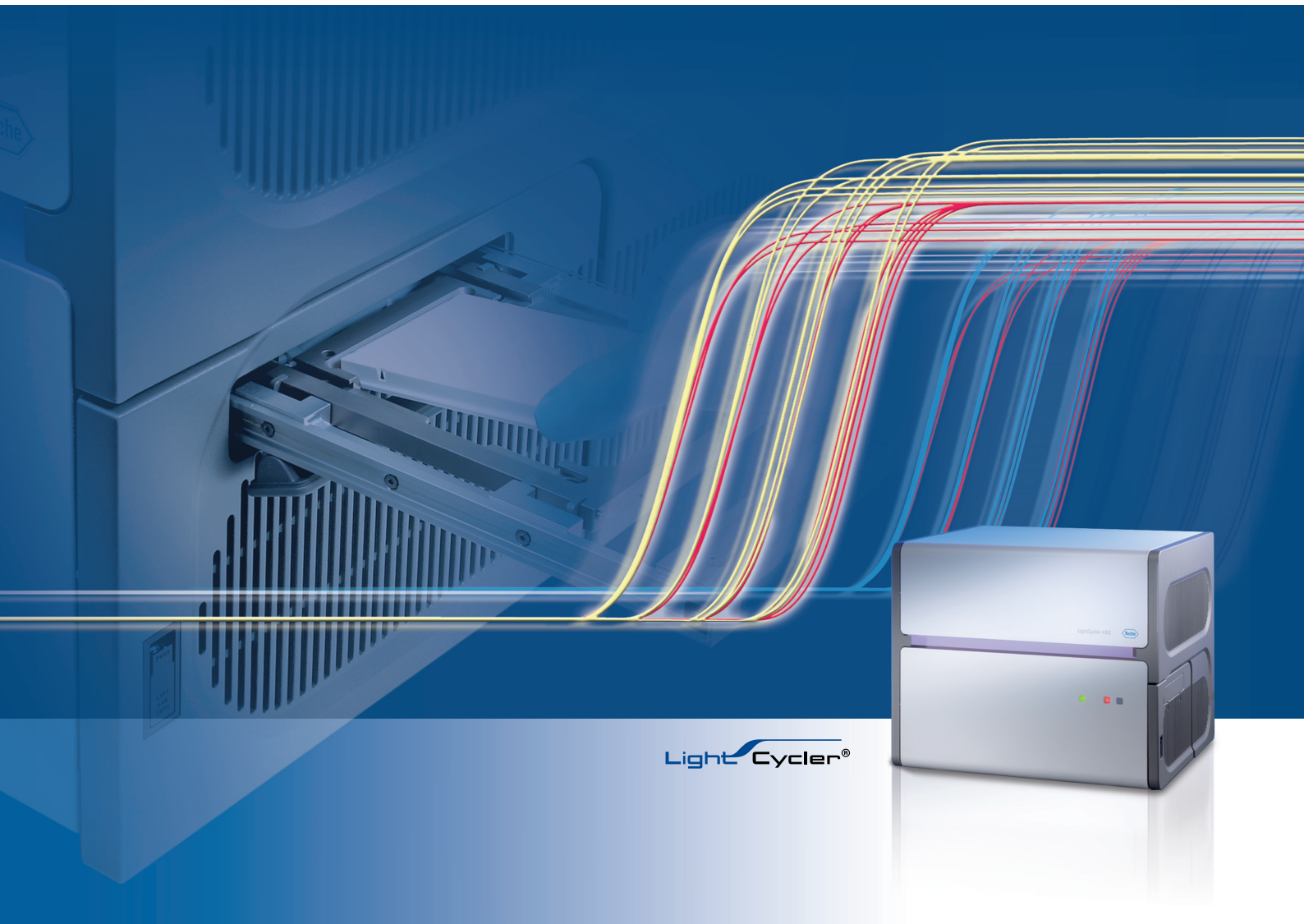
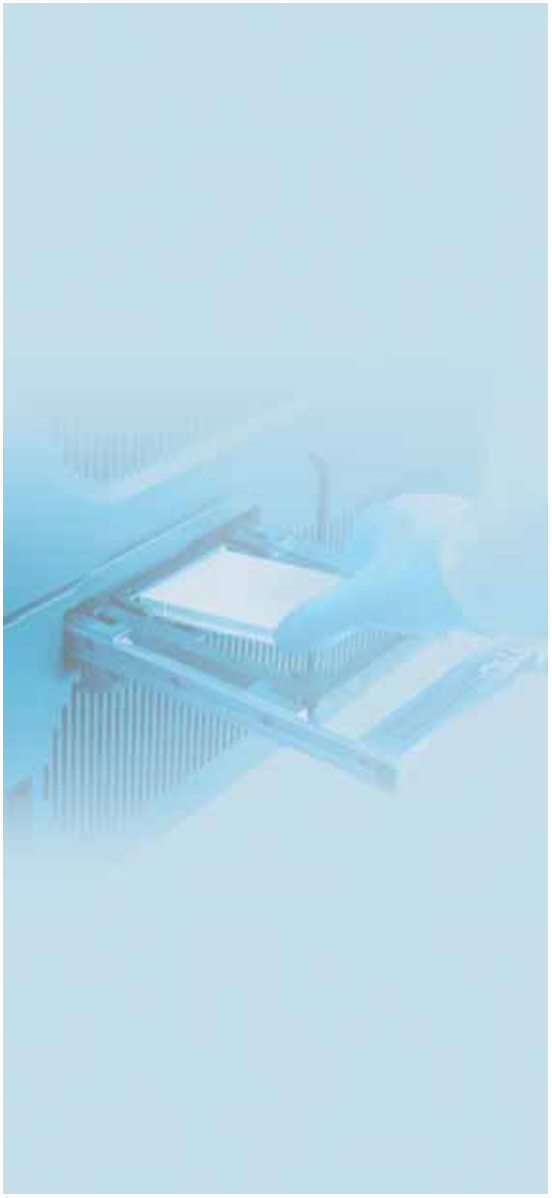
The LightCycler® 480 System

Unleash the Potential of Real-Time PCR



Amplify your success in medium- and high-throughput real-time PCR applications with the innovative LightCycler® 480 System, from Roche Applied Science.

The plate-based LightCycler® 480 System is a highly adaptable and versatile real-time PCR system for the analysis of gene expression and genetic variation. The modern instrument design, outstanding technical and software features, as well as advanced reagents and disposables of the LightCycler® 480 System, exceed the demands for fast, accurate, and most challenging real-time PCR applications.



The LightCycler® 480 Real-Time PCR System

A standard for high-performance real-time PCR

Take your real-time PCR projects to new levels of sensitivity, specificity, and throughput with the accuracy, versatility, and speed of the LightCycler® 480 System.

Compelling reasons for choosing the LightCycler® 480 System…

- Unique silver thermal block cycler technology for exceptional well-to-well data homogeneity.
- Advanced high-performance optical system for accurate data capturing.
- Easily interchangeable 96- and 384-well thermal block cycler units.
- Optional clear or white plates, depending on your workflow and sensitivity needs.
- Excellent PCR sensitivity with high-value LightCycler® 480 reagents and disposables.
- Highest flexibility with fluorescence dyes and detection formats.
- Options for basic and advanced gene expression analysis.
- Genetic variation studies based on HybProbe/SimpleProbe probes, high-resolution melting or endpoint genotyping.
- Intuitive, user-friendly LightCycler® 480 software interface.
- Fast and easy assay setup with the new sample editor.
- One-click experiment setup with options to refine results later.
- Multi-function database with research and traceable modes.
- Multiple plate analysis tools for extended genetic variation and gene expression studies.
- State-of-the-art LIMS connectibility.
- 21 CFR part 11 compliance data protection.
- Premium customer support and instrument service.

