Brilliant III Ultra-Fast QRT-PCR Master Mix

Quick Reference Guide for the Agilent Mx3000P/Mx3005P QPCR Systems

This quick reference guide provides an optimized protocol for using Agilent's Brilliant III Ultra-Fast QRT-PCR Master Mix with the Mx3000P and Mx3005P QPCR Systems. 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 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.*

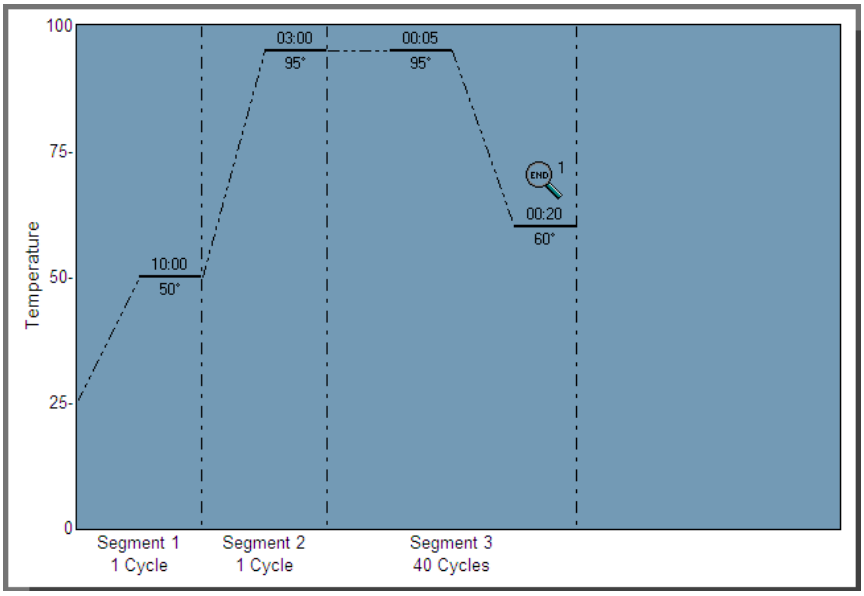
4 Add x µl of experimental RNA to each reaction to bring the final reaction volume to 20 µl. The table below lists a suggested quantity range for different RNA templates.

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. Complete the **Plate Setup** screen for a new experiment as needed, including assigning well types and assay information.
2. On the **Thermal Profile Setup** screen, set the **Thermal Profile Design** selection to **Standard**.
   - Under **Pre-Melt/RT Segment**, click **2 Plateaus**.
   - Under **Amplification Segment**, click **Fast 2 Step**.
3. Adjust the thermal profile according to the image below. The profile includes a 5-second denaturation step. Note that some assays may require a denaturation of up to 20 seconds. The exact denaturation time needs to be optimized for each probe/target system.



Run the PCR Program

1. Place the reactions in the Mx3000P/Mx3005P instrument.
2. On the **Run** screen, 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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