For general laboratory use.
FOR *IN VITRO* USE ONLY.

Roche

Roche Applied Science

LightCycler® DNA Master SYBR Green I

Version September 2005

Easy-to-use Reaction Mix for PCR using the LightCycler® 2.0 System

Cat. No. 12 015 099 001 Kit for 96 reactions
Cat. No. 12 158 817 001 Kit for 480 reactions

Store the kit at −15 to −25°C

⚠ Keep LightCycler® DNA Master SYBR Green I (vial 1, green cap) away from light!

Table of Contents

1. What this Product Does 3

Number of Tests 3
Storage and Stability 3
Additional Equipment and Reagents Required 4
Application 5
Assay Time 5

2. How To Use this Product 6

2.1 Before You Begin 6

Sample Material 6
Negative Control 6
Primers 6
$MgCl_2$ 6

2.2 Experimental Protocol 7

LightCycler® 2.0 System Protocol 7
Fluorescence and Run Setup Parameters 8
Preparation of the PCR Mix 9

2.3 Related Procedures 10

Hot-Start 10
Prevention of Carry-Over Contamination 10
Two-step RT-PCR 11

3. Results 11

Quantification Analysis 11
Melting Curve Analysis 12

4. Troubleshooting 13

5. Additional Information on this Product 15

How this Product Works 15
Test Principle 15
References 16
Quality Control 16

6. Supplementary Information 17

6.1 Conventions 17
6.2 Changes to Previous Version 17
6.3 Ordering Information 17
6.4 Disclaimer of License 19
6.5 Trademarks 19

1. What this Product Does

Number of Tests

The kit is designed for 96 or 480 reactions (depending on pack size) with a final reaction volume of 20 µl each.

Kit Contents

Vial/Cap	Label	Contents/Function a) Cat. No. 12 015 099 001 (96 reactions) b) Cat. No. 12 158 817 001 (480 reactions)
1 green cap	LightCycler® DNA Master SYBR Green I, 10× conc.	a) 3 vials, 64 µl each b) 15 vials, 64 µl each • Ready-to-use reaction mix for PCR • Contains Taq DNA Polymerase, reaction buffer, dNTP mix (with dUTP instead of dTTP), SYBR Green I dye, and 10 mM $MgCl_2$
2 blue cap	$MgCl_2$ stock solution, 25 mM	a) 1 vial, 1 ml b) 2 vials, 1 ml each • To adjust $MgCl_2$ concentration
3 colorless cap	H_2O, PCR-grade	a) 2 vials, 1 ml each b) 7 vials, 1 ml each • To adjust the final reaction volume

Storage and Stability

- Store the kit at −15 to −25°C through the expiration date printed on the label.

⚠ Keep the LightCycler® DNA Master SYBR Green I (vial 1, green cap) away from light.

The kit is shipped on dry ice.

- Once the kit is opened, store the kit components as described in the following table:

Vial	Label	Storage
1 green cap	LightCycler® DNA Master SYBR Green I	• Store at −15 to−25°C • After thawing store at +2 to +8°C for a maximum of 2 weeks. • **Avoid repeated freezing and thawing!** • **Keep vial 1 away from light!**
2 blue cap	$MgCl_2$ stock solution, 25 mM	Store at −15 to −25°C
3 colorless cap	H_2O, PCR-grade	

Additional Equipment and Reagents Required

Additional reagents and equipment required to perform reactions with the LightCycler® DNA Master SYBR Green I using the LightCycler® 2.0 System include:

- LightCycler® 2.0 System* (LightCycler® 2.0 Instrument* or LightCycler® 1.5 Instrument*)
- LightCycler® Capillaries*
- Standard benchtop microcentrifuge containing a rotor for 2.0 ml reaction tubes

The LightCycler® 2.0 System provides adapters that allow LightCycler® Capillaries to be centrifuged in a standard microcentifuge rotor.

or

- LC Carousel Centrifuge 2.0* for use with the LightCycler® 2.0 Sample Carousel (20 µl)(optional)

If you use a LightCycler® Instrument version below 2.0, you need in addition the LC Carousel Centrifuge 2.0 Bucket 2.1*. To adapt the LightCycler® 2.0 Sample Carousel (20 µl) to the former LC Carousel Centrifuge, you need the LC Carousel Centrifuge 2.0 Rotor Set*.

- Nuclease-free, aerosol-resistant pipette tips
- Pipettes with disposable, positive-displacement tips
- Sterile reaction (Eppendorf) tubes for preparing master mixes and dilutions
- Uracil-DNA Glycosylase, heat-labile (optional‡)

‡ For prevention of carry-over contamination; see section Related Procedures for details.

