



**ThermoFisher**  
S C I E N T I F I C

**Comparison of Applied Biosystems™ TaqMan® and  
Roche™ reagents on the LightCycler™ 480 II™  
Real-Time PCR Detection System**

November 12, 2018

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## Objective:

Compare the performance of TaqMan® Master Mixes and Roche™ reagents using TaqMan® Gene Expression Assays on the Roche LightCycler™ 480 II™ real time PCR detection system.

- TaqMan Fast Advanced Master Mix with TaqMan Gene Expression Assays in singleplex and multiplex experiments
  - Sensitivity
  - Dynamic Range
  - Singleplex vs. Duplex Gene Expression
- Applied Biosystems™ TaqPath™ 1-Step Multiplex Master Mix with TaqMan Gene Expression Assays
  - Sensitivity
  - Dynamic Range
  - Singleplex vs. 4-plex Gene Expression

# TaqMan Fast Advanced vs LightCycler Probes Master Comparison

TaqMan Fast Advanced master mix outperforms the LC Probes Master mix on the Light Cycler 480 II system

Attribute	TaqMan Fast Advanced Master Mix	LightCycler Probes Master
7 Log dynamic range	✓	✓
Sensitivity @ 1 copy	✓	✓
Performance in multiplex	✓	-
Includes UNG for carry over contamination	✓	-
Run time	Faster by ~ 10 min	Fast
Benchtop stability	72 hr	24 hr

# TaqMan Fast Adv. and LC 480 Probes Master Comparison

## TaqMan® Fast Advanced Master Mix



VS

## LightCycler™ Probes Master



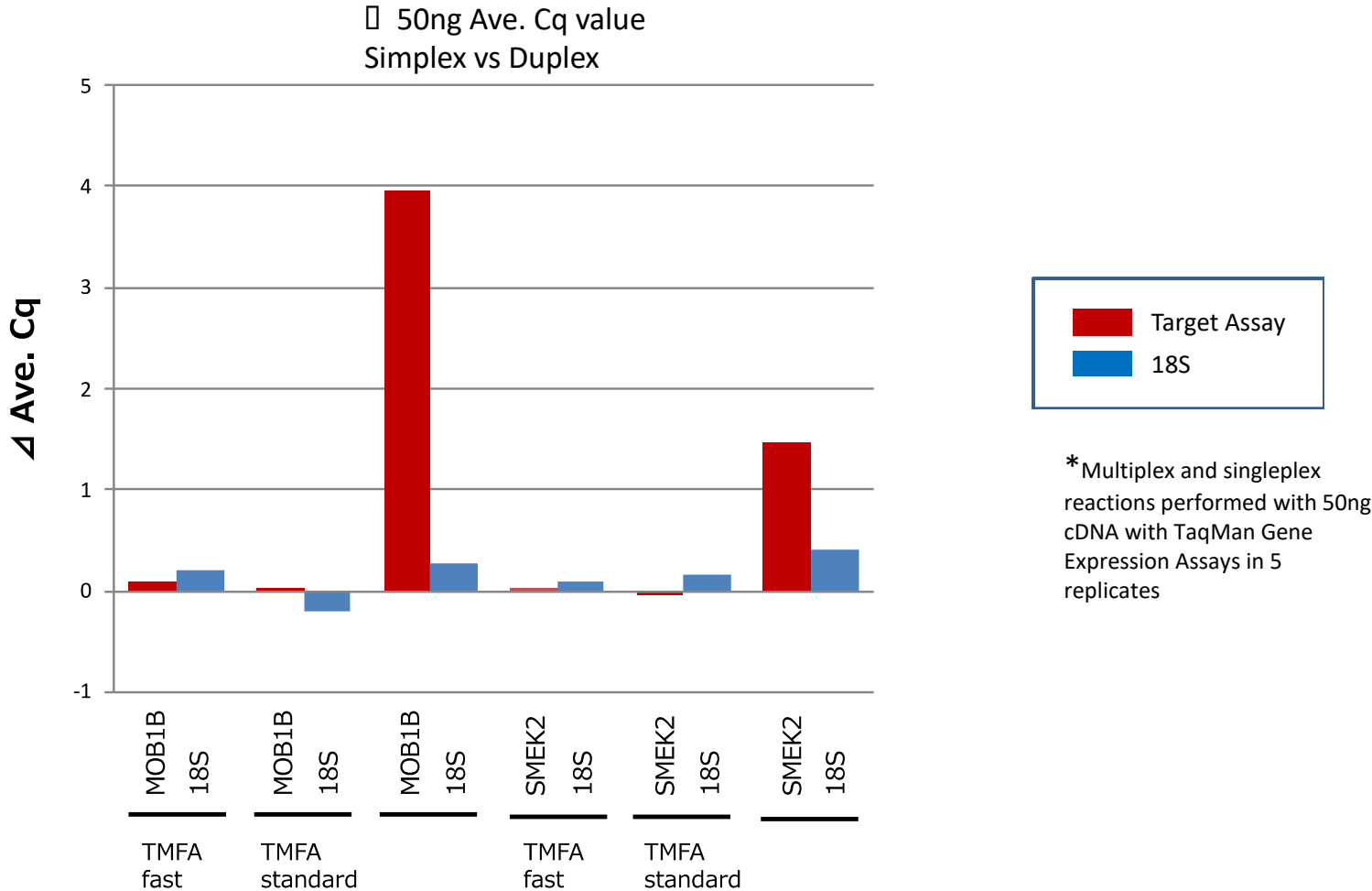
## On the LightCycler™ 480 System II



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# TaqMan Fast Advanced Master Mix: Gene Expression Multiplex

TaqMan<sup>®</sup> Fast Advanced Master Mix outperforms Roche<sup>™</sup> LC 480 Probes Master Mix in multiplexed reactions on the Roche LightCycler<sup>™</sup> 480 II\*.



