Brilliant III Ultra-Fast SYBR® Green QRT-PCR Master Mix

Quick Reference Guide for the ABI 7500 Fast Real-Time PCR System

This quick reference guide provides an optimized protocol for using the Stratagene Brilliant III Ultra-Fast SYBR® Green QRT-PCR Master Mix with the 7500 Fast Real-Time PCR System from Applied Biosystems. For detailed instructions, refer to the full product manual.

Prepare the Reactions

1 Dilute the reference dye 1:500 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.

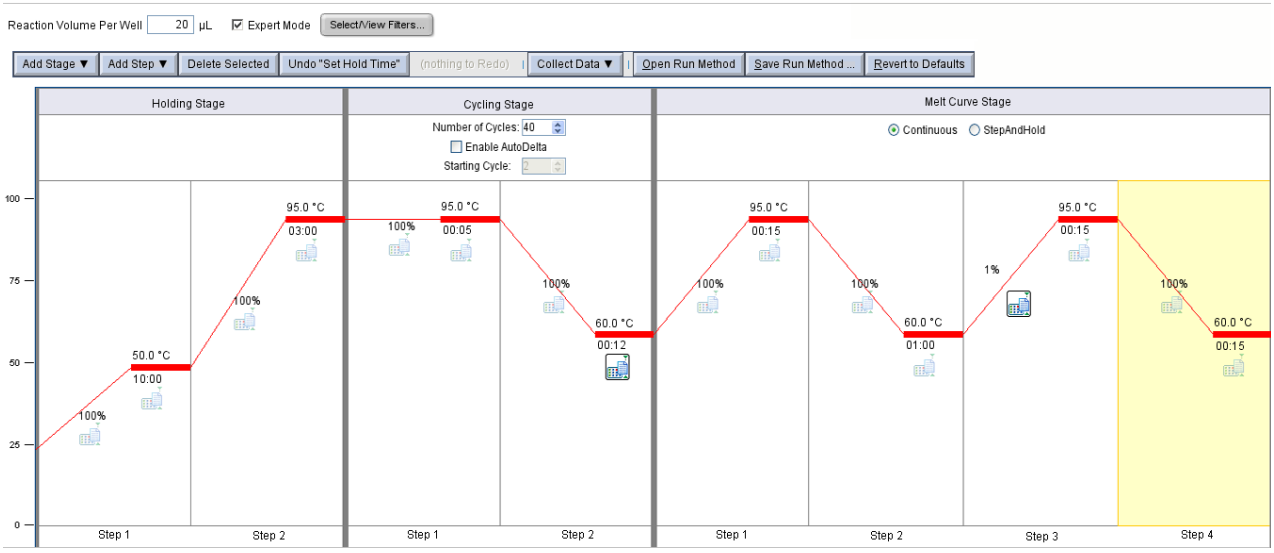
RNA	Quantity per reaction
Total RNA	0.1 pg – 100 ng
mRNA	0.1 pg – 1 ng

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 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 µl 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 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 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.

Product Information	Ordering Information	Technical Services
Catalog #600886, 400 reactions Catalog #600887, 4000 reactions	By phone (US only*): 800-424-5444, x3 On the web: www.genomics.agilent.com	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*

Manual Part Number 5990-3070, Revision A

Printed May 1, 2010
5990-5808EN