

Brilliant III Ultra-Fast SYBR® Green QPCR Master Mix

Quick Reference Guide for the LightCycler® 480 Real-Time PCR System

This quick reference guide provides an optimized protocol for using the Stratagene Brilliant III Ultra-Fast SYBR® Green QPCR Master Mix with the LightCycler 480 Real-Time PCR System from Roche. For detailed instructions, refer to the full product manual.

Prepare the Reactions

1 Prepare the experimental reactions by combining the components of the reagent mixture in the order listed in the table below. Prepare a single reagent mixture for replicate reactions (plus at least one reaction volume excess) using multiples of each component.

Reagent Mixture
Nuclease-free PCR-grade water to bring final volume to 20 μ l (including DNA)
10 μl of 2× SYBR Green QPCR Master Mix
x μl of upstream primer at optimized concentration (200–500 nM)
x μl of downstream primer at optimized concentration (200–500 nM)

- **2** Gently mix the reagent mixture without creating bubbles, then distribute the mixture to the experimental reaction tubes.
- 3 Add x μ l of experimental DNA to each reaction to bring the final reaction volume to 20 μ l. The table below lists a suggested quantity range for different DNA templates.

DNA	Quantity per reaction			
Genomic DNA	5 pg — 50 ng			
cDNA	0.5 pg — 100 ng*			

^{*}Refers to RNA input amount during cDNA synthesis

4 Mix the reactions without creating bubbles, then centrifuge briefly.



Set Up the QPCR Plate and Thermal Profile

- 1 From the main window in the LightCycler 480 software, click **Sample Editor** on the module bar to open the *Sample Editor* module. Enter sample information for your experiment as needed.
- **2** Click **Experiment** on the module bar to open the *Run* module.
- 3 From the Run Protocol tab, enter a reaction volume of 20 µl.
- **4** Set the **Detection Format** to SYBR Green I/HRM Dye.
- **5** Set up the PCR program to run the cycling protocol below:

Program Name	Cycles	Analysis Mode	Acquisition Mode	Ramp Rate (°C/s)	Hold Time	Temperature
Pre-incubation	1	None	None	4.4	3 minutes	95°C
Amplification 45	45	Quantification	None	4.4	5 seconds	95°C
			Single	2.2	10 seconds	60°C
Melting curve	1	Melting curves	None	4.4	5 seconds	95°C
			None	2.2	1 minute	65°C
			Continuous (5 acquisitions/second)	0.11	_	97°C
Cooling	1	None	None	2.2	30 seconds	40°C

Run the PCR

1 Place the reactions in the LightCycler 480 instrument.

Program

2 From the Run Protocol or Data tab, click Start Run.

Analyze Data

1 Analyze the results of the run as needed for your experiment.

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Product Information

Catalog #600882, 400 reactions Catalog #600883, 4000 reactions

Ordering Information

By phone (US only*): 800-424-5444, x3 On the web: www.stratagene.com

Technical Services

By phone (US only*): 800-894-1304, x2 By email: techservices@agilent.com

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