

Brilliant III Ultra-Fast qPCR/QRT-PCR Master Mixes for ABI StepOnePlus Real-Time PCR System

Data Sheet

For faster, improved real-time quantitative PCR (qPCR) on the ABI StepOnePlus Real-Time PCR instrument – choose Agilent

- Novel fast *Taq* mutant for qPCR results in under 40 minutes
- Enhanced rapid hot start capability saves time and reduces primer-dimer formation
- Optimized fast cycling formulation ensures reliable and reproducible data with shorter run times
- Convenient pre-blended formulations compatible with any fluorescent detection chemistry including both sequence-specific probes and SYBR® Green dyes



Figures 1A & 1B
Low Copy Discrimination

Amplification plot and standard curve plot for 2-fold dilution of linearized plasmid run on a StepOnePlus real-time PCR system. Brilliant III Ultra-Fast QPCR master mix exhibits precise detection of 2-fold differences from 1536 copies down to 3 copy equivalents. The novel hot start technology of Brilliant III Ultra-Fast QPCR reagents decreases primer-dimer formation and amplification of unwanted side reactions resulting in superior sensitivity of detection down to very low target concentrations. Efficiency = 95.7%, RSq = 0.992.

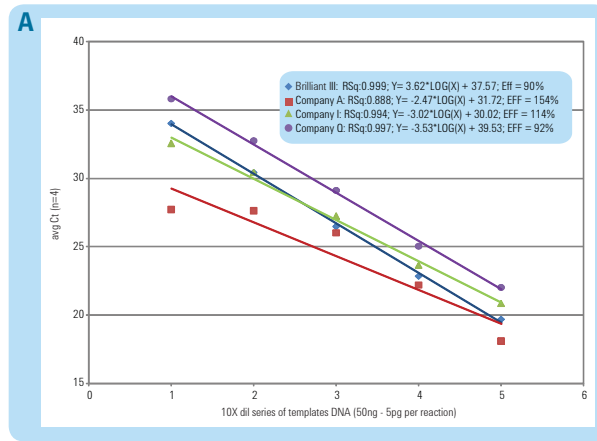
Our next generation Brilliant III Ultra-Fast QPCR and QRT-PCR Master Mix Kits offer significantly reduced cycling time and superior sensitivity without compromising accurate quantification and reproducibility. The Brilliant III QPCR and QRT-PCR master mixes are designed to provide the fastest cycling times on the ABI StepOnePlus real-time PCR instrument (also provide rapid cycling and improved performance on ABI 7900HT and 7500 Fast). The Brilliant III Ultra-Fast kits show the same consistent performance across a wide range of template concentrations and template targets as our Brilliant II reagents. The new ultra-fast reagents allow the completion of real-time experiments in under 40 minutes giving researchers access to their data faster without compromising data quality. These reagents feature a newly engineered *Taq* derived mutant delivering faster extension rate combined with an optimized buffer formulation and novel hot-start technology minimizing non-specific amplification products to increase overall sensitivity. Brilliant III Ultra-Fast QPCR and QRT-PCR Master Mixes provide the benefit of ultra-fast cycling times while maintaining the performance of conventional real-time PCR reagents.

Highly efficient one-step QRT-PCR is performed with our Brilliant III Ultra-Fast QRT-PCR reagents using a Moloney-based RT for 1st strand synthesis with optimal performance at a synthesis temperature of 50°C.

AffinityScript QPCR cDNA Synthesis Kit can be used for 1st strand cDNA synthesis in a 2-step providing flexibility across a wide range of temperatures. Novel hotstart *Taq* DNA polymerase combined with AffinityScript RT, minimizes the potential for primer-dimer formation or other non-specific PCR products and delivers the most reproducible results.

The new Brilliant III Ultra-Fast QPCR Master Mixes can deliver QPCR results in less than 40 minutes on the StepOnePlus Real-Time PCR system. The enhanced sensitivity, specificity and reproducibility within an assay and across multiple assays from high to very low copy number templates makes Brilliant III Ultra-Fast QPCR and QRT-PCR Master Mixes your preferred choice for Real-Time PCR analysis.