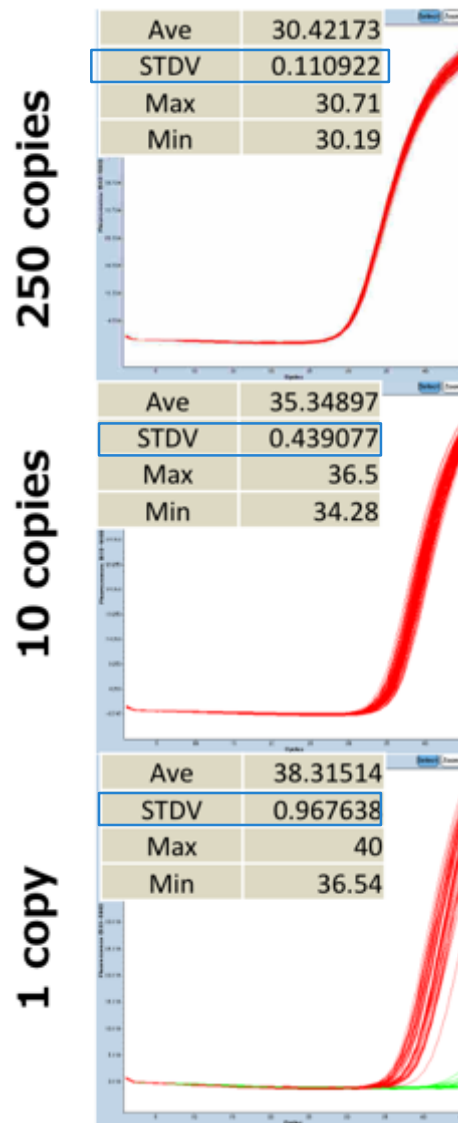
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# TaqMan Fast Advanced Master Mix: Sensitivity

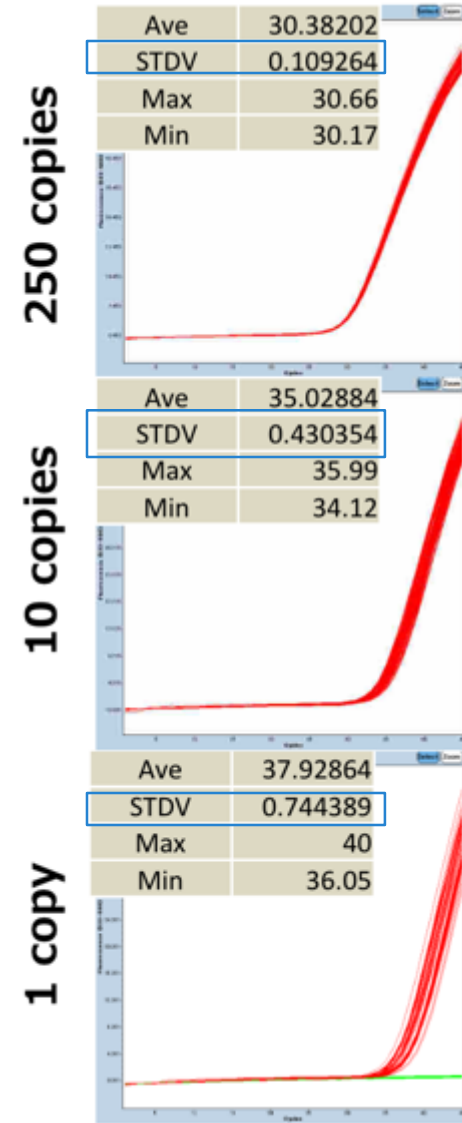
TaqMan<sup>®</sup> Fast Advanced master mix demonstrates sensitivity down to 1 copy on the LightCycler<sup>™</sup> 480 II\*

\*Multiplex and singleplex reactions performed with 50ng cDNA with TaqMan Gene Expression Assays in 5 replicates

