Brilliant III Ultra-Fast SYBR® Green QPCR Master Mix
Quick Reference Guide for the ABI StepOnePlus Real-Time PCR System

This quick reference guide provides an optimized protocol for using Agilent’s Brilliant III Ultra-Fast SYBR® Green QPCR Master Mix with the StepOnePlus Real-Time PCR System from Applied Biosystems. For detailed instructions, refer to the full product manual.

Prepare the Reactions

1. Dilute the reference dye 1:50 using nuclease-free PCR-grade water.
2. 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>
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</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× SYBR Green QPCR Master Mix</td>
</tr>
<tr>
<td>x µl of upstream primer at optimized concentration (200–500 nM)</td>
</tr>
<tr>
<td>x µl of downstream primer at optimized concentration (200–500 nM)</td>
</tr>
<tr>
<td>0.3 µl of diluted reference dye</td>
</tr>
</tbody>
</table>

3. Gently mix the reagent mixture without creating bubbles, then distribute the mixture to the experimental reaction tubes.
4. 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>
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<tbody>
<tr>
<td>Genomic DNA</td>
<td>5 pg – 50 ng</td>
</tr>
<tr>
<td>cDNA</td>
<td>0.5 pg – 100 ng*</td>
</tr>
</tbody>
</table>

*Refers to RNA input amount during cDNA synthesis

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

1. From the Home screen of the StepOnePlus software, click **Advanced Setup**.

2. Complete the Setup screens for a new experiment as needed.

   *On the Experiment Properties screen, select **SYBR Green Reagents** (including a melt curve) and the **Fast** ramp speed.*

   *On the Run Method screen, set the reaction volume to 20 µl and adjust the thermal profile according to the image below.*

![Thermal Profile Diagram]

*Note: If you do not require a high-resolution melt curve, you can increase the ramp rate during the melt segment to 0.5°C per second to shorten the protocol time.*

**Run the PCR Program**

1. Place the reactions in the StepOnePlus instrument.

2. On the Run screen, click **START RUN**.

**Analyze Data**

1. Analyze the results of the run as needed for your experiment.

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