Meet and exceed the capabilities of other real-time PCR systems – take your genomic research to a new level with the LightCycler® 480 System.

The LightCycler® 480 Real-Time PCR System

Proven, high performance LightCycler® Technology

Since their introduction in 1998, the LightCycler® Real-Time PCR Systems from Roche Applied Science have stood for maximum flexibility, high speed, and outstanding data accuracy. In 2005, this tradition of continuous innovation was brought to a new level with the LightCycler® 480 high-throughput plate-based platform.

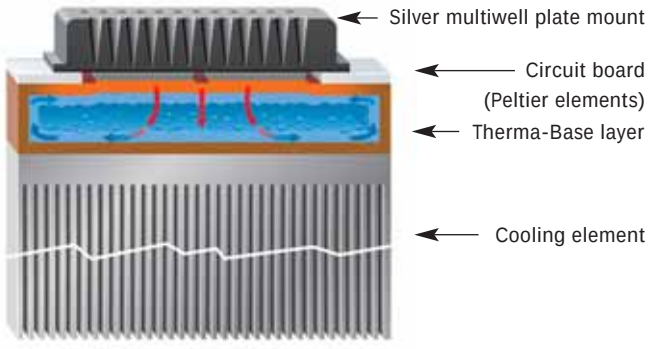
Innovative technological enhancements in the LightCycler® 480 Instrument pave the way for new standards of rapid and accurate real-time PCR data generation and analysis. In particular, the sophisticated design of the silver thermal block cycler unit, optical system, and software deliver the sensitivity, accuracy, and reproducibility one has come to expect from Roche Applied Science instruments.

For maximum flexibility, many components of the LightCycler® 480 Instrument are modular in design. This setup enables users to easily interchange thermal block cyclers (96- and 384-well format), combine various optical filters, and choose between clear or white multiwell plates.

Enhanced software capabilities allow seamless integration of the system into computer-controlled environments and automated workflows. This system setup facilitates data management that complies with 21 CFR Part 11 requirements.

The LightCycler® 480 System comprises versatile instrumentation and software as well as high-performance reagents, customized qPCR assays (Universal ProbeLibrary System) and specially engineered disposable products. This innovative system meets the tough demands of qualitative target detection, quantitative gene expression, and mutation analysis. In addition, its built-in versatility facilitates easy adaptation to new technologies in genomic research.

▲ **Figure 1: Flexibility of the LightCycler® 480 block cycler unit.** The LightCycler® 480 block cycler units (96-well/384-well format) are easily interchangeable by the user, taking no longer than a few minutes. The exchanged block cycler unit is automatically detected and identified by the system, and experiments can be pursued without time-consuming recalibration runs.



▲ **Figure 2: Schematic of the LightCycler® 480 thermal block cycler.** The Therma-Base layer, implemented in the block cycler unit architecture, is a thin cavity lined with a wick structure and filled with fluid. Utilizing a series of condensation and evaporation events, the Therma-Base rapidly adjusts to temperature changes by efficiently dissipating heat.

The LightCycler® 480 Instrument

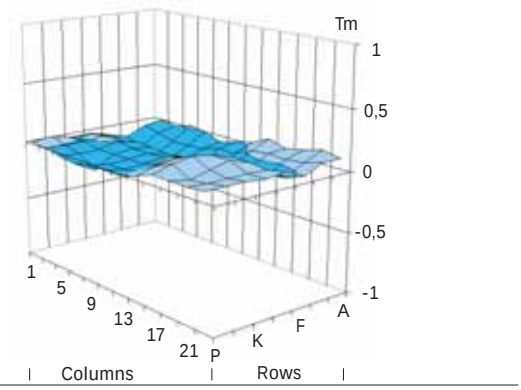
An ideal combination of speed, accuracy and versatility

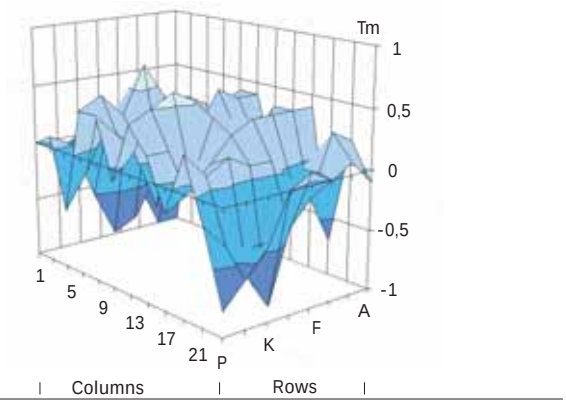
Innovative PCR thermal block cycler design

The LightCycler® 480 System has revolutionized block cycler temperature control through the introduction of a highly efficient heat-equalizing technology (Therma-Base) between the heat block and the cooling element. By removing the effects of spreading resistance, the LightCycler® 480 thermal block cycler provides unparalleled well-to-well temperature homogeneity, as illustrated by minimal inter-well temperature variability across the entire multiwell plate. This innovative temperature control enables exceptional data uniformity, independent of any assay formats or real-time PCR applications, even in fast PCR protocols.

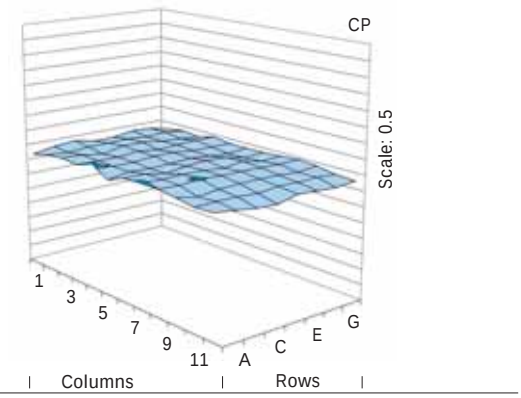
Key benefits of the LightCycler® 480 thermal block cycler:

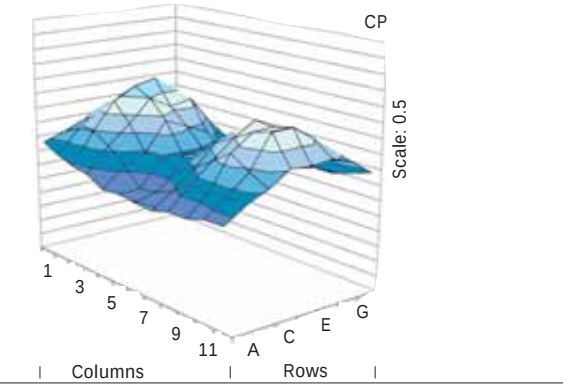
- Run any assay format or application with fast PCR protocols (< 40 minutes for 40 cycles in 384-well plate format).
- Obtain rapid and accurate temperature adjustment.
- Achieve exceptional data homogeneity across the entire multiwell plate.





▲ **Figure 3: Temperature homogeneity across a 384-well plate: a) LightCycler® 480 Instrument; b) another real-time PCR instrument.** The melting temperature (Tm) of a given labeled oligonucleotide was used to demonstrate temperature homogeneity across a multiwell plate (at 50°C). The variation between the measured Tm, and the expected Tm of the oligonucleotide was plotted for all 384 wells using the expected Tm as zero.





