



# Brilliant III Ultra-Fast QRT-PCR 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 the Stratagene Brilliant III Ultra-Fast QRT-PCR 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. *Keep reagent mixture on ice.*

Reagent Mixture
Nuclease-free PCR-grade water to bring final volume to 20 $\mu$ l (including RNA)
10 $\mu$ l of 2 $\times$ QRT-PCR Master Mix
$x$ $\mu$ l of experimental probe at optimized concentration (100–600 nM)
$x$ $\mu$ l of upstream primer at optimized concentration (200–600 nM)
$x$ $\mu$ l of downstream primer at optimized concentration (200–600 nM)
0.2 $\mu$ l of 100 mM DTT
1 $\mu$ l 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  $x$   $\mu$ l of experimental RNA to each reaction to bring the final reaction volume to 20  $\mu$ l. 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 New Run screen, click the **Advanced** tab to access the **Advanced Wizard** options.
- 2 Select the **Two Step** 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 table below.*

Cycles	Duration of Cycle	Temperature
1	10 minutes	50°C
1	3 minutes	95°C
40	5–20 seconds <sup>a</sup>	95°C
	10–20 seconds <sup>b</sup>	60°C

<sup>a</sup> The exact denaturation time needs to be optimized for each probe/target system.

<sup>b</sup> The exact annealing/extension time needs to be optimized for each probe/target system.

## 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 #600884, 400 reactions  
Catalog #600885, 4000 reactions

### Ordering Information

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

### Technical Services

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By email: [techservices@agilent.com](mailto:techservices@agilent.com)

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