Brilliant III Ultra-Fast 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 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 µl (including DNA)
10 µl of 2× QPCR Master Mix
x µl of experimental probe at optimized concentration (150–600 nM)
x µl of upstream primer at optimized concentration (200–600 nM)
x µl of downstream primer at optimized concentration (200–600 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 – 100 ng
cDNA	0.1 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 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 guidelines in the tables below.*

Cycling Protocol for cDNA Targets

Cycles	Duration of Cycle	Temperature
1	3 minutes	95°C
40	5 seconds	95°C
	10 seconds	60°C

Cycling Protocol for Genomic DNA Targets

Cycles	Duration of Cycle	Temperature
1	3 minutes	95°C
40	20 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.

Product Information
Catalog #600880, 400 reactions
Catalog #600881 4000 reactions

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

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