



## QIAGEN Supplementary Protocol:

### LightCycler<sup>®</sup> 480 Software Setup for the QuantiFast<sup>™</sup> Probe PCR +ROX Vial Kit

This protocol shows the necessary parameters that need to be entered into the LightCycler 480 software 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 programs as described in the next few steps.
2. Create two programs and name them, for example, "Reactivation" and "Cycling". Select "Reactivation" and set up the parameters for the initial activation step as shown below.

The screenshot shows the 'Setup' window in the LightCycler 480 software. The 'Detection Format' is set to 'SimpleProbe', 'Block Type' is 384, and 'Reaction Volume' is 10. The 'Programs' table lists two programs: 'Reactivation' (1 cycle, None analysis mode) and 'Cycling' (40 cycles, Quantification analysis mode). The 'Reactivation Temperature Targets' table shows a target of 95°C, None acquisition mode, 00:03:00 hold time, 4.8°C/s ramp rate, 0 acquisitions per °C, 0 secondary target, 0 step size, and 0 step delay.

Reactivation 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	00:03:00	4.8	0	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.

Setup

Detection Format   Block Type  Plate ID  Reaction Volume

Programs		
Program Name	Cycles	Analysis Mode
Reactivation	1	None
▶ Cycling	40	Quantification

  

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	00:00:03	4.8		0	0	0
60	Single	00:00:30	2.5		0	0	0

4. Load your PCR plate and start the program.

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