## LightCycler 480 Probes Master

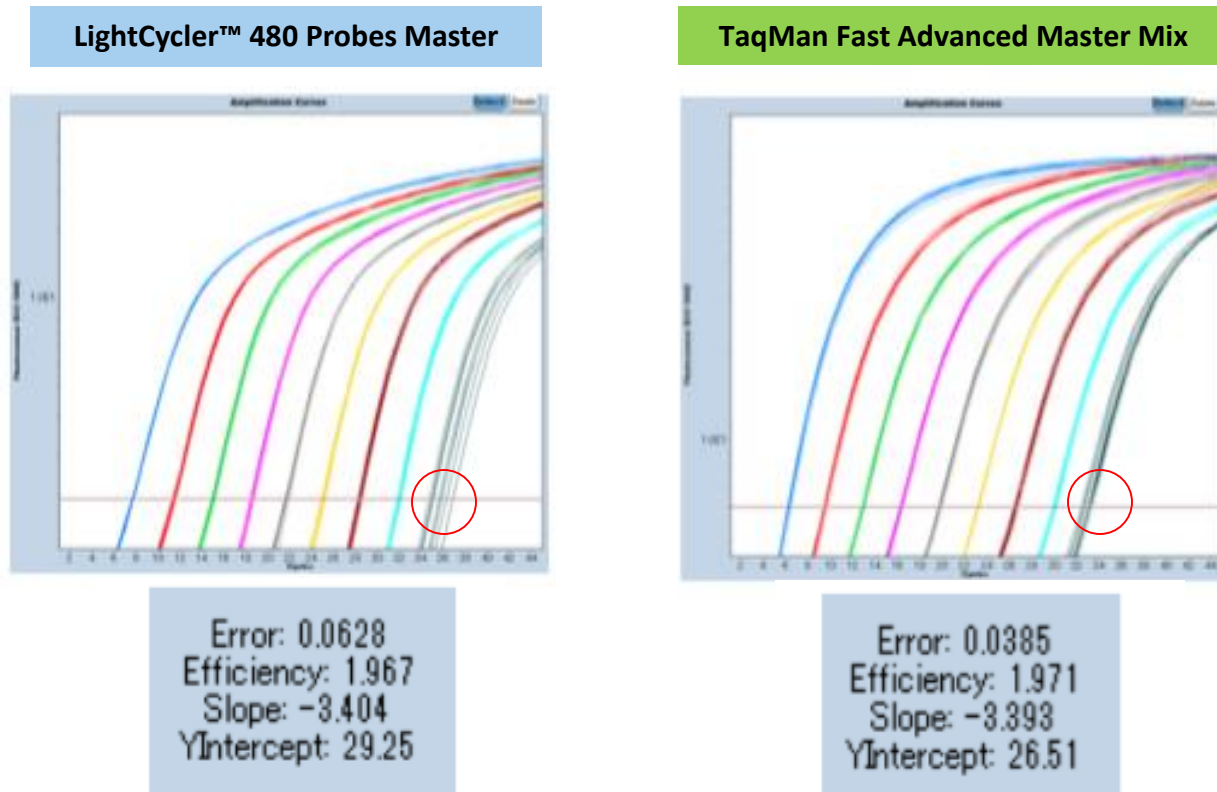


## TaqMan Fast Advanced Master Mix



# TaqMan Fast Advanced Master Mix: Dynamic Range

TaqMan® Fast Advanced master mix yields broad linear dynamic range and reliable quantitation at low copy number



**PCR Eff.=96.685%**

**PCR Eff.=97.117%**

Amplification plots from an 8-log dilution series of 50 ng /  $\mu$ L human cDNA amplified in 5 replicate reactions with an 18S rRNA TaqMan® Gene Expression Assay on a Roche LightCycler 480 II Real-Time PCR Detection System. LightCycler 480 Probes Master (left) was compared to TaqMan Fast Advanced Master Mix (right). Note the more consistent results with TaqMan Fast Advanced Master Mix at the lowest concentration

**cDNA amounts: 50ng, 5ng, 500pg, 50pg, 5pg, 500fg, 50fg, 5fg, 500ag (1/10 dilution), and NTC**

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# TaqPath 1-Step Multiplex Master Mix Comparison

- **TaqPath™ 1-Step Multiplex Master Mix (No ROX)**



## LightCycler™ Multiplex RNA Virus Master Mix



## On the LightCycler™ 480 System II





# TaqPath 1-Step Multiplex vs LC Multiplex RNA Virus Comparison

## TaqPath™ 1-Step Multiplex Master Mix outperforms the LightCycler™ Multiplex RNA Virus Master on the Light Cycler 480 II

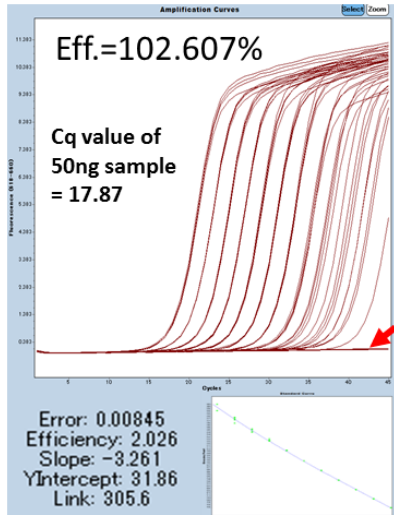
Attribute	TaqPath 1-Step Multiplex Master Mix	LC Multiplex RNA Virus Master
Dynamic range	✓	-
Sensitivity	✓	-
Performance in multiplex	✓	-
PCR efficiency	✓	-
Includes UNG for carry over contamination	✓	-
General Purpose Reagent	✓	-
Run time	✓	✓

# TaqPath 1-Step Multiplex Master Mix: Dynamic range

- TaqPath™ 1-Step Multiplex Master Mix detected at all copy levels on the Roche LightCycler™ 480 II\*

