Roche Applied Science

The LightCycler System

A Proven Standard for Real-Time PCR

Strengthen your chances for success in real-time PCR by adopting the LightCycler System from Roche Applied Science, a recognized leader in genomics and proteomics the world over.

The LightCycler System includes instrumentation, software, reagents, technical support, and application-specific kits to get you up and going quickly, and to maximize your efforts as you expand the boundaries of your research.

The LightCycler System –
A proven standard of excellence

Ever since its introduction in 1998, the Roche Applied Science LightCycler System has stood for outstanding performance and maximum flexibility. Thousands of successful researchers have capitalized on the rapid, accurate, and sensitive nature of the platform to develop thousands of applications in a variety of scientific fields.

The LightCycler System has repeatedly set the standard for real-time PCR. It was the first system to introduce hybridization probes, true melting curve analysis, crossing point determination with 2nd derivative maximum analysis, relative quantification analysis, real-time reaction monitoring, ultra-sensitive glass reaction vessels, assay and analysis in under 45 minutes, 6 channel detection, a multiplex-specific PCR master mix, and more.

We are continuing to break new ground by offering a choice in instruments designed to meet your research needs. Choose the LightCycler 1.5 Instrument for your single dye or duplex probe-based assays, or step up to the LightCycler 2.0 Instrument if you really want to experience the power of multiplexing.

RAPID
Complete your run and analysis in less than half the time required to complete the PCR reaction alone, using a block cycler.

ACCURATE
Rely on ultra-precise hardware components and optimal software algorithms to achieve the most reproducible and accurate results.

SENSITIVE
Depend on a rapid cycling time that increases the signal to noise ratio and guards against the build up of unwanted PCR products.

PROVEN
Trust your colleagues and the thousands of applications that signal success.

The LightCycler 2.0 Instrument

High performance that meets the needs of even the most demanding user

Continuing the success story of proven performance, the new LightCycler 2.0 Instrument offers a multitude of innovative and exciting features, ranging from completely new and enhanced software to additional detection channels and hardware optimizations that result in even higher flexibility. This combination guarantees unmatched performance and reliability for a wide range of applications.

- Six fluorescence detection channels measure fluorescence at 530, 560, 610, 640, 670, and 705 nm.
- Unlimited choice of detection formats.
- Advanced LightCycler Software 4.0, that combines enhanced data analysis with efficient data management.
- Two sizes of reaction capillaries for 20 µl and 100 µl reactions provide flexibility for assay design.
- Optimized hardware components for rapid, ultra-precise temperature regulation for maximum reproducibility.

Large Variety of Detection Formats

- SYBR Green I
- Multicolor HybProbe
- SimpleProbe
- Hydrolysis Probes
- Other formats based on fluorescence resonance energy transfer (FRET)

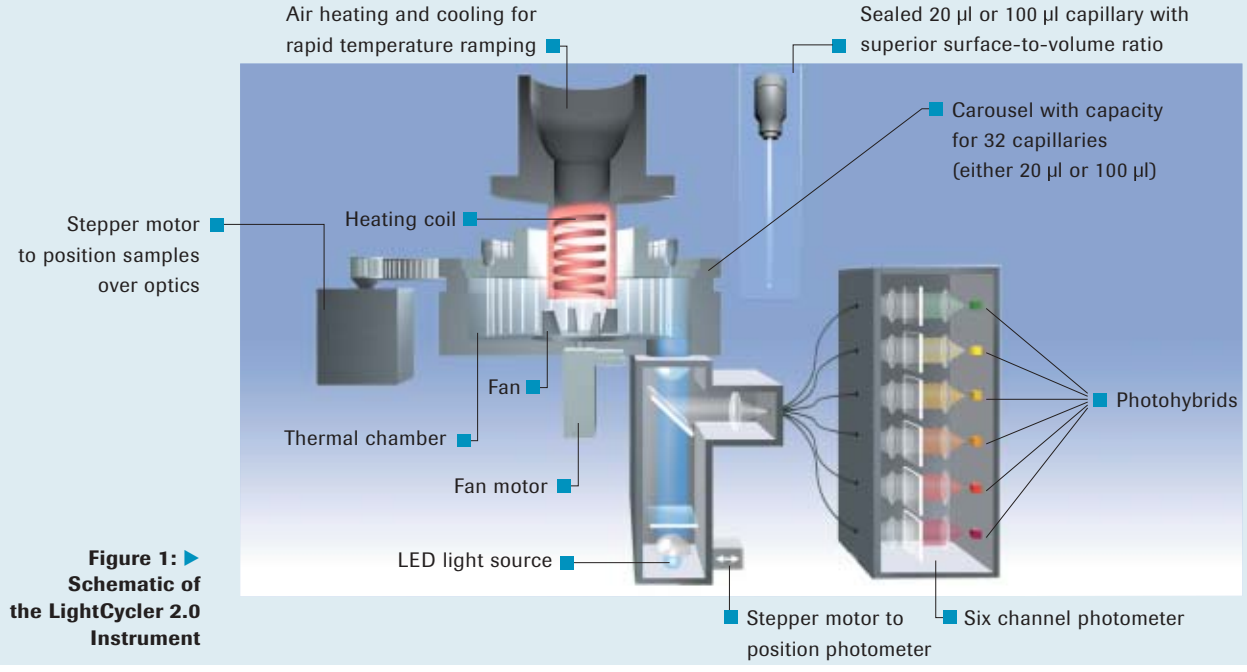


Figure 1: ▶ Schematic of the LightCycler 2.0 Instrument

The LightCycler 1.5 Instrument*

Proven performance that anyone can afford

Based on a six-year history of proven performance, the LightCycler 1.5 Instrument offers a perfect solution for flexible real-time PCR quantification, mutation analysis, and SNP genotyping.

- Three fluorescence detection channels measure fluorescence at 530, 640, and 705 nm for mono- and dual-color assays.
- 20 µl glass capillaries for high-speed cycling.
- Reliable and proven analysis software for accurate quantification and genotyping by melting curve analysis.
- Well established SYBR Green I and HybProbe detection formats.
- SimpleProbe detection formats for SNP analysis.
- Hundreds of peer-reviewed publications.

*** Please ask your local representative for availability.**

Detection Formats

- SYBR Green I
- Dual-color HybProbe
- SimpleProbe
- Hydrolysis Probes

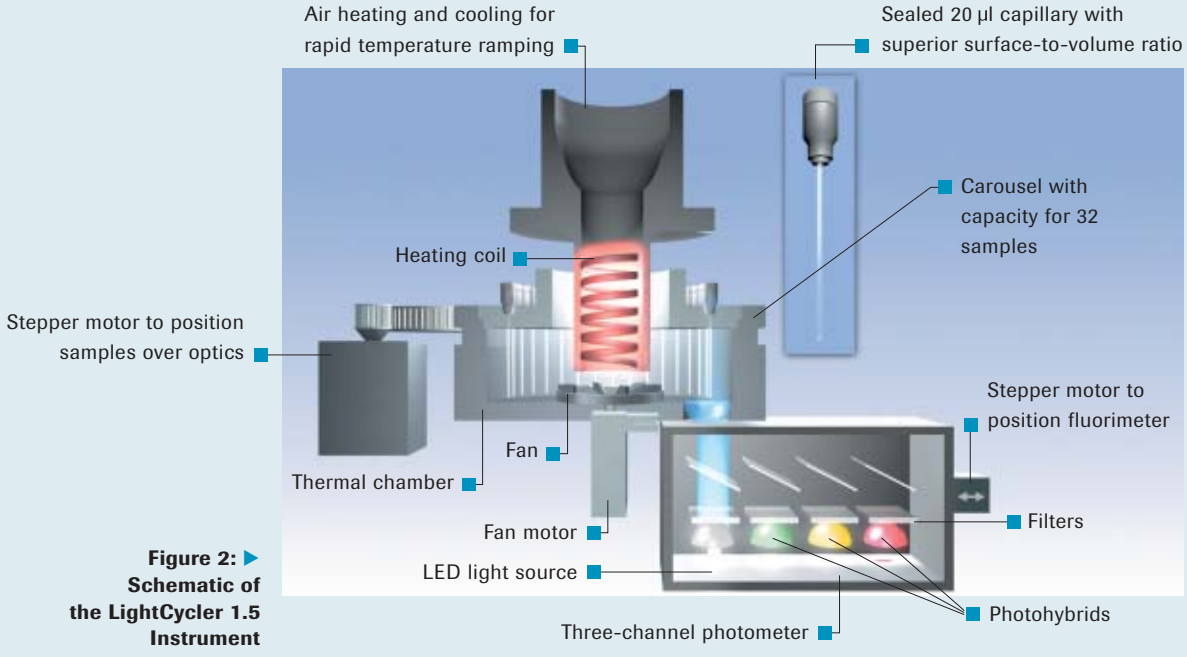


Figure 2: ▶ Schematic of the LightCycler 1.5 Instrument

Performance

Superior dynamic range, speed, sensitivity, and reproducibility

The performance of the LightCycler Instruments maximizes the capabilities of real-time PCR:

- **Broad dynamic range** – measures from 10 to 10^{10} copies in a single run.
- **Superior sensitivity** – detects a single-copy gene in one genome equivalent of DNA.
- **High reproducibility** – CV for crossing points is 0.3% and lower.

Dynamic Range

The LightCycler Instruments detect from 10 to 10^{10} copies in a single run. In Figure 3A, the logarithm of the fluorescence detected during the amplification of a recombinant plasmid containing the 438 bp amplicon of *Chlamydia pneumoniae* 16S rDNA, is plotted against cycle number. In this experiment, all of the samples containing from 10 to 10^{10} copies produce clear signals. Figure 3B is the plot of the resulting crossing points (cycle numbers) versus logarithm of concentration, which shows a linear relationship over the whole concentration range (Data provided by Dr. Udo Reischl, Institute of Medical Microbiology and Hygiene, University Hospital of Regensburg, Germany).

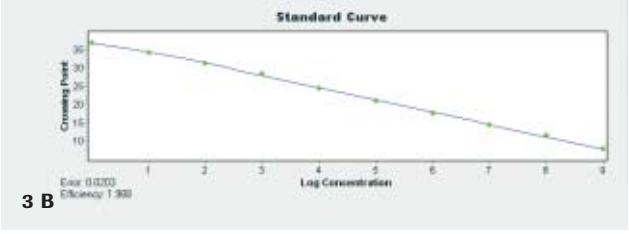
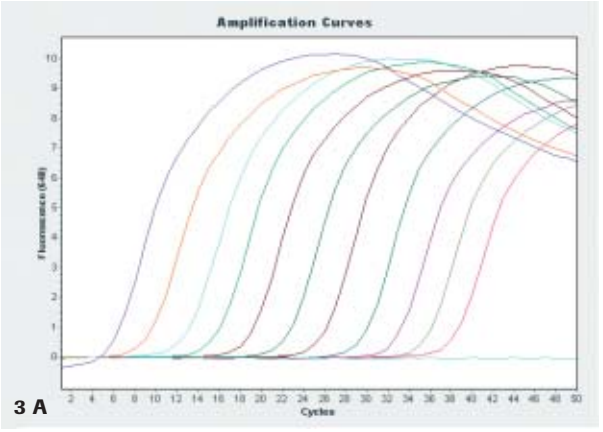


Figure 3 A+B: ▲ Illustration of the dynamic range

Speed

The design of the glass capillaries facilitates high-speed thermal cycling. Their superior surface-to-volume ratio guarantees extremely rapid thermal transfer with an increase in signal-to-noise for the PCR products.

