



Brilliant III Ultra-Fast SYBR[®] Green QRT-PCR Master Mix

Quick Reference Guide for the Stratagene Mx3000P/Mx3005P QPCR Systems

This quick reference guide provides an optimized protocol for using the Stratagene Brilliant III Ultra-Fast SYBR[®] Green QRT-PCR Master Mix with the Stratagene Mx3000P and Mx3005P QPCR Systems from Agilent. 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 the reagent mixture on ice.*

Reagent Mixture
Nuclease-free PCR-grade water to bring final volume to 20 μ l (including RNA)
10 μ l of 2 \times SYBR Green QRT-PCR Master Mix
x μ l of upstream primer at optimized concentration (150–500 nM)
x μ l of downstream primer at optimized concentration (150–500 nM)
0.3 μ l of diluted reference dye
0.2 μ l of 100 mM DTT
1 μ l 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 μ l of experimental RNA to each reaction to bring the final reaction volume to 20 μ l. The table below lists a suggested quantity range for different RNA templates.

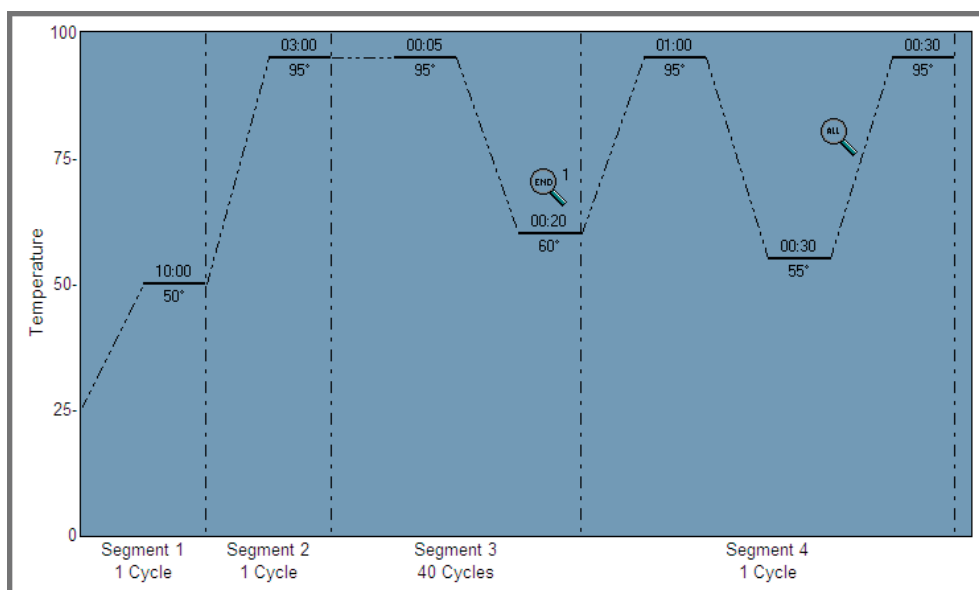
RNA	Quantity per reaction
Total RNA	0.1 pg – 100 ng
mRNA	0.1 pg – 1 ng

- 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**.
 - 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 #600886, 400 reactions
Catalog #600887, 4000 reactions

Ordering Information

By phone (US only*): 800-424-5444, x3
On the web: www.genomics.agilent.com

Technical Services

By phone (US only*): 800-894-1304, x2
By email: techservices@agilent.com

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