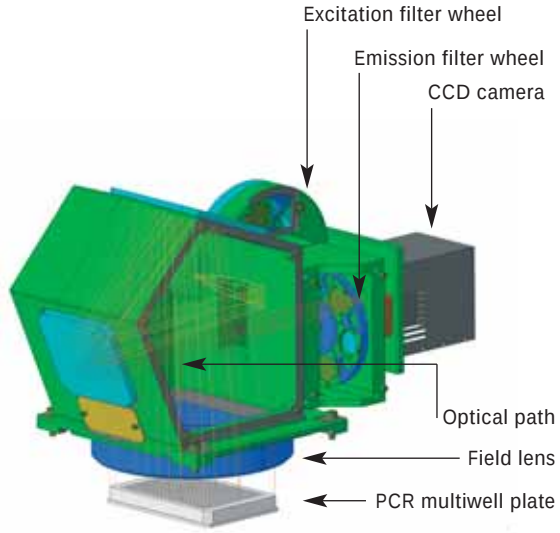
▲ **Figure 4: Crossing point (CP) homogeneity across a 96-well plate: a) LightCycler® 480 Instrument; (b) another real-time PCR instrument.** A low target concentration (100 copies) of a given target sequence (442 bp) was amplified using a fast PCR protocol (20 µl reaction volume, hydrolysis probe format). CP values were plotted for all 96 wells using a 0.5-step CP scale resolution.

The LightCycler® 480 Instrument

An ideal combination of speed, accuracy and versatility

Advanced high-performance optical system

The excellent performance of the LightCycler® 480 Instrument's optical system stems from a high-intensity xenon lamp which emits light across a broad spectrum. A flexible combination of built-in filters for specific excitation and emission facilitates the use of a variety of fluorescent dyes and detection formats for any current real-time PCR application. The special arrangement of the optical components, and the optimum focal length in the LightCycler® 480 Instrument ensure excellent specific signal excitation and uniform data capturing across the entire multiwell plate, independent of sample position. Together with superior signal acquisition rates, this also allows melting curve analysis at high resolution. It also eliminates the need for passive reference dyes (*e.g.*, ROX) for well-to-well signal normalization. As a result, the LightCycler® 480 System gives you the added flexibility to use all channels for target detection, extending multiplexing capabilities.



▲ **Figure 5: Schematic of the LightCycler® 480 detection unit.**

Key benefits of the LightCycler® 480 optical system:

- Enjoy highest flexibility in the choice of fluorescence dyes and detection formats.
- Get advanced accurate data capture across the entire plate without fluorescence signal normalization.
- Work with enhanced multiplexing capabilities.
- Easily access commonly used channel combinations via pre-defined settings.

▼ **Table 1: Overview of excitation and emission filters, dyes and detection formats.** The LightCycler® 480 Instrument employs the high-intensity LightCycler® 480 Xenon Lamp that emits light over a broad wavelength range (430–630 nm). The five excitation and six emission filters of the instrument can be used in any combination.

Xenon lamp (430–630)								
Excitation filters	440	465		498	533			618
Emission filters	488	510		580		610	640	660
Dye	LightCycler® Cyan 500	SYBR Green I ResoLight	Fluorescein FAM		HEX (VIC)	LightCycler® Red 610	LightCycler® Red 640	Cy5
Detection formats Melting Curve		•						
HRM		•						
SimpleProbe probes			•					
HybProbe probes				(•)		•	•	•
Hydrolysis probes 1–3 colors			•		•			•
Hydrolysis probes 4 colors	•			•		•		•

(•) FRET Donor

The LightCycler® 480 Software

Advanced tools to generate high quality data

Advanced high-value software capabilities

The innovative LightCycler® 480 software is characterized by a state-of-the-art design and unique LightCycler® 480 algorithms for fast, highly accurate data generation, without sacrificing comprehensive versatility. Customizable views facilitate intuitive, fast navigation, and a highly sophisticated sample editor allows easy programming, data capturing and analysis. Convenient import and export functionalities enable the seamless integration of the LightCycler® 480 Instrument into computer-controlled environments. Additionally, modern data management and effective data protection capabilities are implemented in the software.

The LightCycler® 480 software provides versatile solutions for the most common real-time PCR applications. The pre-installed software package comprises automated analysis modules for melting curve or endpoint-based genotyping as well as absolute and relative quantification. Additional software modules are available for high-resolution melting and multiple plate analysis. Thus, the LightCycler® 480 System meets all your research needs, from comprehensive customized scientific approaches to streamlined automated routine workflows.

Key benefits of the LightCycler® 480 software:

- Quickly start experiments from ready-to-use macros or templates for all applications
- Apply basic or advanced analysis modes for both gene expression and genotyping studies
- Conveniently define or edit sample information in plate or table views
- Choose between a flexible research mode or a secured traceable database mode

Figure 6: Screenshot of the LightCycler® 480 software interface (opening screen):

- Gain easy access to a broad range of quantification and genotyping methods.
- Quickly start new experiments from macros or templates.

LightCycler® 480 Gene Quantification Solutions

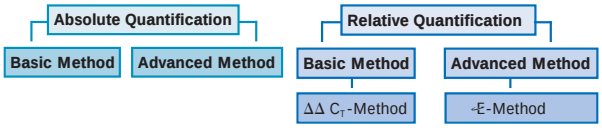
Ultimate innovations for gene expression studies

Highly versatile solutions for gene quantification

The LightCycler® 480 software provides innovative solutions for various quantitative real-time PCR (qPCR) analyses. Absolute and relative quantification analysis methods, and subtypes of these techniques are implemented in the LightCycler® 480 software. Based on unique LightCycler® algorithms, the sophisticated LightCycler® 480 quantification software facilitates reliable quantification data.

Peak performance for gene expression analysis

The versatile LightCycler® 480 Relative Quantification Software provides different relative quantification methods (*e.g.*, basic $\Delta\Delta C_T$-Method, advanced $\mathcal{E}$-Method with standard-curve derived efficiencies) for gene expression and gene dosage studies. This offers you various degrees of quantification reliabilities adaptable to your individual experiment needs. Benefited by the versatile structure of the LightCycler® 480 software, one single PCR result can be refined by guiding it through the different analysis methods. The unique Roche Applied Science $\mathcal{E}$-Method is an innovative, scientifically sound solution for the most demanding relative quantification applications.



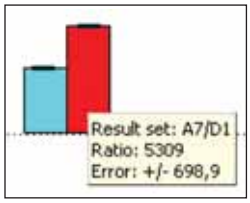
▲ **Figure 7:** Overview of the different PCR quantification principles.

Key benefits of the LightCycler® 480 Quantification Software:

- Speed up PCR analysis with advanced, user-friendly, fast-tracking software tools.
- Choose from quick one-click data checks and in-depth refined analyses for each PCR result; analyze your data the way you want to.
- Get fast results by using basic PCR efficiency assumptions, then achieve ultimate data accuracy with the $\mathcal{E}$-Method.
- Use one or several targets and/or reference genes.
- Analyze targets and references present on same or different plates.
- Choose between Fit Points Method or Second Derivative Maximum Method for Cp calling.

a

b



◀ **Figure 8: Advanced relative quantification analysis ($\mathcal{E}$-method). (a)** Upper part: results in table view, including sample information on chosen references, pairing and Cps. Lower part: Bar-chart display (including errors) of target/reference ratios, with normalized values in red. **(b)** Exact values can be read easily using the mouse-over function.

For detailed information about real-time PCR quantification techniques, please visit: **www.lightcycler.com**

LightCycler® 480 SNP Analysis Solutions

Superior solutions for genetic variation research

Easy access to comprehensive and accurate genotyping information

Genotyping (SNP analysis) on the LightCycler® 480 System can be performed based on two different methods: melting curve analysis with HybProbe probes or SimpleProbe probes (using the LightCycler® 480 Genotyping Master), and endpoint analysis with hydrolysis probes (using the LightCycler® 480 Probes Master). For both methods, software modules for fully automated analysis are included in the preinstalled LightCycler® 480 Software 1.5.