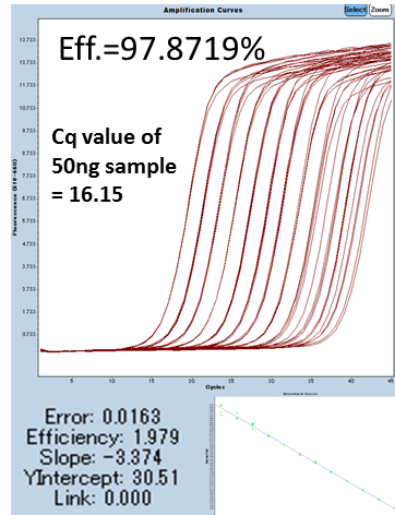
2nd Derivative Max

## LightCycler® Multiplex RNA Virus



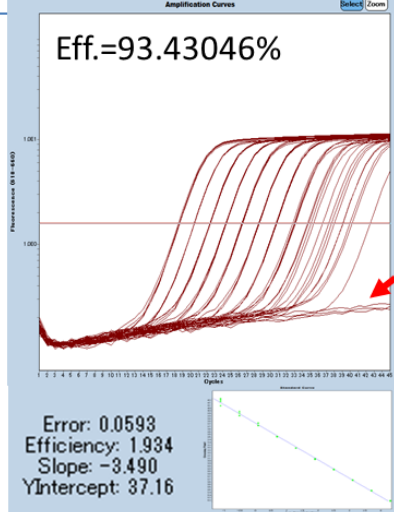
No amplification

## TaqPath™ 1-step Multiplex (no ROX)

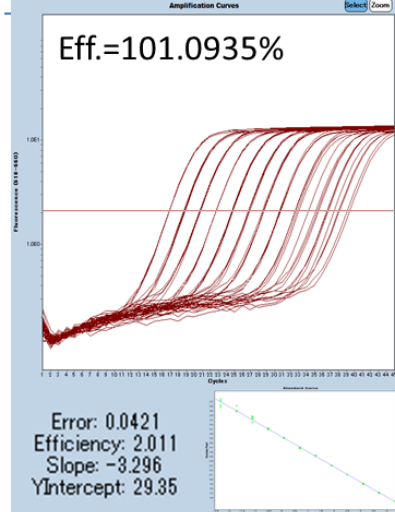


\*Thirty-two TaqMan Gene Expression Assays were run in both 4-plex (5 replicates) and in simplex (3 replicates).

