

LightCycler® 480 and 1536 Real-Time PCR Systems – Powerful Solutions for Different Throughput Levels

A Cross-Platform Comparison

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Introduction

During the past few years real-time PCR has been proven to be a key technology for gene expression analysis, for instance for gene expression profiling and array verification studies and for the genotyping of single nucleotide polymorphisms, both in industrial and public research, with major impact on the fields of drug discovery, molecular medicine and personalized health care [1-3].

Roche Applied Science's family of plate-based LightCycler® Real-Time PCR Instruments offers flexible and reliable solutions for real-time PCR applications from medium (96-well format) to high (384-well format), and even highest throughput needs (1536-well format) per single experiment. This powerful platform of real-time PCR systems combines innovative thermal cycler and optical technologies, unique disposables, streamlined software solutions, and next-generation premium reagents for high-performance gene expression and genotyping studies.

The LightCycler® 480 Real-Time PCR System is equipped to perform either 96 or 384 data points in a single PCR run based on its easily interchangeable thermal cycler units (96-well format: 10–100 µl, or 384-well format: 5–20 µl). The efficient heat-equalizing Thermo-Base technology of the thermal block cycler of the LightCycler® 480 Instrument facilitates the generation of exceptional homogeneous and accurate data with fast protocols. The advanced high-performance optical system enables highest flexibility in choice of detection formats (e.g., HybProbe probes, hydrolysis probes, intercalating green dyes) and fluorescence dyes (e.g., FAM, VIC, LightCycler® Red 610, Cy5). With its cutting-edge, researcher-friendly software this instrument supports a remarkably broad spectrum of basic to advanced analysis options for gene expression and genotyping applications.

The latest innovation of the product line of plate-based LightCycler® Real-Time PCR Systems, the LightCycler® 1536 System, represents an unprecedented breakthrough in real-time PCR technology. This novel instrument com-

binizes miniaturization of individual reaction volumes (0.5–2.0 µl) with high parallelization of sample throughput per experiment (1536-well format), without sacrificing the strengths of classical plate-based real-time PCR applications regarding analytical sensitivity, dynamic range, data quality, speed, and flexibility in plate layout. This means that reagent and sample input can be considerably reduced, with a beneficial impact on the return rate of resources.

The LightCycler® 1536 Instrument builds on the well-established LightCycler® 480 Instrument, supporting mono- and dual-color applications for the detection of green intercalating dyes as well as hydrolysis probes. The streamlined high-throughput LightCycler® 1536 Software generates robust, basic real-time PCR results (e.g., Cps, end-point, and slope values) for gene expression and genotyping analysis, and allocates and manages data conveniently in both network and LIMS environments. Together with the next generation of real-time PCR reagents, the RealTime ready Reagents, the LightCycler® 1536 System supports most demanding applications in automated high-throughput workflows and includes industry-first, pipetting error-tracking features.

The product line of plate-based LightCycler® Systems in combination with the novel RealTime ready Reagents, which have been developed for both the LightCycler® 480 and 1536 System, provide conveniently the possibility of up- and down-scaling of PCR reaction volumes (96-, 384-, 1536-well format) for rapid adaptation to immediate throughput needs. The following cross-platform study represents the sensitivity, reliability, and homogeneity provided across the different throughput formats of the plate-based LightCycler® Systems.

Materials and Methods

To determine whether particular PCR assays optimized on the LightCycler® 480 System were of equal performance when transferred to the high-throughput LightCycler® 1536 System, a defined quantity of target material was

