Roche

LightCycler®

LightCycler® 96 Real-Time PCR System

Super Capabilities Are Now Within Your Reach

Discover the new LightCycler® 96 Real-Time PCR System— A compact and smart device that's the perfect companion for qPCR newbies and experts.

The LightCycler® 96 System embodies Roche's expertise over the last decade in developing and providing high-performance qPCR systems that enable research breakthroughs.

Choose this 96-well qPCR solution to obtain everything top researchers expect from a LightCycler® Instrument: an ideal combination of accuracy, temperature homogeneity, and reproducibility now enhanced by an interface so intuitive that it is accessible to any user in the lab.

In addition to standard analysis methods like endpoint genotying, absolute and relative quantification, dedicated modules for qualitative detection and advanced high resolution melting analysis are also included.

Now the quality and reliability of Roche real-time PCR systems are within every scientist's reach.

For life science research only. Not for use in diagnostic procedures.

Reach New Heights with an All-in-One Amplifier

The quality and features that set your research apart

Have confidence in the data you generate and quickly get publication-ready results.

- Fast precision thermocycling and innovative glass fiber optics for unbiased 96-well data capture.
- Accurate results expected from a LightCycler® System—now including gradient functions.
- Robust multiplex gene expression and HRM assays without the need for passive reference dyes, temperature calibration or color compensation by the user.

Work economically, flexibly adapting your workflow to your assay format and throughput needs.

- Cost-effective value packs of optimized reagents and disposables.
- Choose between multiwell plates and clear or white tube strips provided with caps.
- Avoid unnecessary waiting time during preheating by starting experiments as *stand by.*

Speed time to results with advanced yet easy-to-use software designed for both novice and experienced users.

- Benefit from a large, intuitive touchscreen interface and powerful data analysis.
- Choose your type of connectivity via network or USB stick.
- Create pdf or HTML reports from your data directly on the instrument.
- Conveniently analyze data remotely via email.
- Transfer executed experiments on network shares using the automated backup functionality.

LightCycler® 96 System Hardware

Innovative optics, allowing fast and accurate runs without temperature or color calibration

Achieve the unbiased results your research requires with the innovative optics and thermal block of the LightCycler® 96 System.

Equally excite and simultaneously capture data from 96 wells.

With the LightCycler® 96 System's high-intensity LED and pairs of 96 robust fiber optic cables—half for excitation and half for emission (Figure 1)—you will:

- Eliminate edge effects.
- Avoid variations in signal capture due to lags in acquisition time, common on other systems that use optical scanning.
- Avoid the need for a passive reference dye.
- Perform advanced gene detection, quantification and genotyping experiments with ease

Reduce well-to-well variations through temperature homogeneity.

Maximize data consistency with the LightCycler® 96 System's full silver thermal block cycler, low mass electro-formed silver mount, and heated lid (Figure 2).

- Achieve high temperature homogeneity to reduce well-to-well variation.
- Prevent optical artifacts due to condensation.
- Perform assay optimization across a 20 °C gradient range.

Fluorophore	Excitation filter	Emission filter	Detection format
SYBR Green I	470	514	Intercalating dye
ResoLight			High Resolution Melting
FAM			Hydrolysis probes
VIC HEX Yellow5555	533	572	Hydrolysis probes/ Universal Probe Library probes
Red610 Texas Red	577	620	Hydrolysis probes
Cy5	645	697.5	Hydrolysis probes

Table 1: Overview of LightCycler® 96 excitation and emission filters, dyes and detection formats

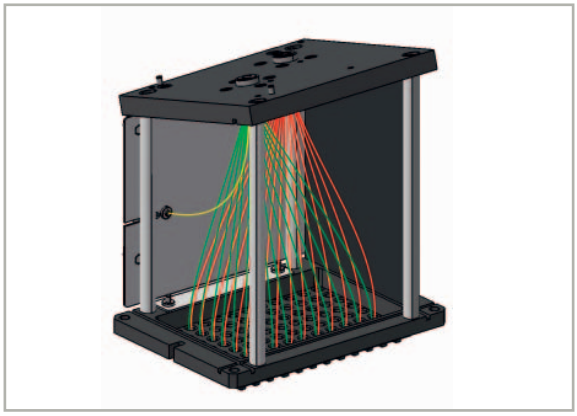


Figure 1: Innovative optics. The LightCycler® 96 patent-pending optics system is comprised of two robust sets of 96 fiber optics, one for providing the excitation light (green) and one for collecting the emitted light (red) to and from each well. The reference channel is shown in yellow.