** available from Roche Applied Science; see Ordering Information for details.*

Application

LightCycler® DNA Master SYBR Green I is designed for research studies. When used with the LightCycler® 2.0 System and suitable PCR primers, this kit allows very sensitive detection and quantification of defined DNA sequences. The kit can also be used to perform two-step RT-PCR.

In principle, the LightCycler® DNA Master SYBR Green I can be used for the amplification and detection of any DNA or cDNA target. To do this, you would need to adapt your detection protocol to the reaction conditions of the LightCycler® 2.0 System, and design specific PCR primers for each target. Refer to the LightCycler® Operator's Manual for general recommendations. LightCycler® DNA Master SYBR Green I can also be used with Uracil-DNA Glycosylase, heat-labile, to prevent carry-over contamination during PCR.

⚠ The amplicon size should not exceed 1 kb in length. For optimal results, select a product length of 700 bp or less.

⚠ The performance of the kit described in this Instruction Manual is warranted only when it is used with the LightCycler® 2.0 System.

Assay Time

Procedure	Time
Optional: dilution of template DNA	5 min
PCR setup	15 min
PCR run (incl. melting curve)	25 min
Total assay time	**45 min**

2. How To Use this Product

2.1 Before You Begin

Sample Material

- Use any template DNA (*e.g.,* genomic or plasmid DNA, cDNA) suitable for PCR in terms of purity, concentration, and absence of inhibitors. For reproducible isolation of nucleic acids use:
  - either the MagNA Pure LC Instrument or the MagNA Pure Compact Instrument together with a dedicated nucleic acid isolation kit (for automated isolation) or
  - a High Pure nucleic acid isolation kit (for manual isolation).

For details see the Roche Applied Science Biochemicals catalog or home page, www.roche-applied-science.com.

- Use up to 50 ng complex genomic DNA or 10^1 – 10^{10} copies plasmid DNA

⚠ Using a too high amount of template DNA might reduce the maximum fluorescence signal by outcompeting the SYBR Green I dye.

If you are using a non-purified cDNA sample from reverse transcription, especially if it contains high background concentrations of RNA and oligonucleotides, you can improve your results by using 2 µl (or less) of that sample in the reaction.

Negative Control

Always run a negative control with the samples. To prepare a negative control, replace the template DNA with PCR-grade water (vial 3, colorless cap).

Primers

Use PCR primers at a final concentration of 0.3–1 µM. The recommended starting concentration is 0.5 µM each.

$MgCl_2$

To ensure specific and efficient amplification with the LightCycler® 2.0 System, you must optimize the $MgCl_2$ concentration for each target. The LightCycler® DNA Master SYBR Green I contains a $MgCl_2$ concentration of 1 mM (final concentration). The optimum concentration for PCR with the LightCycler® 2.0 System may vary from 1 to 5 mM. The table below gives the volumes of the $MgCl_2$ stock solution (vial 2, blue cap) that you must add to a 20 µl reaction (final PCR volume) to increase the $MgCl_2$ concentration to the indicated values.

To reach a final Mg^{2+} concentration (mM) of:	1	2	3	4	5
Add this amount of 25 mM $MgCl_2$ stock solution (µl)	0	0.8	1.6	2.4	3.2

LightCycler® 2.0 System Protocol

The following procedure is optimized for use with the LightCycler® 2.0 System.

⚠ Program the LightCycler® Instrument before preparing the reaction mixes.

A LightCycler® 2.0 System protocol that uses LightCycler® DNA Master SYBR Green I contains the the following programs:

- **Denaturation** of the template DNA
- **Amplification** of the target DNA
- **Melting Curve** for PCR product identification
- **Cooling** the rotor and thermal chamber

For details on how to program the experimental protocol, see the LightCycler® Operator´s Manual.

⚠ [1)] Temperature Transition Rate/Slope is 20°C/sec, except where indicated.

⚠ Set all other protocol parameters not listed in the tables below to '0'.

The following table shows the PCR parameters that must be programmed for a LightCycler® 2.0 System PCR Run with the LightCycler® DNA Master SYBR Green I.

Analysis Mode	Cycles	Segment	Target Temperature [1)]	Hold Time	Acquisition Mode
Denaturation					
None	1	1	95°C	30 s	none
Amplification					
Quantification	45	Denaturation	95°C	0 s	none
		Annealing	primer dependent [2)]	0–10 s [4)]	none
		Extension	72°C [3)]	= (amplicon [bp]/25) s [5)]	single
Melting Curve					
Melting Curves	1	Denaturation	95°C	0 s	none
		Annealing	65°C	15 s	none
		Melting	95°C slope = 0.1°C/sec [1)]	0 s	continuous
Cooling					
None	1		40°C	30 s	none

[2)] For initial experiments, set the target temperature (*i.e.,* the primer annealing temperature) 5°C below the calculated primer T_m. Calculate the primer T_m according to the following formula, based on the nucleotide content of the primer: $T_m = 2°C\ (A+T) + 4°C\ (G+C)$.

