



# Brilliant III Ultra-Fast SYBR<sup>®</sup> 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<sup>®</sup> 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 $\mu$ l (including RNA)
10 $\mu$ l of 2 $\times$ SYBR Green QRT-PCR Master Mix
$x$ $\mu$ l of upstream primer at optimized concentration (150–500 nM)
$x$ $\mu$ l of downstream primer at optimized concentration (150–500 nM)
0.3 $\mu$ l of diluted reference dye
0.2 $\mu$ l of 100 mM DTT
1 $\mu$ l 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  $x$   $\mu$ l of experimental RNA to each reaction to bring the final reaction volume to 20  $\mu$ l. The table below lists a suggested quantity range for different RNA templates.

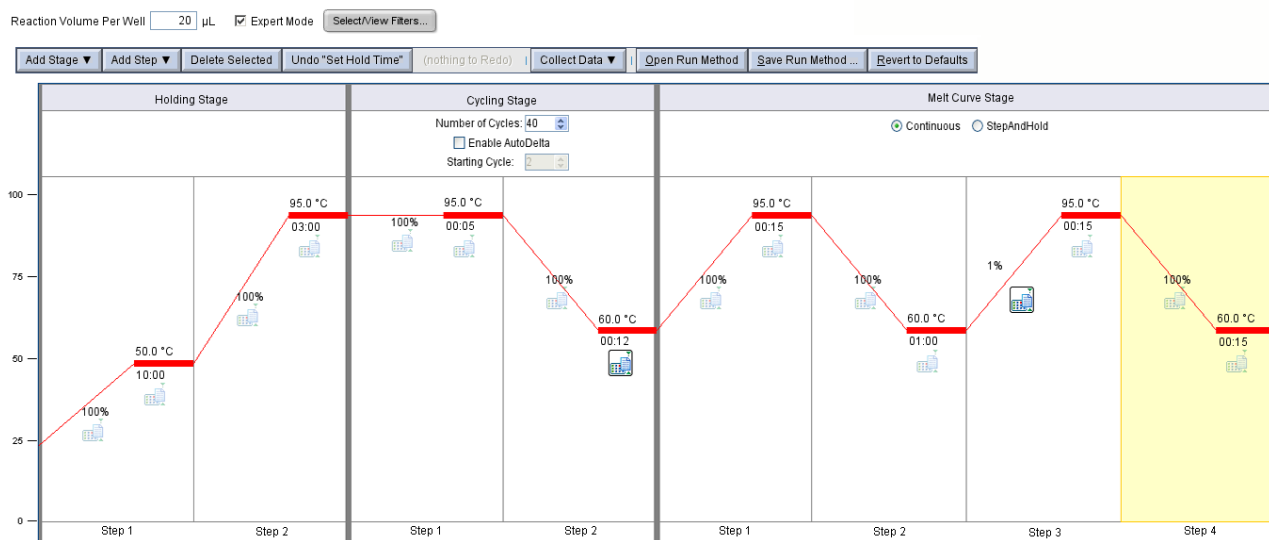
RNA	Quantity per reaction
Total RNA	0.1 pg – 100 ng
mRNA	0.1 pg – 1 ng

- 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  $\mu$ 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 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.

### Notices to Purchaser

Purchase of this product includes an immunity from suit under patents specified in the product insert to use only the amount purchased for the purchaser's own internal research. No other patent rights are conveyed expressly, by implication, or by estoppel. Further information on purchasing licenses may be obtained by contacting the Director of Licensing, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, USA.

SYBR® is licensed for research and development only under patents and patent applications owned by Invitrogen Corporation. SYBR® is a registered trademark of Molecular Probes, Inc.

### Product Information

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

### Ordering Information

By phone (US only\*): 800-424-5444, x3  
On the web: [www.genomics.agilent.com](http://www.genomics.agilent.com)

### Technical Services

By phone (US only\*): 800-894-1304, x2  
By email: [techservices@agilent.com](mailto:techservices@agilent.com)

\*For other countries, please contact your local sales representative at [www.agilent.com/chem/contactus](http://www.agilent.com/chem/contactus)

Manual Part Number 5990-3070, Revision A

©Agilent Technologies, Inc. 2010  
Printed May 1, 2010  
5990-5808EN