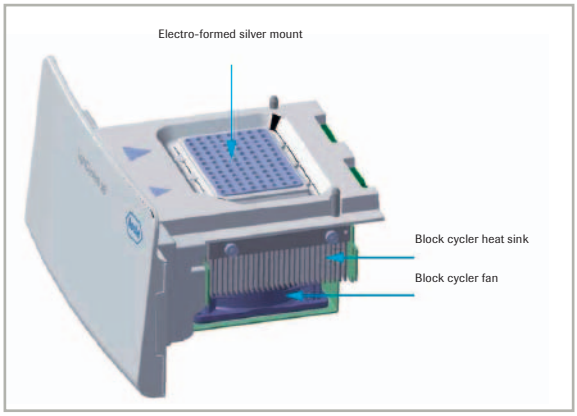


Figure 2: The block cycler unit. The main components of the block cycler unit consist of the silver thermal block cycler, the heated block cycler cover, the block cycler fan, and the electro-formed silver mount.

LightCycler® 96 System Software

As smart and intuitive as you want it to be

Whether you are a qPCR novice or a seasoned expert, the LightCycler® 96 Software can accommodate your needs—without wasting your time learning a new software package. First-time users can easily start generating data for all common applications in gene expression and genetic variation research. Advanced users exploit the system's powerful analytical capabilities and generate publication-ready results.

- Quickly program your run with predefined temperature protocols.
- Simplify routine and advanced tasks through a start-up wizard and shortcuts.
- Easily configure views for added flexibility.
- Meet MIQE requirements* and publish faster by readily generating RDML-formatted data.
- Quickly and automatically save data after run execution on a remote folder, even in dynamic networks.

Novice	*Intermediate*	*Experienced*
Allows guided navigation and easy input.	Generates adaptable predesigned bar-chart diagrams.	Applies auto standard curves and efficiency corrections.
Facilitates analysis with one-view functions.	Offers more flexibility with one-click export options.	Gives access to raw data/ statistics.

Don't slow down your research mission

- Choose your type of connectivity: Control the system and monitor the run progress via the touchscreen, or alternatively, from any connected or network computer.
- Conveniently analyze data remotely: Use any network computer or a USB memory stick to download complete result files or have the instrument send them to you by email, as soon as the run is completed.
- Quickly interpret experiments on standalone instruments via fluorescence heat maps (*e.g.*, to visualize changes in C_q values)—even before the run is finished.

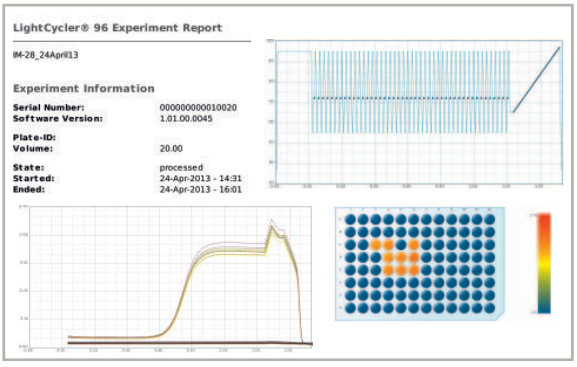


Figure 3: PDF report generated by the instrument software. The report contains experiment and detection format information, temperature profile, raw data amplification curves and heat maps. It can be attached to an email or supplied as a dedicated experiment file.

* The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. Bustin S.A. *et al.* (2009). Clin Chem. 55(4):611-22.

