

# Introducing the LightCycler® 480 System

## The Next Generation of High-Speed, High-Accuracy Real-Time PCR

### Introduction

With the LightCycler® family of PCR systems, Roche Applied Science has set a standard in real-time PCR. LightCycler® Instruments are well known for their speed, accuracy, and flexibility, which are due to the symmetrical “one-well-like” design, air-based heating and cooling, and the use of capillaries as reaction vessels.

Following along the same lines, the new LightCycler® 480 System efficiently facilitates the delivery of higher sample throughputs when performing gene expression or mutation analysis in life-science research. For the first time, a real-time PCR platform now offers the LightCycler® Systems' unique combination of accuracy and speed for multiwell-plate based assays with comparable performance with 96- or 384 sample throughputs, and for all relevant qPCR applications. In addition, the highly modular concept of the LightCycler® 480 System hardware and software allows scientists to customize the system to best suit their laboratory's specific research needs.

### General Design

The LightCycler® 480 System is a compact and versatile benchtop instrument that can hold multiwell plates for either 96 or 384 samples. It is provided with a separate data station based on Windows XP (Figure 1a). The two available thermal blocks (for 96 or 384 samples) can be easily accessed and interchanged within minutes for adaptation to different throughput needs. Calibration is not required after block exchange (Figure 1b).

### LightCycler® 480 System Components

PCR-based gene analysis can only be performed accurately for high sample numbers if signals are generated, captured, and analyzed in a highly reproducible and homogeneous manner. More precisely, PCR cycling conditions (temperature and time), the way dyes are excited and fluorescence is measured, and the algorithms used to characterize genes qualitatively and quantitatively have to be highly precise and reliable. The LightCycler® 480 System meets these requirements at an unprecedented level, with hardware, software, reagents, and disposables all contributing to its high performance.

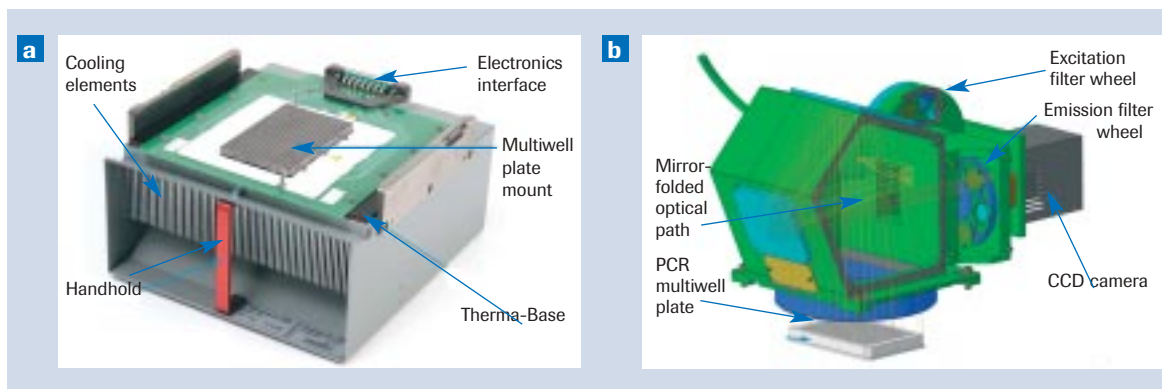
### LightCycler® 480 Instrument's thermal block and disposables

PCR specificity and yield are directly related to the ability of a thermal cycling system to rapidly and accurately reach and maintain reaction temperatures. With the capillary-based LightCycler® Systems, this has been achieved using air-based heating and cooling. To reach the same performance in a multiwell plate-based system, a novel type of thermoblock has now been designed and is an integral part of the LightCycler® 480 System (Figure 2a).

For the first time in a PCR instrument, the LightCycler® 480 Instrument incorporates Therma-Base technology for optimal heat transfer and distribution to all samples in a plate, leading to excellent well-to-well temperature homogeneity and maximized inter-well, inter-cycle reproducibility.

**Figure 1:**  
**(a) LightCycler® 480 Instrument with workstation.**  
**(b) Exchange of thermal block unit.**





**Figure 2:**  
**(a) The LightCycler® Instrument's 480 thermal block.**  
**(b) Optical system.**  
 Note that the xenon lamp is located outside of the shown unit; light is directed to the unit via optical fibers (shown in upper left part of the figure).

