Roche Applied Science

LightCycler® 480 Real-Time PCR System

Rapid by nature,
accurate by design

Planned introduction of the LightCycler® 480 System: September 2005

The LightCycler® 480 Real-Time PCR System

Roche Applied Science brings you one more breakthrough in the real-time PCR analysis of gene expression and genetic variation

Figure 1: LightCycler® 480 Instrument and data station.

Roche Applied Science's cutting-edge gene analysis technology is now available in a high-throughput, microwell plate-based cycler format (96- or 384-well).

Combining amazing speed with exceptional accuracy, the LightCycler® 480 Real-Time PCR System is a novel platform for gene-expression and melting curve-based mutation analysis. The compact yet versatile LightCycler® 480 benchtop instrument can be interfaced with robotics to create an automated high-throughput solution.

Based on innovative hardware and software, the LightCycler® 480 Instrument is a modular online PCR device for qualitative or quantitative detection of nucleic acids, genotyping, and mutation analysis. It will meet the needs of a broad range of scientific applications in genomics research, such as array validation, gene-knockdown studies, or SNP analysis.

Offering the sensitivity and accuracy one has come to expect only from the Roche Applied Science LightCycler® System, the LightCycler® 480 Real-Time PCR Instrument goes one step further in providing enhanced throughput and multiplexing capabilities.

Supporting all current probe formats and applications in gene-expression and mutation analysis, its built-in versatility allows adaptation to future technological advances in genomic research.

Important:

Please note that all information contained in this document related to features and specifications of the LightCycler® 480 System (instrument, reagents, software) is preliminary. Characteristics of each component of the LightCycler® 480 family of products may change until official introduction to the market (planned for September 2005).

The LightCycler® 480 Real-Time PCR System –

An ideal combination of speed, accuracy, and versatility

Hardware –
eliminate the "edge-effect"

Innovative temperature control

- The 96- or 384-well LightCycler® 480 thermoblock is based on improved heat-transfer technology, leading to extraordinary well-to-well temperature homogeneity and maximized inter-well, inter-cycle reproducibility.
- Easily adapt the system to changes in your throughput needs: thermoblocks can be readily exchanged by the user in minutes, and do not require any time-consuming recalibration.

High-performance optical system

- Fluorescence excitation by a high-intensity xenon lamp, and a specially developed signal-collection system, result in maximal sensitivity for any assay format in all channels.
- Special arrangement of optical components ensures the uniform collection of signals across the plate and makes analysis independent of sample position or differences in reaction volume.
- The LightCycler® 480 System is suitable for many assay formats: HybProbe (FRET, hybridization) probes, DNA-binding dyes (*e.g.*, SYBR Green I), SimpleProbe probes, hydrolysis probes, Molecular Beacons, and others.
- Freely combine built-in filters (excitation: 450 nm, 483 nm, 523 nm, 558 nm, 615 nm; detection: 500 nm, 533 nm, 568 nm, 610 nm, 640 nm, 670 nm) to fully exploit multiplexing and multicolor capabilities.

Software –
pay for what you need

Customizable modular system concept

- Building on the principles of the well-established, performance-proven LightCycler® Software 4.0, the LightCycler® 480 platform comes equipped with modules for run control, T_m calling, and absolute quantification.
- Further customize your system to your specific needs by adding advanced analysis modules as required (offered separately):
  – Qualitative (yes/no) detection
  – Absolute quantification with controls
  – Relative quantification with controls
  – Genotyping with controls
  – Statistical analysis tools

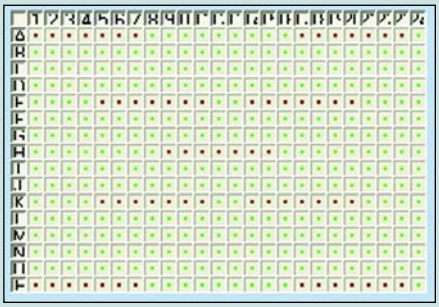
Reagents – especially developed for the LightCycler® 480 System

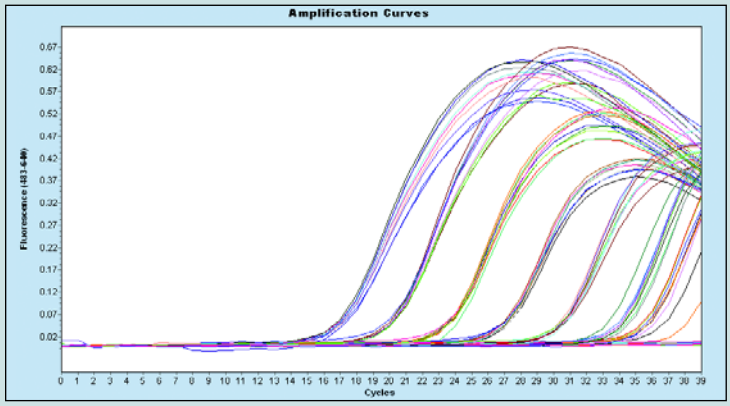
Application-specific master mixes

- A tailor-made hot-start PCR enzyme master mix is available for each type of LightCycler® 480 application (quantification or genotyping, generic or specific dyes). Built on Roche's performance-proven FastStart technology, these reagents ensure optimal reproducibility and high dynamic ranges:
  – LightCycler® 480 SYBR Green I Master
  – LightCycler® 480 Probes Master
  – LightCycler® 480 Genotyping Master, specially designed for HybProbe probe or SimpleProbe probe assays, and optimized for melting curve performance.