LightCycler® 96 Performance

Generate the quality data that will leave your competitors in awe

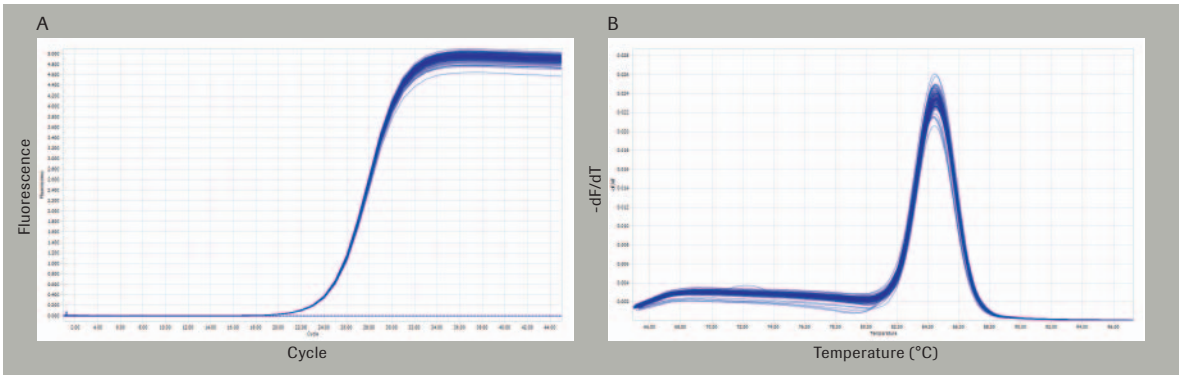


Figure 4: Excellent amplification and homogeneity of reaction products.
Real-time PCR was done using 30 ng of human DNA in each of the 96 block positions. (**A**) A 110 bp amplicon of the beta globin gene was amplified using SYBR Green I detection. (**B**) The reaction product was also subjected to melting curve analysis. In summary, low variation C_q values (C_q range = 0.16 and SD = 0.033) and overlapping melt curves of the amplicon (T_M range of 0.28 °C and SD = 0.063) in each of the 96 positions demonstrated temperature homogeneity and equal treatment of all samples—independent of block position.

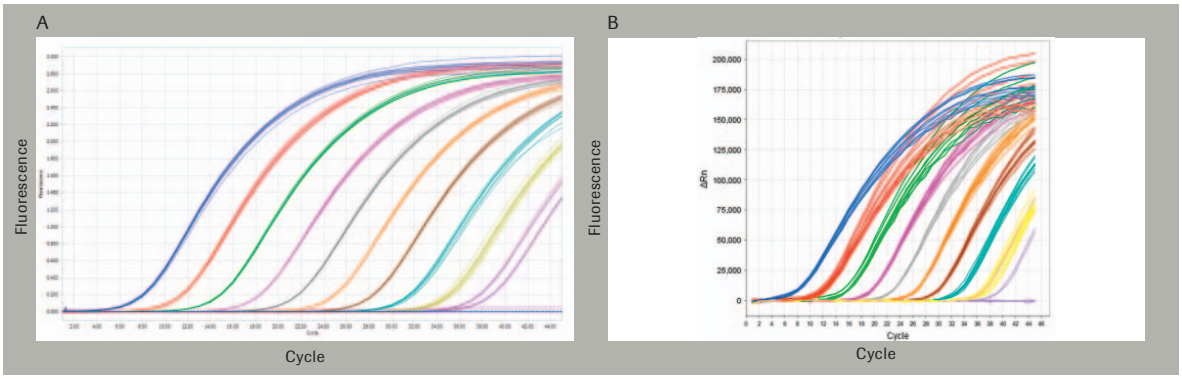


Figure 5: Dynamic range of gene quantification at low dilutions.
A Parvo B19 gene fragment was amplified in ten serial 1:10 dilutions ranging from 10^9 to 10^0 copies per well, and detected with Universal ProbeLibrary (UPL) Probe #137. Ten replicates were run for each dilution (only 9 for the 4 highest concentrations). (**A**) Results on the LighCycler® 96 System show excellent reproducibility and resolution down to very low copy numbers. (**B**) On a competitor instrument, C_q standard deviation values for a given concentration were much higher and C_q differences between dilution steps varied more across the whole dilution range.

Qualitative Detection

Easily add a new quality check to your gene detection

The LightCycler® 96 Software's Qualitative Detection Module allows for reliable analysis of target genes and controls, using two or even more colors. By using an internal control (IC) with the target in the same or a different well, inhibition of the PCR reaction (*e.g.*, impurities originating from sample preparation) can be monitored.