Fit Points

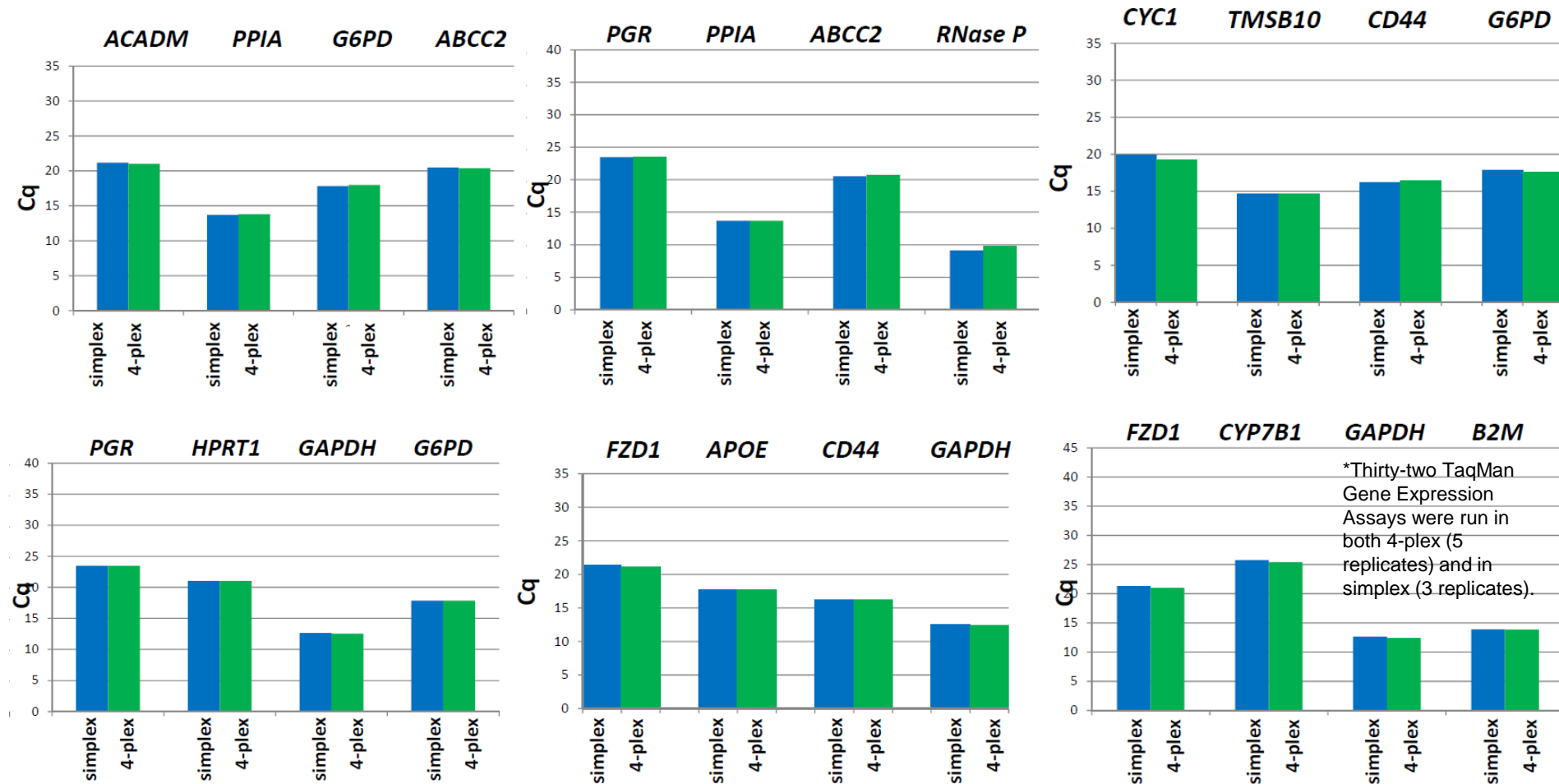


No amplification



# TaqPath™ 1-Step Multiplex: Gene Expression Multiplex

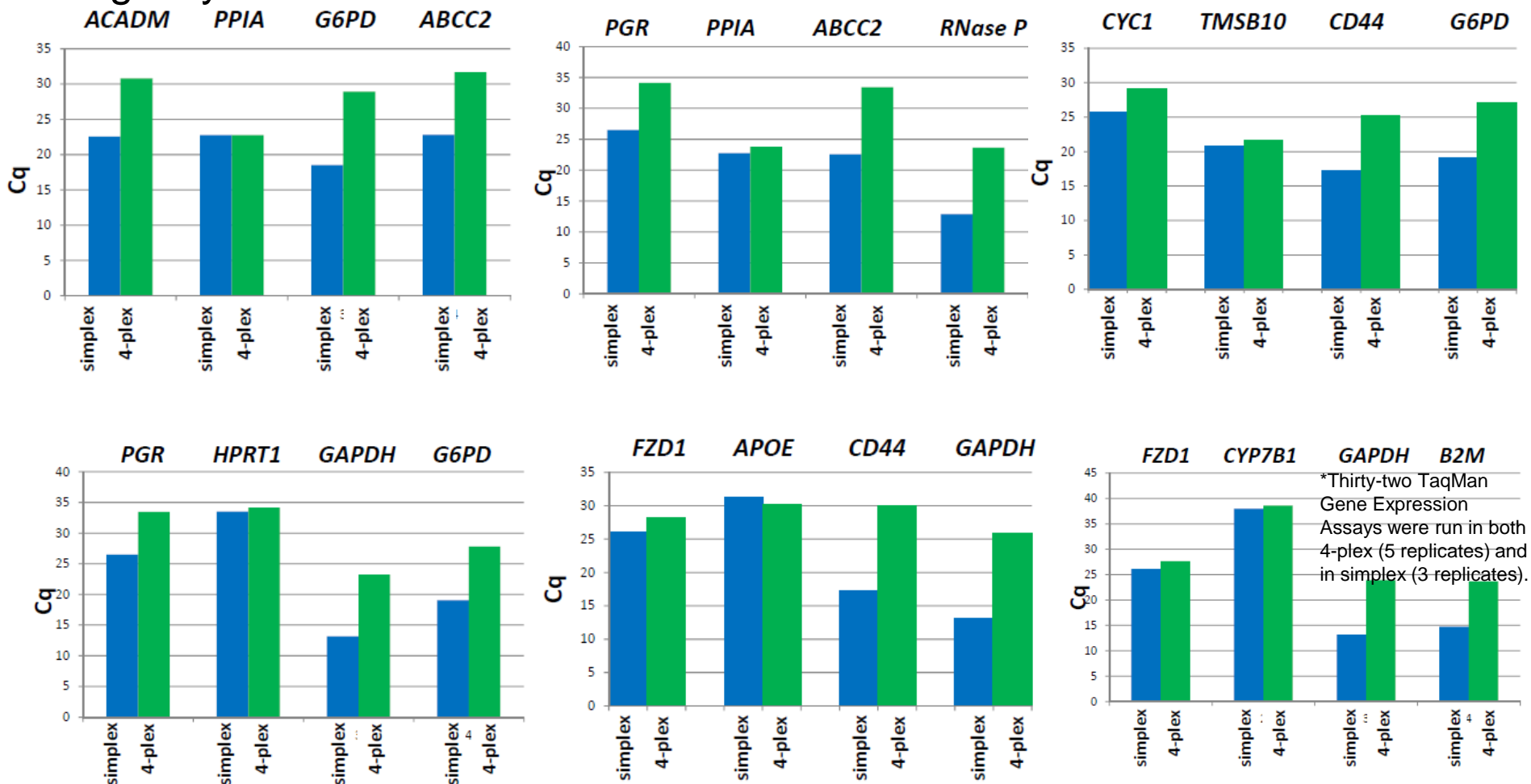
- TaqPath 1-step Multiplex Master Mix provides consistent, reliable results in **singleplex** and **multiplex** gene expression on the Roche™ LightCycler™ 480 II\*



\*Thirty-two TaqMan Gene Expression Assays were run in both 4-plex (5 replicates) and in simplex (3 replicates).

