

Brilliant III Ultra-Fast QPCR 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 QPCR 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.

Reagent Mixture

Nuclease-free PCR-grade water to bring final volume to 20 μI (including DNA)

10 μ l of 2× QPCR Master Mix

x µl of experimental probe at optimized concentration (150-600 nM)

x μ l of upstream primer at optimized concentration (200–600 nM)

x µl of downstream primer at optimized concentration (200–600 nM)

 $0.3 \ \mu$ 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 $x \mu l$ 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.

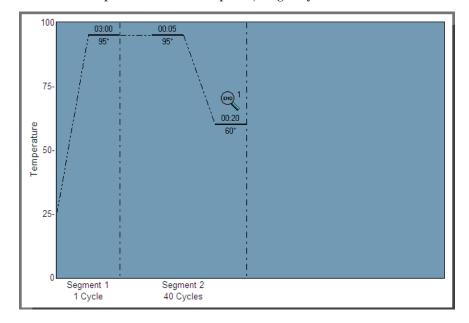


Stratagene Products

Set Up the
R Plate and1 Complete the Plate Setup screen for a new experiment as needed,
including assigning well types and assay information.

QPCR Plate and Thermal Profile

- 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
Program1Place the reactions in the Mx3000P/Mx3005P instrument.2On 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 #600880, 400 reactions Catalog #600881, 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

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

Manual Part Number 5990-7204, Revision A

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