



Brilliant III Ultra-Fast SYBR[®] Green QPCR Master Mix with Low ROX

Quick Reference Guide for the ABI 7500 Fast 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 Low ROX (Catalog #600892) with the 7500 Fast Real-Time PCR System from Life Technologies. 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 μ L (including DNA)
10 μ L of 2 \times SYBR Green QPCR Master Mix
x μ L 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 μ 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 μ g – 50 ng
cDNA	0.5 μ g – 100 ng*

*Refers to RNA input amount during cDNA synthesis

- 4 Mix the reactions without creating bubbles, then centrifuge briefly.

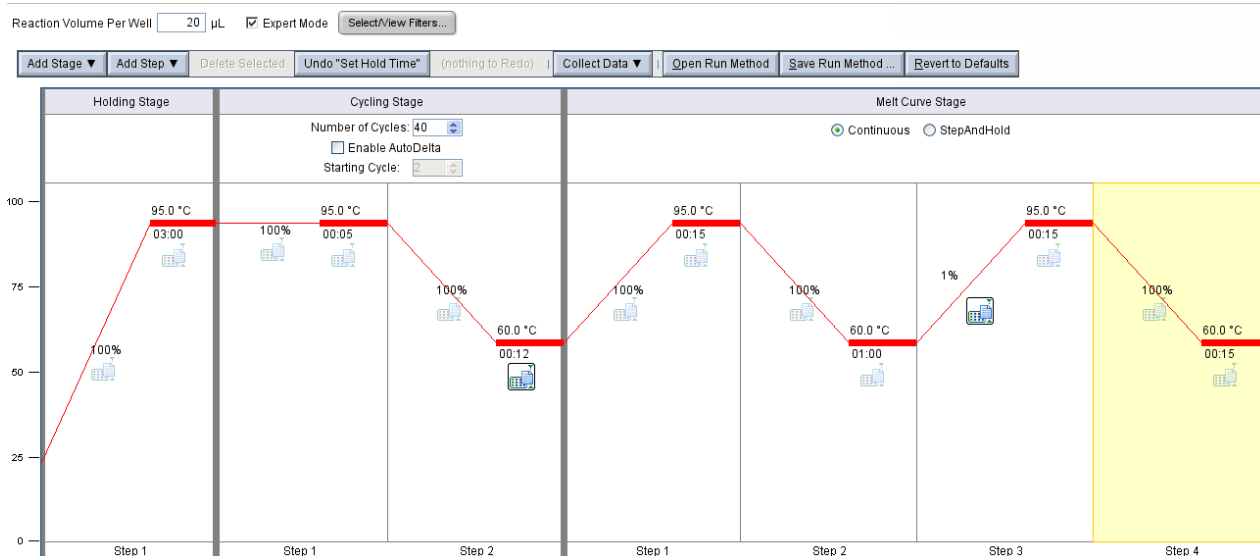


Set Up the QPCR Plate and Thermal Profile

- 1 From the Home screen of the 7500 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.*

- 3 On the **Run Method** screen, set the reaction volume to 20 μ L and mark the **Expert Mode** check box. Click **Select/View Filters** and deselect any filters not in use in the experiment.
- 4 Adjust the thermal profile according to the image below.



Note: If you do not require a high-resolution melt curve, you can select the **StepAndHold** option for the melt curve stage and then increase the ramp rate to 0.5°C per second to shorten the protocol time.

Run the PCR Program

- 1 Place the reactions in the 7500 instrument.
- 2 Click **START RUN**.

Analyze Data

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

Endnote: SYBR[®] Green is a registered trademark of Molecular Probes, Inc.

Product Information

Catalog #600892, 400 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*): 800-227-9770
By email: techservices@agilent.com

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

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