



Brilliant III Ultra-Fast SYBR® Green QRT-PCR 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 QRT-PCR 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. *Keep the reagent mixture on ice.*

Reagent Mixture
Nuclease-free PCR-grade water to bring final volume to 20 µl (including RNA)
10 µl of 2× SYBR Green QRT-PCR Master Mix
x µl of upstream primer at optimized concentration (150–500 nM)
x µl of downstream primer at optimized concentration (150–500 nM)
0.2 µl of 100 mM DTT
1 µl of RT/RNase Block

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

RNA	Quantity per reaction
Total RNA	0.1 pg – 100 ng
mRNA	0.1 pg – 1 ng

- 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
Reverse transcription	1	None	None	4.4	10 minutes	50°C
Denaturation	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.

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Product Information

Catalog #600886, 400 reactions
Catalog #600887, 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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