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 Agilent's 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 µl (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 µl of experimental DNA to each reaction to bring the final reaction volume to 20 µl. 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	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 Program

1. Place the reactions in the LightCycler 480 instrument.
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.

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

Ordering Information
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5990-6144EN