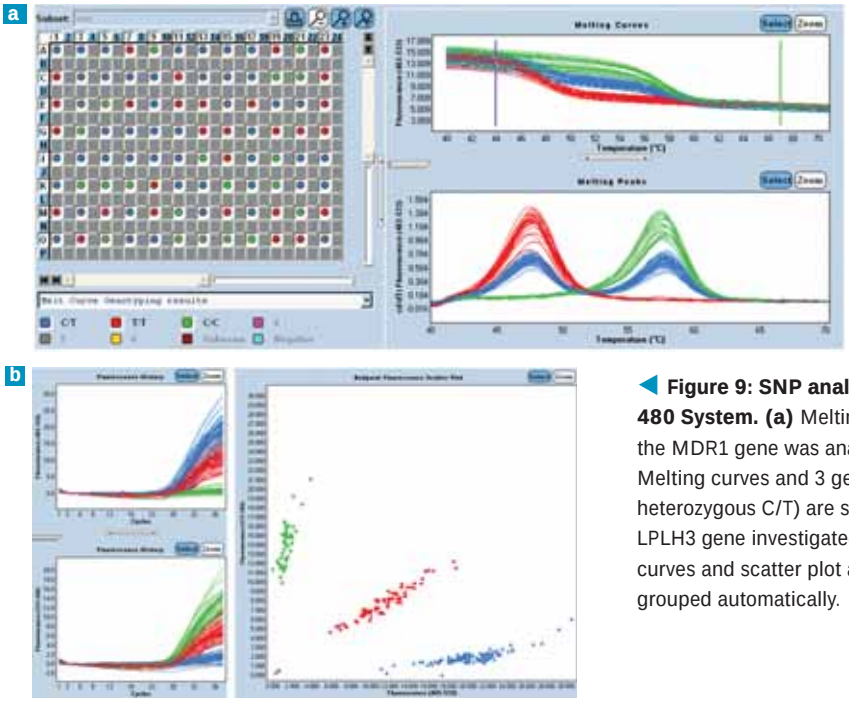
In melting curve analysis, different alleles or allele combinations are identified due to the different strength of interaction they have with the probe. Allele-specific primers or probes are not needed; the same sequence is used for all alleles of an investigated SNP. This reduces reagent costs and enables straightforward reaction mulitplexing. As a post-PCR process, melting curve analysis depends neither on the efficiency of the amplification process nor on the cleavage of a substrate, and is therefore very robust.

The LightCycler® 480 genotyping algorithm groups samples with similar melting curve shape either by auto-calling or via included standards of known genotypes (Figure 9).

Endpoint genotyping is based on the use of dual color hydrolysis probe assays, *e.g.*, with commercially available predefined SNP genotyping assays. Genotypes can be called automatically and easily visualized in scatter plots (Figure 9b).

Key benefits of LightCycler® 480 Genotyping Solutions:

- Choose between HybProbe-/SimpleProbe probes or hydrolysis probe based methods.
- Perform endpoint genotyping to set up experiments quickly without optimization.
- Get more insight into complex genetic setups by running highly flexible melting curve assays.



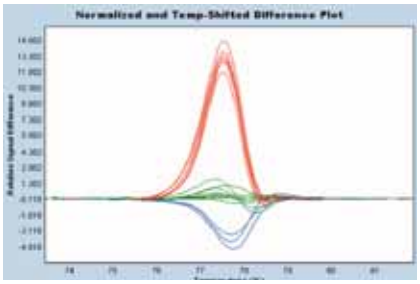
◀ **Figure 9: SNP analysis methods on the LightCycler® 480 System. (a)** Melting curve analysis: A polymorphism in the MDR1 gene was analyzed with SimpleProbe probes. Melting curves and 3 genotypes (homozygous C/C and T/T, heterozygous C/T) are shown. **(b)** Endpoint analysis of the LPLH3 gene investigated with hydrolysis probes. Amplification curves and scatter plot analyses can be displayed and samples grouped automatically.

The LightCycler® 480 Gene Scanning Software

Integrating PCR and High-Resolution Melting on multiwell plates

Highly robust and convenient mutation discovery

High-Resolution Melting is a homogeneous, closed-tube, post-PCR technique enabling researchers to rapidly and efficiently discover genetic variations (*e.g.*, SNPs, mutations, methylations) in DNA fragments. High-Resolution Melting provides outstanding specificity and sensitivity with high sample throughput. It also saves time and reduces costs compared to non-homogeneous (gradient- or gel-based) mutation screening methods (*e.g.*, dHPLC) that require PCR and analysis on separate instruments.



▲ **Figure 10: LightCycler® 480 Gene Scanning Software reveals differences between wild types and variants by grouping of HRM curves.** A fragment of the human TNF SF18 gene with the A/T polymorphism rs723858 was amplified by PCR using the LightCycler® 480 High Resolution Melting Master from genomic DNA isolated from blood samples. Difference curve analysis enables differentiation between wild type samples (AA, green), homozygous mutants (TT, blue), and heterozygotes (AT, red.).

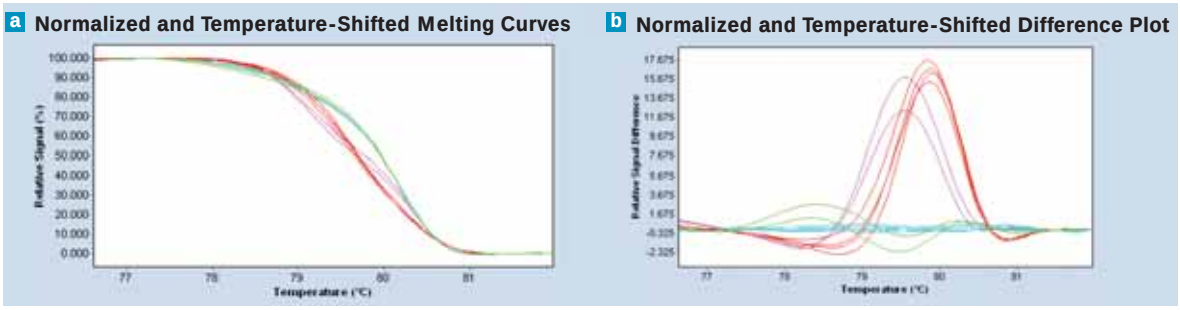
The LightCycler® 480 Real-Time PCR System has the capability to generate and analyze High-Resolution Melting curves at high throughput.

In a LightCycler® 480 Gene Scanning experiment, sample DNA is first amplified in the presence of ResoLight, a novel type of saturating DNA dye contained in the LightCycler® 480 High Resolution Melting Master. Using the instrument's high data acquisition rate, a melting curve is generated, and the resulting data analyzed using the LightCycler® 480 Gene Scanning Software Module.

In this analysis, signal differences between each curve and one chosen reference curve are plotted, allowing the automatic clustering of samples into distinct groups that have similar melting curve shapes (*e.g.*, heterozygotes versus homozygotes).

Key benefits of the LightCycler® 480 Gene Scanning Solution:

- The only currently available one-instrument solution for PCR and High-Resolution Melting on 96- and 384-well plates.
- Melting Master includes novel High-Resolution Melting dye with improved signal dynamics and PCR compatibility.
- Automated grouping algorithm efficiently identifies new variants with high sensitivity.



▲ **Figure 11: Genetic variation in the human CFTR gene analyzed by high resolution melting. a)** A 198 bp fragment of the human CFTR gene was amplified using the LightCycler® 480 High Resolution Melting Master and subjected to amplicon melting at high resolution. **b)** Difference plot analysis revealed three different groups of heterozygotes (red, pink, and green) in addition to the homozygous samples (blue). Data courtesy of Dr. Peter Bauer and Dr. Stefanie Beck-Wödl, University Hospital of Tübingen, Germany.