[3)] If the primer annealing temperature is low (< +55°C), reduce the transition rate/slope to 2–5°C/s.

[4)] For typical primers, choose an incubation time of 0 – 10 s for the annealing step. To increase the specificity of primer binding, use an incubation time of < 5 s.

[5)] For greater precision in target quantification experiments, it can be advantageous (in some cases) to choose longer extension times for the amplification cycles.

Fluorescence and Run Setup Parameters

Parameter	Setting	
All LightCycler® Software Versions		
Seek Temperature	30°C	
LightCycler® Software prior to Version 3.5		
Display Mode	fluorescence channel F1	
Fluorescence Gains	**Fluorimeter**	**Gain Value**
	Channel 1 (F1)	3
	Channel 2 (F2)	1
	Channel 3 (F3)	1
LightCycler® Software Version 3.5		
Display Mode	fluorescence channel F1	
Fluorescence Gains	not required In data created with LightCycler® Software Version 3.5, all fluorescence values are normalized to a fluorescence gain of "1". This produces a different scale on the Y-axis than that obtained with previous LightCycler® software versions. This difference does not affect the crossing points nor any calculated concentrations obtained.	
LightCycler® Software Version 4.0		
Default Channel	fluorescence channel 530	
Fluorescence Gains	not required	
"Max. Seek Pos"	Enter the number of sample positions the instrument should look for.	
"Instrument Type"	• "6 Ch.": for LightCycler® 2.0 Instrument (selected by default) • "3 Ch.": for LightCycler® 1.5 Instrument and instrument versions below	
"Capillary Size"	Select "20 µl" as the capillary size for the experiment. ⚠ For the "6 Ch." instrument type only.	

Preparation of the PCR Mix

Proceed as described below for a 20 µl standard reaction.

⚠ Do not touch the surface of the capillaries. Always wear gloves when handling the capillaries.

❶ Depending on the total number of reactions, place the required number of LightCycler® Capillaries in precooled centrifuge adapters or in a LightCycler® Sample Carousel in a precooled LC Carousel Centrifuge Bucket.

❷ Prepare a 10× conc. solution of the PCR primers.

If you are using the recommended final concentration of 0.5 µM for each primer, the 10x conc. solution would contain a 5 µM concentration of each primer.

❸ In a 1.5 ml reaction tube on ice, prepare the PCR Mix for one 20 µl reaction by adding the following components in the order mentioned below:

Component	Volume	Final conc.
H_2O, PCR-grade (vial 3, colorless cap)	x µl	
$MgCl_2$ stock solution (vial 2, blue cap)	y µl	Use concentration that is optimal for the target.
PCR Primer, 10× conc.	2 µl	0.3–1.0 µM each (recommended conc. is 0.5 µM)
LightCycler® DNA Master SYBR Green I, 10× conc. (vial 1)	2 µl	1×
Total volume	**18 µl**	

To prepare the PCR Mix for more than one reaction, multiply the amount in the "Volume" column above by *z*, where *z* = the number of reactions to be run + one additional reaction.

❹
- Mix carefully by pipetting up and down. Do not vortex.
- Pipet 18 µl PCR mix into each precooled LightCycler® Capillary.
- Add 2 µl of the DNA template
- Seal each capillary with a stopper.

continued on next page

❺	• Place the adapters (containing the capillaries) into a standard benchtop microcentrifuge. ⚠ Place the centrifuge adapters in a balanced arrangement within the centrifuge. • Centrifuge at 700 × *g* for 5 s (3,000 rpm in a standard benchtop microcentrifuge). • Alternatively, use the LC Carousel Centrifuge centrifuge for spinning the capillaries.
❻	Transfer the capillaries into the LightCycler® Sample Carousel.
❼	Cycle the samples as described above.

2.3 Related Procedures

Hot-Start

If the reaction components are thoroughly mixed prior to the initial heat denaturation step, non-specific annealing and primer elongation events may occur. Conventional manual hot-start or wax techniques cannot be used with the LightCycler® 2.0 System due to the small capillaries. However, you can run PCR applications that use the SYBR Green I detection format in hot-start mode by using the LightCycler® FastStart DNA Master SYBR Green I or LightCycler® FastStart DNA Master^{PLUS} SYBR Green I, which contain a chemically modified Taq DNA Polymerase, FastStart Taq DNA polymerase, that is activated by heat.

Prevention of Carry-Over Contamination

Uracil-DNA N-Glycosylase (UNG) is suitable for preventing carry-over contamination in PCR. This carry-over prevention technique involves incorporating deoxyuridine triphosphate (dUTP, a component of the reaction mixes of all LightCycler® reagent kits) into amplification products, then pretreating later PCR mixtures with UNG. If a dUTP-containing contaminant is present in the later PCRs, it will be cleaved by a combination of the UNG and the high temperatures of the initial denaturation step; it will not serve as a PCR template.