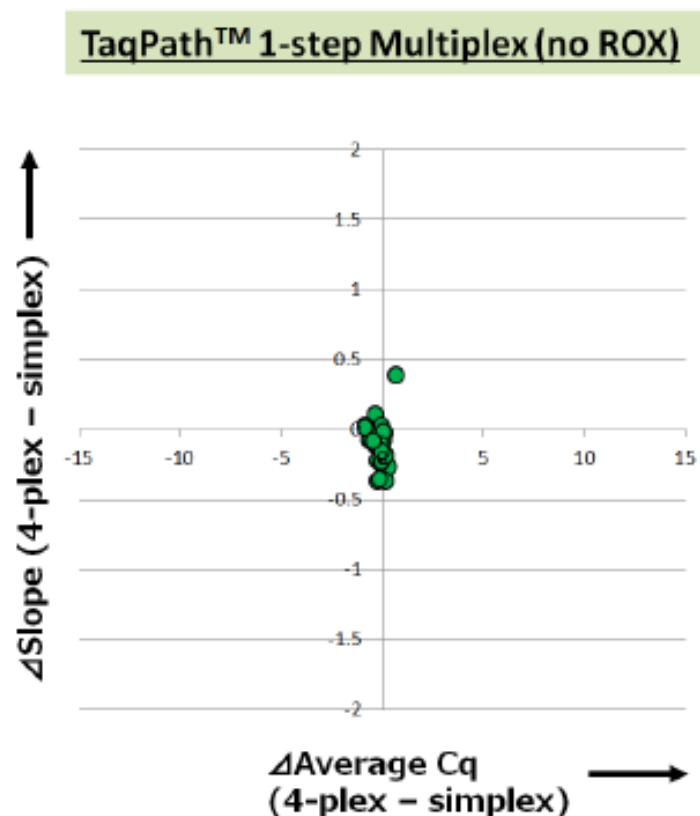
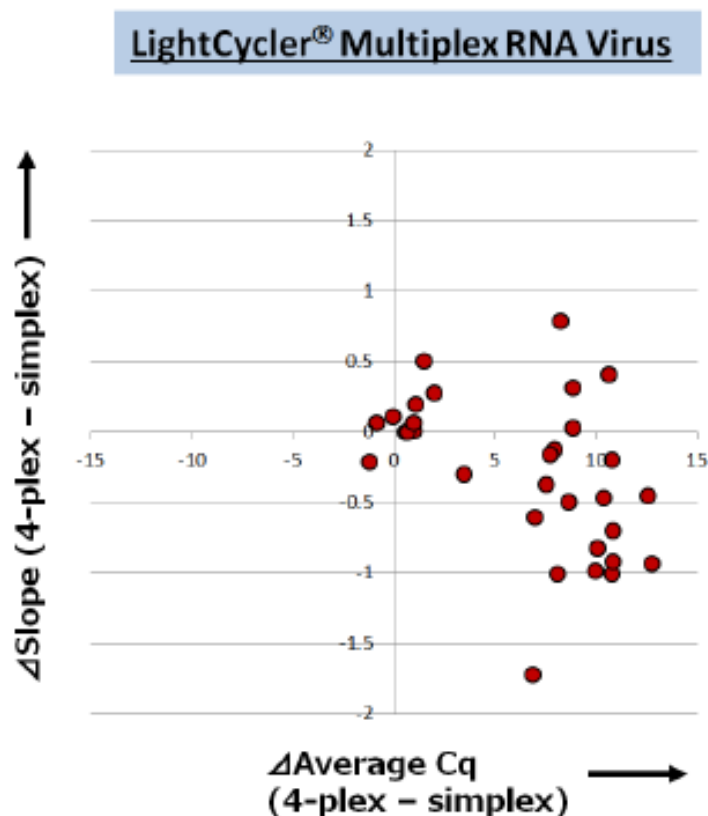
# LightCycler Multiplex RNA Virus: Gene Expression Multiplex

- LightCycler™ Multiplex RNA Virus Master Mix shows **inconsistent** results between **singleplex** and **multiplex** gene expression on the Roche™ LightCycler™ 480 II\*



# TaqPath 1-Step Multiplex Master Mix: Multiplexing

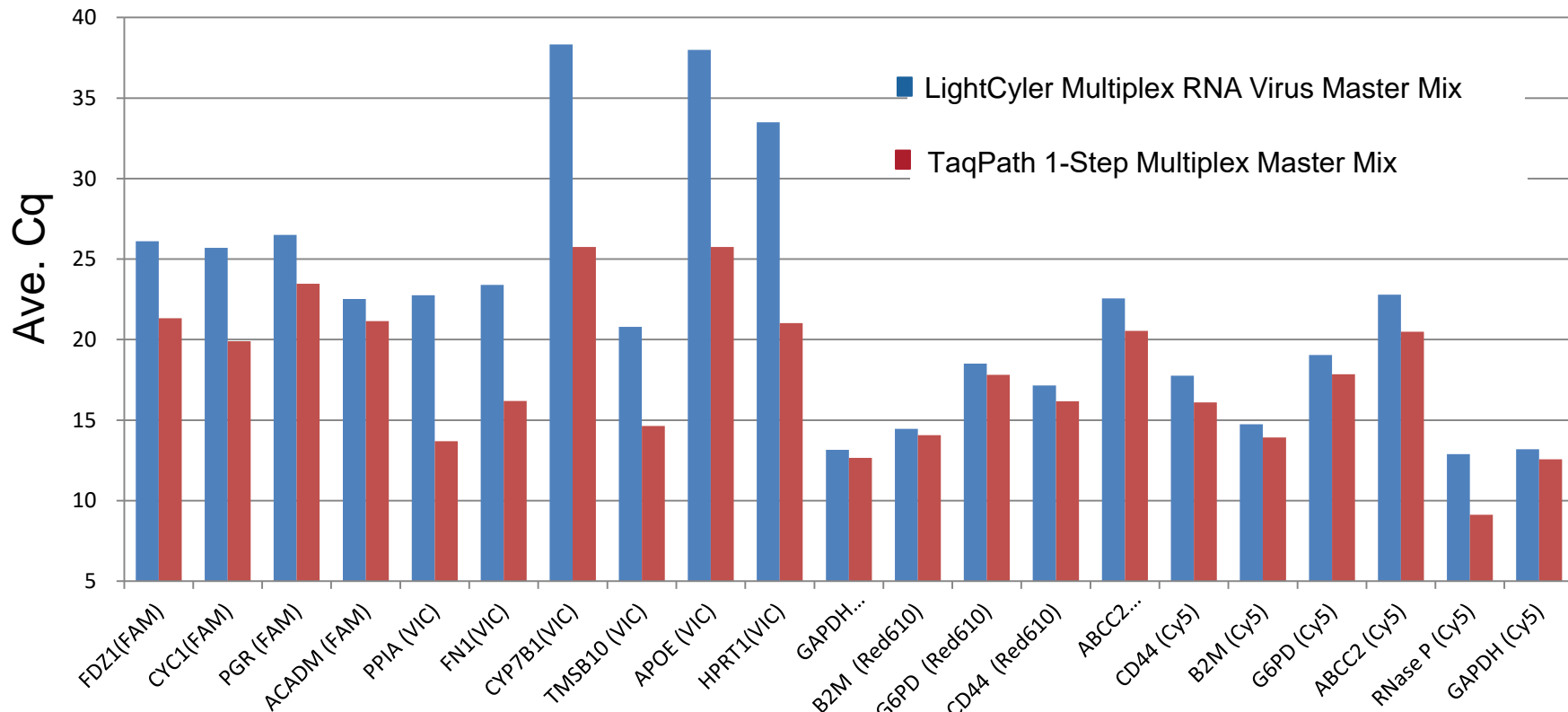
- TaqPath™ 1-step Multiplex outperforms LightCycler™ Multiplex RNA Virus Master Mix in multiplex qPCR on the Roche LightCycler 480 II



