

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
Reactions1Prepare 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 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 $x \ \mu$ 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*

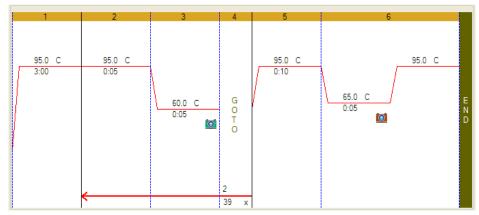
*Refers to RNA input amount during cDNA synthesis

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
Program1Place the reactions in the CFX96 instrument.2From the Start Run tab, start the PCR program.
- **Analyze Data 1** Analyze the results of the run as needed for your experiment.

Notices to Purchaser

Purchase of this product includes an immunity from suit under patents specified in the product insert to use only the amount purchased for the purchaser's own internal research. No other patent rights are conveyed expressly, by implication, or by estoppel. Further information on purchasing licenses may be obtained by contacting the Director of Licensing, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, USA.

SYBR® is licensed for research and development only under patents and patent applications owned by Invitrogen Corporation. SYBR® is a registered trademark of Molecular Probes, Inc.

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 **Technical Services**

By phone (US only*): 800-894-1304, x2 By email: techservices@agilent.com

©Agilent Technologies, Inc. 2010

*For other countries, please contact your local sales representative at www.agilent.com/chem/contactus

Manual Part Number 5990-3057, Revision A

Brilliant III Ultra-Fast SYBR® Green OPCR Master Mix