⚠ Use Uracil-DNA Glycosylase, heat-labile* in combination with LightCycler® DNA Master SYBR Green I. Proceed as described in the package insert. LightCycler® Uracil-DNA Glycosylase is to be used in combination with LightCycler® FastStart DNA Masters only.

Since your target DNA template contains thymidine rather than uridine, it is not affected by this procedure.

The use of UNG lowers the melting temperature (T_m) in melting curve analysis by approx. 1°C.

Two-step RT-PCR The LightCycler® DNA Master SYBR Green I can also be used to perform two-step RT-PCR. In two-step RT-PCR, the reverse transcription of RNA into cDNA is separated from the other reaction steps and is performed outside the LightCycler® 2.0 System. Subsequent amplification and online monitoring is performed according to the standard LightCycler® 2.0 System procedure, using the cDNA as the starting sample material. One of the following reagents is required for reverse transcription of RNA into cDNA:

- Transcriptor Reverse Transcriptase*
- Transcriptor First Strand cDNA Synthesis Kit*
- First Strand cDNA Synthesis Kit for RT-PCR (AMV)*

Synthesis of cDNA is performed according to the detailed instructions provided with the cDNA synthesis reagent.

⚠ Do not use more than 8 µl of undiluted cDNA template per 20 µl final reaction volume because greater amounts may inhibit PCR. For initial experiments, we recommend running undiluted, 1:10 diluted, and 1:100 diluted cDNA template in parallel to determine the optimum template amount.

3. Results

Quantification Analysis The following amplification curves were obtained using the LightCycler® DNA Master SYBR Green I in combination with the LightCycler® Control Kit DNA targeting human β-globin gene. The fluorescence values versus cycle number are displayed. 30 pg (approx. 10 genome equivalents) of human genomic DNA can be reproducibly detected by amplifying in the LightCycler® 2.0 System and using SYBR Green I as detection format. 3 pg (approx. 1 genome equivalent) are sporadically detected due to statistical fluctuations.

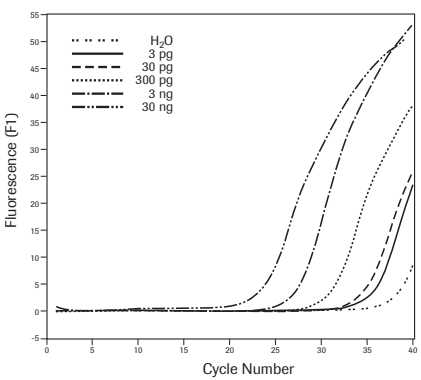

Fig. 1: Serially diluted samples containing 30 ng, 3 ng, 300 pg, 30 pg, or 3 pg human genomic DNA as starting template were amplified using the LightCycler® DNA Master SYBR Green I. As a negative control, template DNA was replaced by PCR-grade water. Arithmetic background subtraction was applied and the fluorescence channel was set to F1/1.

Melting Curve Analysis

Specificity of the amplified PCR product was assessed by performing a melting curve analysis. The resulting melting curves allow discrimination between primer-dimers and specific product. The specific β-globin product melts at a higher temperature than the primer-dimers. The melting curves display the specific amplification of the β-globin gene when starting from 30 ng, 3 ng, 300 pg, 30 pg, or 3 pg human genomic DNA. Additionally, small amounts of PCR by-products are detected when starting from 30 pg and 3 pg human genomic DNA. In the negative control sample, only primer-dimers were amplified.

⚠ Smaller reaction volumes may result in melting temperature variations.

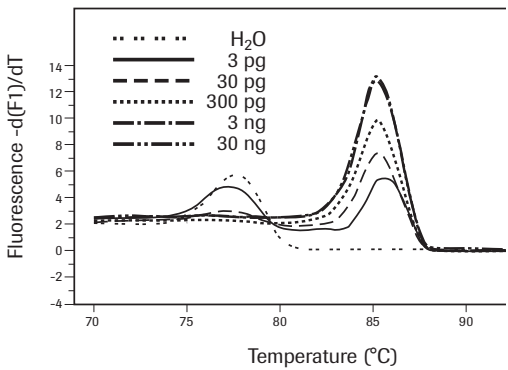

Fig. 2: Melting curve analysis of amplified samples containing 30 ng, 3 ng, 300 pg, 30 pg or 3 pg human genomic DNA as starting template. As a negative control, template DNA was replaced by PCR-grade water.

