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 Agilent's 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 µl (including RNA)
10 µl of 2× QRT-PCR Master Mix
x µl of experimental probe at optimized concentration (100–600 nM)
x µl of upstream primer at optimized concentration (200–600 nM)
x µl of downstream primer at optimized concentration (200–600 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 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 [a]	95°C
	10 –20 seconds [b]	60°C

[a] The exact denaturation time needs to be optimized for each probe/target system.
[b] 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.

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

Ordering Information
By phone (US and Canada*): 800-227-9770
On the web: www.agilent.com/genomics

Technical Services
By phone (US and Canada*): 800-227-9770
By email: techservices@agilent.com

*For other countries, please contact your local sales representative at *www.agilent.com/genomics/contactus*

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Manual Part Number 5990-7215, Revision B0
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PR7000-0447
5990-6151EN