# 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 the Stratagene 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.

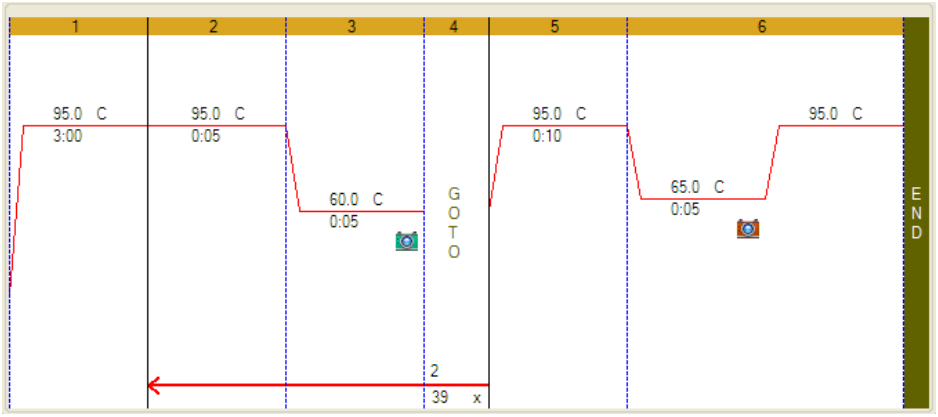
| DNA | Quantity per reaction |
|---|---|
| Genomic DNA | 5 pg – 50 ng |
| cDNA | 0.5 pg – 100 ng* |

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

**Ordering Information**
By phone (US only*): 800-424-5444, x3
On the web: www.stratagene.com

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By phone (US only*): 800-894-1304, x2
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