Brilliant III Ultra-Fast SYBR® Green QPCR Master Mix with High ROX

Quick Reference Guide for the ABI 7900HT 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 High ROX *(Catalog #600889) with the* 7900HT 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 µL (including DNA)
10 µL of 2× SYBR Green QPCR Master Mix
x µL of upstream primer at optimized concentration (200–500 nM)
x µL 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 µL of experimental DNA to each reaction to bring the final reaction volume to 20 µL. The table below lists a suggested quantity range for different DNA templates.

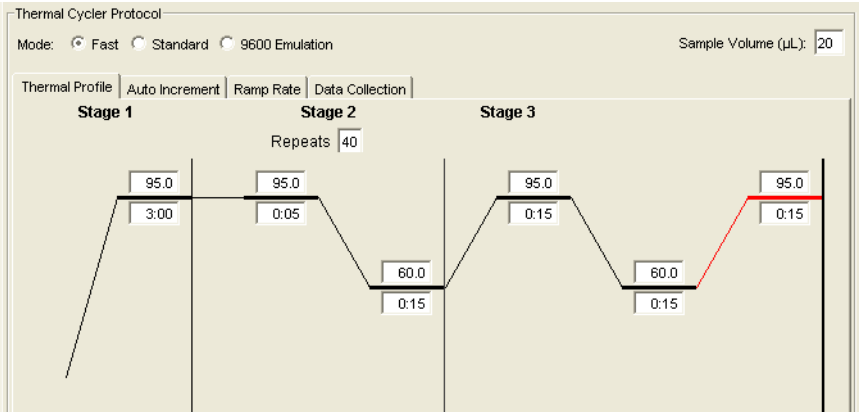
DNA	Quantity per reaction
Genomic DNA	5 pg – 50 ng
cDNA	0.5 pg – 100 ng*

*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 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.

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

Product Information	Ordering Information	Technical Services
Catalog #600889, 400 reactions	By phone (US and Canada*): 800-227-9770 On the web: www.agilent.com/genomics	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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