



# Brilliant III Ultra-Fast SYBR<sup>®</sup> Green QPCR Master Mix

## Quick Reference Guide for the QIAGEN Rotor-Gene Q Real-Time PCR Cycler

*This quick reference guide provides an optimized protocol for using Agilent's Brilliant III Ultra-Fast SYBR<sup>®</sup> Green QPCR Master Mix with the Rotor-Gene Q Real-Time PCR Cycler from QIAGEN. 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 $\mu$ l (including DNA)
10 $\mu$ l of 2 $\times$ SYBR Green QPCR Master Mix
x $\mu$ l of upstream primer at optimized concentration (200–500 nM)
x $\mu$ l 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  $x$   $\mu$ l of experimental DNA to each reaction to bring the final reaction volume to 20  $\mu$ l. The table below lists a suggested quantity range for different DNA templates.

DNA	Quantity per reaction
Genomic DNA	5 $\mu$ g – 25 ng
cDNA	0.5 $\mu$ g – 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 New Run screen, click the **Advanced** tab to access the **Advanced Wizard** options.
- 2 Select the **Two Step with Melt** template and click **New**.
- 3 Use the boxes of the wizard to make selections appropriate for your experiment.

*In the Temperature Profile box, click **Edit** to open the **Profile Editor**. Adjust the cycling protocol according to the guidelines in the tables below.*

### Cycling Protocol for cDNA Targets

Cycles	Duration of Cycle	Temperature
1	3 minutes	95°C
40	10 seconds	95°C
	10 seconds	60°C

### Cycling Protocol for Genomic DNA Targets

Cycles	Duration of Cycle	Temperature
1	3 minutes	95°C
40	10 seconds	95°C
	20 seconds	60°C

## Run the PCR Program

- 1 Place the reactions in the Rotor-Gene Q instrument.
- 2 On the last screen of the wizard 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 and Canada\*): 800-227-9770  
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