



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

Quick Reference Guide for the ABI 7900HT 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 7900HT 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 1:50 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 μ l (including RNA) |
| 10 μ l of 2 \times SYBR Green QRT-PCR Master Mix |
| x μ l of upstream primer at optimized concentration (150–500 nM) |
| x μ l of downstream primer at optimized concentration (150–500 nM) |
| 0.3 μ l of diluted reference dye |
| 0.2 μ l of 100 mM DTT |
| 1 μ 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 μ l of experimental RNA to each reaction to bring the final reaction volume to 20 μ l. The table below lists a suggested quantity range for different RNA templates.

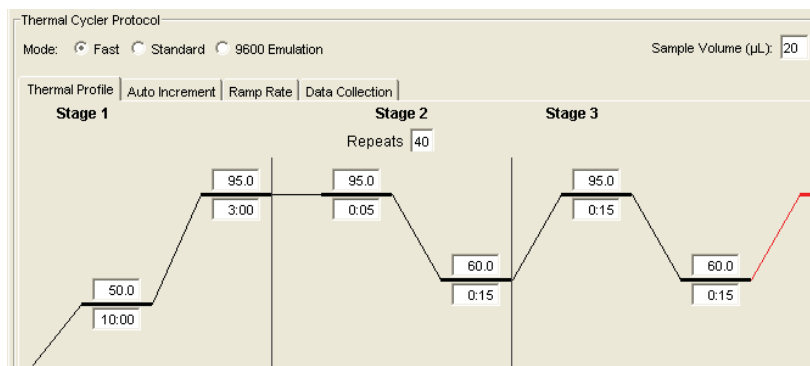
| RNA | Quantity per reaction |
|-----------|-----------------------|
| Total RNA | 0.1 pg – 100 ng |
| mRNA | 0.1 pg – 1 ng |

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



Set Up the QPCR Plate and Thermal Profile

- 1 From the SDS software, click **File > New** to open the Plate Document Wizard.
- 2 Enter the appropriate assay and well information for a new experiment.
- 3 Click **OK**. The Wizard will close and the plate document will appear in the main software window.
- 4 Click **Add Detector**, and select the correct reporter for the assay. Click **Copy to Plate Document**, then click **Done**.
- 5 Highlight the wells that will contain samples and check the selected reporter dye.
- 6 On the Instrument/Thermal Profile tab, enter a sample volume of 20 μL and select the *Fast* run mode. Adjust the thermal cycling conditions according to the image below, and set the instrument to report fluorescence during the 60°C step of each cycle.



Run the PCR Program

- 1 Place the reactions in the 7900HT instrument.
- 2 On the Instrument/Real Time tab, 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

Technical Services

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 5990-3074, Revision A
For Research Use Only. Not for use in diagnostic procedures.

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

©Agilent Technologies, Inc. 2010, 2016
PR7000-0445
Printed May 1, 2016
5990-5812EN