Brilliant III Ultra-Fast QRT-PCR Master Mix
Quick Reference Guide for the Bio-Rad CFX96 Real-Time PCR Detection System

This quick reference guide provides an optimized protocol for using Agilent’s Brilliant III Ultra-Fast QRT-PCR Master Mix with the CFX96 Real-Time PCR Detection System from Bio-Rad. 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 the reagent mixture on ice.

<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 RNA)</td>
</tr>
<tr>
<td>10 µl of 2× QRT-PCR Master Mix</td>
</tr>
<tr>
<td>x µl of experimental probe at optimized concentration (100–600 nM)</td>
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<tr>
<td>x µl of upstream primer at optimized concentration (200–600 nM)</td>
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<tr>
<td>x µl of downstream primer at optimized concentration (200–600 nM)</td>
</tr>
<tr>
<td>0.2 µl of 100 mM DTT</td>
</tr>
<tr>
<td>1 µl of RT/RNase Block</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>RNA</th>
<th>Quantity per reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total RNA</td>
<td>0.1 pg – 100 ng</td>
</tr>
<tr>
<td>mRNA</td>
<td>0.1 pg – 1 ng</td>
</tr>
</tbody>
</table>

4. Mix the reactions without creating bubbles, then centrifuge briefly.
Set Up the QPCR Plate and Thermal Profile

1. In the CFX Manager software, click File > New > Experiment.
2. From the Express Load drop-down menu, select CFX_RT_qPCR.
3. On the Protocol tab of the software, click Edit Selected to open the Protocol Editor.
4. Specify a sample volume of 20 µl and edit the protocol parameters to match those shown below.

5. Click OK to close the Protocol Editor window.
6. On the Plate tab of the software, click Edit Selected to open the Plate Editor. Edit the contents of the wells as needed, and click OK to close the Plate Editor window.

Run the PCR Program

1. Place the reactions in the CFX96 instrument.
2. From the Start Run tab, start the PCR program.

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
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