	Possible cause	Recommendation
Amplification reaches plateau phase before the program is complete	Starting amount of nucleic acid is very high.	Stop the program by clicking on the **End Program** button. The next cycle program will start automatically.
	The number of cycles is too high.	Reduce the number of cycles in the cycle program.
Log-linear phase of amplification just starts as the amplification program finishes	Starting amount of nucleic acid is very low.	• Improve PCR conditions (*e.g.*, $MgCl_2$ concentration, primer and probe design). • Use more starting material • Repeat the run.
	The number of cycles is too low.	Increase the number of cycles in the cycle program.
No amplification occurs	Using wrong channel to display amplification onscreen.	Change the channel setting on the programming screen. (The data obtained up to this point will be saved.)
	Pipetting errors or omitted reagents.	• Check for missing reagents. • Titrate $MgCl_2$ concentration. • Check for missing or defective dye.
	Chosen gain settings are too low.	Optimize gain setting using the **Real Time Fluorimeter** function. Then repeat the run, using the optimal gain settings in the cycle programs.
	Scale of axes on graph are unsuitable for analysis.	Change the values for the x- and y-axis by double-clicking on the maximum and/or minimum values, then changing to suitable values.
	Measurements do not occur.	Check the cycle programs. For SYBR Green I detection format, choose "single" as the acquisition mode at the end of the elongation phase.
	Amplicon length is >1 kb.	Do not use amplicons >1 kb. Optimal results are obtained with amplicons of 700 bp or less
	Impure sample material inhibits reaction.	• Do not use more than 8–10 µl of DNA per 20 µl PCR reaction mixture. • Repurify the nucleic acids to ensure removal of inhibitory agents
Fluorescence intensity is too high and reaches overflow	Gain settings are too high.	Gain settings cannot be changed during or after a run, so you must repeat the run. Before repeating the run, use the **Real Time Fluorimeter** option to find suitable gain settings. For SYBR Green I, the background fluorescence at measuring temperature should not exceed 10. ⚠ Use an extra sample for this procedure, so the dyes in your experimental samples will not be bleached. LightCycler® software versions 3.5 and higher do not require a gain setting.
Fluorescence intensity is too low	Deterioration of dye in reaction mixtures; dyes not stored properly	• Store the dye containing reagents at −15 to −25°C, and keep them away from light. • Avoid repeated freezing and thawing. • After thawing, store the LightCycler® DNA Master SYBR Green I at +2 to +8°C for a maximum of 2 weeks and keep it away from light.
	Reaction conditions are not optimized, leading to poor PCR efficiency	• Titrate $MgCl_2$ concentration. • Primer concentration should be between 0.3 and 1.0 µM • Check annealing temperature of primers. • Check experimental protocol. • Always run a positive control along with your samples.

continued on next page

	Possible cause	Recommendation
Fluorescence intensity varies	PCR mix is still in the upper part of the capillary. Air bubble is trapped in the capillary tip.	Repeat capillary centrifugation step.
	Skin oils on the surface of the capillary tip	Always wear gloves when handling the capillaries.
Amplification curve reaches plateau at a lower signal level than the other samples	Starting amount of genomic DNA is too high; DNA captures dye, producing a high background signal. There is not enough dye left to monitor the increase of fluorescence signal during amplification.	• Do not use more than 50 ng of complex genomic DNA in a 20 µl reaction. • Use the HybProbe format (which allows analysis of up to 500 ng DNA) instead of SYBR Green I.
	Dye bleached.	Make sure the reagents containing the dye are stored away from light. Avoid repeated freezing and thawing.
Negative control samples give a positive signal	Contamination, or presence of primer-dimers.	• Remake all critical solutions. • Pipet reagents on a clean bench. • Close lid of the negative control reaction immediately after pipetting it. • Use heat-labile UNG to eliminate carry-over contamination.
Melting peak is very broad and peaks cannot be differentiated	°C to Average setting is too high.	Reduce the value of **°C to Average** (only applicable for LightCycler® software version 3.5).
Double melting peak appears for one product	Two products of different length or GC content have been amplified (e.g., due to pseudogenes or mispriming)	• Check products on an agarose gel • Elevate the reaction stringency by: – redesigning the primers, – checking the annealing temperature, – performing a "touch-down" PCR, or – using HybProbes for better specificity.
Melting temperature of a product varies from experiment to experiment	Variations in reaction mixture (*e.g.*, salt concentration).	• Check purity of template solution. • Reduce variations in parameters such as $MgCl_2$, heat-labile UNG, hot-start antibody, and program settings.
Only a primer-dimer peak appears, with no specific PCR product peak seen; or very high primer-dimer peaks	Primer-dimers have out-competed specific PCR product for available primers.	• Keep all samples at +2 to +8°C until the run is started. • Keep the time between preparing the reaction mixture and starting the run as short as possible. • Increase starting amount of DNA template. • Titrate $MgCl_2$. • Increase annealing temperature in order to enhance stringency.
	Quality of primer is poor.	• Purify primer more thoroughly. • Use a hot-start method.
	Sequence of primer is inappropriate.	Redesign primer.
Primer-dimer and product peaks are very close together	Unusually high GC-content of the primers.	• Redesign primers. • Run melting curve at lowest ramping rate (0.1 °C/sec with continuous measurement) • Expand scale of the x-axis. • Reduce the value of°C to Average' (only applicable for LightCycler® software version 3.5).
Very broad primer-dimer peak with multiple peaks	Heterogeneous primers with primer-dimer variations (*e.g.*, concatamers, loops)	• Redesign primers. • Use hot-start method.
One peak of the same height occurs in all samples	Contamination in all samples.	• Close capillaries during centrifugation step. • Use fresh solutions.

