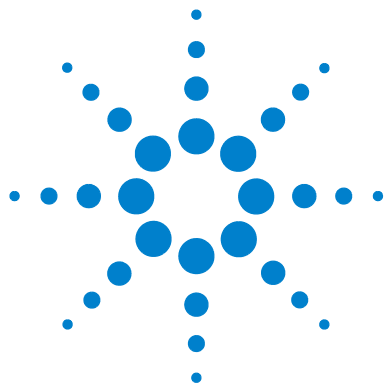




Brilliant III Ultra-Fast QRT-PCR Master Mix

Quick Reference Guide for the ABI 7500 Fast Real-Time PCR System

This quick reference guide provides an optimized protocol for using the Stratagene Brilliant III Ultra-Fast QRT-PCR 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. *Keep reagent mixture on ice.*

Reagent Mixture
Nuclease-free PCR-grade water to bring final volume to 20 μ l (including RNA)
10 μ l of 2 $\times$ QRT-PCR Master Mix
x μ l of experimental probe at optimized concentration (100–600 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
0.2 μ l of 100 mM DTT
1 μ l 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 μ l of experimental RNA to each reaction to bring the final reaction volume to 20 μ l. 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.

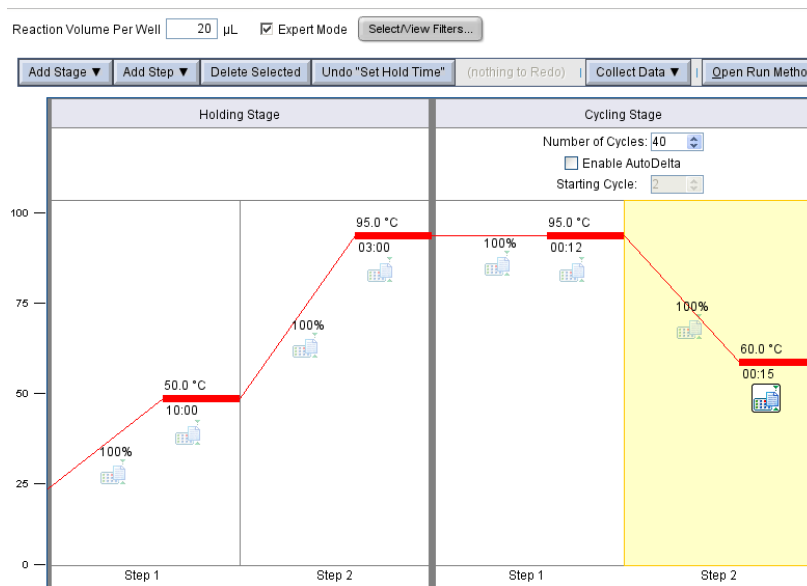


Set Up the QPCR Plate and Thermal Profile

- 1 From the 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. *Note that a new step needs to be added to the beginning of the profile for the 50°C incubation.*



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 #600884, 400 reactions
Catalog #600885, 4000 reactions

Ordering Information

By phone (US only*): 800-424-5444, x3
On the web: www.genomics.agilent.com

Technical Services

By phone (US only*): 800-894-1304, x2
By email: techservices@agilent.com

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