This quick reference guide provides an optimized protocol for using Agilent’s Brilliant III Ultra-Fast SYBR® Green QRT-PCR Master Mix with the LightCycler 480 Real-Time PCR System from Roche. 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.*

   **Reagent Mixture**
   - Nuclease-free PCR-grade water to bring final volume to 20 µl (including RNA)
   - 10 µl of 2× SYBR Green QRT-PCR Master Mix
   - x µl of upstream primer at optimized concentration (150–500 nM)
   - x µl of downstream primer at optimized concentration (150–500 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 main window in the LightCycler 480 software, click **Sample Editor** on the module bar to open the **Sample Editor** module. Enter sample information for your experiment as needed.

2. Click **Experiment** on the module bar to open the **Run** module.

3. From the **Run Protocol** tab, enter a reaction volume of 20 μl.

4. Set the **Detection Format** to **SYBR Green I/HRM Dye**.

5. Set up the PCR program to run the cycling protocol below:

<table>
<thead>
<tr>
<th>Program Name</th>
<th>Cycles</th>
<th>Analysis Mode</th>
<th>Acquisition Mode</th>
<th>Ramp Rate (°C/s)</th>
<th>Hold Time</th>
<th>Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reverse transcription</td>
<td>1</td>
<td>None</td>
<td>None</td>
<td>4.4</td>
<td>10 minutes</td>
<td>50°C</td>
</tr>
<tr>
<td>Denaturation</td>
<td>1</td>
<td>None</td>
<td>None</td>
<td>4.4</td>
<td>3 minutes</td>
<td>95°C</td>
</tr>
<tr>
<td>Amplification</td>
<td>45</td>
<td>Quantification</td>
<td>None</td>
<td>4.4</td>
<td>5 seconds</td>
<td>95°C</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Single</td>
<td>2.2</td>
<td>10 seconds</td>
<td>60°C</td>
</tr>
<tr>
<td>Melting curve</td>
<td>1</td>
<td>Melting curves</td>
<td>None</td>
<td>4.4</td>
<td>5 seconds</td>
<td>95°C</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>None</td>
<td>2.2</td>
<td>1 minute</td>
<td>65°C</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Continuous (5 acquisitions/second)</td>
<td>0.11</td>
<td>—</td>
<td>97°C</td>
</tr>
<tr>
<td>Cooling</td>
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<td>None</td>
<td>None</td>
<td>2.2</td>
<td>30 seconds</td>
<td>40°C</td>
</tr>
</tbody>
</table>

Run the PCR Program

1. Place the reactions in the LightCycler 480 instrument.

2. From the **Run Protocol** or **Data** tab, click **Start Run**.

Analyze Data

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

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Product Information

Catalog #600886, 400 reactions
Catalog #600887, 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