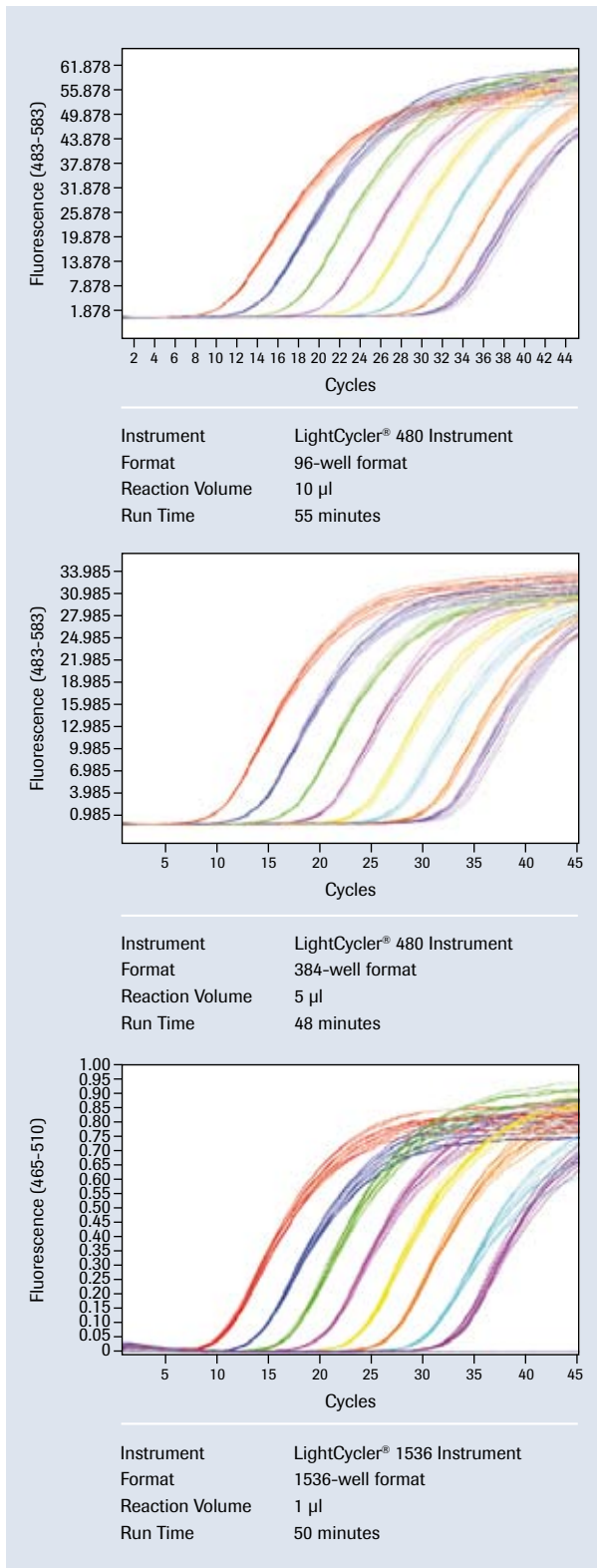


Figure 1: Linearity and reproducibility of the LightCycler® 480 and 1536 Instruments (96-, 384-, 1536-well format) depicted based on the respective amplification curves. Serial 1:10 dilutions of 10^7 - 10 copies/reaction of a plasmid DNA target detected by a FAM-labeled hydrolysis probe (UPL).

tested as a tenfold dilution series in both the LightCycler® 480 System (96- and 384-well format) and the LightCycler® 1536 System (1536-well format).

A plasmid containing an inserted sequence of the human HPRT (Hypoxanthine-PhosphoRibosyl-Transferase) gene was used for serial dilution from 10^7 - 10 copies/ μ l. Stock solutions of either dilution step were prepared and used for the PCR setup (ten replicates each) of the different plate formats. Each PCR reaction contained RealTime ready DNA Probes Master, 500 nM of forward- and reverse-primers and 700 nM of a FAM-labelled UPL probe (Universal Probe Library Human HPRT Gene Assay) in the following different multiwell formats: 96-well format (10 μ l total reaction volume), 384-well format (5 μ l total reaction volume), 1536-well format (1 μ l total reaction volume). Concentrations of all reaction components were identical, independent of the particular multiwell plate format.

The PCR setups for the LightCycler® 480 Multiwell Plates (96- and 384-well format) were manually conducted, whereas the LightCycler® 1536 Multiwell Plate was automatically loaded using a low-volume liquid handler (Innovadyne™ Nanodrop™ Express, 16-tip head, IDEX Health & Science LLC).

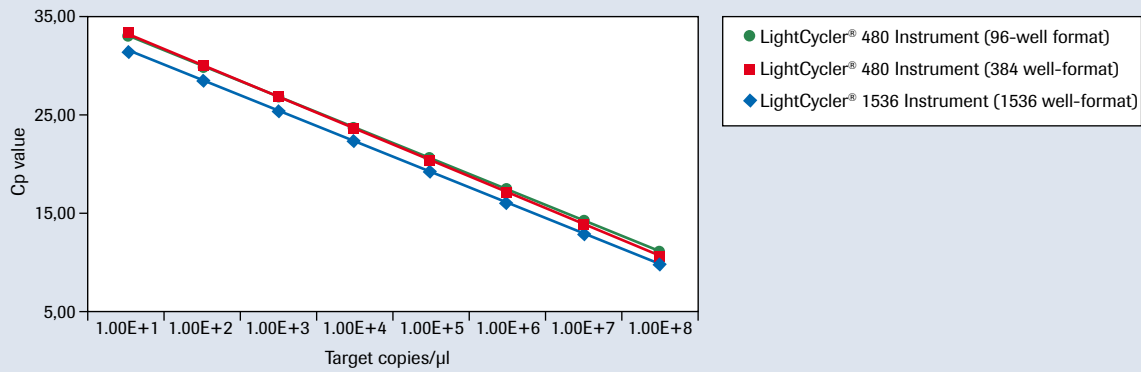
The real-time PCR runs were performed on the LightCycler® Instruments according to the manufacturer's instructions.