The LightCycler® 480 Reagents and Disposables

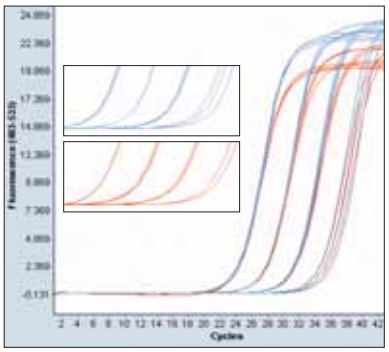
Maximized convenience in fast real-time PCR

High-performance reagents for all PCR application needs

The LightCycler® 480 reagents are based on Roche Applied Science's improved hot-start PCR enzyme formulation, delivering exceptional sensitivity and specificity for real-time PCR assays. The convenient ready-to-use LightCycler® 480 master mixes are specially designed to support each of the main real-time PCR applications (*e.g.*, gene quantification, genotyping) for both standard and fast ramping PCR run protocols. Furthermore, these optimized master mixes offer extended room temperature stability for maximum robustness on automated high-throughput workflows, and improved storage conditions for added convenience with daily use.

Key benefits of the LightCycler® 480 reagents:

- Enjoy exceptional detection sensitivity and specificity for all standard and fast PCR protocols.
- Get maximum enzyme stability for automated high-throughput workflows at room temperature.
- Save time with ready-to-use one-component master mixes.



▲ **Figure 12: Stability of the LightCycler® 480 SYBR Green I Master.** Serial 1:10 dilutions (10,000 – 10 copies/reaction, three replicates) of a human DNA target sequence were assayed either immediately after PCR setup (blue curves) or after 24 hours standing in a loading robot at room temperature (red curves). The shape of the amplification curves demonstrates that the PCR performance was not affected by prolonged pre-PCR standing.

Reagents	Formats	Applications	Hot-start PCR	PCR Speed	
				Standard	Fast
LightCycler® 480 SYBR Green I Master (2× concentrated)	SYBR Green I	Qualitative/ Quantitative	✓	✓	✓
LightCycler® 480 High Resolution Melting Master (2× concentrated)	ResoLight dye	Qualitative/ Quantitative	✓	✓	✓
LightCycler® 480 Probes Master (2× concentrated)	Hydrolysis probes, UPL probes, HybProbe probes, SimpleProbe probes	Qualitative/ Quantitative	✓	✓	✓
LightCycler® 480 Genotyping Master* (5× concentrated)	HybProbe probes, SimpleProbe probes	Melting curve-based genotyping	✓	✓	✓

* Optimized for multiplex applications. Not suitable for end-point genotyping with hydrolysis probes because enzyme lacks 5´-exonuclease activity.

Sequence-independent DNA detection
Sequence-specific DNA detection

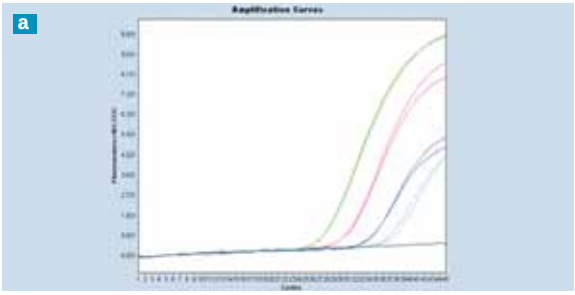
▲ **Table 2: Application areas of the LightCycler® 480 reagents.** All LightCycler® 480 reagents prevent carry-over contamination by employing dUTP for UNG (Uracil-DNA-Glycosylase)-mediated decontamination. Extended storage conditions enable storage at +4 to +8°C for up to four weeks, in addition to the usual long-term storage conditions at -15 to -25°C.

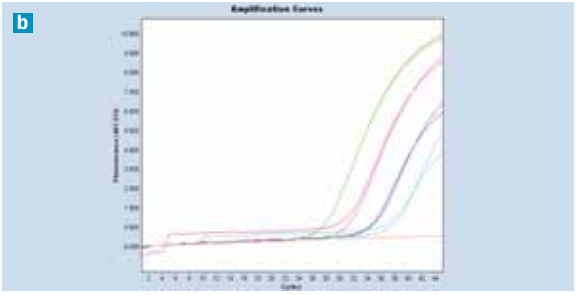
Optimized disposables

Specially designed LightCycler® 480 Multiwell Plates fit perfectly in the thermal block cycler mount, ensuring maximum heat transfer and therefore maximum PCR sensitivity and reproducibility. The opaque, white-colored plate design provides excellent optical sensitivity. These plates ensure consistent PCR results without the need for routine decontamination of the thermal block cycler mount (*e.g.*, removal of fluorescence-labeled probes). Clear LightCycler® 480 multiwell plates are available as an alternative for SYBR Green I and hydrolysis probe assays on instruments with LightCycler® 480 Software 1.5 installed. Bar-codes on each plate enable simple and user-friendly workflow tracking.

Key benefits of LightCycler® 480 Multiwell Plates:

- Achieve high PCR sensitivity and reproducibility with the specialized plate design.
- Eliminate the risk of false-positive influences in PCR results.
- Benefit from bar-code labeled multiwell plates for fast workflow tracking.
- Choose from clear and white plates, depending on your workflow and sensitivity needs.





Step	Temp. °C	Time (a)	Time (b)	Cycles
Reverse transcription	63	3 min	3 min	1
Denaturation	95	30 sec	30 sec	1
Denaturation	95	**10 sec**	**1 sec**	**45**
Annealing	60	**30 sec**	**10 sec**	
Elongation	72	**1 sec**	**1 sec**	

▲ **Table 3: One-step RT-PCR assay with the fast cycling protocol using the LightCycler® 480 RNA Master Hydrolysis Probes.** Dilutions of total RNA (DNA-free) from HeLa cells (from 100 pg to 0.1 pg) were amplified in duplicate in 96-well plates in a total volume of 20µl and detected with a Universal ProbeLibrary assay for beta-actin. Addition of the special enhancer solution generated equivalent crossing point values with the conventional protocol **a)** and the fast protocol **b)**.

Ultra-fast and sensitive RNA analysis

For one-step RT-PCR with hydrolysis probe detection, the LightCycler® 480 System includes a master mix that is specially adapted to the rapid and accurate cycling environment of the LightCycler® 480 Instrument. Optimized buffers maintain sensitivity while allowing significantly shorter reverse transcription than with traditional methods. Highly accurate qPCR data can thus be generated in less than 45 minutes. Multiplex assays can be set up to analyze target and reference genes together, or to study several DNA or RNA viruses in a single well. The LightCycler® 480 RNA Master is also an ideal companion to Universal ProbeLibrary probes when assays have to be designed quickly and results are needed fast.

The Universal ProbeLibrary System

A fast-track approach to gene quantification analysis

Customized qPCR assay

Extraordinarily fast, flexible and accurate gene expression analysis can be achieved with the integration of the sophisticated LightCycler® 480 System, advanced RT-PCR reagents (*e.g.*, Transcriptor First Strand cDNA Synthesis Kit) and the innovative Universal ProbeLibrary qPCR assays.

The unique combination of prevalidated Universal ProbeLibrary (UPL) real-time PCR probes, and the online assay design software [www.universalprobelibrary.com] allow rapid and flexible quantification of virtually any transcript in the transcriptomes of a large number of organisms. The standardized UPL qPCR assays that work first time enhance throughput and efficiency without compromising on sensitivity and specificity.