5. Additional Information on this Product

How this Product Works

LightCycler® DNA Master SYBR Green I is a ready-to-use PCR reaction mix. This product is designed specifically for real-time PCR assays in 20 µl glass capillaries using the SYBR Green I detection format on the LightCycler® 2.0 System. LightCycler® DNA Master SYBR Green I provides convenience, excellent performance and reproducibility, as well as minimizing contamination risk. All you have to supply is template DNA, PCR primers, and additional $MgCl_2$ (if necessary).

Test Principle

Generation of PCR products can be detected by measurement of the SYBR Green I fluorescence signal. SYBR Green I intercalates into the DNA double helix. In solution, the unbound dye exhibits very little fluorescence; however, fluorescence (wavelength, 530 nm) is greatly enhanced upon DNA-binding. Therefore, during PCR, the increase in SYBR Green I fluorescence is directly proportional to the amount of double-stranded DNA generated. Since SYBR Green I dye is very stable (only 6% of the activity is lost during 30 amplification cycles) and the LightCycler® Instruments' optical filter set matches the wavelengths of excitation and emission, it is the reagent of choice when measuring total DNA.

The basic steps of DNA detection by SYBR Green I during real-time PCR on the LightCycler® 2.0 System are:

① At the beginning of amplification, the reaction mixture contains the denatured DNA, the primers, and the dye. The unbound dye molecules weakly fluoresce, producing a minimal background fluorescence signal which is subtracted during computer analysis.
② After annealing of the primers, a few dye molecules can bind to the double strand. DNA binding results in a dramatic increase of the SYBR Green I molecules to emit light upon excitation.
③ During elongation, more and more dye molecules bind to the newly synthesized DNA. If the reaction is monitored continuously, an increase in fluorescence is viewed in real-time. Upon denaturation of the DNA for the next heating cycle, the dye molecules are released and the fluorescence signal falls.
④ Fluorescence measurement at the end of the elongation step of every PCR cycle is performed to monitor the increasing amount of amplified DNA.

To prove that only your desired PCR product has been amplified, you may perform a melting curve analysis after PCR. In melting curve analysis the reaction mixture is slowly heated to 95°C, which causes melting of double-stranded DNA and a corresponding decrease of SYBR Green I fluorescence. The instrument continuously monitors this fluorescence decrease and displays it as melting peaks. Each melting peak represents the characteristic melting temperature (T_m) of a particular DNA product (where the DNA is 50% double-stranded and 50% single-stranded). The most important factors that determine the T_m of dsDNA are the length and the GC-content of that fragment. If PCR generated only one amplicon, melting curve analysis will show only one melting peak. If primer-dimers or other non-specific products are present, they will be shown as additional melting peaks. Checking the T_m of a PCR product can thus be compared with analyzing a PCR product by length in gel electrophoresis.

References

1 Plachy, R et al. (2005). McRAPD as a new approach to rapid and accurate identification of pathogenic yeasts. Journal of Microbiological Methods 60, 107-13.

2 Seino, A et al. (2005). Translational control by internal ribosome entry site in Saccharomyces cerevisiae. *Biochimica Et Biophysica Acta (BBA) - Gene Structure and Expression* **1681**, 166-74.

3 Fasshauer, M et al. (2004). Interleukin-6 is a positive regulator of tumor necrosis factor [alpha]-induced adipose-related protein in 3T3-L1 adipocytes. *FEBS Letters* **560**, 153-7.

4 Hartmann, J et al. (2004). Distinct Roles of G{alpha}q and G{alpha}11 for Purkinje Cell Signaling and Motor Behavior. *J Neurosci* **24**, 5119-30.

5 Jiang, MZ et al. (2004). Effects of antioxidants and nitric oxide on TNF-[alpha]-induced adhesion molecule expression and NF-[kappa]B activation in human dermal microvascular endothelial cells. *Life Sciences* **75**, 1159-70.

