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 µl (including DNA)
10 µl of 2× SYBR Green QPCR Master Mix
x µl of upstream primer at optimized concentration (200–500 nM)
x µl 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 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.

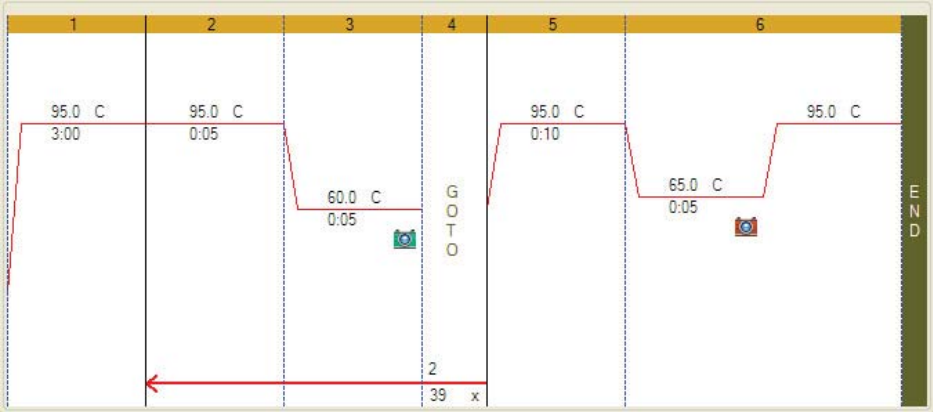
DNA	Quantity per reaction
Genomic DNA	5 pg – 50 ng
cDNA	0.5 pg – 100 ng*

*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 µl 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.

Product Information	Ordering Information	Technical Services
Catalog #600882, 400 reactions Catalog #600883, 4000 reactions	By phone (US and Canada*): 800-227-9770 On the web: www.agilent.com/genomics	By phone (US and Canada*): 800-227-9770 By email: techservices@agilent.com

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