Together, the glass capillaries and the air-driven temperature control of the LightCycler Instruments ensure rapid PCR. An entire 35-cycle run can be performed in as little as 30 minutes (with 20 µl capillaries) or 60 minutes (with 100 µl capillaries).



- 10^{10} copies/reaction
- 10^{9} copies/reaction
- 10^{8} copies/reaction
- 10^{7} copies/reaction
- 10^{6} copies/reaction
- 10^{5} copies/reaction
- 10^{4} copies/reaction
- 10^{3} copies/reaction
- 10^{2} copies/reaction
- 10^{1} copies/reaction
- 1 copy/reaction
- No template control

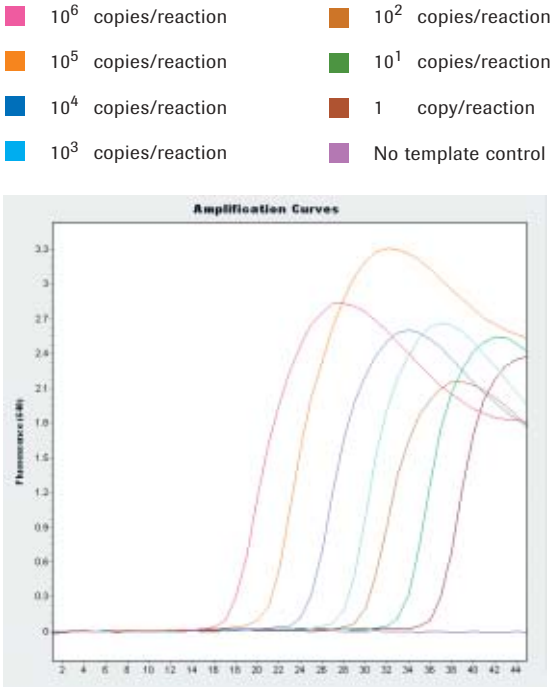
Sensitivity

The sensitivity of a LightCycler Instrument experiment depends on several factors that are common to conventional PCR (*i.e.*, the quality of template, primer design, and optimization of PCR conditions).

Experiments have shown that, under optimal conditions, a single-copy gene can be detected in 3 pg of human genomic DNA (approximately one human genome equivalent). At this concentration, statistics significantly affect sensitivity and reproducibility. According to a Poisson distribution, the probability that a target is actually present in a given sample is approximately 67%.

Other types of DNA, such as plasmid DNA, are detected when 1–10 copies are present in a sample.

Figure 4 shows the profile of a DNA amplification. From this result, it is clear that one copy can be detected in a single reaction.



▲ **Figure 4: Profile of a DNA amplification with the LightCycler 2.0 Instrument.**

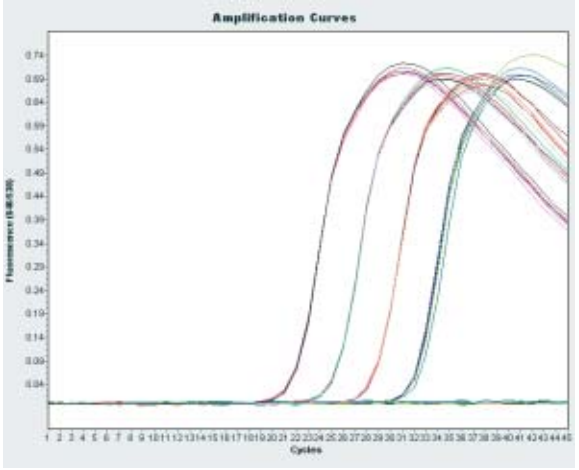
Reproducibility

The ability of a real-time PCR instrument to differentiate starting concentrations depends primarily on the quality of individual instrument components.

In the LightCycler Instruments, starting concentrations can be determined with very high resolution. Variability from sample to sample is extremely low. This outstanding reproducibility is primarily due to the following instrument components:

- continuously rotating sample carousel in a single thermal chamber, which ensures identical PCR conditions for every sample position.
- a single optical unit to ensure identical measurements.

Reproducibility of the LightCycler 2.0 Instrument is illustrated in Figure 5. The data shows a six-fold determination of plasmid DNA dilutions containing 10^5 to 10^2 copies. The crossing point of 1000 copies showed a coefficient of variation (CV) of 0.15%, and for 10,000 copies, a CV of 0.09% was determined.



▲ **Figure 5: Six-fold determination of DNA plasmid dilutions containing 10^5 to 10^2 copies.**

LightCycler Software 4.0

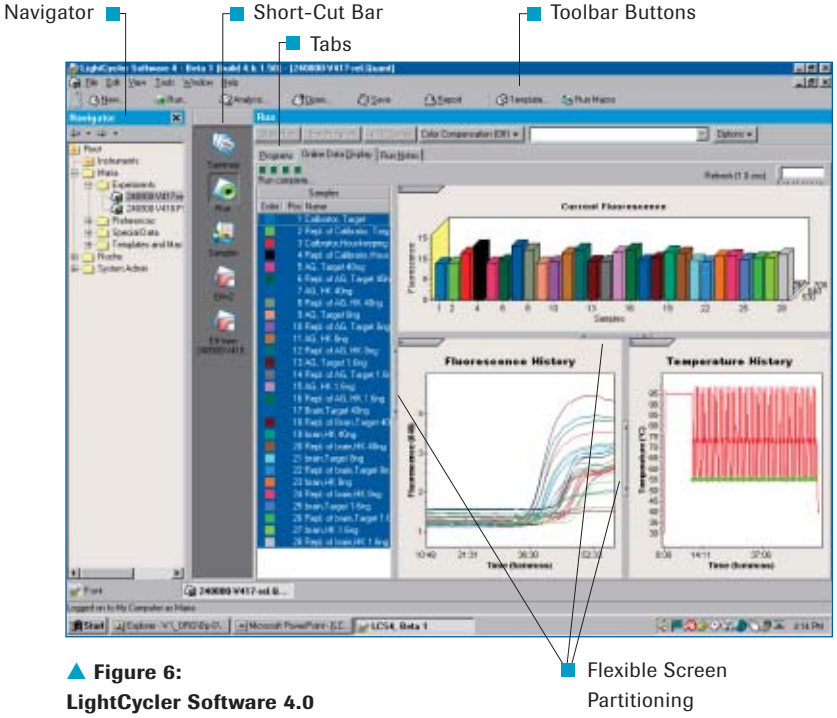
Advanced data management, analysis, and protection

LightCycler Software 4.0 meets the demanding standards of researchers by providing advanced data analysis modules, efficient data management, and effective data protection. This sophisticated software lets you choose to automate your analysis for routine use, or customize your assay in many ways. The redesigned user interface is easy to navigate and features:

- Improved workflow
- Enhanced analysis module options
- Integrated data and user management

Improved Workflow

The modular structure of the LightCycler Software 4.0 makes LightCycler experiments and data analysis extremely easy to perform. You can now create experimental protocols more quickly by using templates and macros. These allow you to store individual items, such as a protocol or sample list from an old experiment, and then apply them to a new experiment.



▲ **Figure 6: LightCycler Software 4.0 user interface**

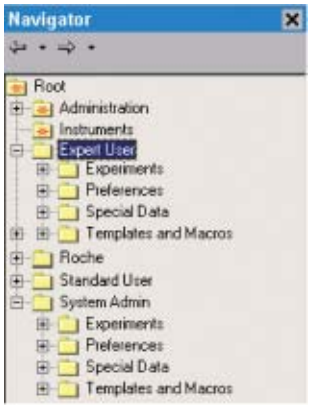
Integrated Data and User-Management

Data generated with the LightCycler Software 4.0 are safely stored in a database, thus providing enhanced data security.

In addition, the software offers a flexible user-management system that enables you to create an account for each user that contains the log-in name, password, and level of access to the software. The software allows three levels of user access, as defined by three assignable user roles:

- Standard User
- Expert User
- Local Administrator

Each user level is allowed to perform different software operations, such as creating and modifying experimental protocols, using macros, and viewing folders and objects.



Enhancement of Analysis Module Options

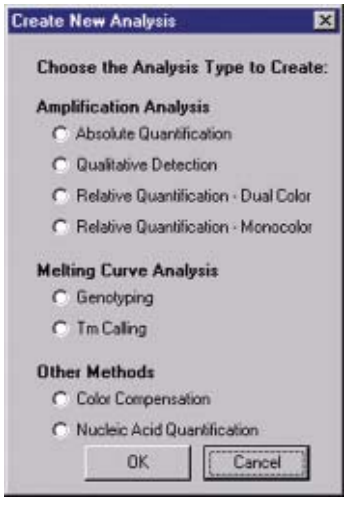
Optimize your experiment with improved algorithms that ensure precise data evaluation. View all your results at a glance by taking advantage of the software's "Call" option that lets you compare samples with a variety of controls. Streamline your analysis by using one of the enhanced analysis modules:

Qualitative Detection
Determine whether a target sequence is present in unknown samples.

Absolute and Relative Quantification
Calculate the concentration of your target DNA or RNA in unknown samples based on the concentration of standard samples (as described in "Perform Absolute Quantification") or a comparison of the ratio of two nucleic acid sequences (as described in "Perform Relative Quantification").

Melting Curve Analysis
Analyze sample melting temperatures and profiles to obtain sequence information from the amplified PCR product.

Nucleic Acid Quantification
Determine the concentration of nucleic acids without doing an amplification reaction.



Data Analysis and Interpretation

Perform Qualitative Detection

The Qualitative Detection module detects the presence of a particular target in unknown samples, based on whether the target shows amplification or not. However, the module does not quantify the amount of target DNA present. LightCycler Software 4.0 allows you to perform a simple Qualitative Detection experiment without controls, or a more complex Qualitative Detection experiment with positive, negative, or internal controls. The results of a Qualitative Detection analysis are clearly displayed, as shown in Figure 7. The "Combined" value is based on the "Target" value and the "Control" value. The displayed "Target" is based on the observed amplification profile (amplification or no amplification) of each sample. The "Control" result displays the "Success" or "Failure" for all controls that are relevant to the particular sample. If any of those controls fail, the "Control" column will read "Failure" for the affected sample.

Samples				Calls		
Include	Color	Pos	Name	Combined	Target	Control
☑		1	no template control	Success	Negative	Success
☑		2	Negative Control	Success	Negative	Success
☑		3	Positive Control	Success	Positive	Success
☑		4	Sample 1	Positive	Positive	Success
☑		5	Sample 2	Positive	Positive	Success
☑		6	Sample 3	Negative	Negative	Success
☑		7	Sample 4	Positive	Positive	Success
☑		8	Sample 5	Positive	Positive	Success

▲ **Figure 7: Data display of a Qualitative Detection analysis.**

Perform Quantification

To analyze quantification data, LightCycler Instruments measure the fluorescent signals during the exponential increase (log-linear) phase of PCR. The LightCycler Instruments use the crossing point (CP) of the sample (*i.e.*, the cycle at which the fluorescence of a sample rises above background fluorescence), to determine the presence, concentration, or relative concentration of target DNA in unknown samples.