6 Kilian, O et al. (2004). Expression of EDA+ and EDB+ fibronectin splice variants in bone. *Bone* **35**, 1334-45.

7 Ohtani, S et al. (2004). Quantitative analysis of p53-targeted gene expression and visualization of p53 transcriptional activity following intratumoral administration of adenoviral p53 in vivo. *Mol Cancer Ther* **3**, 93-100.

8 Schalk, JAC et al. (2004). Estimation of the number of infectious measles viruses in live virus vaccines using quantitative real-time PCR. *Journal of Virological Methods* **117**, 179-87.

9 Broberg, EK et al. (2003). Low copy number detection of herpes simplex virus type 1 mRNA and mouse Th1 type cytokine mRNAs by Light Cycler quantitative real-time PCR. *Journal of Virological Methods* **112**, 53-65.

10 Moeller, F et al. (2003). New tools for quantifying and visualizing adoptively transferred cells in recipient mice. Journal of Immunological Methods 282, 73-82.

11 Wu, CC et al. (2003). Nuclear Factor of Activated T Cells c Is a Target of p38 Mitogen-Activated Protein Kinase in T Cells. *Mol Cell Biol* **23**, 6442-54.

12 Odemis, V et al. (2002). Interleukin-6 and cAMP Induce Stromal Cell-derived Factor-1 Chemotaxis in Astroglia by Up-regulating CXCR4 Cell Surface Expression. Implications for Brain Inflammation. *J Biol Chem* **277**, 39801-8.

13 Worm, J et al. (2001). In-Tube DNA Methylation Profiling by Fluorescence Melting Curve Analysis. *Clin Chem* **47**, 1183-9.

Quality Control

The LightCycler® DNA Master SYBR Green I is function tested using the LightCycler® 2.0 System.

6. Supplementary Information

6.1 Conventions

Text Conventions

To make information consistent and memorable, the following text conventions are used in this Instruction Manual:

Text Convention	Usage
Numbered stages labeled ①, ②, etc.	Stages in a process that usually occur in the order listed.
Numbered instructions labeled ❶, ❷, etc.	Steps in a procedure that must be performed in the order listed.
Asterisk *	Denotes a product available from Roche Applied Science.

Symbols

In this Instruction Manual, the following symbols are used to highlight important information:

Symbol	Description
	Information Note: Additional information about the current topic or procedure.
	Important Note: Information critical to the success of the procedure or use of the product.

Abbreviations

In this Instruction Manual the following abbreviations are used:

Abbreviation	Meaning
CP	crossing point
dsDNA	double-stranded DNA
RT	room temperature
T_m	melting temperature
UNG	Uracil-DNA N-Glycosylase

6.2 Changes to Previous Version

Editorial changes only

6.3 Ordering Information

Roche Applied Science offers a large selection of reagents and systems for life science research. For a complete overview of related products and manuals, please visit and bookmark our home page, www.roche-applied-science.com, and our Special Interest Sites including:

- The LightCycler® 2.0 System family for real-time, online PCR:
  http://www.lightcycler-online.com
- the MagNA Pure Family for automated nucleic acid isolation:
  http://www.magnapure.com

	Product	Pack Size	Cat. No.
Instrument and Accessories	LightCycler® 2.0 Instrument	1 instrument plus accessories	03 351 414 001
	LightCycler® 1.5 Instrument	1 instrument plus accessories	04 484 495 001
	LightCycler® Capillaries (20 µl)	1 pack (8 boxes, each with 96 capillaries and stoppers)	11 909 339 001
	LC Carousel Centrifuge 2.0 Rotor Set	1 rotor + 2 buckets	03 724 697 001
	LC Carousel Centrifuge 2.0 Bucket 2.1	1 bucket	03 724 689 001
	LC Carousel Centrifuge 2.0	1 centrifuge plus rotor and bucket	03 709 507 001 (115 V) 03 709 582 001 (230 V)
	MagNA Pure LC Instrument	1 instrument plus accessories	12 236 931 001
LightCycler® Kits for PCR	LightCycler® DNA Master HybProbe	1 kit (96 reactions) 1 kit (480 reactions)	12 015 102 001 2 158 825
	LightCycler® FastStart DNA Master HybProbe	1 kit (96 reactions) 1 kit (480 reactions)	03 003 248 001 12 239 272 001
	LightCycler® FastStart DNA Master^{PLUS} HybProbe	1 kit (96 reactions) 1 kit (480 reactions)	03 515 575 001 03 515 567 001
	LightCycler® FastStart DNA Master^{PLUS} HybProbe, 100 µl Reactions	1 kit (384 reactions)	03 752 178 001
	LightCycler® FastStart DNA Master SYBR Green I	1 kit (96 reactions) 1 kit (480 reactions)	03 003 230 001 12 239 264 001
	LightCycler® FastStart DNA Master^{PLUS} SYBR Green I	1 kit (96 reactions) 1 kit (480 reactions)	03 515 869 001 03 515 885 001
	LightCycler® FastStart DNA Master^{PLUS} SYBR Green I, 100 µl Reactions	1 kit (384 reactions)	03 752 186 001
cDNA Synthesis Reagents	Transcriptor Reverse Transcriptase	250 U 500 U 2000 U	03 531 317 001 03 531 295 001 03 531 287 001
	Transcriptor First Strand cDNA Synthesis Kit	1 kit	04 379 012 001
	First Strand cDNA Synthesis Kit for RT-PCR (AMV)	1 kit (30 reactions)	11 483 188 001
Associated Kits and Reagents	Uracil-DNA Glycosylase, heat-labile	100 U	11 775 367 001
	LightCycler® Control Kit DNA	1 kit (50 reactions)	12 158 833 001
	High Pure PCR Template Preparation Kit	1 kit (100 purifications)	11 796 828 001
	High Pure Plasmid Isolation Kit	1 kit (50 purifications) 1 kit (250 purifications)	11 754 777 001 11 754 785 001