The internal control can be either an endogenous target gene (ß-globin in humans) or an exogenous target gene (viral nucleic acid or synthetic target).

For easy analysis, the LightCycler® 96 qualitative detection module includes a control concept: based on the individual calls for target gene(s) and internal control, a combined call is automatically generated and the overall result visualized via an intuitive heatmap (see Figure 6):

Figure 6: The Qualitative Detection Module provides a Combined Call by combining the individual calls of a target gene and an internal control (IC).

Three basic result types are reported:

- Positive combined call: target call positive, IC call positive or negative (*e.g.*, wells A2, A3, B2; positions labeled 1,2,4)
- Negative combined call: Target call negative, IC call positive (*e.g.*, well F2; positions labeled 6)
- Invalid combined call: Target call negative, IC call negative (*e.g.*, well G2; positions labeled 7)

High Resolution Melting Analysis

Detect known or unknown mutations with ease

High Resolution Melting (HRM) is a homogeneous, closed-tube, post-PCR technique enabling rapidly and efficient discovery of genetic variations (*e.g.*, SNPs, insertions, deletions, methylated regions).

In LightCycler® HRM experiments, sample DNA is first amplified in the presence of ResoLight, a special 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 is analyzed in four steps (see Fig. 7, left part). Signal differences between each curve and a chosen reference are plotted, allowing the automatic clustering of samples into distinct groups that have similar melting curve shapes (*e.g.*, heterozygotes versus homozygotes).