Absolute Quantification

The Absolute Quantification module uses external standards to calculate an absolute concentration value for an unknown sample. The LightCycler Software 4.0 allows you to run a standard curve within your experiment or to import a previously determined standard curve. Furthermore, you may spike each sample with an internal control. Absolute Quantification without internal controls can be performed as a mono-color analysis. However, if you use internal controls, you will need two pairs of HybProbe probes (*e.g.*, one labeled with LightCycler Red 640 and one labeled with LightCycler Red 705) to simultaneously detect and differentiate the target and the control.

Figure 8 shows an Absolute Quantification analysis with internal controls. In addition to the "Results" section, a "Calls" section is included. The Calls section provides a quick overview of whether the samples and the controls performed as expected.

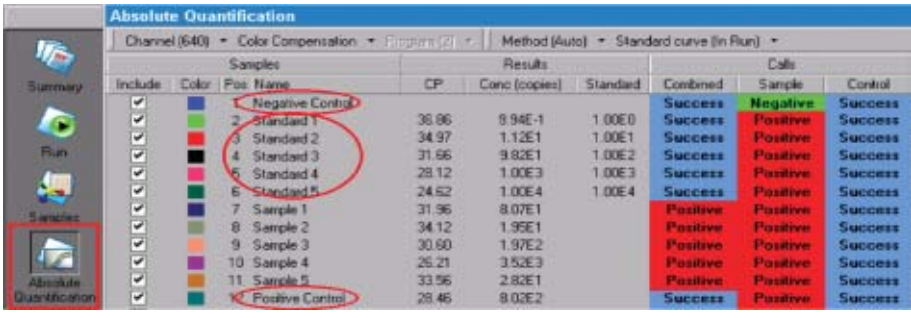


Figure 8: ▶ Data display of an Absolute Quantification analysis with internal control.

Relative Quantification

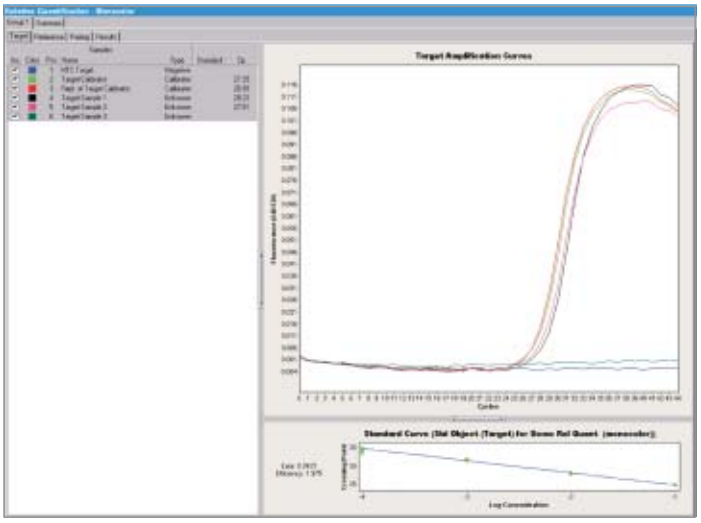
Relative Quantification analysis compares two ratios: the ratio of the target DNA sequence to a reference DNA sequence (*e.g.*, a housekeeping gene) in an unknown sample, is compared with the ratio of the same two sequences in a standard sample (*e.g.*, a cell line) called a calibrator. The calibrator contains typical proportions of the target and the reference sequences. The result of a Relative Quantification analysis is therefore expressed as a normalized ratio. You can perform Relative Quantification analysis as a single channel experiment "Relative Quantification-Monocolor" or a multichannel experiment "Relative Quantification-Dual Color". If you perform a dual-color experiment, each pair of target and reference samples must be in the same capillary.

$$\text{Normalized Ratio} = \frac{\text{concentration target (sample)}}{\text{concentration reference (sample)}} : \frac{\text{concentration target (calibrator)}}{\text{concentration reference (calibrator)}}$$

Efficiency Correction

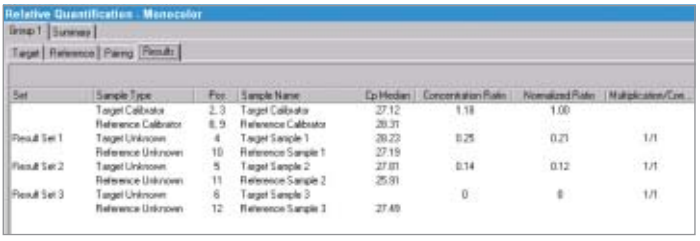
When you perform a Relative Quantification analysis, you must specify a PCR efficiency value for the targets and the references. This may be done by including standards in your experiment to generate a standard curve, or by importing a previously generated standard curve, or merely setting the efficiency value at 2 (E=2) for both target and reference samples. Accuracy of results of a Relative Quantification depends on PCR efficiency of target and reference (being identical, or on a correction of differences). Whereas other approaches to Relative Quantification rely on PCR efficiencies being always at E=2, Roche's approach acknowledges and corrects differences in PCR efficiency.

Figure 9 shows a calibrator-normalized Relative Quantification analysis. The efficiency correction was determined by using predefined standard curve objects for target and reference. Results of the analysis are summarized in Figure 10.



▲ **Figure 9: Target amplification curve and target standard curve in a Relative Quantification analysis with efficiency correction.**

▼ **Figure 10: Results tab of the Relative Quantification analysis.**



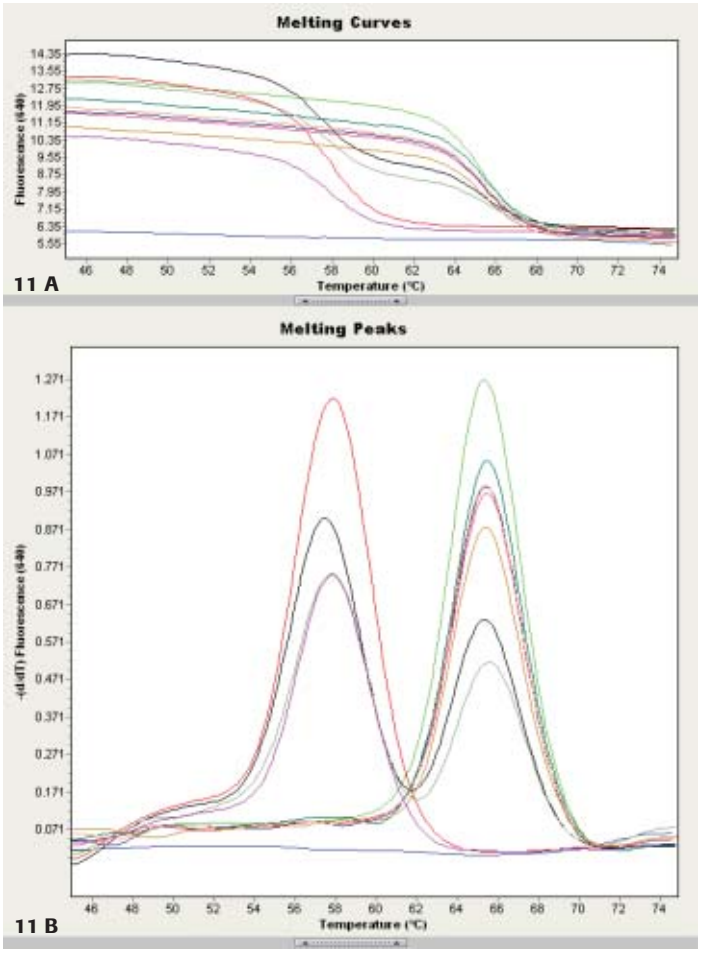
Perform Melting Curve Analysis

Use Melting Curve Profiles to Identify DNA Products and to Genotype Samples

The temperature at which DNA strands separate or melt when heated can vary largely, depending on the sequence, length, and GC content. Even single-base differences in heterozygous DNA can change its melting temperature. Thus, melting temperature profiles can be used to identify and genotype DNA products.

To determine melting temperature profiles, the temperature is steadily increased while the fluorescence of the sample is monitored. Sample fluorescence decreases as the temperature increases. For the SYBR Green I dye, this is because the double DNA strands separate and release SYBR Green I molecules. For HybProbe probes, it happens because target-probe hybrids melt out, resulting in the spatial separation of the dye molecules and a drop in fluorescence. For SimpleProbe probes, fluorescence decreases during heating because the signal from the probe is quenched when the probe is displaced.

Figure 11 shows a melting curve chart and a melting peak chart from a HybProbe probes experiment. The melting curve chart (Figure 11A) displays sample fluorescence versus temperature. The chart shows the downward curve in fluorescence for the samples as they melt. The melting peak chart (Figure 11B) plots the first negative derivative of the sample fluorescence versus temperature, and displays the melting temperature of each sample as a peak. This second chart makes it easier to distinguish each sample's characteristic melting peak and to reveal differences between samples.



▲ **Figure 11:**
Melting chart of a HybProbe probes experiment.
In the example shown, some sample curves have two peaks. These dual peaks indicate heterozygous samples. After DNA amplification, a heterozygous sample contains two DNA sequences, each of which melts at a different temperature, resulting in a two-peak curve.

LightCycler Software 4.0 Offers Two Types of Melting Curve Analysis

Genotyping analysis automatically groups samples with similar melting profiles together and identifies each group as a genotype. Use Genotyping analysis for genotyping and mutation detection.

Tm Calling analysis automatically calculates the melting temperature, the melting peak, height, width, and the area under each peak for each sample. Use Tm Calling analysis to identify characteristic melting profiles in DNA products.

Perform a Genotyping Analysis

Use genotyping analysis in every experiment that contains a melt program. The Genotyping analysis module determines the genotypes of unknown samples by analyzing the shapes of the melting curves of all samples and then grouping curves with similar shapes together. The median curve from each group is designated the "genotype standard" for that group. Moreover, the software allows you to include your own standards with known genotypes in the experiment or import previously determined standards. LightCycler Software 4.0 generates two numerical values that are used to describe how well individual sample curves fit with similar curves (Figure 12):

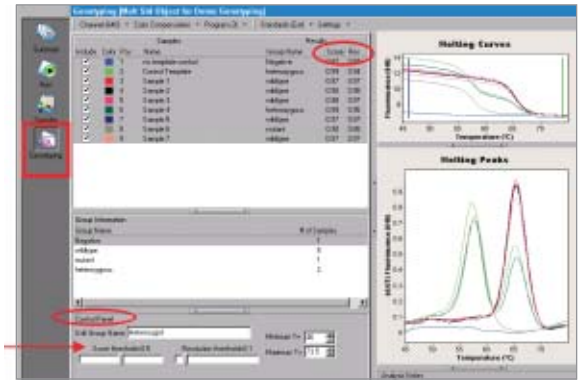
The score value measures the similarity between the sample and the standard that is closest to the sample. A score of 1 indicates a sample with a melting curve identical to the melting curve of a standard, while a score of nearly 0 indicates a sample with a melting curve unlike the shape of the melting curve of any of the standards.

