

QIAGEN Supplementary Protocol

LightCycler[®] 480 software setup for the QuantiFast[®] Probe RT-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 RT-PCR + ROX Vial Kit.

IMPORTANT: Please read the *QuantiFast Probe RT-PCR Handbook*, paying careful attention to the “Safety Information” section, before beginning this procedure.

Procedure

1. Launch the software and set up the programs as described in the next few steps.
2. Create 4 programs and name them, for example, “RT”, “Reactivation”, “Cycling”, and “Cooling”. Select “RT” and set up the parameters for the reverse-transcription step as shown below.

The screenshot displays the software interface for setting up programs and temperature targets. At the top, the 'Detection Format' is set to 'Multi Color Hydrolysis Probe', 'Block Size' is 384, and 'Reaction Volume' is 10. Below this, the 'Programs' table is visible:

Program Name	Cycles	Analysis Mode
RT	1	None
Reactivation	1	None
Cycling	40	Quantification
Cooling	1	None

Below the programs table, the 'RT Temperature Targets' table is shown:

Target (°C)	Acquisition Mode	Hold (hh:mm:ss)	Ramp Rate (°C/s)	Acquisitions (per °C)	Sec Target (°C)	Step Size (°C)	Step Delay (cycles)
50	None	00:10:00	4.8	0	0	0	0



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

Detection Format: Multi Color Hydrolysis Probe | Customize | Block Size: 384 | Plate ID: | Reaction Volume: 10

Color Comp ID: | Lot No: | Test ID: |

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

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	00:05:00	4.8	0	0	0	0

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.

Detection Format: Multi Color Hydrolysis Probe | Customize | Block Size: 384 | Plate ID: | Reaction Volume: 10

Color Comp ID: | Lot No: | Test ID: |

Programs			
Program Name	Cycles	Analysis Mode	
RT	1	None	
Reactivation	1	None	
Cycling	40	Quantification	
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	0
60	Single	00:00:30	2.5	0	0	0	0

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

Detection Format: Multi Color Hydrolysis Probe | Customize | Block Size: 384 | Plate ID: | Reaction Volume: 10

Color Comp ID: | Lot No: | Test ID: |

Programs			
Program Name	Cycles	Analysis Mode	
RT	1	None	
Reactivation	1	None	
Cycling	40	Quantification	
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	0

6. Load your PCR plate 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.

Material safety data sheets (MSDS) for any QIAGEN product can be downloaded from www.qiagen.com/Support/MSDS.aspx.

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