The LightCycler® 480 System is capable of running PCRs over a very broad range of volumes, ranging from 5 to 20  $\mu\text{l}$  (384-well plate) and 20 to 100  $\mu\text{l}$  (96-well plate). This allows for a highly flexible assay design, for example, when sensitivity is crucial because of low target concentrations. Special multiwell plates to optimally fit the LightCycler® 480 thermal block are an integral part of the system. They are used in combination with sealing foils that prevent evaporation and contamination. Bar codes on each plate allow easy sample tracking.

#### LightCycler® 480 Instrument's optical system

For fluorescence excitation, the LightCycler® 480 Instrument uses a high-intensity xenon lamp. The broad spectrum of this light source supports the use of a wide range of dyes and at the same time provides maximal sensitivity for a broad range of assay formats in all channels.

To make excitation as specific as possible and to reduce crosstalk between channels when more than one dye is used, the LightCycler® 480 Instrument includes filters (five different wavelength maxima - 450, 483, 523, 558 and 615 nm - for excitation; six wavelength maxima - 500, 533, 568, 610, 640, 670 nm - for detection). These filters can be flexibly combined according to the requirements of the probe format and dyes used in an experiment (Figure 2b).

For signal capture, a large field lens and a charge-coupled device (CCD) camera are specifically arranged to attain uniform collection of signals across the plate. Fluorescence detection is therefore largely independent of sample position and can be done in the absence of additional dyes (e.g., ROX) used otherwise to compensate for two-dimensional differences in intensity.

#### LightCycler® 480 System's software concept

LightCycler® 480 Instruments are provided with a basic software module to enable users to easily set up reaction protocols and run absolute quantification or melting-curve analysis. Additional software modules are available

and can be purchased according to the user's application needs (e.g., for relative quantification and genotyping). Building on the principles of Roche Applied Science's LightCycler® Software 4.05, the analysis modules are based on highly robust and accurate methods, including whole melting curve-based genotyping and second-derivative crossing point (Cp) determination.

No matter which application, the software interface is designed to make assay setup, user and data management convenient and straightforward. All modules support the use of in-run or imported standards, the definition and storage of subsets and templates. Since raw data remain accessible after an instrument run, analysis can be done very flexibly. Options for easy data transfer, such as through batch export, are provided.

Compared to the classical LightCycler® systems, the LightCycler® 480 System goes one step further in offering enhanced compatibility with automated high-throughput workflows. For integrated workflow monitoring, the LightCycler® 480 System offers connectivity to standard LIMS (laboratory information management systems) and compatibility with CFR 21 Part 11 standards. Combined with an integrated bar-code reader, the LIMS/barcode software module (optional) allows the instrument's use downstream of robotic equipment for automated sample preparation, reaction setup or plate loading.

#### LightCycler® 480 System's reagents

The LightCycler® 480 System includes generic master mixes specific for each of the main LightCycler® 480 applications (gene detection, gene or cDNA quantification, genotyping). Since all reagents are provided as one-component master mixes, reaction setup requires only the addition of template DNA, primers and (except for experiments with SYBR Green I) probes. No adjustment in the  $\text{MgCl}_2$  concentration is generally needed to amplify different sequences. The mixes are all compatible with

**Table 1: LightCycler® 480 System detection formats, dyes, and applications.**

Excitation (nm)	Detection (nm)	Assay format	Dyes	Applications
483	533	SYBR Green I	SYBR Green I	Characterization Quantification
483	610	HybProbe probes	LightCycler® Red 610	Quantification
483	640		LightCycler® Red 640	SNP analysis
483	670		Cy5	
450	500	Hydrolysis probes	LightCycler® Cyan 500	Quantification
483	533		FAM	SNP analysis
523	568		VIC / HEX	
558	610		LightCycler® Red 610	
558	640		LightCycler® Red 640	
615	670		Cy5	
483	533	SimpleProbe probes	Fluorescein	SNP analysis

hot-start protocols, and can be used in two-step RT-PCR applications (e.g., downstream of a Transcriptor Reverse Transcriptase reaction).