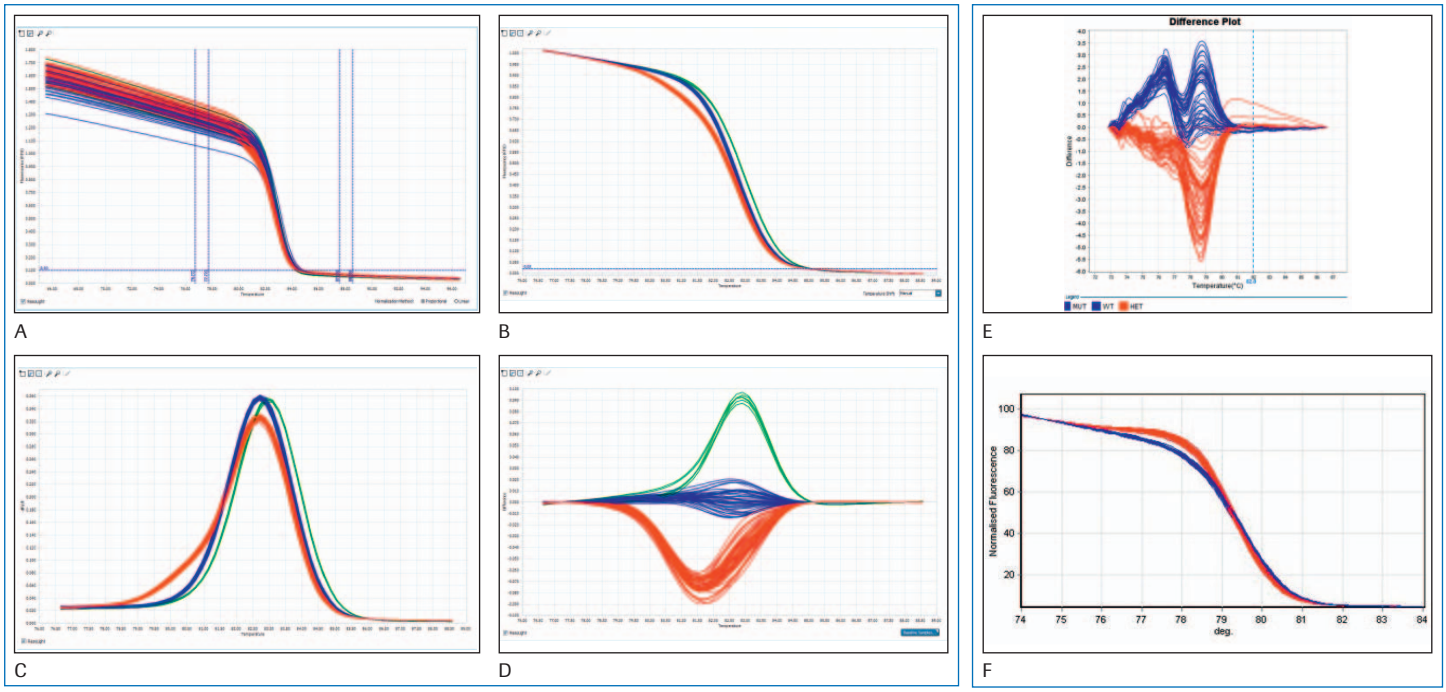
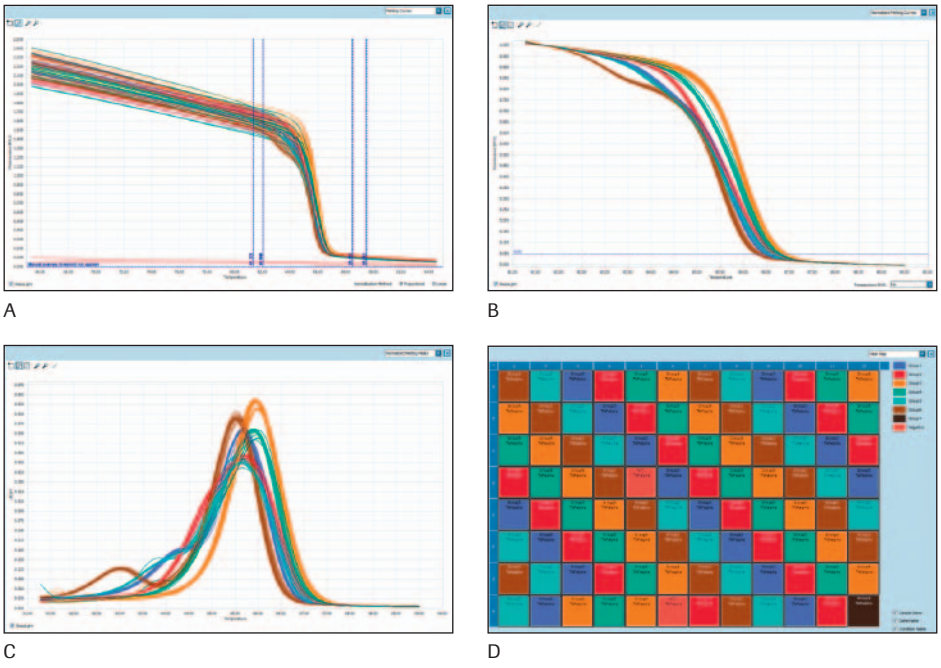


Figure 7: High Resolution Melting on the LightCycler® 96 System.
Left part: A 72 bp fragment of the HFE gene including a class III SNP was amplified from 10 ng of human genomic DNA using the LightCycler® 480 High Resolution Melting Master containing ResoLight dye. Analysis of generated HRM data was done in four steps: **A**: original melting curves, **B**: normalized melting curves, **C**: normalized melting peaks, **D**: difference plot.
Right part: Two different competitor instruments tested with the same assay failed in detecting all present groups of sequence variation (green group seen in D is missing in E and F).

Notably, with the LightCycler® 96 Software's HRM module,

- analyses are always generated in a *gene-specific* manner (*e.g.*, when multiple genes are contained in different wells of the same run).
- an automated algorithm calculates groups based on automated normalization slider and sensitivity settings.
- the *"Normalized Melting Peaks"* chart enables improved discrimination of complex groupings compared, for example, to the difference plot. (see example with 6 genotypes of TNF alpha in Figure 8, C)
- automatic and manual annotation are supported
- heat maps are provided as an additional option for result visualization (see Figure 8, D)



A B C D

Figure 8: HRM analysis of a 111bp fragment of the human TNF alpha gene present in six different sequence variants. Human genomic DNA isolated from different donor samples was used as template in technical replicates. PCR was done using the LightCyclerR 480 High Resolution Melting Master, including ResoLight dye for detection and subsequent high resolution melting analysis. **A**: original melting curves, **B**: normalized melting curves, **C**: normalized melting peaks, **D**: color-coded overview of observed variants.

