

# QIAGEN Supplementary Protocol

## LightCycler<sup>®</sup> 2.0 software setup for the QuantiFast<sup>®</sup> Probe RT-PCR + ROX Vial 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 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 (for dual-labeled probes)

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”, “Dual-Labeled Probe Cycling”, and “Cooling”. 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				
Dual Labeled Probe Cycling	40	Quantification				
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		
Dual Labeled Probe Cycling				40	Quantification		
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 "Dual-Labeled Probe 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		
▶ Dual Labeled Probe Cycling				40	Quantification		
Cooling				1	None		

  

Dual Labeled Probe 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 "Cooling" and set up the parameters for cooling as shown below.

Programs							
Program Name				Cycles	Analysis Mode		
RT				1	None		
Reactivation				1	None		
Dual Labeled Probe Cycling				40	Quantification		
▶ 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	
▶ 40	00:00:30	20	0	0	0	None	

6. Load your PCR capillaries and start the program.

## Procedure (for FRET probes)

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", "FRET Probe Cycling", and "Cooling". 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					
FRET Probe Cycling	40	Quantification					
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					
FRET Probe Cycling	40	Quantification					
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 "FRET Probe 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					
▶ FRET Probe Cycling	40	Quantification					
Cooling	1	None					

  

FRET Probe 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	00:00:15	20	0	0	0	Single	
95	00:00:15	20	0	0	0	None	

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

Programs							
Program Name	Cycles	Analysis Mode					
RT	1	None					
Reactivation	1	None					
FRET Probe Cycling	40	Quantification					
▶ 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	
▶ 40	00:00:30	20	0	0	0	None	

6. Load your PCR capillaries and start the program.

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

The QuantiFast Probe RT-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.

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