### Assay Formats and Experimental Design

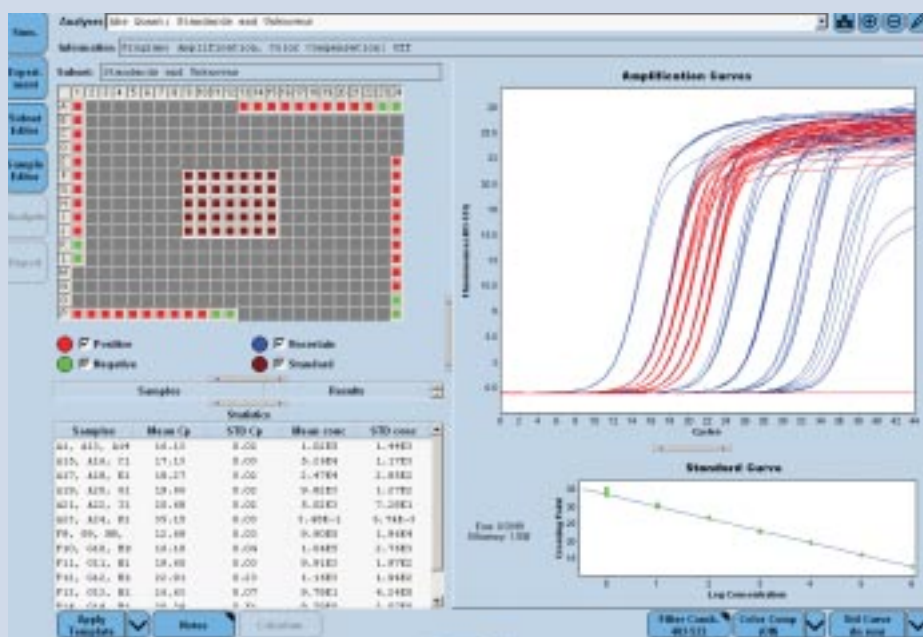
Offering a broad range of wavelengths with a high light intensity for signal generation and detection, the LightCycler® 480 System is compatible with most currently available dyes and assay formats. According to Roche Applied Science's time-tested and performance-proven gene expression and genotyping methods, the system has been optimized in view of each of these applications for

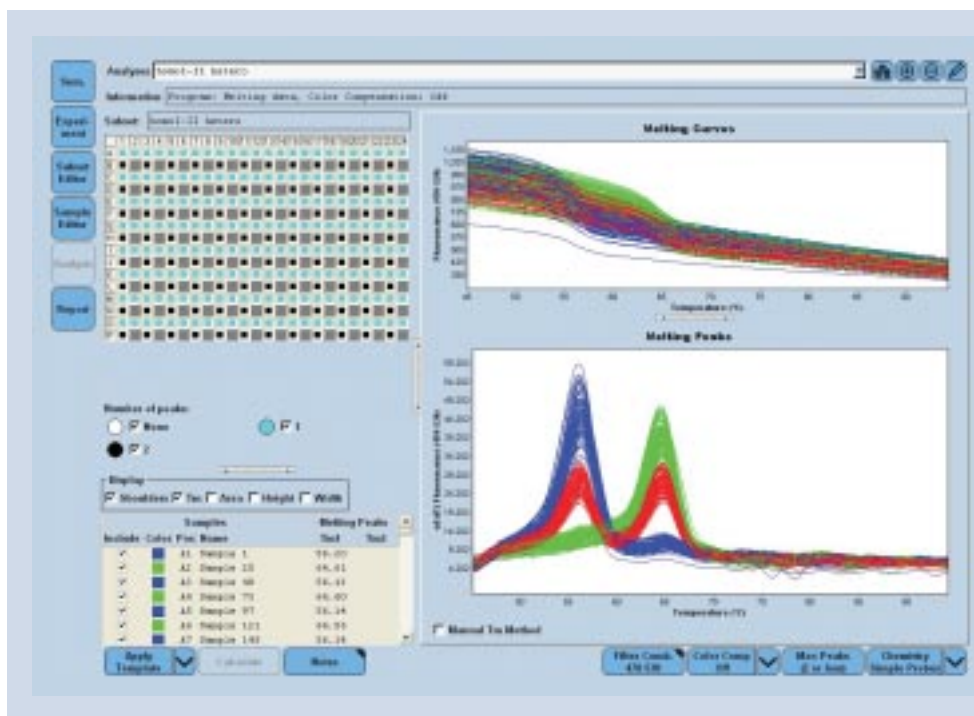
the use of hydrolysis probes and HybProbe (FRET) probes, respectively. A summary of LightCycler® 480 System detection formats, dyes, and applications is provided in Table 1.