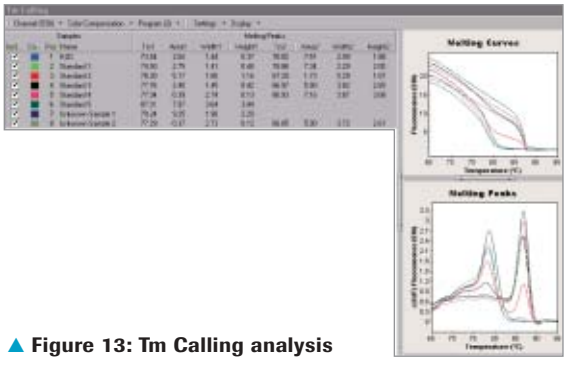
The resolution value measures the dissimilarity between the melting curve shape of the sample and the shape of the second closest standard to the sample. If the resolution of a sample is nearly equal to the score of that sample, the melting curve of that sample is similar to the melting curve of only one standard. Alternatively, if the resolution of a sample is close to 0, the melting curve of the sample is similar to the shapes of the melting curves of two standards.

Perform Tm Calling Analysis

Use Tm Calling analysis in any experiment that includes a melt program. The melting temperature, or Tm, is defined as the point at which half the probes (or dye) have melted off the DNA. Figure 13 shows the results from a Tm Calling analysis, which includes the melting temperature of each sample, the height and width of its melting peak, and the size of the area under the melting peak.

▼ **Figure 12: Genotyping analysis**





▲ **Figure 13: Tm Calling analysis**

LightCycler Probe Design Software 2.0

Automatically finds the optimal primers and probes for all real-time PCR applications, including multiplexing

The LightCycler Probe Design Software 2.0 is Optimized to Design:

- Primer/Probe sets for quantitative PCR.
- Primer/Probe sets for detection of mutations using Melting Curve analysis with the LightCycler Instruments.
- Primers only for PCR using SYBR Green I detection.
- SimpleProbe probes for genotyping.
- Up to 4 different HybProbe probe pairs for multiplex quantitative PCR and mutation detection with the LightCycler 2.0 Instrument.

Major Benefits of the LightCycler Probe Design Software 2.0

- Easily import sequence data and select design parameters for specific applications.
- Take advantage of automated analysis and scoring of sequences according to design parameters.
- Experience a ranked display of Primer/Probe research results.
- Use advanced tools, such as "Cross-Complementarity", or directly submit probe sequences to the NBCI Blast Web site.
- Benefit from the detailed software manual that specifies each step and provides valuable hints and guidelines for optimized primer-probe design, and how they work in specific applications.

The "Sequence Tab" Facilitates Import of Sequence Data and Selection of Design Parameters for Specific Applications

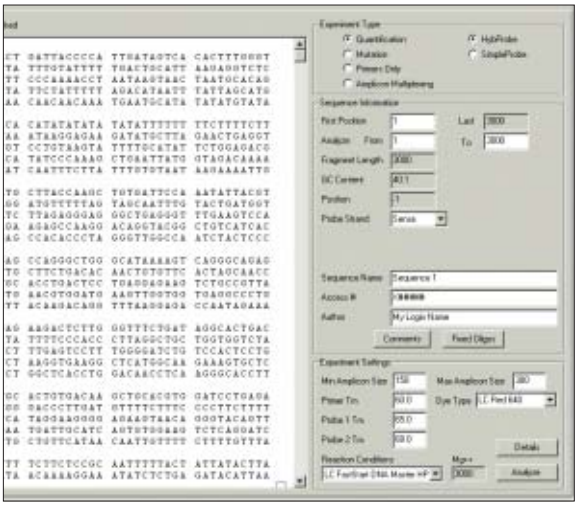
The areas on the right side of the "Sequence tab" (Figure 14) let you specify design parameters according to:

Experiment Type
Specifies the type of primer or probe you want to design.

Sequence Information
Defines the portion of the sequence you want to analyze. The options available depend on the type of primer or probe you specified in the "Experiment Type" section.

Experiment Settings
Specifies design constraints, such as the desired amplicon size range or melting temperature of a primer or probe. Experiment settings can also be specified in a "Settings" dialog box, displayed when you click the "Details" button.

▼ **Figure 14: LightCycler Probe Design Software Sequence tab.**

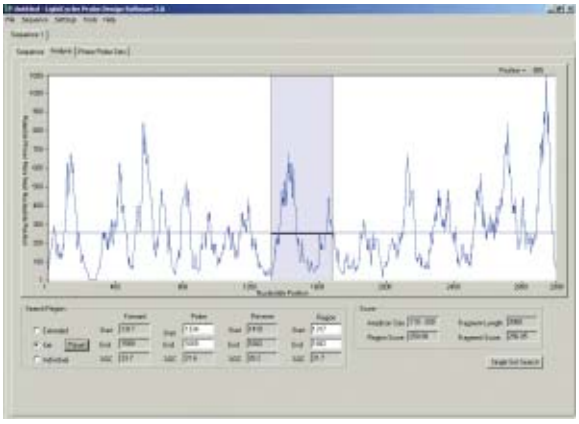


Automated Analysis and Scoring of Sequences According to Design Parameters on the "Analysis Tab"

The software scores each base in the sequence fragment according to various criteria and displays the results on the "Analysis tab" (Figure 15). Use this tab to further limit the search area for primers and probes.

On the Analysis tab, the scores are displayed as a graph with score values on the Y axis and nucleotide positions on the X axis. Higher scores indicate more promising sites, lower scores indicate less desirable sites.

The shaded area is the search area. This area represents a possible location for the primer/probe sites.



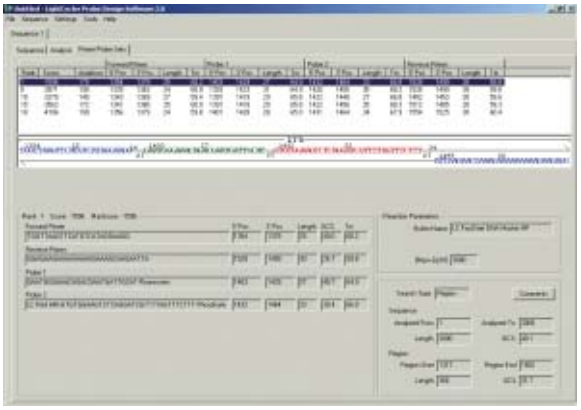
▲ **Figure 15: Analysis tab**

Ranked Display of Primer/Probe Research Results on the "Primer/Probe Sets Tab"

The Primer/Probe Sets tab displays the results of the analysis (Figure 16). The designs generated by the software are displayed in a ranked list, with the highest scoring designs at the top.

"Selecting a design" displays its sequence details directly below the list. Information about the selected design is also displayed in text boxes on the bottom left. Reaction conditions and search parameters are summarized on the right.

After you have made your decision for a specific primer/probe set, you can analyze it further using the **Cross-Complementarity** tool. This tool allows you to view the cross-complementarities (including self-complementarities) between any two oligonucleotides in a selected design. You can see the number, length, and location of the cross-complementarities at a glance in a graphical display. The tool also scores the cross-complementarities according to their number or their stability.



▲ **Figure 16: Primer/Probe Set tab**

Primer/Probes for Multiplex Analysis

When analysing multiple targets in one PCR reaction, primer/probe design becomes the most critical step, because cross-complementarities between the multiple primer/probe pairs can lead to:

- Formation of primer-dimers
- Primer binding to the false target
- Formation of byproducts

With the LightCycler 2.0 Probe Design Software, multiple primer/probe sets, optimized for multiplex reactions, are designed and analyzed in detail for cross-complementarities. In addition, they are checked for nonspecific binding by submitting probe sequences to the NBCI Blast Web site.

LightCycler System Reagents

The LightCycler System offers optimized and specifically designed master mixes or kits for real-time PCR and RT-PCR assays

Expression analysis of low-abundance messages or mutation detection and SNP analysis, require highly specific and efficient reaction conditions, optimal primer design, and absence of contaminants. Highly specific PCR avoids accumulation of side-products or artifacts, and prevents primer-dimer formation. This is guaranteed by the unique combination of the ultra-rapid cycling of the LightCycler System on one hand and the master mixes containing **FastStart Taq DNA Polymerase**, the optimal enzyme for hot-start reactions.

Ultra-rapid cycling is the result of air-driven temperature control and the use of our **LightCycler Capillaries**, providing an excellent surface-to-volume ratio for ultra-rapid temperature shifts. Rapid PCR avoids the accumulation of side products.

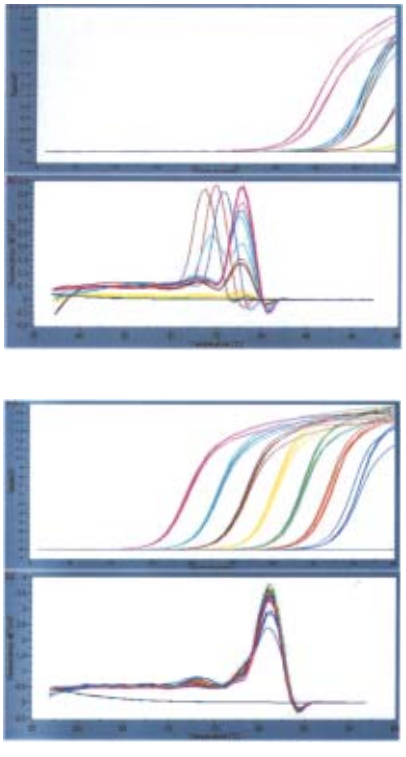
FastStart Taq DNA Polymerase is a modified form of thermostable recombinant Taq DNA Polymerase. Due to heat-labile blocking groups on some of the amino acid residues, it is inactive at room temperature, thus, preventing nonspecific elongation during the period when primers can bind less stringently. FastStart Taq DNA Polymerase is activated during a short pre-incubation step at 95°C.

PCR Reactions

The classical **LightCycler FastStart DNA Master HybProbe or SYBR Green I** is a hot-start reaction mix optimized for the use in the LightCycler Capillaries, ensuring rapid and specific DNA amplification.

With the new **LightCycler FastStart DNA Master^{PLUS} HybProbe or SYBR Green I**, hot start PCR enters into a new dimension of convenience and performance.

