



QIAGEN Supplementary Protocol:

LightCycler[®] 1.x Software Setup for the QuantiFast[™] Probe PCR + ROX Vial 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 Probe PCR + ROX Vial Kit.

IMPORTANT: Please read the *QuantiFast Probe PCR Handbook*, paying careful attention to the safety information, before beginning this procedure. The QuantiFast Probe PCR + ROX Vial 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" in "Analysis Mode" and set up the parameters for the initial activation step as shown below.

The screenshot displays the 'Cycle Program Data' window in the LightCycler 1.x software. The 'Analysis Mode' is set to 'None'. The 'Cycles' field is set to 1. The 'Temperature Targets' section shows the following parameters: Target Temperature (95.0 °C), Incubation Time (3:00), Temperature Transition Rate (20.00 °C/s), Secondary Target Temperature (0.0 °C), Step Size (0.0 °C), Step Delay (0 cycles), and Acquisition Mode (NONE). The 'Ins' button is highlighted in green, and the 'Del' button is highlighted in red.

Parameter	Value
Cycles	1
Analysis Mode	None
Target Temperature (°C)	95.0
Incubation Time (hrs:min:sec)	3:00
Temperature Transition Rate (°C/s)	20.00
Secondary Target Temperature (°C)	0.0
Step Size (°C)	0.0
Step Delay (cycles)	0
Acquisition Mode	NONE

3. If using dual-labeled probes, 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

Cycles: 40

Analysis Mode: None, Quantification, Melting Curves

Temperature Targets

Target Temperature (°C): 95

Incubation Time (hrs:min:sec): 3

Temperature Transition Rate (°C/s): 20.00

Secondary Target Temperature (°C): 60

Step Size (°C): 30

Step Delay (cycles): 20.00

Acquisition Mode: NONE, SINGLE

Ins

If using FRET probes, 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

Cycles: 40

Analysis Mode: None, Quantification, Melting Curves

Temperature Targets

Target Temperature (°C): 95

Incubation Time (hrs:min:sec): 10

Temperature Transition Rate (°C/s): 20.00

Secondary Target Temperature (°C): 60

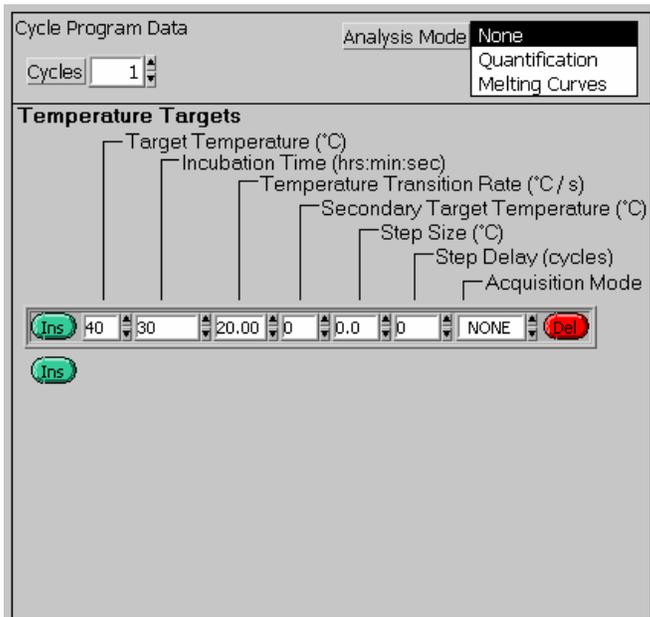
Step Size (°C): 15

Step Delay (cycles): 20.00

Acquisition Mode: NONE, SINGLE, NONE

Ins

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



5. Load your PCR capillaries 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.

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