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 Agilent's 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. Dilute 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.

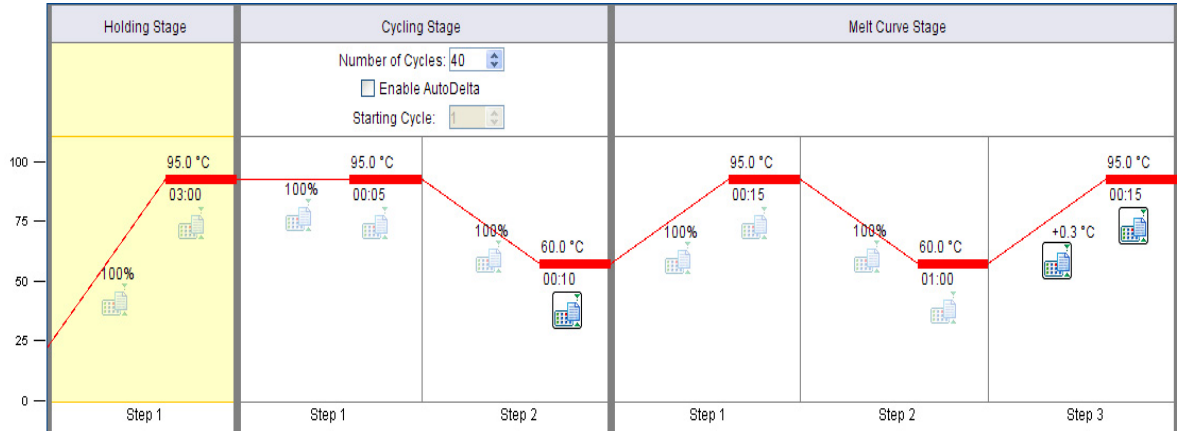
Set Up the QPCR Plate and Thermal Profile

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 #600882, 400 reactions
Catalog #600883, 4000 reactions

Ordering Information
By phone (US and Canada*): 800-227-9770
On the web: www.agilent.com/genomics

Technical Services
By phone (US and Canada*): 800-8227-9770
By email: techservices@agilent.com

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

Manual Part Number 5990-3056, Revision B0
For Research Use Only. Not for use in diagnostic procedures.