6.4 Disclaimer of License

LIMITED LICENSE: A license under the non-U.S. counterparts of U.S. Patents Nos. 4,683,202, 4,683,195 and 4,965,188 owned by F. Hoffmann-La Roche Ltd, for use in research and development, has an up-front fee component and a running-royalty component. The purchase price of the product includes a limited, non-transferable license under the running-royalty component to use only this amount of product to practice the Polymerase Chain Reaction (PCR) and related processes described in said patents solely for the research and development activities of the purchaser when this product is used in conjunction with a thermal cycler whose use is covered by the up-front fee component. Rights to the up-front fee component must be obtained by the end user in order to have a complete license. These rights under the up-front fee component may be purchased from Applied Biosystems or obtained by purchasing an Authorized Thermal Cycler. This product is also an authorized reagent and may also be used under service sublicenses from Applied Biosystems under the foregoing patents.

The purchase price of this product includes a limited, non-transferable license under U.S. Patent Nos. 5,994,056 and 6,171,785 and corresponding patent claims outside the United States, owned by Roche Molecular Systems, Inc. and F. Hoffmann-La Roche Ltd ("Roche"), and under U.S. Patent No. 6,569,627 and corresponding patent claims outside the United States, licensed from Idaho Technology Inc., for using only this amount of the product for dsDNA-binding dye processes covered by said patent solely for the purchaser's own internal research and development activities. This product is also a Licensed Dye Binding Kit for use with service sublicenses available from Applied Biosystems.

No right under any other patent claims (such as apparatus or system claims in U.S. Patent No. 6,814,934) and no right to use this product for any other purpose or for commercial services of any kind, including without limitation reporting the results of purchaser's activities for a fee or other commercial consideration, is hereby granted expressly, by implication, or by estoppel. This product is for research use only. Diagnostic uses require a separate license from Roche.

Further information on purchasing licenses to practice real-time PCR processes may be obtained by contacting the Director of Licensing at Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, USA.

The technology used for the LightCycler® System is licensed from Idaho Technology Inc., Salt Lake City, UT, USA.

Purchase of this product includes a limited non-transferable end-user license to the purchaser under the SYBR Green I Technology owned by Idaho Technology under U.S. Patents 6,569,627 and foreign counterparts to use this product for any purpose.

The product is covered in-part by US 5,871,908, co-exclusively licensed from Evotec OAI AG.

6.5 Trademarks

LIGHTCYCLER, LC, FASTSTART, HYBPROBE, MAGNA PURE, and HIGH PURE are trademarks of Roche.

SYBR is a Trademark of Molecular Probes, Inc., Eugene, OR, USA. SYBR Green I is manufactured by Molecular Probes, Inc., and is provided under license from Molecular Probes, Inc., for direct research use for PCR, where the dye is present during the PCR.

Contact and Support

If you have questions or experience problems with this or any Roche Applied Science (RAS) product, please contact our Technical Support staff. Our scientists commit themselves to providing rapid and effective help.
We also want you to contact us if you have suggestions for enhancing RAS product performance or using our products in new or specialized ways. Such customer information has repeatedly proven invaluable to RAS and the world-wide research community.

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support** Site at:

www.roche-applied-science.com/support

To call, write, fax, or email us, visit the Roche Applied Science home page, www.roche-applied-science.com, and select your home country.
Country-specific contact information will be displayed.
On the Roche Applied Science home page select **Printed Materials** to find:

- in-depth Technical Manuals
- Lab FAQS: Protocols and references for life science research
- our quarterly Biochemica Newsletter
- Material Safety Data Sheets
- Pack Inserts and Product Instructions

or to request hard copies of printed materials.

0905.**12036827001**Ⓒ

Roche Diagnostics

Roche Diagnostics GmbH
Roche Applied Science
68298 Mannheim
Germany