Brilliant III Ultra-Fast 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 Agilent's Brilliant III Ultra-Fast 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 reagent mixture on ice.*

Reagent Mixture
Nuclease-free PCR-grade water to bring final volume to 20 µl (including RNA)
10 µl of 2× QRT-PCR Master Mix
x µl of experimental probe at optimized concentration (100–600 nM)
x µl of upstream primer at optimized concentration (200–600 nM)
x µl of downstream primer at optimized concentration (200–600 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.*

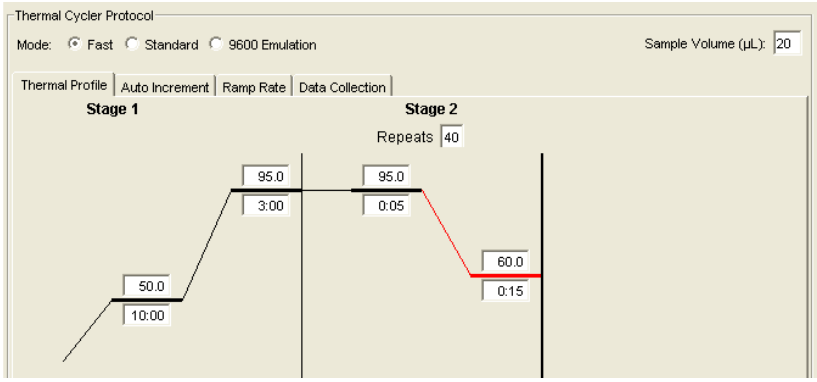
4 Add x µl of experimental RNA to each reaction to bring the final reaction volume to 20 µl. The table below lists a suggested quantity range for different RNA templates.

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 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	Ordering Information	Technical Services
Catalog #600884, 400 reactions Catalog #600885, 4000 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

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5990-5815EN