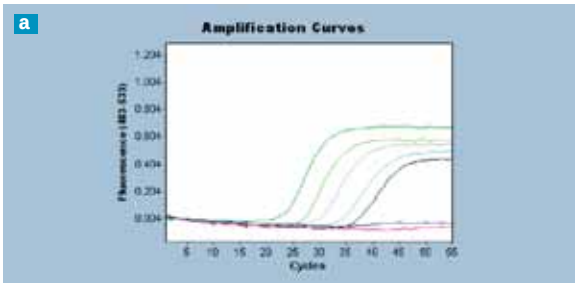
Key benefits of the Universal ProbeLibrary System:

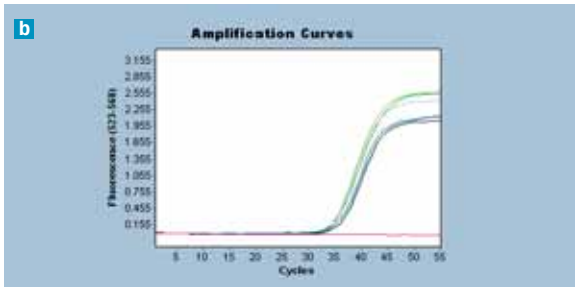
- Significantly reduce the assay design time for any target.
- Enjoy excellent flexibility, specificity and convenience.
- Simplify multitarget analysis and dual color assays with the UPL standard PCR protocol.

▲ **Figure 13: The Universal ProbeLibrary System.**

Ready-to-use reference genes for dual color assays

Quantifying the expression of any human gene of interest is easy using a endogenous control in a dual-color assay. In addition to the 165 FAM-labeled Universal ProbeLibrary probes, assays for four human reference genes (ACTB, HPRT, PGK1, and PBDD) are available. The assays contain primers and UPL probes, labeled with LightCycler® Yellow 555 and a dark quencher, specific for the respective reference gene. When dual-color qPCR assays for a pair of target and selected reference gene are designed with the ProbeFinder software, the assay combination is validated *in silico* to ensure uniqueness and specificity (Figure 14).



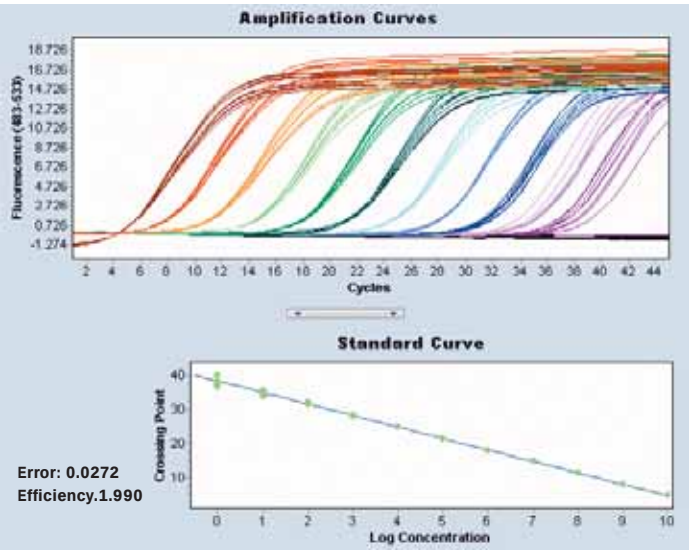


▲ **Figure 14: Typical dual-color qPCR results using the Universal ProbeLibrary Human PBGD Gene Assay:**
a) Sequential 1:10 dilutions for a target gene assay, measured in the FAM channel of the LightCycler® 480 Instrument.

▲ **b)** 10^3 copies of a reference gene (PBGD) were spiked into each dilution of the target gene assay, detected with a reference gene labeled with a different dye, and measured in a second channel.

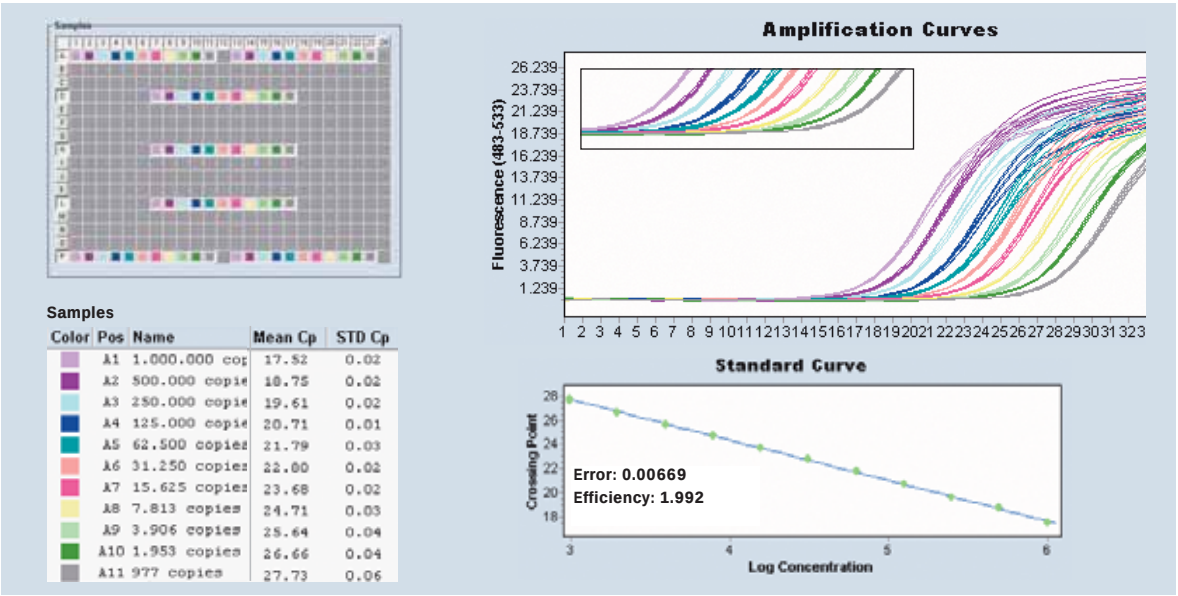
The LightCycler® 480 System Performance

Excellent dynamic range, sensitivity, and reproducibility

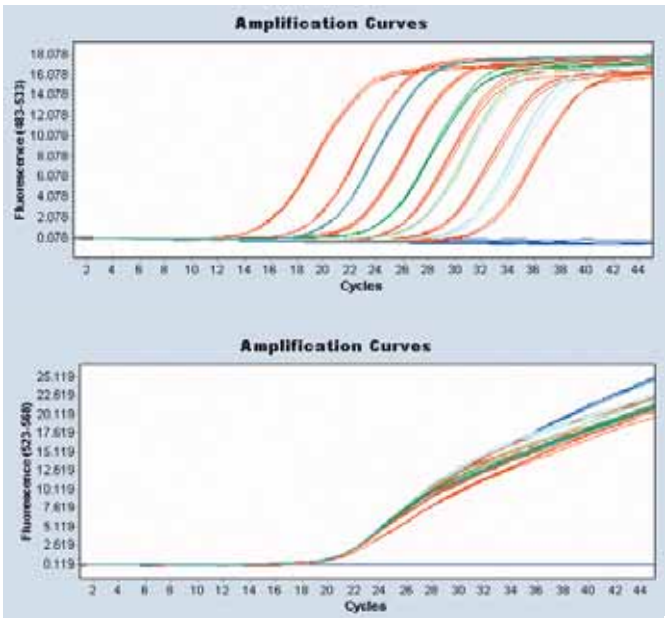


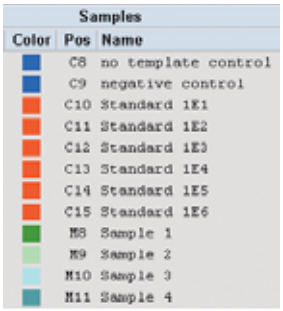
◀ Figure 15: Linear range of the LightCycler® 480 System. Serial 1:10 dilutions (nine replicates each) of a plasmid DNA sequence were amplified with the LightCycler® 480 Probes Master and detected with a UPL probe. The PCR result shows a log-linear relationship over a broad dynamic range (10 log-intervals) and highly reproducible CP values for replicates of each dilution.