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# TaqPath 1-Step Multiplex Master Mix: Sensitivity

- TaqPath™ 1-step Multiplex shows superior sensitivity compared to LightCycler Multiplex RNA Virus Master Mix on the Roche™ LightCycler™ 480 II\*

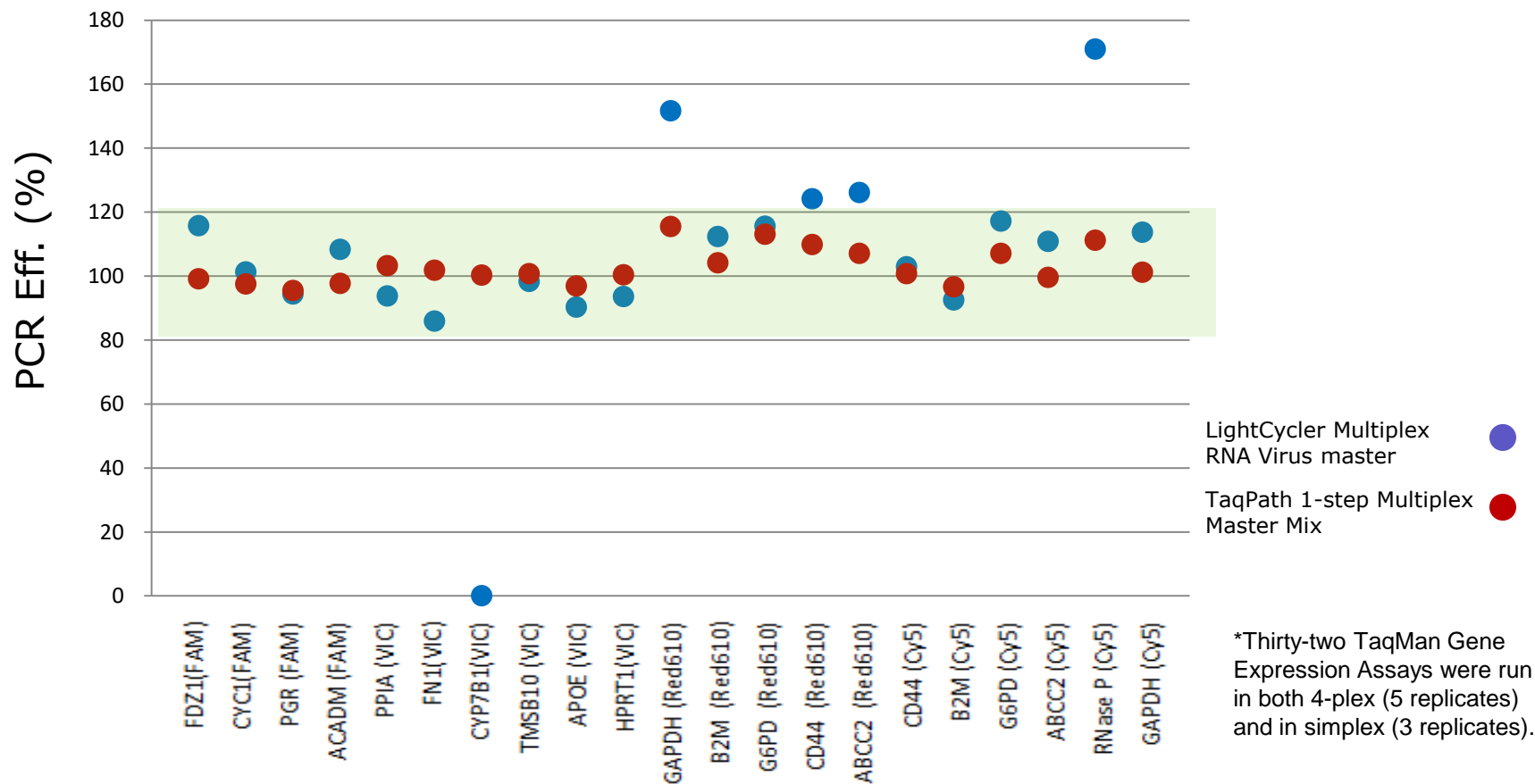


**ΔAve Cq of 50ng (TaqPath vs. LightCycler)**

\*Thirty-two TaqMan Gene Expression Assays were run in both 4-plex (5 replicates) and in simplex (3 replicates).