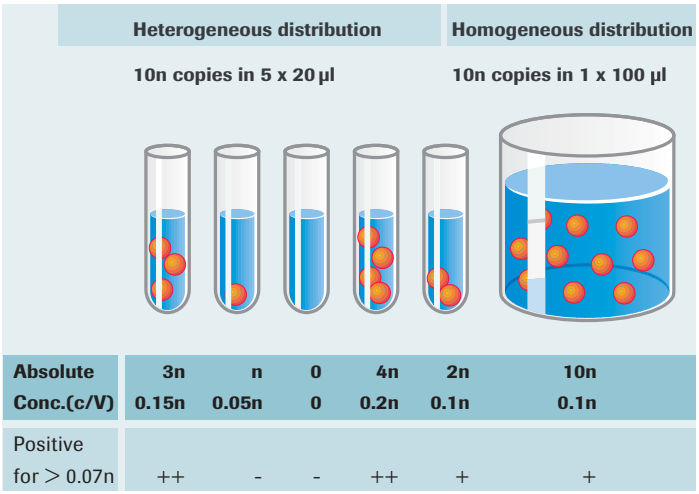
- Reaction buffer and enzyme were optimized for robustness and render the reaction less susceptible to PCR inhibitors.
- Optimal $MgCl_2$ concentration, tested on a large variety of targets is preadjusted in the reaction buffer and spares tedious $MgCl_2$ titration.
- More PCR product and higher sensitivity than with any other hot-start reaction mix.



◀ Figure 17: Competitor PCR Master versus LightCycler FastStart DNA Master^{PLUS} (10^6 to 1 copy/µl).

Competitor

LightCycler FastStart DNA Master^{PLUS}



Absolute Conc.(c/V)	3n 0.15n	n 0.05n	0 0	4n 0.2n	2n 0.1n	10n 0.1n
Positive for > 0.07n	++	-	-	++	+	+

▲ **Figure 18: Assumption of distribution of low-abundance targets.**

LightCycler Fast Start DNA Master^{PLUS} HybProbe or SYBR Green I, 100 µl Reaction for larger volume PCR in the 100 µl LightCycler Capillaries, provides utmost sensitivity and flexibility.

Even though a reaction volume of 20 µl provides sufficient sensitivity for a vast majority of applications, a reaction volume of 100 µl can even increase the probability to detect extremely low-abundance targets by increasing the probability of the presence of a target copy in a reaction after a random distribution over a number of reactions (Figure 18). Experimental data confirm this theoretical model, showing that the probability of success increases with the reaction volume (Table 1).

Concentration [Copies/ µl reaction]	Positively identified samples (total number of analyzed samples)		Level of positive hits (%)	
	20 µl PCR	100 µl PCR	20 µl PCR	100 µl PCR
0.01	4 (30)	11 (20)	13.3	55
0.025	13 (70)	38 (45)	19	84
0.05	17 (30)	14 (15)	56	93

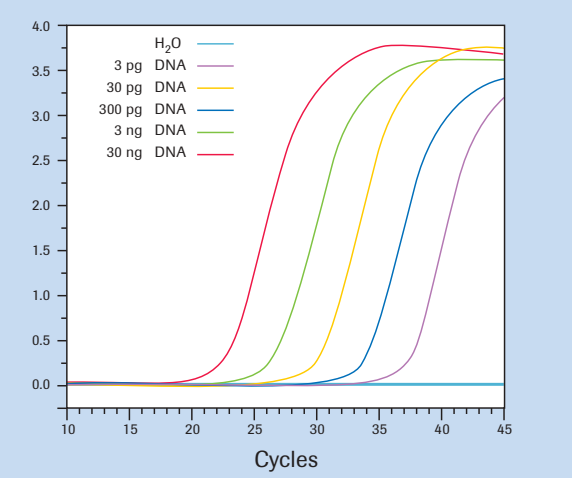
▲ **Table 1: Comparison of PCR sensitivity in 20 µl and 100 µl capillaries, calculated both in absolute numbers and in percentages.**

LightCycler TaqMan Master is a ready-to-use reaction mix designed for the TaqMan (hydrolysis probe) detection format in glass capillaries using the LightCycler System. It contains FastStart Taq DNA Polymerase for hot start PCR and an optimal $MgCl_2$ concentration.

Use the LightCycler TaqMan Master for quantitative PCR or two-step RT-PCR with the LightCycler Instruments in combination with suitable PCR primers and a hydrolysis probe.

- Save time with a convenient, ready-to-use 5x concentrated hot start master mix.
- Perform sensitive and quantitative PCR or RT-PCR using the LightCycler Instruments with TaqMan (hydrolysis) probes.
- Eliminate time-consuming $MgCl_2$ titration.
- Achieve consistent, high-quality performance with the LightCycler Instruments.

Figure 19 shows the amplification of the human cyclophiline A gene from dilutions of genomic DNA, with a set of primers and a FAM/TAMRA-labeled hydolysis probe on the LightCycler 2.0 Instrument.



▲ **Figure 19: Cyclophiline A amplification curves in the analysis module for absolute quantification of LightCycler Software 4.0.**

Multiplex PCR Reactions

With its 6 fluorescence detection channels, the LightCycler 2.0 Instrument is ideally suited for multiplex reactions with up to 4 different targets. Optimal primer/probe pairs are designed with the LightCycler Probe Design Software 2.0.

Multiplex reactions demand a higher degree of specificity and sensitivity than mono- or dual-color reactions. Competition of parameters, different target concentrations, and specificity of primer/probe pairs require an utmost effective buffer/enzyme system for reliable amplification and sensitive detection.

This is why the **LightCycler Multiplex DNA Master HybProbe** contains a special reaction mix, and even a specifically optimized Taq DNA Polymerase.

To validate the LightCycler Multiplex DNA Master HybProbe, we performed cDNA amplification assays from four housekeeping genes: ß2 microglobulin (ß2M), porphobilinogen deaminase (PBGD), hypoxanthine phosphoribosyl transferase (HPRT), and glucose-6-phosphate dehydrogenase (G6PDH), in two different cell lines, DAUDI and MCF-7.

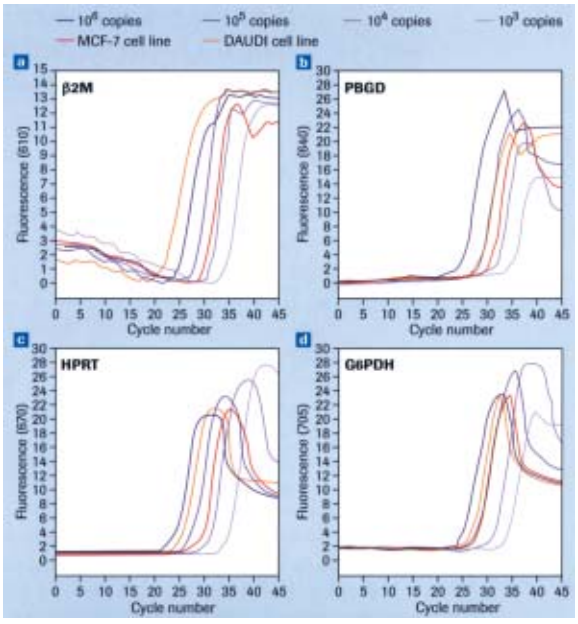
As a positive control, a reference control RNA from the LightCycler h-Housekeeping Gene Selection Set was diluted from 10^6 to 10^3 copies/µl for each target. No $MgCl_2$ adjustment of the master mix was performed.

For each sample, the amount of the four different housekeeping gene RNAs was analyzed in a single capillary by performing multiple color detection. For each concentration, the controls were amplified in one reaction (Figure 20).

The results show that the LightCycler Multiplex DNA Master HybProbe enables the measurement of four parameters with varying expression profiles in one reaction.

LightCycler Multiplex DNA Master HybProbe

- Hot-start reaction mix, especially developed for multiplex applications.
- Contains a 5'– 3' exo-minus, N-terminal deleted form of recombinant Taq DNA Polymerase for higher processitivity and specificity.
- Buffer conditions, including optimal enzyme and $MgCl_2$ concentrations, were specifically designed and evaluated for multiplex reactions.
- Ready-to-use, 5x reaction mix, which provides the flexibility to be adjusted to a specific $MgCl_2$ concentration, if required.



▲ **Figure 20: Amplification curves of the four housekeeping genes.**
(a) β2M (Channel 610), **(b)** PBGD (Channel 640), **(c)** HPRT (Channel 670) and **(d)** G6PDH (Channel 705)

RT-PCR Reactions

Two strategies can be used for RNA quantification with a LightCycler Instrument: one- or two-step RT-PCR, each having its advantages, depending on the experimental setup. Dedicated master mixes are offered for each version.

One-step RT-PCR

The main advantage of one-step RT-PCR is, that the reaction setup includes fewer processing steps and thus minimizes loss of sample and contamination risks.

LightCycler RNA Master, HybProbe, or SYBR Green I ready-to-use hot-start reaction mix uses Tth DNA Polymerase in combination with **Aptamers.**

- **Tth DNA Polymerase**, a thermostable enzyme with RNA-dependent reverse transcriptase activity and DNA-dependent polymerase activity, is the ideal enzyme for the combination of RT and PCR reactions in a single tube without compromizing one activity for the other. The higher reaction temperature of Tth DNA Polymerase allows efficient transcription of sequences with high GC content or secondary structures.
- **Aptamers** are dedicated oligonucleotides that bind to the active center of the Tth DNA Polymerase and prevent attachment to nucleic acid targets at temperatures below the optimal reaction temperature of the Tth DNA Polymerase. They are released from the enzyme at higher temperatures, thus, enabling a hot-start reaction.

  Also available for one-step RT-PCR are the **LightCycler RNA Amplification Kits HybProbe or SYBR Green I** for flexible experimental setup.

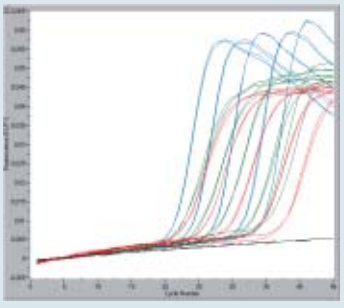
Two-step RT-PCR

When high yields of full-length cDNA are required, use the Transcriptor Reverse Transcriptase combined with the LightCycler FastStart DNA Master^{PLUS}, or the dedicated LightCycler Multiplex DNA Master HybProbe.

Transcriptor First Strand cDNA Synthesis Kit
Contains all reagents required, including a random hexamer primer and an anchored-oligo(dT)$_{18}$ primer. The new recombinant enzyme, Transcriptor Reverse Transcriptase, has an unwinding activity, and RNase H activity that degrades RNA in RNA-DNA hybrids. This spares the additional time-consuming RNase H incubation step after reverse transcription.

Unbiased, high-fidelity transcription of even problematic sequences (GC-rich, high secondary structure) guarantees high yields of full-length cDNA, ideally suited for RT-PCR assays on the LightCycler System.

--- Transcriptor: 10 U / 20 µl cDNA synthesis reaction
--- RNase H- M-MuLV - Supplier A - 200 U / 20 µl cDNA synthesis reaction
--- rec. AMV - Supplier A - 15 U / 20 µl cDNA synthesis reaction



▲ **Figure 21: 10^6 to 10^2 copies of PBGD RNA were reverse transcribed with Transcriptor Reverse Transcriptase or competitor reverse transcriptase enzymes, and amplified using the LightCycler 2.0 Instrument with LightCycler FastStart DNA Master^{PLUS} HybProbe.**

For a complete listing of LightCycler System Reagents, please refer to pages 27-31, or visit www.lightcycler-online.com

Fluorescence Detection Formats

More choices to monitor real-time PCR

Fluorescence Detection Formats

Real-time PCR with LightCycler System allows quantification of starting target material by correlating the increase of fluorescent signal, measured at each amplification cycle, to the amount of PCR product formed. Fluorescent detection of PCR products can be achieved with two different methods, using either an intercalating fluorescent dye, SYBR Green I, or target-specific fluorescently labeled probes. The use of sequence-specific probes largely increases the unambigous identification of PCR products, thus, adding to specificity and sensitivity, provided that reaction conditions are chosen in a way to prevent the competing formation of nonspecific side products.

