Brilliant III Ultra-Fast SYBR® Green 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 SYBR® Green 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× SYBR Green QPCR Master Mix
x µl of upstream primer at optimized concentration (200–500 nM)
x µl of downstream primer at optimized concentration (200–500 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.

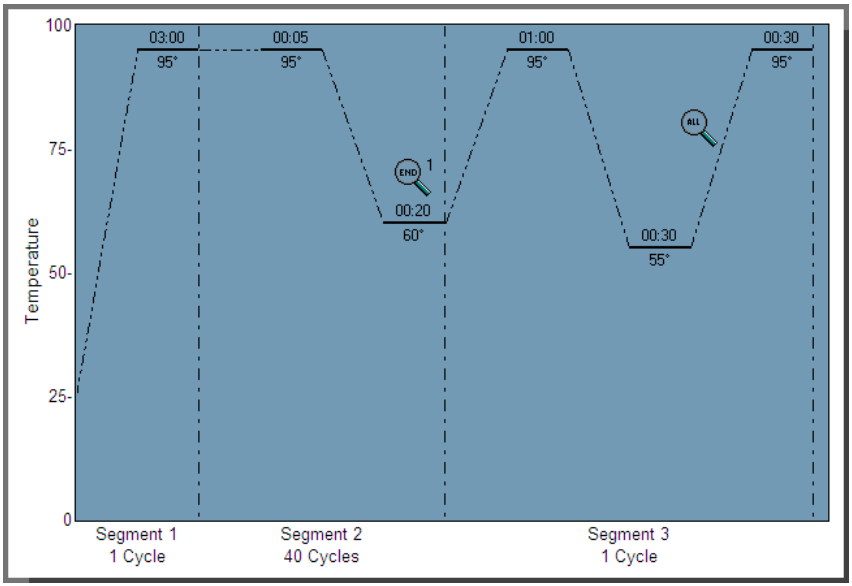
DNA	Quantity per reaction
Genomic DNA	5 pg – 50 ng
cDNA	0.5 pg – 100 ng*

*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**.
   - Under **Dissociation/Melt Segment**, click **Dissociation/Melt**.
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 target.



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
Catalog #600882, 400 reactions
Catalog #600883, 4000 reactions

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

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