



Brilliant III Ultra-Fast 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 QPCR Master Mix with High ROX (Catalog #600888) 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 μ L (including DNA)
10 μ L of 2 \times QPCR Master Mix
x μ L of experimental fluorescent probe at optimized concentration (150–600 nM)
x μ L of upstream primer at optimized concentration
x μ L of downstream primer at optimized concentration

- 2 Gently mix the reagent mixture without creating bubbles, then distribute the mixture to the experimental reaction tubes.
- 3 Add x μ L of experimental DNA to each reaction to bring the final reaction volume to 20 μ L. The table below lists a suggested quantity range for different DNA templates.

DNA	Quantity per reaction
Genomic DNA	5 pg – 100 ng
cDNA	0.1 pg – 100 ng*

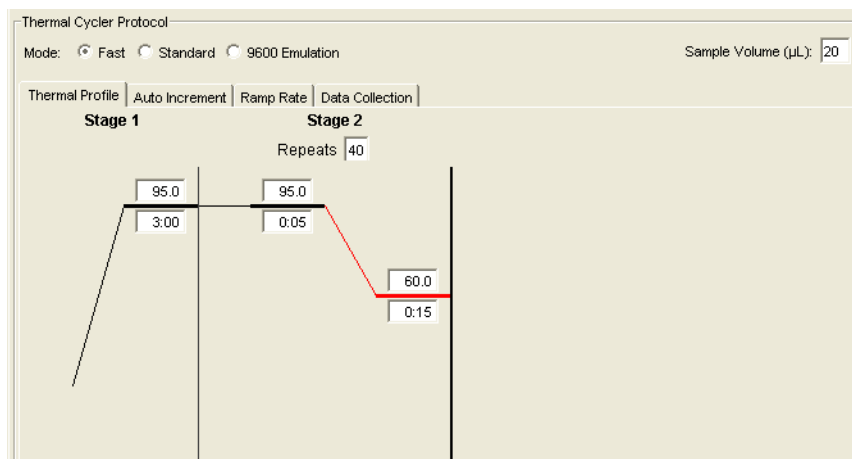
*Refers to RNA input amount during cDNA synthesis

- 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.

Product Information

Catalog #600888, 400 reactions

Ordering Information

By phone (US and Canada*): 800-227-9770
On the web: www.agilent.com/genomics

Technical Services

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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