



Brilliant III Ultra-Fast SYBR[®] Green QPCR 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 SYBR[®] Green QPCR 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.

Reagent Mixture
Nuclease-free PCR-grade water to bring final volume to 20 μ l (including DNA)
10 μ l of 2 \times SYBR Green QPCR Master Mix
x μ l of upstream primer at optimized concentration (200–500 nM)
x μ l of downstream primer at optimized concentration (200–500 nM)

- 2 Gently mix the reagent mixture without creating bubbles, then distribute the mixture to the experimental reaction tubes.
- 3 Add x μ l of experimental DNA to each reaction to bring the final reaction volume to 20 μ l. The table below lists a suggested quantity range for different DNA templates.

DNA	Quantity per reaction
Genomic DNA	5 pg – 50 ng
cDNA	0.5 pg – 100 ng*

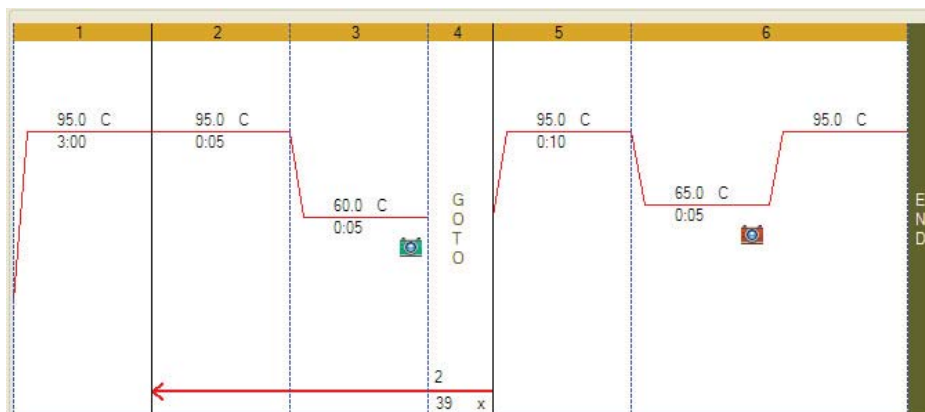
*Refers to RNA input amount during cDNA synthesis

- 4 Mix the reactions without creating bubbles, then centrifuge briefly.



Set Up the QPCR Plate and Thermal Profile

- 1 In the CFX Manager software, click **File > New > Experiment**.
- 2 From the **Express Load** drop-down menu, select **CFX_2StepAmp+Melt**.
- 3 On the **Protocol** tab of the software, click **Edit Selected** to open the **Protocol Editor**.
- 4 Specify a sample volume of 20 μ l and edit the protocol parameters to match those shown below.



Note: Increasing the annealing/extension step to 10 seconds is recommended for especially challenging applications, e.g. amplification of low-abundant targets.

- 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 #600882, 400 reactions
Catalog #600883, 4000 reactions

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