisition Mode: SINGLE

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

Cycle Program Data

Analysis Mode: None, Quantification, **Melting Curves**

Cycles: 1

Temperature Targets

Target Temperature (°C) | Incubation Time (hrs:min:sec) | Temperature Transition Rate (°C / s) | Secondary Target Temperature (°C) | Step Size (°C) | Step Delay (cycles) | Acquisition Mode

Ins 95	15	20.00	0	0.0	0	NONE	Del
Ins 60	15	20.00	0	0.0	0	NONE	Del
Ins 95	0	0.10	0	0.0	0	CONT	Del

6. Select "None" and set up the parameters for cooling as shown below.

Cycle Program Data

Analysis Mode: None, Quantification, Melting Curves

Cycles: 1

Temperature Targets

Target Temperature (°C) | Incubation Time (hrs:min:sec) | Temperature Transition Rate (°C / s) | Secondary Target Temperature (°C) | Step Size (°C) | Step Delay (cycles) | Acquisition Mode

Ins 40	30	20.00	0	0.0	0	NONE	Del
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7. Load your PCR capillaries and start the program.

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