# Brilliant III Ultra-Fast QRT-PCR Master Mix

## Quick Reference Guide for the LightCycler® 480 Real-Time PCR System

*This quick reference guide provides an optimized protocol for using Agilent's Brilliant III Ultra-Fast QRT-PCR Master Mix with the LightCycler 480 Real-Time PCR System from Roche. 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. *Keep reagent mixture on ice.*

| Reagent Mixture |
|---|
| Nuclease-free PCR-grade water to bring final volume to 20 µl (including RNA) |
| 10 µl of 2× QRT-PCR Master Mix |
| x µl of experimental probe at optimized concentration (100–600 nM) |
| x µl of upstream primer at optimized concentration (200–600 nM) |
| x µl of downstream primer at optimized concentration (200–600 nM) |
| 0.2 µl of 100 mM DTT |
| 1 µl of RT/RNase Block |

2 Gently mix the reagent mixture without creating bubbles, then distribute the mixture to the experimental reaction tubes. *Keep the reactions on ice.*

3 Add $x$ µl of experimental RNA to each reaction to bring the final reaction volume to 20 µl. 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 |

4 Mix the reactions without creating bubbles, then centrifuge briefly.

## Set Up the QPCR Plate and Thermal Profile

1. From the main window in the LightCycler 480 software, click **Sample Editor** on the module bar to open the *Sample Editor* module. Enter sample information for your experiment as needed.
2. Click **Experiment** on the module bar to open the *Run* module.
3. From the **Run Protocol** tab, enter a reaction volume of 20 µl.
4. Set the **Detection Format** to *Mono Color Hydrolysis Probe* or *Multi Color Hydrolysis Probe* as approrpriate for your experiment.
5. Set up the PCR program to run the cycling protocol below:

| Program Name | Cycles | Analysis Mode | Acquisition Mode | Ramp Rate (°C/s) | Hold Time | Temperature |
|---|---|---|---|---|---|---|
| Reverse transcription | 1 | None | None | 4.4 | 10 minutes | 50°C |
| Denaturation | 1 | None | None | 4.4 | 3 minutes | 95°C |
| Amplification | 45 | Quantification | None | 4.4 | 5 seconds | 95°C |
| | | | Single | 2.2 | 10 seconds | 60°C |
| Cooling | 1 | None | None | 2.2 | 30 seconds | 40°C |

## Run the PCR Program

1. Place the reactions in the LightCycler 480 instrument.
2. From the **Run Protocol** or **Data** tab, click **Start Run**.

## Analyze Data

1. Analyze the results of the run as needed for your experiment.



### Product Information
Catalog #600884, 400 reactions
Catalog #600885, 4000 reactions

### Ordering Information
By phone (US and Canada*): 800-227-9770
On the web: www.agilent.com/genomics

### Technical Services
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