Brilliant III Ultra-Fast QPCR 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 QPCR 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.

Reagent Mixture
Nuclease-free PCR-grade water to bring final volume to 20 µl (including DNA)
10 µl of 2× QPCR Master Mix
x µl of experimental probe at optimized concentration (150–600 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

3. Gently mix the reagent mixture without creating bubbles, then distribute the mixture to the experimental reaction tubes.
4. 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.

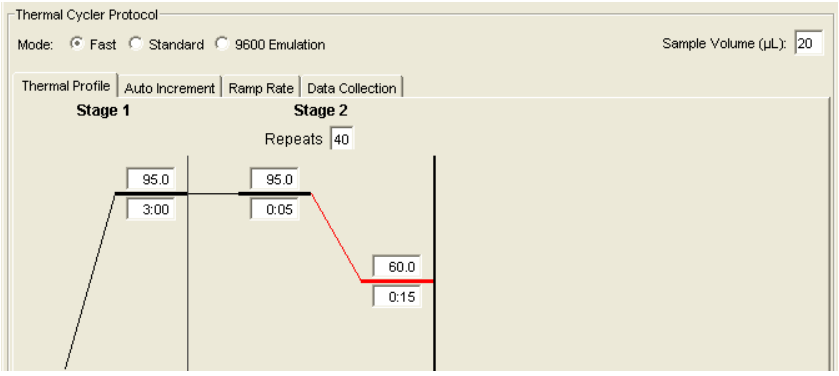
DNA	Quantity per reaction
Genomic DNA	5 pg – 100 ng
cDNA	0.1 pg – 100 ng*

*Refers to RNA input amount during cDNA synthesis

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.

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Product Information
Catalog #600880, 400 reactions
Catalog #600881, 4000 reactions

Ordering Information
By phone (US only*): 800-424-5444, x3
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Technical Services
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