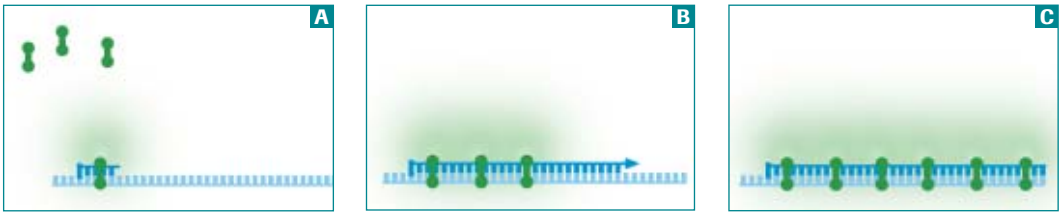
The LightCycler Instruments are compatible with a wide variety of dyes and fluorescent detection formats, including:

- **SYBR Green I**
- **HybProbe Probes**
- **SimpleProbe Probes**
- **Hydrolysis Probes**

Monitor PCR with the SYBR Green I Dye

When the SYBR Green I dye intercalates into dsDNA, its fluorescence greatly increases. During the different stages of PCR, the intensity of the fluorescent signal will vary, depending on the amount of dsDNA that is present.

▼ **Figure 22: Schematic diagram of the SYBR Green I format.**



A During annealing, PCR primers hybridize to the target and form small regions of dsDNA where SYBR Green I intercalates; the fluorescent signal slightly increases.

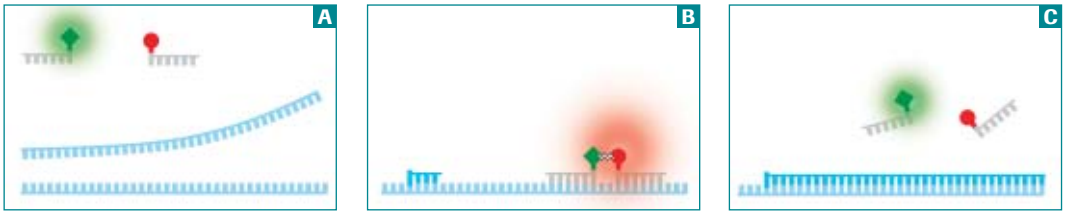
B In the elongation phase, more dsDNA is formed and more SYBR Green I dye can intercalate; higher fluorescent signal.

C At the end of the elongation phase, all DNA has become double-stranded and the maximum amount of SYBR Green I is intercalated. The fluorescence is measured (530 nm) at the end of each elongation phase.

Monitor PCR with the LightCycler HybProbe Format

The unique LightCycler HybProbe format is based on the principle of fluorescence resonance energy transfer (FRET). Two sequence-specific oligonucleotide probes are labeled with different dyes (donor and acceptor), and are added to the reaction mix in addition to the PCR primers. HybProbe probes hybridize to the target sequences on the amplified DNA fragment during the annealing phase in a head-to-tail arrangement, thereby bringing the two dyes into close proximity.

The donor dye (fluorescein) is excited by the blue light LED source. When the two dyes are close to each other (within 15 nucleotides), the energy emitted by the donor dye excites the acceptor dye attached to the second HybProbe probe, which then emits fluorescent light at a different wavelength. The amount of fluorescence is directly proportional to the amount of target DNA generated during the PCR process. HybProbe probes are displaced during the elongation and denaturation steps.



◀ Figure 23: Schematic diagram of the HybProbe format.

A The donor-dye probe is labeled with fluorescein at the 3′ end and the acceptor-dye probe is labeled with LightCycler Red at the 5′ end. Hybridization does not take place during the denaturation phase of PCR and, thus, the distance between the dyes is too large to allow energy transfer to occur.

B During the annealing phase, the probes hybridize to the amplified DNA fragment in a close head-to-tail arrangement. When fluorescein is excited by the light from the LED, it emits green fluorescent light, transferring the energy to LightCycler Red, which then emits red fluorescent light. This red fluorescence is measured at the end of each annealing step, when the fluorescence intensity is highest.

C After annealing, the temperature is raised and the HybProbe probe is displaced during elongation. At the end of this step, the PCR product is double-stranded and the displaced HybProbe probes are again too far apart to allow FRET to occur.

Advantages of the HybProbe Format:

- In a HybProbe analysis, the presence of a specific amplification product is quantitatively recorded by an increase in fluorescence.
- No fluorescence is recorded in the absence of specific template.
- Increase in specificity because two probes hybridize to the target in a defined, sequence-specific mode.
- Primer-dimers are not detected because the sequence-specific probes do not recognize them.
- Since both HybProbe probes are still intact at the end of the amplification, they may be used in a subsequent melting curve experiment, such as mutation detection or SNP analysis.

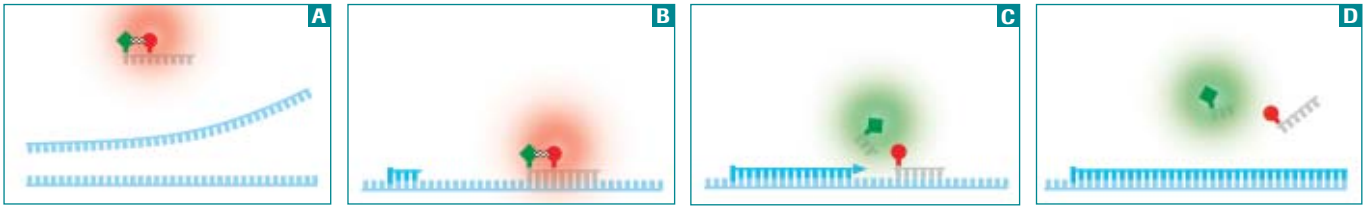
Choose HybProbe Format to:

- Benefit from maximum detection sensitivity, ease-of-use, and sequence-specific product identification.
- Experience the widest choice of applications (*e.g.*, SNP genotyping and qPCR).
- Perform multiplex assays.

Monitor PCR with Hydrolysis Probes

Hydrolysis probe assays, conventionally called "TaqMan" assays, can technically be described as homogenous 5´ nuclease assays, since a single 3´ non-extendable Hydrolysis probe, which is cleaved during PCR amplification, is used to detect the accumulation of a specific target DNA sequence. This single probe contains two labels, a fluorescence reporter and a fluorescence quencher, in close proximity to each other. When the probe is intact, the quencher dye is close enough to the reporter dye to suppress the reporter fluorescent signal (fluorescence quenching takes place via FRET). During PCR, the 5´ nuclease activity of the polymerase cleaves the Hydrolysis probe, separating the reporter and quencher. In the cleaved probe, the reporter is no longer quenched and can emit a fluorescence signal when excited.

▼ **Figure 24: Schematic diagram of the Hydrolysis Probes format.**



A The probe carries two fluorescent dyes in close proximity, with the quencher dye suppressing the reporter fluorescence signal. The 3' end of the Hydrolysis probe is dephosphorylated, so it cannot be extended during PCR. During denaturation the target double-stranded DNA is separated.

B In the annealing phase of PCR, primers and probes specifically anneal to the target sequence.

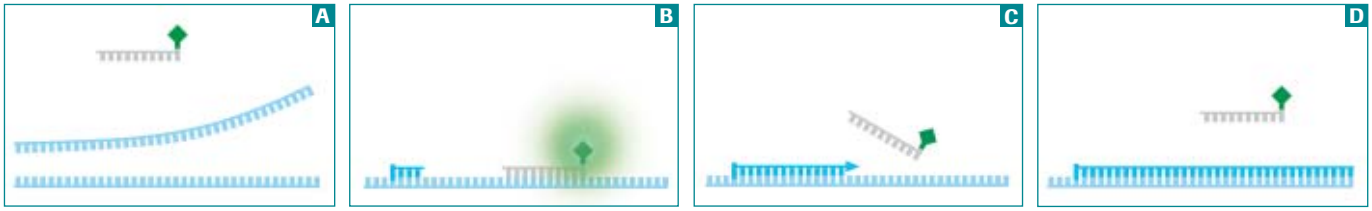
C As the DNA polymerase extends the primer, it encounters the probe. The polymerase then cleaves the probe with its inherent 5´ nuclease activity, displaces the probe fragments from the target, and continues to polymerize the new amplicon.

D In the cleaved probe, the reporter dye is no longer quenched and therefore can emit fluorescent light that can be measured by one channel of the LightCycler optical unit Thus, the increase in fluorescence from the reporter dye directly correlates to the accumulation of PCR products.

SNP Genotyping and Mutation Detection with SimplePrObe Probes

SimpleProbe probes are a special type of hybridization probes. They differ from HybProbe probes in one important way: instead of two probes working together, only a single probe is needed. This single probe hybridizes specifically to a target sequence that contains the SNP of interest. Once hybridized, the SimpleProbe probe emits a greater fluorescent signal than it does when it is not hybridized to its target. As a result, changes in fluorescent signal depend solely on the hybridization status of the probe.

▼ **Figure 25: Schematic diagram of the SimpleProbe format.**



A During the denaturation phase no hybridization takes place, thus, only a low fluorescence background is detected at 530 nm.

B During the annealing phase, the probe hybridizes to the amplified DNA fragment and is no longer quenched. Fluorescein, when excited by the LightCycler LED, emits green fluorescent light which is measured only at the end of each annealing step at maximum intensity.

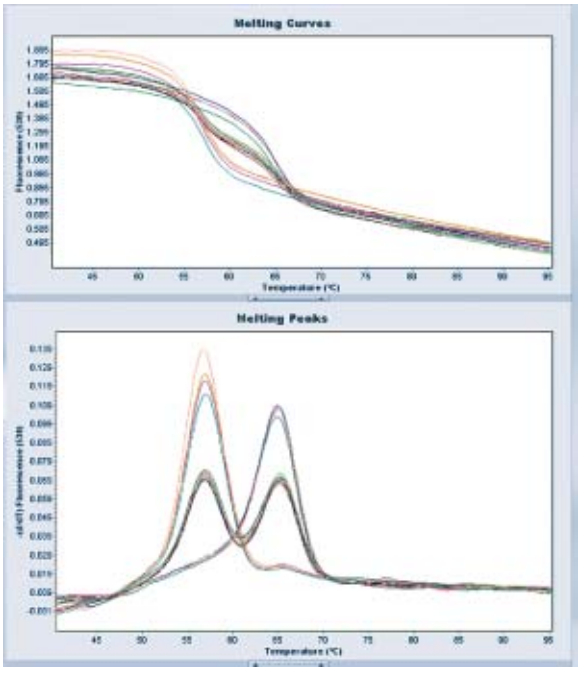
C During the subsequent elongation step, the SimpleProbe probe is displaced.