# TaqPath 1-Step Multiplex: PCR Efficiency

- TaqPath™ 1-step Multiplex master mix shows superior PCR efficiency compared to LightCycler Multiplex RNA Virus Master Mix on the Roche™ LightCycler™ 480 II\*



# Conclusions and Next Steps

- Applied Biosystems™ TaqMan® / TaqPath™ assays and reagents outperform the Roche™ LightCycler™ reagents on the Roche LightCycler 480 II qPCR instrument.
- **TaqMan® Fast Advanced Master Mix** shows better reproducibility, dynamic range and PCR efficiency on the LightCycler 480 II for gene expression singleplex and multiplex assays than LightCycler Probe Master.  
<https://www.thermofisher.com/order/catalog/product/4444556>
- **TaqPath™ 1-Step Multiplex Master Mix** demonstrates improved dynamic range, PCR efficiency, and sensitivity than LightCycler Multiplex RNA Virus Master Mix on Roche LightCycler 480 II for gene expression for both simplex and multiplex applications  
<https://www.thermofisher.com/taqpath>
- To learn more about Applied Biosystems qPCR master mixes or to request a free sample, please visit [www.thermofisher.com/qpcrmm](http://www.thermofisher.com/qpcrmm)
- Did you know TaqMan assays are guaranteed to perform? To learn more about TaqMan assays or purchase an extra small size, please visit [www.thermofisher.com/taqman](http://www.thermofisher.com/taqman)



- TaqMan<sup>®</sup> Fast Advanced Master Mix product information
- TaqPath<sup>™</sup> 1-Step Multiplex Master Mix product information
- Thermal cycling conditions

# TaqMan Fast Advanced Master Mix

TaqMan<sup>®</sup> 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 is achieved today.

- **Best-in-class performance**
- **Enhanced benchtop stability**
- **Optimization for multiplexing**
- **Reduced run times on fast and standard instrumentation**
- **Includes UNG**

Verified with TaqMan Assays for gene expression and microRNAs, and TaqMan array microfluidic cards



Use with:  
**TaqMan<sup>®</sup> Gene  
Expression  
Assays**  
and  
**TaqMan  
Advanced  
miRNA Assays**

# TaqPath 1-Step Multiplex Master Mix

A 1-step RT-qPCR master mix optimized specifically for multiplex applications that delivers superior performance and enhanced data confidence.

- **Inhibitor tolerant**
- **Excellent sensitivity and reproducibility**
- **Optimized for multiplexing**
- **Easy-to-use 4X mix**

Manufactured in an ISO 13485 certified facility



TaqPath™ 1-Step  
Multiplex Master Mix  
includes MUSTANG PURPLE™  
passive reference dye

TaqPath™ 1-Step  
Multiplex Master Mix  
(No ROX)  
no passive reference dye

# Thermal Cycling Conditions

	LightCycler™ 480 Probe Master	TaqMan® Fast Advanced Master Mix <i>Standard</i>	TaqMan® Fast Advanced Master Mix <i>Fast</i>
Format	384 well plate	384 well plate	384 well plate
# of Cycles	45	45	45
Ramp Rate	LC 480 II default *	LC 480 II default *	LC 480 II default *
Pre-Incubation			
Enzyme Activation	5:00 @ 95°	0:20 @ 95°	0:20 @ 95°
Amplification			
Denature	0:10 @ 95°	0:15 @ 95°	0:03 @ 95°
Anneal	0:30 @ 50°	1:00 @ 60°	0:30 @ 60°
Extend	0:01 @ 72°	-	-
Cooling	0:10 @ 40°	-	-
<b>Total Run Time</b>	<b>~ 58 min</b>	<b>~ 79 min</b>	<b>~ 47 min</b>

\* 384 well plate default ramp rate (up 4.8° C / down 2.5° C )

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# Thermal Cycling Conditions

	LightCycler™ Multiplex RNA Virus Master	TaqPath™ 1-Step Multiplex Master Mix
Format	384 well plate	384 well plate
# of Cycles	45	45
Ramp Rate	LC 480 II default *	LC 480 II default *
UNG incubation	N/A	2:00 @ 25°C**
Pre-Incubation	10:00 @ 50°C	10:00 @ 53°C
Enzyme Activation	0:30 @ 95°C	2:00 @ 95°C
Amplification		
Denature	0:05 @ 95°C	0:03 @ 95°C
Anneal	0:30 @ 60°C	0:30 @ 60°C
Cooling	0:30 @ 40°C	0:10 @ 40°C
Total Run Time	~60 min	~ 60 min (+2 min UNG incubation)

\*384 well plate default ramp rate (up 4.8° C / down 2.5°C )

\*\*on the bench (the lowest block temp of LC480II is 37°C