Brilliant III Ultra-Fast QPCR 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 QPCR 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.

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.

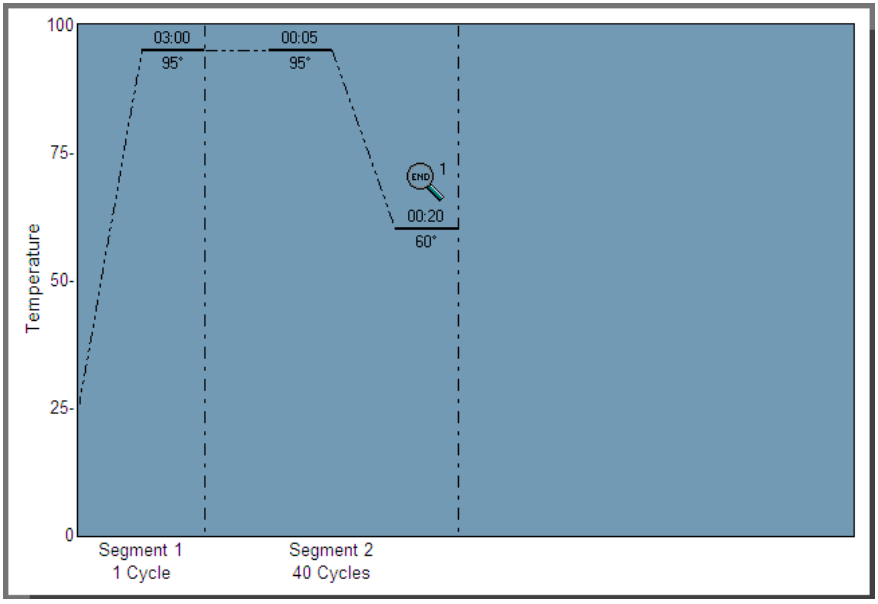
DNA	Quantity per reaction
Genomic DNA	5 pg – 100 ng
cDNA	0.1 pg – 100 ng*

*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. 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 **1 Plateau**.
   - 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 #600880, 400 reactions Catalog #600881, 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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