D At the end of the elongation step, the PCR product is double stranded and the displaced SimpleProbe probe is again quenched.

During SNP analysis, the melting behavior of the SimpleProbe probes is monitored. The more stable the hybridization between the SimpleProbe probe and the target sequence, the higher the melting temperature. Mutations, such as SNP, weaken the binding of the SimpleProbe probe. As the temperature increases, the LightCycler Instruments monitor the melting of the SimpleProbe probes by measuring the decrease in fluorescence.

The experimental example in Figure 26 shows the genotyping of a C/T polymorphism. The upper graph displays sample fluorescence versus temperature. A clear drop in signal fluorescence can be seen for each sample as the temperature increases. The lower graph plots the first negative derivative of the sample fluorescence versus temperature, showing the melting temperature of each sample as a peak.

SimpleProbe probes are an excellent tool for SNP genotyping and mutation detection because they readily identify wild type, mutant, or heterozygous samples, yet are as simple to design and use as standard PCR primers. When combined with the LightCycler Software 4.0 Genotyping or Tm Calling module, these probes can easily distinguish the various types of mutations.



▲ **Figure 26: Genotyping of a C/T polymorphism with SimpleProbe probes.**

Other Fluorescent Formats to Use with the LightCycler 2.0 Instrument

The LightCycler 2.0 Instrument also supports other fluorescent-detection formats *(e.g.,* molecular beacons, which use fluorescein as the FRET donor dye). However, the fluorescent dyes used must be compatible with the optical unit of the LightCycler Instruments.

CHANNEL	SUITABLE DYES
530	SYBR Green I and Fluorescein
560	HEX/VIC
610	LightCycler Red 610
640	LightCycler Red 640
670	LightCycler Red 670
705	LightCycler Red 705

▲ **Table 2: Specifications of the LightCycler 2.0 Instrument optical unit.**

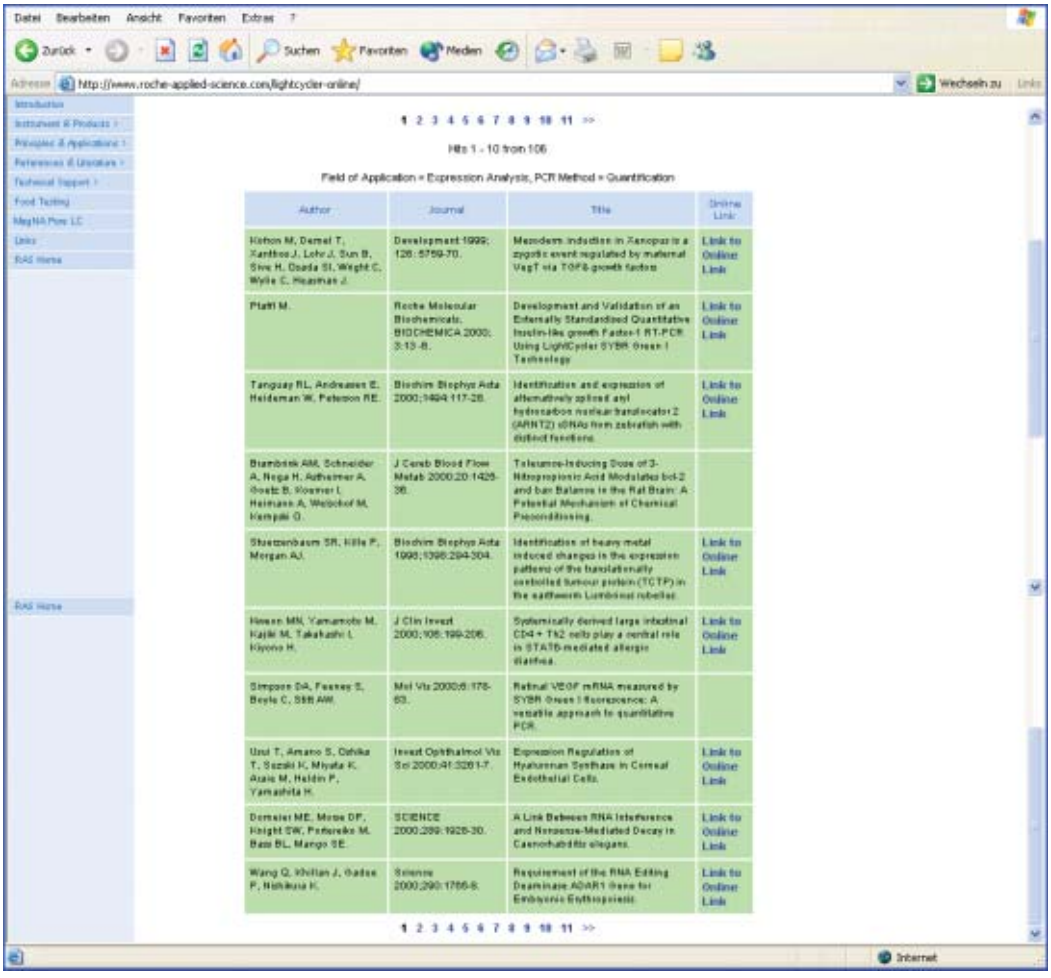
The LightCycler System Reference List

Referenced publications that prove performance

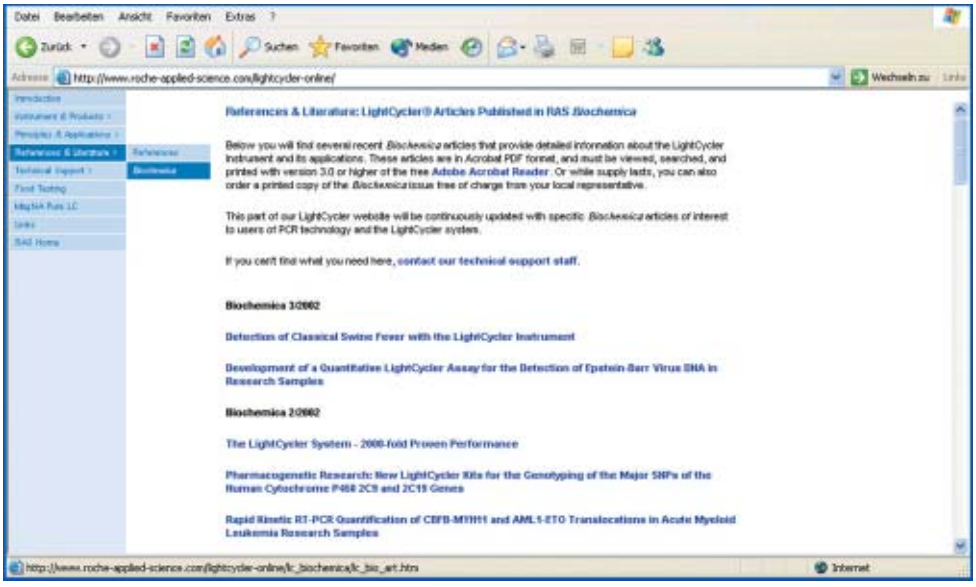
The LightCycler System provides a unique combination of high-performance instruments, advanced software for quantitative and mutation analysis, dedicated master mixes, and detection formats. These features render the LightCycler System to be the ideal choice for real-time PCR assays.

More than a thousand published reports of leading scientists in international journals, and a large number of articles sent in by satisfied customers for publication in the Roche *BIOCHEMICA* journal, prove the outstanding performance of this real-time PCR system. Visit our website to browse through the list of publications or scroll through the *BIOCHEMICA* articles provided online.

www.lightcycler-online.com



▲ **Figure 27: Example of a publications list on: www.lightcycler-online.com**



▲ **Figure 28: Example of *BIOCHEMICA* articles on: www.roche-applied-science.com**

LightCycler 2.0 Instrument Characteristics

Hardware dimensions and weight w/o computer	28 x 28.5 x 50.5 cm approximately 22 kg
Computer	Pentium PC with Windows 2000
Sample number	32
Reaction vessel	Glass capillary
Reaction volume	20 µl or 100 µl
Fluorescence excitation	Blue LED with peak emission of 470 nm
Detection channels	530 nm, 560 nm, 610 nm, 640 nm, 670 nm, and 710 nm
Measuring mode	Real-time, online (continuous display of readings)
Typical run time	< 30 minutes for 35 cycles
Temperature ramp rate (Settings)	0.1 – 20°C/second
Temperature accuracy	+/- 0.4°C

LightCycler 1.5 Instrument Characteristics

Hardware dimensions and weight w/o computer	45 x 30 x 40 cm 20 kg
Computer	Pentium PC with Windows NT
Sample number	32
Reaction vessel	Glass capillary
Reaction volume	10 – 20 µl
Fluorescence excitation	Blue LED with peak emission of 470 nm
Detection channels	530 nm, 640 nm, and 710 nm
Measuring mode	Real-time, online (continuous display of readings)
Typical run time	< 30 minutes for 35 cycles
Temperature ramp rate (Settings)	0.1 – 20°C/second
Temperature accuracy	+/- 0.4°C

Components, Consumables, and System Reagents

LightCycler Instruments and Additional Products

	Cat. No.	Pack Size
LightCycler 2.0 Instrument ●	03 531 414 201	1 Instrument*
LightCycler 1.5 Instrument ✢ ●	04 484 495 001	1 Instrument**
LightCycler Probe Design Software 2.0 ▲	04 342 054 001	1 Pack containing a CD and a Manual
LightCycler Capillaries (20 µl) ▲	11 909 339 001	8 boxes, 96 capillaries per box
LightCycler Capillaries (100 µl) ▲	03 337 090 001	8 boxes, 96 capillaries per box
LightCycler 2.0 Sample Carousel (20 µl) ▲	03 603 962 001	1 Carousel
LightCycler 2.0 Sample Carousel (100 µl) ▲	03 603 954 001	1 Carousel
LightCycler Sample Carousel (20 µl) ▲	11 909 282 001	1 Carousel
LightCycler 2.0 Capillary Releaser ▲	03 603 920 001	1 Releaser
LightCycler Carousel Centrifuge 2.0	03 709 582 001 03 709 507 001	1 Instrument (230 Volt) 1 Instrument (115 Volt)
LightCycler Centrifuge Adapters ▲	11 909 312 001	1 Set containing 32 adapters in an aluminum cooling block
LightCycler Capping Tool	03 357 317 001	1 Capping Tool
LightCycler Sample Carousel O-Ring	03 603 989 001	1 Set (2 O-Rings)

✢ **Please ask your local representative for availability.**

* System package includes LightCycler 2.0 Sample Carousel (20 µl), LightCycler 2.0 Sample Carousel (100 µl), 1 box LightCycler Capillaries (20 µl), 1 box LightCycler Capillaries (100 µl), LightCycler Centrifuge Adapters, LightCycler Capping Tool, LightCycler Capillary Releaser, Operator's Manual, LightCycler Software 4.0.
A desktop or notebook PC is supplied with the Instrument.

** System package includes LightCycler Sample Carousel, LightCycler Software, LightCycler Centrifuge Adapters, 1 box LightCycler Capillaries (20 µl), LightCycler Capping Tool, LightCycler Capillary Releaser, Operator's Manual.
A desktop or notebook PC is supplied with the Instrument.