■	NTC	■	10^5
■	1	■	10^6
■	10	■	10^7
■	10^2	■	10^8
■	10^3	■	10^9
■	10^4	■	10^{10}

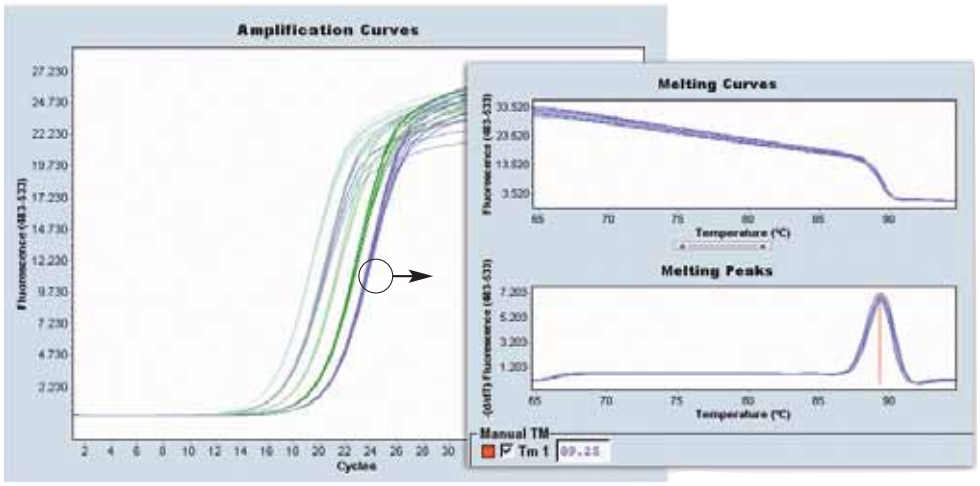


▲ Figure 16: Sensitivity and reproducibility of the LightCycler® 480 System. Serial 1:2 dilutions (seven re-plicates each) of a viral target sequence were assayed with the LightCycler® 480 SYBR Green I Master. A special pipetting scheme was used to distribute the samples across the entire plate. Results obtained from every position demonstrate the outstanding resolution, sensitivity, reproducibility and data homogeneity of the LightCycler® 480 System. Reproducibility is shown by the uniformity of CP values within replicate groups and low coefficients of variation (CV < 0.2 %).





◀ **Figure 17: Dual-color detection with the Light Cycler® 480 System.** These data illustrate an absolute quantification assay. Serially diluted standards (1:10 dilutions, three replicates each) were assayed along with unknown samples. In addition, an internal PCR control (IC) was added to each sample to prevent misinterpretation of negative PCR results. The specific sequences were amplified with the LightCycler® 480 Probes Master and detected with hydrolysis probes (target: FAM-labeled, IC: VIC-labeled).



▲ **Figure 18: Melting curve analysis for DNA product identification with the LightCycler® 480 System.** Serial 1:2 dilutions (eight replicates each) of a plasmid DNA sequence were amplified with the LightCycler® 480 SYBR Green I Master. Post-PCR melting-curve analysis was performed to identify the amplified products by their melting temperatures. The melting curve result illustrates a pure, homogenous PCR product that depicts the highest dilution step (purple line, Tm of 89.25°C). The LightCycler® 480 software conveniently supports Tm analysis by displaying melting curves (sample fluorescence versus temperature) and melting peak charts (first negative derivative of the sample fluorescence versus temperature) and calculating the Tm.

Key benefits of the LightCycler® 480 System performance:

- Work in a broad dynamic range from 10^{10} down to one copy in a single run.
- Obtain exceptional two-fold resolution below 1000 copies.
- Experience new standards for well-to-well consistency and low copy-detection sensitivity.
- Achieve high reproducibility (CV < 0.3).

The LightCycler® 480 Real-Time PCR System

Advanced automated high-throughput workflows

True workflow capabilities

The LightCycler® 480 System can be seamlessly integrated into a computer-controlled environment or automated laboratory workflow with the optional LightCycler® 480 LIMS Interface module. This module facilitates two-way information exchange between the LightCycler® 480 System and a Laboratory Information Management System (LIMS). Furthermore, this module can control system loading procedures, the PCR run, and data analysis, thereby enabling integration of the LightCycler® 480 System into a completely automated workflow. The ability to recognize bar-code labeled LightCycler® 480 Multiwell Plates (via the internal bar-code reader) is pre-installed on all LightCycler® 480 Instruments with Software Version 1.5.

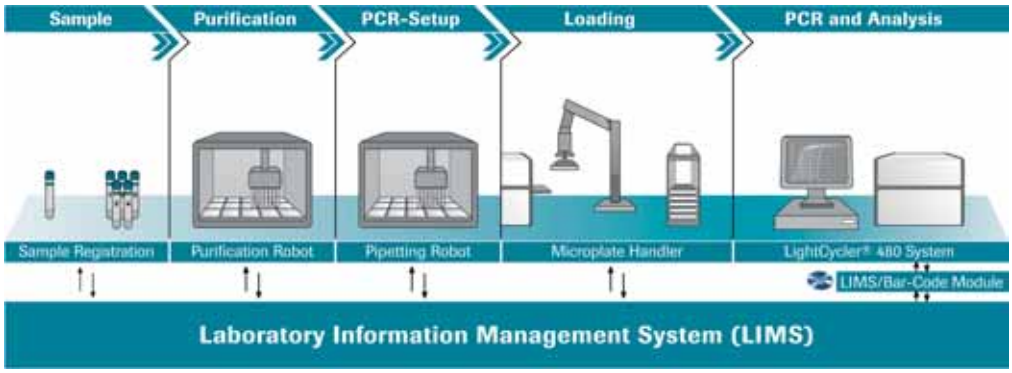
Dependable data management

The LightCycler® 480 System supports 21 CFR Part 11, and meets the general regulatory data management requirements.

Key benefits of the LightCycler® 480 System workflow capabilities:

- Work with true walk-away and automation work flow capabilities.
- Get easy LIMS connectivity with innovative software design.
- Ensure regulatory data management requirements.

▼ **Figure 19: Schematic overview of the LightCycler® 480 System's integration into an automated laboratory workflow.**



▲ **Figure 20: Automated LightCycler® 480 Instrument loading process.**

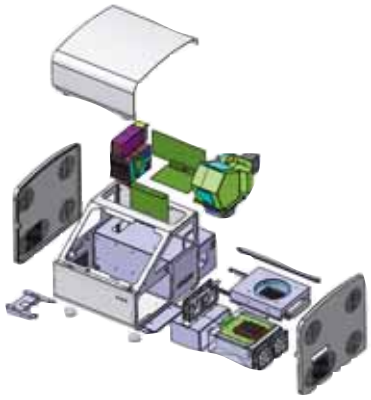
The LightCycler® 480 Real-Time PCR System

It's service all around …

Roche Applied Science, part of Roche Diagnostics, can rely on 30 years success in laboratory diagnostics instrumentation, and nearly a decade of innovative development in real-time PCR instruments. Based on this vast expertise, we can offer a highly professional service concept that meets your most demanding needs.

