



Brilliant III Ultra-Fast SYBR® Green QPCR Master Mix with Low ROX

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 Low ROX (Catalog #600892) with the Mx3000P and Mx3005P QPCR Systems. For detailed instructions, refer to the full product manual.

Prepare the Reactions

- 1 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)

- 2 Gently mix the reagent mixture without creating bubbles, then distribute the mixture to the experimental reaction tubes.
- 3 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*

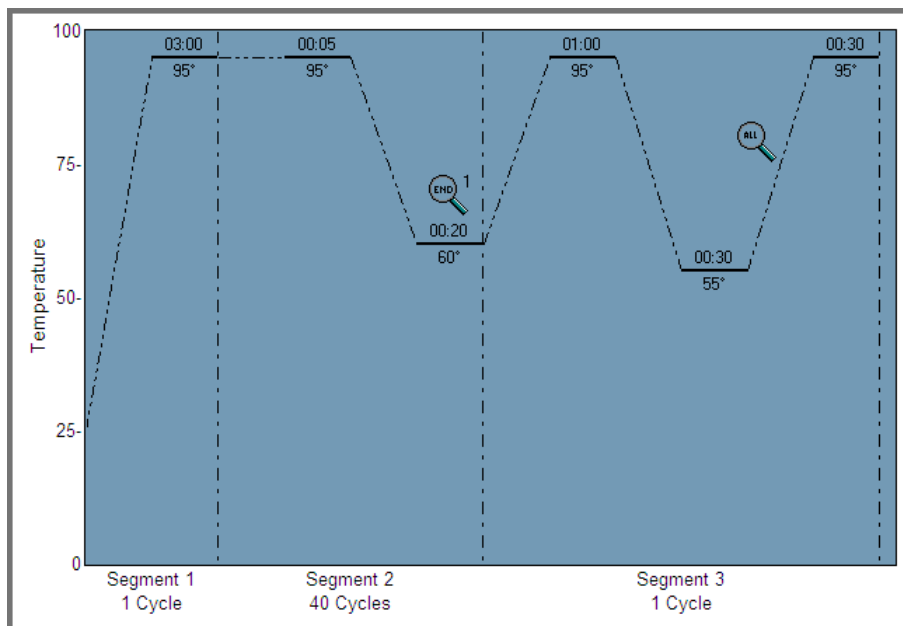
*Refers to RNA input amount during cDNA synthesis

- 4 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.

Endnote: SYBR® Green is a registered trademark of Molecular Probes, Inc.

Product Information

Catalog #600892, 400 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
By email: techservices@agilent.com

*For other countries, please contact your local sales representative at www.agilent.com/genomics/contactus