LightCycler Kits for PCR

	Applications	Cat. No.	Pack Size
LightCycler FastStart DNA Master^{PLUS} SYBR Green I ❖ ■	Easy-to-use hot start reaction mix for highly sensitive PCR, containing SYBR Green I dye and $MgCl_2$ for real-time detection and quantification of the PCR product.	03 515 869 001 03 515 885 001	1 kit (96 reactions) 1 kit (480 reactions)
LightCycler FastStart DNA Master^{PLUS} HybProbe ◆ *	Easy-to-use hot start reaction mix containing $MgCl_2$ for highly sensitive PCR applications with sequence-specific probes.	03 515 575 001 03 515 567 001	1 kit (96 reactions) 1 kit (480 reactions)
LightCycler FastStart DNA Master^{PLUS} SYBR Green I, 100µl ❖ ■	Easy-to-use hot start reaction mix for highly sensitive PCR, containing SYBR Green I dye and $MgCl_2$ for real-time detection and quantification of the PCR product.	03 752 186 001	1 kit (384 reactions)
LightCycler FastStart DNA Master^{PLUS} HybProbe, 100µl ◆ *	Easy-to-use hot start reaction mix containing $MgCl_2$ for highly sensitive PCR applications with sequence-specific probes.	03 752 178 001	1 kit (384 reactions)
LightCycler Multiplex DNA Master HybProbe ◆ *	Easy-to-use hot start reaction mix for multiplex PCR with sequence-specific HybProbe probes.	04 340 019 001	1 kit (96 reactions)
LightCycler FastStart DNA Master SYBR Green I ❖ ■	Easy-to-use hot start reaction mix for PCR, containing SYBR Green I dye for real-time detection and quantification of the PCR product.	03 003 230 001 12 239 264 001	1 kit (96 reactions) 1 kit (480 reactions)
LightCycler FastStart DNA Master HybProbe ◆ *	Easy-to-use hot start reaction mix for amplification, real-time detection and quantification of a specific PCR product with sequence-specific probes.	03 003 248 001 12 239 272 001	1 kit (96 reactions) 1 kit (480 reactions)
LightCycler TaqMan Master ◆	Easy-to-use hot start reaction mix for PCR with TaqMan probes using the LightCycler Instruments.	04 535 286 001	1 kit (96 reactions)
LightCycler DNA Master SYBR Green I ❖ ■	Easy-to-use reaction mix for PCR, containing SYBR Green I dye for real-time detection and quantification of the PCR product.	12 015 099 001 12 158 817 001	1 kit (96 reactions) 1 kit (480 reactions)
LightCycler DNA Master HybProbe ◆ *	Easy-to-use reaction mix for amplification, real-time detection and quantification of a specific PCR product with sequence-specific probes.	12 015 102 001 12 158 825 001	1 kit (96 reactions) 1 kit (480 reactions)
LightCycler Control Kit DNA ▲ *	Easy-to-use reaction mixes that provide performance controls for assays with either the LightCycler DNA Master SYBR Green I or HybProbe probes.	12 158 833 001	1 kit (50 control reactions)
LightCycler Uracil-DNA Glycosylase ▲	Prevent carryover contamination between PCRs. Use with FastStart-enzyme-based LightCycler Kits.	03 539 806 001	100 U (50 µl)

LightCycler Kits for RT-PCR

	Applications	Cat. No.	Pack Size
LightCycler RNA Master SYBR Green I ❖ ■	Easy-to-use hot start reaction mix for one-step RT-PCR, and detection of product with SYBR Green I dye.	03 064 760 001	1 kit (96 reactions)
LightCycler RNA Master HybProbe ◆ *	Easy-to-use hot start reaction mix for one-step RT-PCR, and detection of product with HybProbe probes.	03 018 954 001	1 kit (96 reactions)
LightCycler RNA Amplification Kit SYBR Green I ❖ ■	Easy-to-use reaction mix containing SYBR Green I for real-time detection and quantification of amplified RNA.	12 015 137 001	1 kit (96 reactions)
LightCycler RNA Amplification Kit HybProbe ◆ *	Easy-to-use reaction mix for real-time detection and quantification of a specific amplified product from RNA using HybProbe probes.	12 015 145 001	1 kit (96 reactions)
LightCycler Control Kit RNA ▲ *	Easy-to-use reaction mixes that provide performance controls for assays with the LightCycler RNA Amplification Kit SYBR Green I or HybProbe probes.	12 158 841 001	1 kit (50 control reactions)

LightCycler Reagents for Labeling and Detection of Hybridization Probes

	Applications	Cat. No.	Pack Size
LightCycler Fluorescein CPG ▲	Suitable for 3'-end labeling of an oligonucleotide. Use with a 5'-LC Red-labeled oligonucleotide in a HybProbe probe detection assay.	03 138 178 001 03 113 906 001	1 gram 5 columns for the synthesis of 5 oligonucleotides labeled at the 3' end (0.2 µmol scale)
LightCycler Red 610-N-hydroxysuccinimide ester ▲	Suitable for 5'-end labeling of one HybProbe probe. Use with a second HybProbe probe that is 3'-end labeled with fluorescein.	03 561 488 001	1 vial for at least 5 x 50 nmol oligonucleotides
LightCycler Red 640-N-hydroxysuccinimide ester ▲	Suitable for 5'-end labeling of one HybProbe probe. Use with a second HybProbe probe that is 3'-end labeled with fluorescein.	12 015 161 001	1 vial for at least 5 x 50 nmol oligonucleotides
LightCycler Multicolor Demo Set ▲	Reagent set for the demonstration of 4-channel multicolor detection and generation of color compensation data using the LightCycler 2.0 Instrument.	03 624 854 001	1 Set for 5 color compensation runs and 20 multicolor reactions
LightCycler Color Compensation Set ▲	Ready-to-use reagents to generate color compensation files that can efficiently compensate for inter-channel cross-talk during dual-color experiments using the 640 and 705 nm detection channels.	12 158 850 001	1 Set for 5 calibration runs

LightCycler Kits for Accurate Relative Quantification of Gene Expression Levels

	Applications	Cat. No.	Pack Size
LightCycler h-PBGD Housekeeping Gene Set ▲ *	Ready-to-use Primer/HybProbe probe mixture and RNA standards to detect mRNA from the PBGD gene, which is used as reference gene in the relative quantification of an unknown target gene.	03 146 073 001	1 Set (96 reactions)
LightCycler h-ß2M Housekeeping Gene Set ▲ *	Ready-to-use Primer/HybProbe probe mixture and RNA standards to detect mRNA from the ß2M gene, which is used as reference gene in the relative quantification of an unknown target gene.	03 146 081 001	1 Set (96 reactions)
LightCycler h-G6PDH Housekeeping Gene Set ▲ *	Ready-to-use Primer/HybProbe probe mixture and RNA standards to detect mRNA from the G6PDH gene, which is used as reference gene in the relative quantification of an unknown target gene.	03 261 883 001	1 Set (96 reactions)
LightCycler h-HPRT Housekeeping Gene Set ▲ *	Ready-to-use Primer/HybProbe probe mixture and RNA standards to detect mRNA from the HPRT gene, which is used as reference gene in the relative quantification of an unknown target gene.	03 261 891 001	1 Set (96 reactions)
LightCycler h-ALAS Housekeeping Gene Set ▲ *	Ready-to-use Primer/HybProbe probe mixture and RNA standards to detect mRNA from the ALAS gene, which is used as reference gene in the relative quantification of an unknown target gene.	03 302 504 001	1 Set (96 reactions)
LightCycler h-Housekeeping Gene Selection Set ▲ *	Ready-to-use Primer/HybProbe probe mixtures and positive controls *(in vitro* transcribed RNAs encoding fragments of the respective human Housekeeping Gene) to detect human mRNA for ß-2M, G6PDH, ALAS, HPRT, and PBGD.	03 310 159 001	1 Set (5 x 16 reactions)

LightCycler Kits for Detection and Quantification of Genetically Modified Organisms (GMO)

	Applications	Cat. No.	Pack Size
LightCycler GMO Screening Kit **	Kit containing all components to qualitatively detect genetically modified plants.	03 267 199 001	1 kit (for a max. of 56 samples)
LightCycler GMO Soya Quantification Kit **	Kit containing all components to determine the relative content of genetically modified Roundup-Ready Soya in food samples and raw material. A calibrator is provided to calibrate the run and to be used as a positive control.	03 267 164 001	1 kit (for a max. of 56 samples)
LightCycler GMO Maize Quantification Kit **	Kit containing all components to determine the relative content of genetically modified Bt-176 Maize in food samples and raw material.	03 267 172 001	1 kit (for a max. of 56 samples)

LightCycler Kits for Amplification and Detection of Food-borne Pathogens

	Applications	Cat. No.	Pack Size
LightCycler foodproof *Salmonella* Detection Kit ▼*	Kit containing all components to qualitatively detect *Salmonella* DNA in food samples and raw material. An internal control is provided.	03 357 449 001	1 kit (for a max. of 90 samples)
LightCycler *Listeria monocytogenes* Detection Kit ▼*	Kit containing all components to qualitatively detect *Listeria monocytogenes* DNA in food samples and raw material. An internal control is provided.	03 357 457 001	1 kit (for a max. of 90 samples)
LightCycler foodproof *Listeria* Genus Detection Kit ▼*	Kit containing all components to qualitatively detect *Listeria* Genus DNA in food samples and raw material. An internal control is provided.	03 535 614 001	1 kit (for a max. of 90 samples)
LightCycler foodproof Beer Screening Kit ▼*	Kit containing all components to qualitatively detect beer spoilage bacterial DNA and the identification of *Lactobacillus brevis, Lactobacillus linderi, Pediococcus damnosus, Pediococcus inopinatus* and *Megaspherea cerevisiae* in beer samples.	03 610 888 001	1 kit (for a max. of 90 samples)
LightCycler foodproof *E. coli* 0157 Detection Kit ▼*	Kit containing all components to qualitatively detect *E. coli* 0157 (including *E. coli* 0157:H7) DNA in food samples and raw material.	03 671 110 001	1 kit (96 reactions for a max. of 90 samples)

LightCycler Kits for Microbiology Research

	Applications	Cat. No.	Pack Size
LightCycler *Bacillus anthracis* Detection Kit ◗*	Kit containing all components to qualitatively detect the encapsulation B *(capB)* and protective antigen *(pagA)* genes of *Bacillus anthracis* in research samples.	03 303 411 001	1 kit (for a max. of 90 samples)

Related Products

Transcriptor Reverse Transcriptase	Fast recombinant reverse transcriptase expressed in *E coli* for synthesis of first strand cDNA for use in subsequent amplification reactions using LightCycler Instruments.	03 531 317 001 03 531 295 001 03 531 287 001	250 U for 25 reactions 500 U for 50 reactions 4 x 500 U for 200 reactions
Transcriptor First Strand cDNA Synthesis Kit	Ready-to-use reagents for performing first strand cDNA synthesis reactions from RNA which can be used for subsequent PCR amplification in LightCycler Instruments.	04 379 012 001	1 kit (50 reactions)

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