



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 μ l (including DNA)
10 μ l of 2 \times SYBR Green QPCR Master Mix
x μ l of upstream primer at optimized concentration (200–500 nM)
x μ l of downstream primer at optimized concentration (200–500 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 – 50 ng
cDNA	0.5 pg – 100 ng*

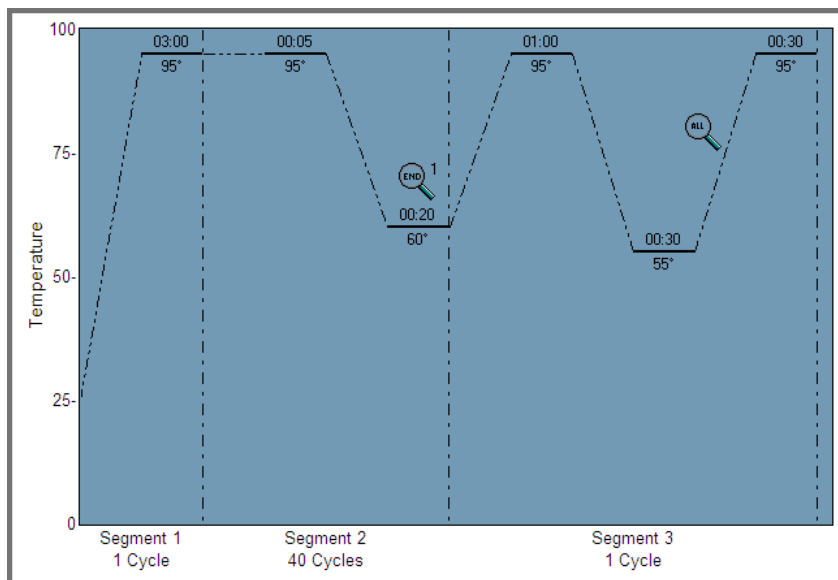
*Refers to RNA input amount during cDNA synthesis

- 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.

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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

Technical Services

By phone (US and Canada*): 800-227-9770
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Brilliant III Ultra-Fast SYBR® Green QPCR Master Mix

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