



Brilliant III Ultra-Fast QPCR Master Mix

Quick Reference Guide for the ABI 7500 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 the 7500 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 1:500 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 μ l (including DNA)
10 μ l of 2 \times QPCR Master Mix
x μ l of experimental probe at optimized concentration (150–600 nM)
x μ l of upstream primer at optimized concentration (200–600 nM)
x μ l of downstream primer at optimized concentration (200–600 nM)
0.3 μ l 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 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

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

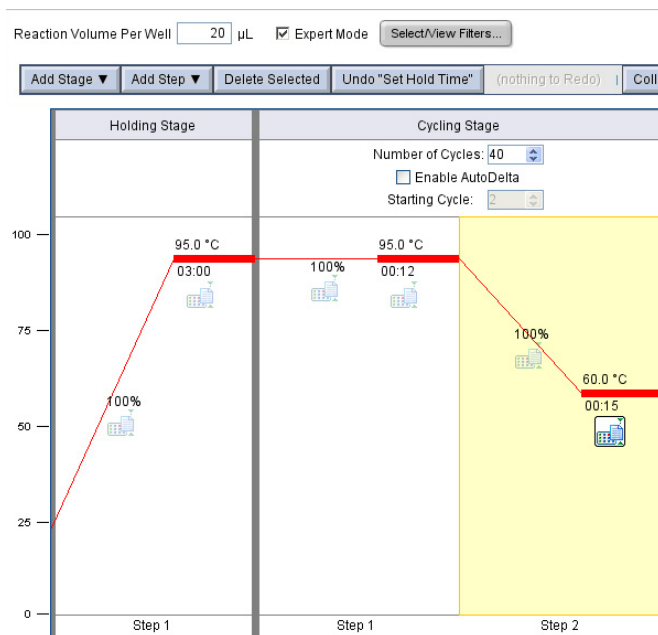


Set Up the QPCR Plate and Thermal Profile

- 1 From the Home screen of the 7500 software, click **Advanced Setup**.
- 2 Complete the Setup screens for a new experiment as needed.

On the **Experiment Properties** screen, select **TaqMan Reagents** and the **Fast** ramp speed.

- 3 On the **Run Method** screen, set the reaction volume to 20 μL and mark the **Expert Mode** check box. Click **Select/View Filters** and deselect any filters not in use in the experiment.
- 4 Adjust the thermal profile according to the image below.



Run the PCR Program

- 1 Place the reactions in the 7500 instrument.
- 2 Click **START RUN**.

Analyze Data

- 1 Analyze the results of the run as needed for your experiment.

Notice to Purchaser

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Product Information

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

Ordering Information

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

Technical Services

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