



Brilliant III Ultra-Fast SYBR® Green QRT-PCR 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 QRT-PCR 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. *Keep the reagent mixture on ice.*

Reagent Mixture
Nuclease-free PCR-grade water to bring final volume to 20 µl (including RNA)
10 µl of 2× SYBR Green QRT-PCR Master Mix
x µl of upstream primer at optimized concentration (150–500 nM)
x µl of downstream primer at optimized concentration (150–500 nM)
0.3 µl of diluted reference dye
0.2 µl of 100 mM DTT
1 µl of RT/RNase Block

- 3 Gently mix the reagent mixture without creating bubbles, then distribute the mixture to the experimental reaction tubes. *Keep the reactions on ice.*
- 4 Add x µl of experimental RNA to each reaction to bring the final reaction volume to 20 µl. The table below lists a suggested quantity range for different RNA templates.

RNA	Quantity per reaction
Total RNA	0.1 pg – 100 ng
mRNA	0.1 pg – 1 ng

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



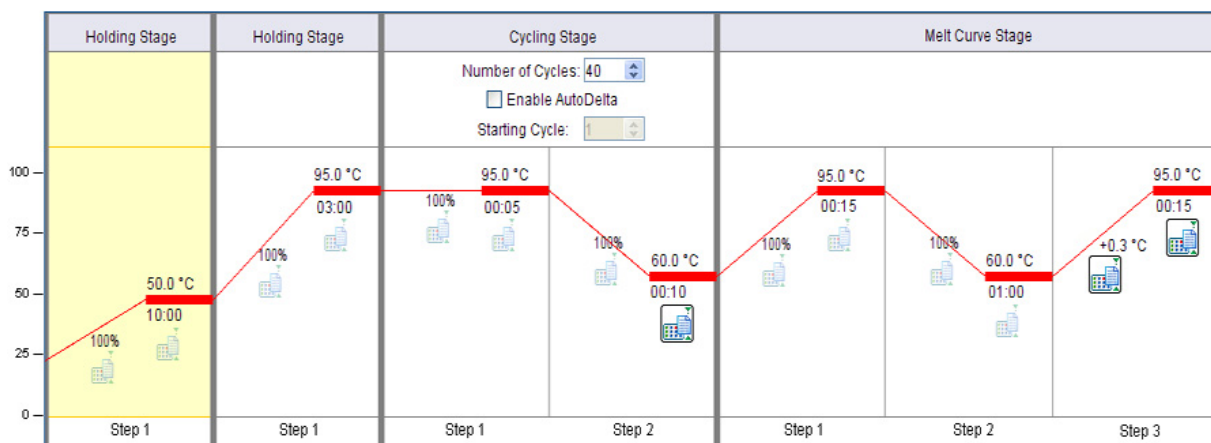
Set Up the QPCR Plate and Thermal Profile

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.*

3 *On the Run Method screen, set the reaction volume to 20 μ l and adjust the thermal profile according to the image below. Note that a new step needs to be added to the beginning of the profile for the 50°C incubation.*



Note: If you do not require a high-resolution melt curve, you can increase the ramp rate during the melt curve stage 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.

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Product Information

Catalog #600886, 400 reactions
Catalog #600887, 4000 reactions

Ordering Information

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Manual Part Number 5990-3060, Revision B0

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