For gene expression analysis with the LightCycler® 480 System, Roche Applied Science's novel Universal ProbeLibrary probes offer an ideal complementary approach. Organism-specific ready-to-use Universal ProbeLibrary sets are available, containing a combination of only 90 pre-validated detection probes to cover the entire transcriptome of the chosen species. Supported by a free online assay design center ([www.universalprobelibrary.com](http://www.universalprobelibrary.com)), probes

#### Figure 3: Intra-assay reproducibility of an absolute gene quantification experiment using the LightCycler® 480 Instrument.

Serial dilutions of a 390-bp amplicon derived from the G6PDH gene (seven steps,  $10^2$  to  $10^6$  copies/20  $\mu$ l) were detected with SYBR Green I. The whole dilution series was assayed in five replicates positioned in the center of the plate (brown) and used to establish a standard curve (lower right). Five samples with concentrations different from the standards were analyzed as duplicates on four edges of the plate (red). Non-template controls are shown in green. The graphic illustration of the amplification curves (standards in blue, unknowns in red) as well as the standard deviation values demonstrate that the position of a sample in the plate has no significant influence on the results, thus demonstrating very high well-to-well homogeneity over the entire block.





**Figure 4:**  
**High-throughput melting-curve analysis of samples containing different variants of the polymorphic mammalian *MDR-1* gene.**

Amplification products were subjected to melting-curve analysis with HybProbe probes using the LightCycler® 480 Instrument. Each sample was measured in 96 replicates distributed evenly across a 384-well plate (wells with *turquoise squares*: one melting peak; wells with *black squares*: two melting peaks; empty wells shown in *grey*). As shown in the graphs and table, highly reproducible melting curves and  $T_m$  (melting temperature) values were obtained (*green curves*: homozygous wild type; *blue curves*: homozygous mutant; *red curves*: heterozygous samples).

can be selected from the library and primers designed in seconds. Using this approach, whole gene expression assays can be accomplished within only two days.

## Performance Data

As illustrated by the features of its individual components (hardware, software and reagents) and by the following experimental examples, the LightCycler® 480 System as a whole provides optimal conditions for highly accurate and reproducible real-time PCR applications. When sample replicates are distributed evenly across a multi-well plate ("walk-around-the-block" experiments), assay reproducibility and accuracy can be demonstrated both for gene quantification (Figure 3) and for melting-curve analysis (Figure 4). Such experiments also demonstrate the excellent resolution and dynamic range of the LightCycler® 480 System.

## Summary

The LightCycler® 480 System is a novel modular online PCR platform for highly accurate qualitative and quantitative detection of nucleic acids and genotyping. Building on the benefits of Roche's LightCycler® 1.5 and 2.0 Systems, it goes one step further in offering enhanced throughput, compatibility with automation equipment and maximum flexibility regarding hard- and software. Providing novel ways to combine speed and accuracy without compromises, it meets the needs of a broad range of applications in research fields such as SNP analysis, gene-expression studies, or array data validation. ■

Product	Pack Size	Cat. No.
<b>LightCycler® 480 Instrument</b>	384-well	04 545 885 001
	96-well	04 640 268 001
<b>LightCycler® 480 Block Kit</b>	384-well	04 643 631 001
	96-well	04 643 640 001
<b>Generic reagents</b>		
<b>LightCycler® 480 SYBR Green I Master</b>	1 kit (500 reactions)	04 707 516 001
<b>LightCycler® 480 Probes Master</b>	1 kit (500 reactions)	04 707 494 001
<b>LightCycler® 480 Genotyping Master</b>	1 kit (384 reactions)	04 707 524 001
<b>Other reagents</b>		
<b>LightCycler® 480 Control Kit</b>	1 kit	04 710 924 001
<b>Disposables</b>		
<b>LightCycler® 480 Multiwell Plate 96</b>	50 plates + foils	04 729 692 001
<b>LightCycler® 480 Multiwell Plate 384</b>	50 plates + foils	04 729 749 001
<b>LightCycler® 480 Sealing Foil</b>	50 foils	04 729 757 001
<b>Software</b>		
<b>LightCycler® 480 LIMS/Barcode Module</b>	1 software package	04 727 886 001

