Brilliant III Ultra-Fast SYBR® Green QPCR Master Mix

Quick Reference Guide for the ABI StepOnePlus Real-Time PCR System

This quick reference guide provides an optimized protocol for using the Stratagene Brilliant III Ultra-Fast SYBR® Green QPCR Master Mix with the StepOnePlus Real-Time PCR System from Applied Biosystems. For detailed instructions, refer to the full product manual.

Prepare the Reactions

1 Dilue the reference dye 1:50 using nuclease-free PCR-grade water.

2 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)
0.3 µl of diluted reference dye

3 Gently mix the reagent mixture without creating bubbles, then distribute the mixture to the experimental reaction tubes.

4 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

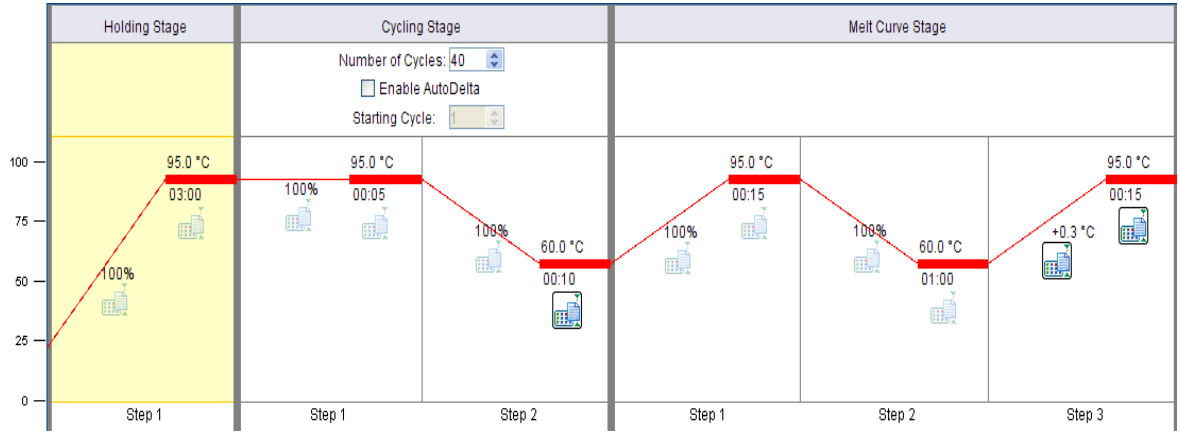
5 Mix the reactions without creating bubbles, then centrifuge briefly.

Set Up the QPCR Plate and Thermal Profile

1. From the Home screen of the StepOnePlus software, click **Advanced Setup**.
2. Complete the Setup screens for a new experiment as needed.

   On the Experiment Properties *screen, select* ***SYBR Green Reagents*** *(including a melt curve) and the* ***Fast*** *ramp speed.*

   On the Run Method *screen, set the reaction volume to 20 µl and adjust the thermal profile according to the image below.*



Note: If you do not require a high-resolution melt curve, you can increase the ramp rate during the melt segment to 0.5°C per second to shorten the protocol time.

Run the PCR Program

1. Place the reactions in the StepOnePlus instrument.
2. On the Run screen, click **START RUN**.

Analyze Data

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

Product Information
Catalog #600805, 400 reactions
Catalog #600816, 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

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

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