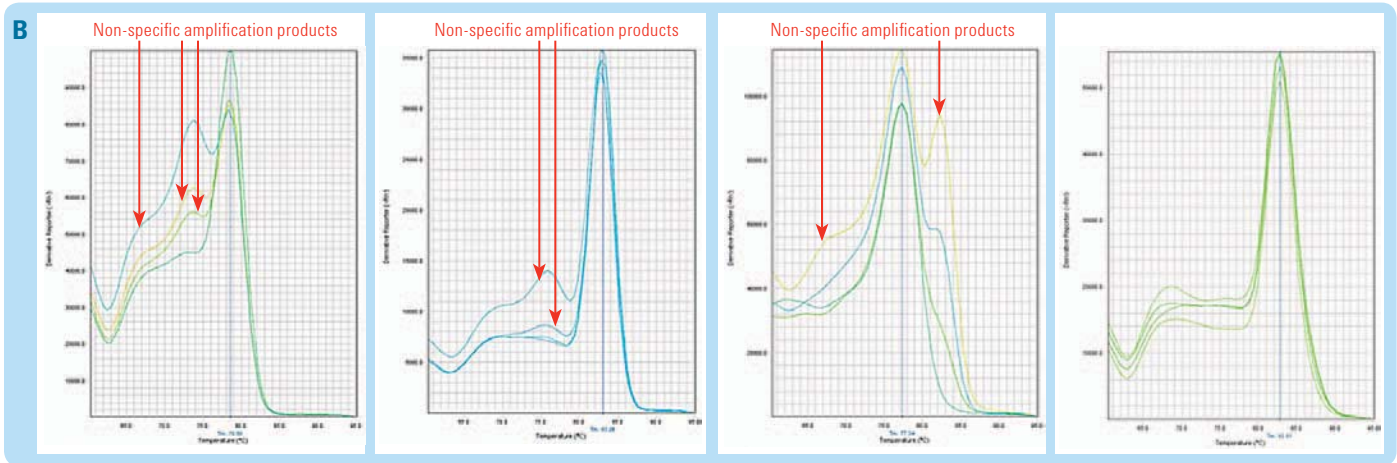


Company A
Tm full prod: 78.6

Company Q
Tm full prod: 83.1

Company I
Tm full prod: 82.6

Agilent Brilliant III
Tm full prod: 78.6



Figures 2A & 2B
Minimizing Primer Dimerization Delivers Superior Sensitivity

Brilliant III Ultra-Fast qPCR Master Mixes prevent generation of non-specific secondary products, to provide a greater degree of confidence in your qPCR results. Standard curve (A) showing 5-fold dilution series of 50 ng to 5 pg of human genomic DNA to detect Numb-1. The Dissociation Curve (B) shows primer-dimers or secondary non-specific PCR artifacts for all competitor master mixes. Although Company A and Company I generate earlier Ct's, the efficiency of the reaction is compromised by formation of these artifacts competing with the specific product amplification, reducing the assay limit of detection and dynamic range. The novel *Taq* mutant and new hot start technology of Brilliant III Ultra-Fast qPCR Master Mixes reduces the need for more extensive assay validation and provides more reliable and consistent data across a wider range of different assays.

Ordering Information

Description	Qty	Rxn*	Cat Nos.
Brilliant III Ultra-Fast qPCR Master Mix for ABI StepOnePlus	2 x 2 ml	400	600880
Brilliant III Ultra-Fast qPCR Master Mix for ABI StepOnePlus (10 pack)	20 x 2 ml	4000	600881
Brilliant III Ultra-Fast QRT-PCR Master Mix for ABI StepOnePlus	2 x 2 ml	400	600884
Brilliant III Ultra-Fast QRT-PCR Master Mix for ABI StepOnePlus (10 pack)	20 x 2 ml	4000	600885
Brilliant III Ultra-Fast SYBR® Green qPCR Master Mix for ABI StepOnePlus	2 x 2 ml	400	600882
Brilliant III Ultra-Fast SYBR® Green qPCR Master Mix for ABI StepOnePlus (10 pack)	20 x 2 ml	4000	600883
Brilliant III Ultra-Fast SYBR® Green QRT-PCR Master Mix for ABI StepOnePlus	2 x 2 ml	400	600886
Brilliant III Ultra-Fast SYBR® Green QRT-PCR Master Mix for ABI StepOnePlus (10 pack)	20 x 2 ml	4000	600887

*assumes 20 µl reaction volume

Learn more:

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

Quick Reference Guide for the ABI StepOnePlus Real-Time PCR System

This quick reference guide provides an optimized protocol for using the Stratagene Brilliant III Ultra-Fast SYBR[®] Green QPCR Master Mix with the StepOnePlus Real-Time PCR System from Applied Biosystems. For detailed instructions, refer to the full product manual.

Prepare the Reactions

- 1 Dilute the reference dye 1:50 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.

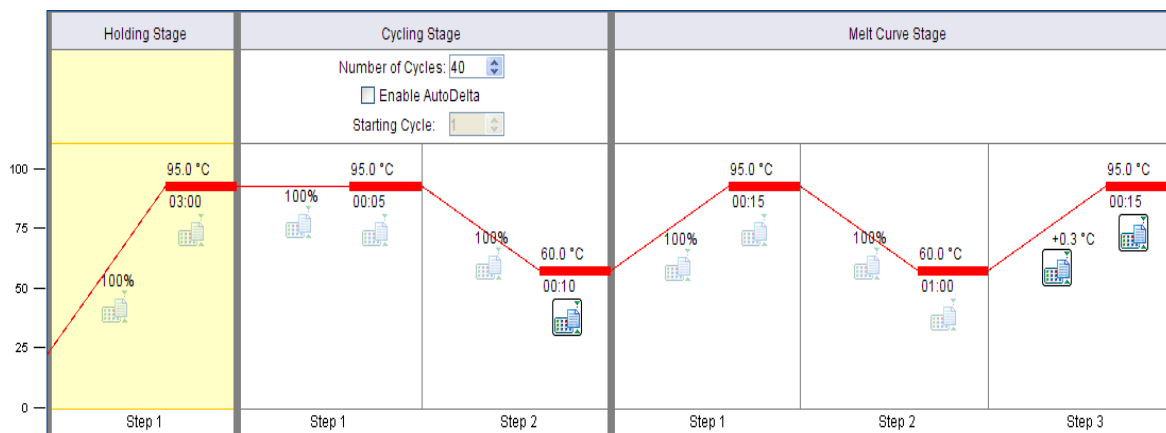


Set Up the QPCR Plate and Thermal Profile

- 1 From the Home screen of the StepOnePlus software, click **Advanced Setup**.
- 2 Complete the Setup screens for a new experiment as needed.

On the Experiment Properties screen, select **SYBR Green Reagents** (including a melt curve) and the **Fast** ramp speed.

On the Run Method screen, set the reaction volume to 20 μ l and adjust the thermal profile according to the image below.



Note: If you do not require a high-resolution melt curve, you can increase the ramp rate during the melt segment to 0.5°C per second to shorten the protocol time.

Run the PCR Program

- 1 Place the reactions in the StepOnePlus 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 #600805, 400 reactions
Catalog #600816, 4000 reactions

Ordering Information

By phone (US only*): 800-424-5444, x3
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Technical Services

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By email: techservices@agilent.com

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