Results and Conclusions

As illustrated by the results for this respective UPL real-time PCR assay in combination with the RealTime ready DNA Probes Master, up- and down-scaling of PCR reaction volumes using the LightCycler® 480 and 1536 Systems can be conveniently conducted to adjust them to changing throughput needs (96-, 384-, 1536-well format; Figure 1 and 2).

The fairly early Cp (crossing point) values which have been observed for the results obtained with the LightCycler® 1536 Instrument can be attributed to the smaller reaction volume in combination with the proprietary LightCycler® 1536 Multiwell Plate. Due to its unique Thermaxis® technology, the LightCycler® 1536 Multiwell Plate enables unsurpassed thermal performance, which finally results in earlier Cps in comparison to data generated by the LightCycler® 480 System (LightCycler® Multiwell Plates, 96-, 384-well format).

In conclusion, we have shown that all three plate-based formats (LightCycler® 480 System, 96- and 384-well for-



Target copies/µl	● LightCycler® 480 Instrument (96-well format)		■ LightCycler® 480 Instrument (384-well format)		◆ LightCycler® 1536 Instrument (1536-well format)	
1.00E+08	110,3 (0.04)	0.14	10,53 (0.03)	0.10	9,85 (0.09)	0.26
1.00E+07	10,03 (0.05)	0.20	13,87 (0.05)	0.15	13,17 (0.08)	0.23
1.00E+06	17,68 (0.06)	0.23	17,30 (0.08)	0.32	16,33 (0.11)	0.40
1.00E+05	20,98 (0.06)	0.18	20,70 (0.10)	0.31	19,44 (0.09)	0.29
1.00E+04	24,30 (0.09)	0.32	24,15 (0.22)	0.63	22,72 (0.12)	0.46
1.00E+03	27,48 (0.11)	0.35	27,40 (0.19)	0.61	26,00 (0.11)	0.39
1.00E+02	30,72 (0.11)	0.36	30,53 (0.15)	0.50	29,29 (0.19)	0.70
1.00E+01	32,94 (0.36)	1.31	32,52 (0.43)	1.33	31,53 (0.38)	1.23
Slope	-3,20		-3,22		-3,15	
Correlation	0,998		0,997		0,999	

Figure 2: Linearity and reproducibility of the LightCycler® 480 and 1536 Instruments (96-, 384-, 1536-well format) depicted based on calculated standard curves. Standard curves were calculated from the mean value of ten replicates which were performed for each dilution step. The PCR efficiency and precision were calculated based on the data of the standard curve. For all platforms the correlation coefficients are excellent (above 0.99).

mat; LightCycler® 1536 System, 1536-well format) correlate well with each other. Assays originally optimized for larger real-time PCR reaction volumes, for instance using the LightCycler® 480 Multiwell Plates (96- or 384-well format), can be conveniently transferred and miniaturized to low-volume format (LightCycler® 1536 Multiwell Plates) without the need for time-consuming and cost-extensive revalidation studies. ■

References

1. Lyon E, Wittwer CT (2009) J Mol Diagn 11:93-101
2. Brazeau DA (2004) Drug Discov Today 9:838-845
3. Kim S, Misra A (2007) Annu Rev Biomed Eng 9:289-320

For detailed information, please visit:
www.lightcycler1536.com, www.lightcycler480.com,
 and www.universalprobelibrary.com.



Product	Pack Size	Cat. No.
LightCycler® 1536 Instrument	1 instrument	05 334 276 001
LightCycler® 1536 Multiwell Plate	10x 10 plates	05 358 639 001
LightCycler® 480 Instrument, 96-well	1 instrument	05 015 278 001
LightCycler® 480 Instrument, 384-well	1 instrument	05 015 243 001
LightCycler® 480 Multiwell Plate 96, white	5x 10 plates / 50 foils	04 729 692 001
LightCycler® 480 Multiwell Plate 384, white	5x 10 plates / 50 foils	04 729 749 001
RealTime ready DNA Probes Master	5x conc., 5x 1 ml (12.500x 2 µl rxns)	05 502 381 001
Universal ProbeLibrary Human HPRT Gene Assay	1 set (200 reactions of 50 µl or 500 reactions of 20 µl)	05 0461 57 001