The LightCycler® 480 Real-Time PCR System –
Applications that meet your complex needs

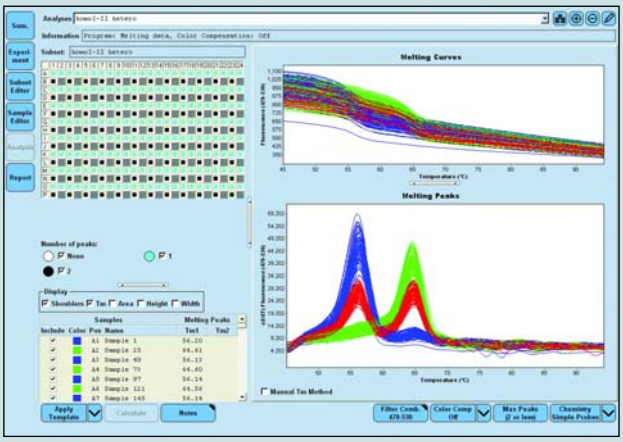
Quantification analysis





▲ **Figure 2: Reproducibility and sensitivity of real-time PCR on the LightCycler® 480 Instrument.** Serial dilutions of a viral target sequence (seven steps, 10^6 to 10^0 copies/20 µl) were amplified via PCR and detected with HybProbe probes. The whole dilution series was assayed in nine replicates positioned on different areas of the plate (*e.g.*, wells A1 to A7 corresponding to one of the nine replicates). The graphic illustration of the amplification curves shows 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 (*e.g.*, standard deviation for 10^2 copies: 0.11).

Melting curve analysis



	T_m(1)°C	T_m(2)°C
average	56.19	64.76
minimum	55.77	64.48
maximum	56.56	65.23
standard deviation	0.18	0.17

◀ **Figure 3: High-throughput melting curve analysis of samples containing different variants of the polymorphic mammalian MDR-1 gene.** Target gene amplification products were subjected to melting curve analysis with HybProbe probes on 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).

Data generated by Roche Applied Science, Penzberg, Germany.

Benefits of the LightCycler® 480 Real-Time PCR System –

The speed and accuracy of the LightCycler® 2.0 System with the throughput, versatility, and compatibility to get the job done

Speed – rapid temperature control for superb specificity and yield

PCR specificity and yield are directly related to the ability of the thermal-cycling system to rapidly and accurately arrive at and maintain reaction temperatures.

- The LightCycler® 480 System utilizes a novel block technology to allow the rapid heating and cooling required to complete a 40-cycle, 384-well qPCR run in less than 40 minutes.
- The LightCycler® 480 System includes reagents that have been optimized for exceptional sensitivity and specificity in this rapid cycling environment.

Accuracy – unbiased conditions for unambiguous results

The key to accurate results is the elimination of uncontrolled variables so that data within and between assays may be relied upon for consistency and reproducibility.

- The LightCycler® 480 System eliminates the "edge-effect" found in so many block-based thermal-cycling systems. In addition, superior optics and algorithms accounting for instrument-specific parameters ensure the integrity of the data over the entire plate for reaction volumes ranging from 3 to 100 µl.
- The LightCycler® 480 System uses a time-tested, publication-proven software algorithm for efficiency-corrected relative quantification. When necessary, non-linear fit functions for standards are applied. Given the nature of PCR, this method produces more accurate and consistent results than with conventional calculation algorithms.

Versatility – utmost flexibility for maximum efficiency

Why pay for things you don't need or clutter your system with modules you never use?

- Regardless of your work environment, the LightCycler® 480 platform allows you to build a system that best suits your needs with respect to sample load, automation capabilities, analysis tools, and assay chemistry. Easily combine hardware and software modules according to your current application needs while keeping all options for easy, stepwise upgrades as your projects evolve.
- Five excitation and six emission filters allow you to work with the assay format of your choice, including SYBR Green I, hydrolysis probes, HybProbe probes, SimpleProbe probes, and others.

Compatibility – uncompromising adaptability for a perfect fit

Whether you need a system that can grow with you or one that will interface with your current systems, the LightCycler® 480 System was designed with you in mind.

- The integrated bar-code reader is compatible with upstream use of automated sample-preparation workstations. Automated loading is possible in combination with robotic equipment. For integrated workflow monitoring, the LightCycler® 480 System offers connectivity to laboratory information management systems (LIMS).

Inspiring Discovery

LightCycler® 480 System Specifications

Dimensions	W 60 cm x D 60 cm x H 54.5 cm W 24 in x D 24 in x H 21.5 in
Weight	55 kg (121 lbs)
Sample numbers	Blocks for either 96 or 384 samples, easily exchangeable by user
Reaction volumes	5 µl - 20 µl (384 well), 20 µl -100 µl (96 well)
Temperature control	• Peltier-based heating/cooling from 40°C - 95°C • Heated lid, PCR without any overlay (*e.g.*, wax or oil) • Passive post-run cooling to < 40°C
Excitation	5 excitation filters, high-intensity broad-spectrum xenon lamp
Detection	6 detection filters, signals detected online by CCD camera
Run time	< 60 minutes (96-well plate) < 40 minutes (384-well plate)
Dynamic range	1 to 10^8 copies/well detectable (viral target)
Pre-installed software	• Run software • Analysis software (absolute quantification, T_m calling)
Additional software	• Relative quantification including efficiency correction • Genotyping via automated sample grouping and allele calling
Computer	Pentium PC with Windows XP
Automation capabilities	• Integrated bar-code reader • LIMS connectivity • 21 CFR Part 11 compatibility • Drawer controllable by software for plate loading

In development. For research use only.
Not for use in diagnostic procedures.

Roche Diagnostics

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