

TaqMan® Fast Advanced Master Mix on the Roche LightCycler® 480 Real-Time PCR System

Introduction

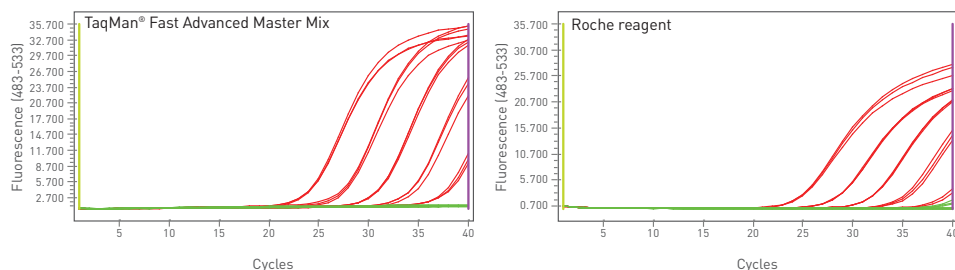
TaqMan® Fast Advanced Master Mix has been designed to match or exceed the performance of standard master mixes, delivering shorter run times (<40 minutes) with results equal to or better than what you achieve today.

Highlighted here is the performance of TaqMan® Fast Advanced Master Mix on the Roche LightCycler® 480 System with TaqMan® Gene Expression Assays. The results demonstrate the superior performance obtained using TaqMan® Fast Advanced Master Mix compared to that obtained using Roche's recommended probe-based reagent on the Roche LightCycler® 480 System.

Benefits of TaqMan® Fast Advanced Master Mix: not just fast cycling (<40 min)

- Best-in-class performance—superior sensitivity, accuracy, dynamic range, and specificity compared to standard mixes in standard mode
- Enhanced benchtop stability for high-throughput handling and convenience—stable at room temperature for up to 72 hours in preassembled reactions
- Optimized for multiplexing—validated for duplexing with exogenous and endogenous internal positive control assays
- Seamlessly transitions into experiments—validated with Applied Biosystems® TaqMan® Assays for gene expression and microRNAs

A. Standard assay (amplicon <30% GC)



B. GC-rich assay (amplicon >70% GC)

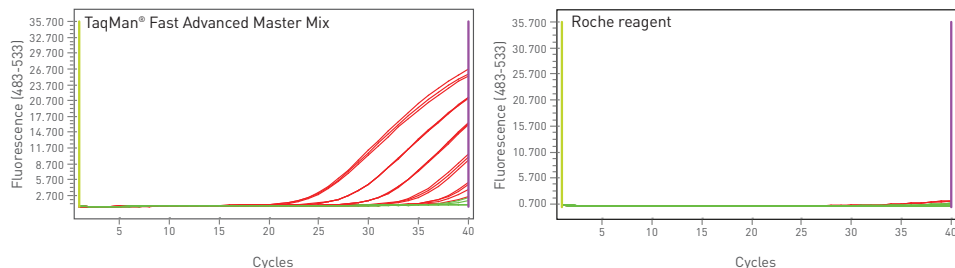


Figure 1. Comparison data for TaqMan® Fast Advanced Master Mix and Roche's recommended probe-based reagent on the Roche LightCycler® 480 System. PCR reactions were performed on the Roche LightCycler® 480 System using a dilution series containing 100 ng to 0.01 ng of template cDNA. The amplification plots demonstrate the superiority of TaqMan® Fast Advanced Master Mix over Roche's recommended probe-based reagent. Each reagent was used according to the manufacturers' respective recommended protocols.

Run the experiment

1. Prepare the PCR reaction mix.
 1. Thoroughly mix TaqMan® Fast Advanced Master Mix.
 2. Thaw frozen samples and frozen TaqMan® Assays on ice. Resuspend by vortexing, then briefly centrifuge.
 3. Calculate the total volume required for each component: *volume for 1 reaction x total number of reactions*.
 4. Mix gently. Do not vortex. Centrifuge briefly and then prepare the PCR reaction plate.

Component	Volume (µL) for 1 reaction		Final concentration
	384-well plate	96-well plate	
TaqMan® Fast Advanced Master Mix (2X)*	5.0	10.0	1X
TaqMan® Assay primer/probe (20X)	0.5	1.0	1X
cDNA template	1.0	2.0	0.001–100 ng/well
Nuclease-free water	3.5	7.0	
Total volume per reaction	10.0	20.0	

* ROX™ dye is included in the TaqMan® Fast Advanced Master Mix. The LightCycler® 480 Real-Time PCR System can detect this dye, but it will not be used for fluorescence normalization in the LightCycler® 480 system software.

2. Set up the run method.

See your instrument's user manual for detailed instructions on how to configure the run method.

Roche LightCycler® 480 System	UNG activation	Polymerase activation	PCR (40 cycles)	
	Hold	Hold	Denature	Anneal/extend
Temperature	50°C	95°C	95°C	60°C
Time (min:sec)	02:00	00:20	00:03	00:30

* Before setup, check your primer annealing temperature. If the primer T_m is <60°C, we recommend using a 3-step protocol.

3. Run the PCR reaction plate.

Load the reaction plate into the instrument and start the run. See your instrument's user manual for detailed instructions on how to load and run the plate.

4. Analyze the results.

Data analysis varies depending on the instrument. See the TaqMan® Fast Advanced Master Mix Protocol (PN 4444605) and your instrument's user manual for detailed instructions on how to analyze the data.

ORDERING INFORMATION

Product	Size	Quantity	Number of 20 µL reactions	Cat. No.
TaqMan® Fast Advanced Master Mix	Mini-Pack	1 x 1 mL	100	4444556
	1-Pack	1 x 5 mL	500	4444557
	2-Pack	2 x 5 mL	1,000	4444963
	5-Pack	5 x 5 mL	2,500	4444964
	10-Pack	10 x 5 mL	5,000	4444965
	Bulk-Pack	1 x 50 mL	5,000	4444558

For safety and biohazard guidelines, refer to the MSDS and follow the handling instructions. Work only in a suitably equipped laboratory space (including, without limitation, proper ventilation, electrical installation, hazardous waste disposal) and wear appropriate protective eyewear, clothing, and gloves.

This document shows (1) simplified procedures for using TaqMan® Fast Advanced Master Mix on the Roche LightCycler® 480 Real-Time PCR System, and (2) comparison data for TaqMan® Fast Advanced Master Mix and Roche's recommended probe-based reagent on the Roche LightCycler® 480 System. The TaqMan® Fast Advanced Master Mix Protocol (Part Number 4444605) provides detailed instructions.

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NOTICE TO PURCHASER: Please refer to the TaqMan® Fast Advanced Master Mix Protocol (PN 4444605) and user's manual FOR LIMITED LABEL LICENSE OR DISCLAIMER INFORMATION.

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Headquarters

5791 Van Allen Way | Carlsbad, CA 92008 USA | Phone +1.760.603.7200 | Toll Free in the USA 800.955.6288

www.lifetechnologies.com