Technical Specifications

General

Weight	approx. 27 kg
Dimensions	W x D x H: 40 x 40 x 53 cm
Noise level during run	43 dB(A)
Electrical approvals	CE, ICE, UL
Reaction volumes	10–50 µl
Sample format	96-well plates, 8-tube strips
Runtime	< 40 min for 3-step 40 cycles PCR < 1,5 h for HRM

Hardware

Thermal cycling system	Peltier-based, 96-well block calibration-free for all applications including HRM
Max ramp rate heating	4.4 °C
Average ramp rate cooling	2.2 °C
Programmable temperature range	37–98 °C
Temperature accuracy	±0.2 °C of target temperature
T_M uniformity	Range (max–min) 0.4 °C, SD<0.1 °C
Gradient operational range	37–98 °C
Gradient programmable span	Max. 20 °C
Excitation	High-power broad spectrum LED
Measurement (integration time)	Simultaneous data acquisition for all positions in 10–1000 ms
Detection	CCD camera
Optical system	Fixed fiber optics with four excitation and four emission filters No moving scanning elements
C_q uniformity	Range (max–min) 0.8, SD < 0.2 (enabling resolution of 2-fold concentration differences)
Maintenance and Support	No routine maintenance required IQOQ available on request

Analysis Software

Operating systems	Win XP, Win 7 and Win 8
Data analysis	Absolute and Relative Quantification T_M Calling Endpoint Genotyping High Resolution Melting (HRM) Qualitative Detection
Data import and export	from and to .txt or .csv files
Failure flagging	Automated flagging for critical controls (*e.g.*, positive control is negative)

Run Mode

Stand alone	Large 10" touchscreen, adjustable for optimal pressure points Flexible experiment programming and execution Online fluorescence display Generation of instrument pdf report including heat maps Storage of up to 50 runs on the instrument Auto backup function to network server
PC connected	Programming, monitoring and analysis Visualization of C_q values as bar chart with standard deviations
LAN connected	Support of online monitoring using LAN connection Support of remote Roche service
External devices	Support of external barcode scanner using USB connection
Instrument active communication	Email notification, with success or failure messaging and optional experiment file attachment

Applications

Dynamic range	10 orders of magnitude
Range of excitation/ emission wave-lengths (nm)	470/514 (SYBR, FAM, ResoLight dye) 533/572 (VIC, Hex, Yellow555) 577/620 (Red610, Texas Red), 645/697 (Cy5)
Detection formats	Intercalating dyes; UPL probes
Multiplex analysis	Up to 4 channels; pre-calibrated color compensation (no user interaction necessary)
Passive reference dyes	Not necessary

Order Information

Product	*Cat. No.*	*Pack Size*
LightCycler® 96 Instrument	05 815 916 001	1 instrument
LightCycler® 8-Tube Strips (white)	06 612 601 001	120 strips (white) and caps (clear); 10 unit packs with 12 tube and cap strips each
LightCycler® 96 DNA Green Value Pack S	06 713 092 001	5 packs FastStart Essential DNA Green Master (25 ml); 1 pack LightCycler® 480 Multiwell Plates 96, white (50 plates)
LightCycler® 96 DNA Green Value Pack L	06 713 106 001	20 packs FastStart Essential DNA Green Master (100 ml); 2 packs LightCycler® 480 Multiwell Plates 96, white (100 plates)
LightCycler® 96 DNA Probes Value Pack S	06 713 076 001	5 packs of FastStart Essential DNA Probes Master (25 ml); 1 pack LightCycler® 480 Multiwell Plates 96, white (50 plates)
LightCycler® 96 DNA Probes Value Pack L	06 713 122 001	20 packs of FastStart Essential DNA Probes Master (100 ml); 2 packs LightCycler® 480 Multiwell Plates 96, white (100 plates)
LightCycler® 480 High Resolution Melting Master	04 909 631 001	5 x 1 ml for up to 500 reactions, 20 µl each

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