Built for serviceability

The LightCycler® 480 Instrument's excellent modular design facilitates easy maintenance and optimal serviceability. In addition, the instrument has the added advantage that no routine maintenance is required (*e.g.*, standard instrument calibration runs). Replacement parts, like the affordable LightCycler® 480 Xenon Lamp, can be easily exchanged by the user in minutes without any recalibration.



LightCycler® 480 services

Roche Applied Science is committed to providing innovative systems with highly professional service channels, worldwide. Furthermore, our ISO 9001 certified local and central support organizations can offer you solutions to meet your specific LightCycler® 480 needs.

The LightCycler® 480 service includes:

- Up-to-date web-based LightCycler® 480 System information.
- Comprehensive LightCycler® 480 System online support (*e.g.*, webinars, e-learning tools).
- Custom-made convenient LightCycler® 480 System hotline.
- On-site LightCycler® 480 System technical support.
- Premium LightCycler® 480 Instrument Q³ Qualification Service Package.[1,2)]
- Customized LightCycler® 480 System service plans and contracts.[2)]

1) The LightCycler® 480 Instrument Q³ Qualification Service Package consists of three service modules performed at your laboratory at three different times: IQOQ (Installation Qualification Operational Qualification), OQ (Operational Qualification) and PQ (Performance Qualification). Furthermore, the service provides all the necessary documentation, including a detailed report and instrument certification.

2) For further details on these optional services, please contact your local representative.

LightCycler® 480 Instrument Characteristics

Dimensions	W 57.4 cm × D 58.8 cm × H 49.7 cm
Weight	55.6 kg
Power consumption	200–240 Vac (50/60 Hz, 1500 VA)
Reaction volumes	5 µl–20 µl (384-well), 10 µl–100 µl (96-well)
Temperature control	Peltier-based heating/cooling from 37°C-95°C (20° starting temperature to perform specific melting curve analyses)
Heating rate	96-well block: 4.4°C 384-well block: 4.8°C
Cooling rate	96-well block: 2.2°C 384-well block: 2.5°C
Excitation	LightCycler® 480 Xenon Lamp (430 - 630 nm)
Detector	Cooled monochrome CCD camera
Filters	Excitation (nm): 440, 465, 498, 533, 618 Detection (nm): 488, 510, 580, 610, 640, 660
Computer	Pentium PC with Windows XP
Preinstalled software configuration	• Absolute Quantification • Tm Calling • Absolute Quantification Analysis • Relative Quantification Analysis • Endpoint Genotyping • Melting Curve Genotyping
Accessory software analysis modules	• Gene Scanning Software • Multiple Plate Analysis Software
Automation	- Interface with LightCycler® 480 LIMS Interface Module - Bar-code assisted multiwell plate scanning - Plate loading capability
Data management	21 CFR Part 11 compatibility

Order a LightCycler® 480 System, and additionally receive:

- On-site LightCycler® 480 System installation.
- Customized LightCycler® 480 System start-up training.
- Comprehensive LightCycler® 480 Operator's Manual.
- Supreme LightCycler® 480 System user support.

Ordering Information

LightCycler® 480 Instruments and Additional Products

Product	Cat. No.	Pack Size
LightCycler® 480 Instrument II, 96-well ∗●▼	05 015 278 001	1 Instrument[1)]
LightCycler® 480 Instrument II, 384-well ∗●▼	05 015 243 001	1 Instrument[1)]
LightCycler® 480 Block Kit 96 Silver ●▼	05 015 219 001	1 Kit[2)]
LightCycler® 480 Block Kit 384 Silver ●▼	05 015 197 001	1 Kit[2)]
LightCycler® 480 Bar-Code Scanner	04 710 606 001	1 Scanner
LightCycler® 480 Xenon Lamp	04 686 136 001	1 Lamp
LightCycler® 480 Software, Version 1.5 ∗●	04 994 884 001	1 Software Package
LightCycler® 480 LIMS Interface Module ∗●	05 066 310 001	1 Software Package
LightCycler® 480 Gene Scanning Software ∗●	05 103 908 001	1 Software Package
LightCycler® 480 Multiple Plate Analysis Software ∗●	05 075 122 001	1 Software Package
LightCycler® 480 Multiwell Plate 96, white ●‹	04 729 692 001	50 Plates / 50 Foils
LightCycler® 480 Multiwell Plate 384, white ●‹	04 729 749 001	50 Plates / 50 Foils
LightCycler® 480 Multiwell Plate 96, clear ●‹	05 102 413 001	50 Plates / 50 Foils
LightCycler® 480 Multiwell Plate 384, clear ●‹	05 102 430 001	50 Plates / 50 Foils
LightCycler® 480 Sealing Foil ●‹	04 729 757 001	50 Foils

[1)] Instrument package includes LightCycler® 480 Instrument, LightCycler® 480 thermal block cycler unit (96- or 384-well), LightCycler® 480 software, LightCycler® 480 Instrument Operator's Manual, LightCycler® 480 Xenon Lamp (spare lamp). A Pentium desktop PC is supplied with the instrument.

[2)] Kit package includes LightCycler® 480 thermal block cycler unit (96- or 384-well), block cycler cover, storage box.

Ordering Information

LightCycler® 480 Reagents and RT-PCR Products

Product	Cat. No.	Pack Size
LightCycler® 480 SYBR Green I Master ✶●✚❍✧ (2× concentrated)	04 707 516 001 04 887 352 001	5 × 1 ml (500 × 20 µl reactions) 10 × 5 ml (5000 × 20 µl reactions)
LightCycler® 480 Probes Master ●◆❍ (2× concentrated)	04 707 494 001 04 887 301 001 04 902 343 001	5 × 1 ml (500 × 20 µl reactions) 10 × 5 ml (5000 × 20 µl reactions) 1 × 50 ml (5000 × 20 µl reactions)
LightCycler® 480 Genotyping Master ✶●✖ (5× concentrated)	04 707 524 001	4 × 384 µl (384 × 20 µl reactions)
LightCycler® 480 High Resolution Melting Master ✶✚	04 909 631 001	5 × 1ml (500 × 20 µl reactions)
LightCycler® 480 RNA Master Hydrolysis Probes ◆✣	04 991 885 001	500 x 20µl reactions
LightCycler® 480 CYAN 500 Labeling Reagent ✭	04 764 153 001	1 vial
LightCycler® 480 Control Kit ✶●✣	04 710 924 001	1 Kit (3 control runs)
Transcriptor First Strand cDNA Synthesis Kit[1)]	04 379 012 001 04 896 866 001 04 897 030 001	1 Kit (50 reactions) 1 Kit (100 reactions) 1 Kit (200 reactions)
Universal ProbeLibrary Set, Human ✣❍◗	04 683 633 001	1 Set[2)]
Universal ProbeLibrary Set, Mouse ✣❍◗	04 683 641 001	1 Set[2)]
Universal ProbeLibrary Set, Rat ✣❍◗	04 683 650 001	1 Set[2)]
Universal ProbeLibrary Extension Set ✣❍◗	04 869 877 001	1 Set[2)]
Universal ProbeLibrary, Human PBGD Gene Assay ✣❍◗	05 046 149 001	500 reactions
Universal ProbeLibrary, Human HPRT Gene Assay ✣❍◗	05 046 157 001	500 reactions
Universal ProbeLibrary, Human ACTB Gene Assay ✣❍◗	05 046 165 001	500 reactions
Universal ProbeLibrary, Human PGK1 Gene Assay ✣❍◗	05 046 173 001	500 reactions

[1)] For detailed information, visit **www.roche-applied-science.com/pcr**
[2)] For detailed information, visit **www.universalprobelibrary.com**

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