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.

<table>
<thead>
<tr>
<th>Reagent Mixture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuclease-free PCR-grade water to bring final volume to 20 μl (including DNA)</td>
</tr>
<tr>
<td>10 μl of 2× QPCR Master Mix</td>
</tr>
<tr>
<td>x μl of experimental probe at optimized concentration (150–600 nM)</td>
</tr>
<tr>
<td>x μl of upstream primer at optimized concentration (200–600 nM)</td>
</tr>
<tr>
<td>x μl of downstream primer at optimized concentration (200–600 nM)</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>DNA</th>
<th>Quantity per reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genomic DNA</td>
<td>5 pg – 100 ng</td>
</tr>
<tr>
<td>cDNA</td>
<td>0.1 pg – 100 ng*</td>
</tr>
</tbody>
</table>

*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.

<table>
<thead>
<tr>
<th>Cycling Protocol for cDNA Targets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cycles</td>
</tr>
<tr>
<td>--------</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>40</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cycling Protocol for Genomic DNA Targets</th>
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<tr>
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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 #600880, 400 reactions
Catalog #600881 4000 reactions

Ordering Information
By phone (US and Canada*): 800-227-9770
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