For general laboratory use. Not for use in diagnostic procedures. FOR *IN VITRO* USE ONLY.

LightCycler® DNA Master SYBR Green I

Version April 2008

Easy-to-use Reaction Mix for PCR using the LightCycler® Carousel-Based 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!

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PROTOCOL

1. What this Product Does

Number of Tests

The kit is designed for 96 or 480 reactions (depeding 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® Carousel-Based System include:

- LightCycler® Carousel-Based System* (LightCycler® 2.0 Instrument* or LightCycler® 1.5 Instrument*, or an instrument version below)
- LightCycler® Capillaries*
- Standard benchtop microcentrifuge containing a rotor for 2.0 ml reaction tubes

The LightCycler® Carousel-Based System provides Centrifuge 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.

Application

LightCycler® DNA Master SYBR Green I is designed for research studies. When combined with the LightCycler® Carousel-Based 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® Carousel-Based 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® Carousel-Based System.

Assay Time

Procedure	Time
Optional: dilution of template DNA	5 min
PCR setup	15 min
LightCycler® 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 one of the following:
  - the MagNA Pure LC Instrument with a dedicated MagNA Pure LC reagent kit (for medium throughput automated isolation)
  - the MagNA Pure Compact Instrument with a dedicated MagNA Pure Compact reagent kit (for low throughput automated isolation).
  - a High Pure nucleic acid isolation kit (for manual isolation)

For further information, consult the Roche Applied Science Biochemicals catalog or home page: www.roche-applied-science.com. See Ordering Information for selected products, recommended for isolation of template DNA.

- 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.

Q 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.

Primers

Use PCR primers at a final concentration of 0.2 to 1 µM. The recommended starting concentration is 0.5 µM each.

$MgCl_2$

To ensure specific and efficient amplification with the LightCycler® Carousel-Based 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 optimal concentration for PCR with the LightCycler® Carousel-Based 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

The volume of water in the PCR reaction must be reduced, accordingly.

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).

2.2 Experimental Protocol

LightCycler® Carousel-Based System Protocol

The following procedure is optimized for use with the LightCycler® Carousel-Based System.

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

A LightCycler® Carousel-Based System protocol that uses the 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/amplicon analysis
- **Cooling** the rotor and the thermal chamber

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

⚠ 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® Carousel-Based 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		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

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

[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 to 5°C/s.

[4] For typical primers, choose an incubation time of 0 to 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 4.1		
Default Channel	fluorescence channel 530	
Fluorescence Gains	not required	
"Max. Seek Pos."	Enter the number of sample positions for which the instrument should look.	
"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 pre-cooled centrifuge adapters or in a LightCycler® Sample Carousel in a pre-cooled LC Carousel Centrifuge Bucket.

❷
- Thaw the solutions and for maximal recovery of contents, briefly spin vials in a microcentrifuge before opening.
- Mix carefully by pipetting up and down and store on ice.

❸ 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 10× conc. solution would contain a 5 µM concentration of each primer.

❹ 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	0.2 to 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.

❺
- Mix gently by pipetting up and down. Do not vortex.
- Pipette 18 µl PCR mix into each pre-cooled LightCycler® Capillary.
- Add 2 µl of the DNA template
- Seal each capillary with a stopper.

❻
- Place the centrifuge 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 for spinning the capillaries.

❼ Transfer the capillaries into the LightCycler® Sample Carousel and then into the LightCycler® Instrument.

❽ 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® Carousel-Based 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.

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

Prevention of Carry-Over Contamination

Uracil-DNA Glycosylase, heat-labile (UNG, heat-labile) 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 and in the table below to prevent carry-over contamination. LightCycler® Uracil-DNA Glycosylase is to be used in combination with LightCycler® FastStart DNA Masters only.

❶	Add 1 µl UNG, heat-labile to the master mix per 20 µl final reaction volume.
❷	Add template DNA and incubate the completed reaction mixture for 5 min at room temperature.
❸	Destroy any contaminating template and inactivate the UNG enzyme by performing the initial denaturation step for 2 min at 95°C.

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

When performing the Melting Curve analysis, the use of UNG might lower the melting temperature (T_m) 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® Carousel-Based System. Subsequent amplification and online monitoring is performed according to the standard LightCycler® Carousel-Based 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 optimal 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. Thirty pg (approx. 10 genome equivalents) of human genomic DNA can be reproducibly detected by amplifying in the LightCycler® Carousel-Based System and using the SYBR Green I detection format. Three pg (approx. 1 genome equivalent) are sporadically detected, due to statistical fluctuations.

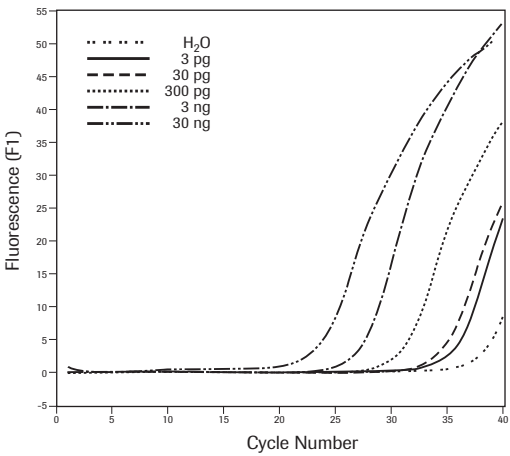

Fig. 1: Serially diluted samples containing 30 ng, 3 ng, 300 pg, 30 pg, or 3 pg human genomic DNA as starting template were amplified using the LightCycler® DNA Master SYBR Green I. As a negative control, template DNA was replaced by PCR-grade water.

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 PCR 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.

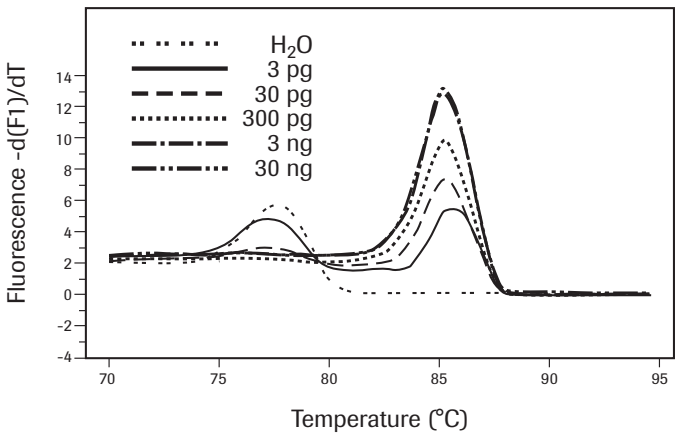

Fig. 2: Melting Curve analysis of amplified samples containing 30 ng, 3 ng, 300 pg, 30 pg or 3 pg human genomic DNA as starting template. As a negative control, template DNA was replaced by PCR-grade water.

4. Troubleshooting

	Possible cause	Recommendation
Amplification reaches plateau phase before the program is complete.	Very high starting amount of nucleic acid.	The program can be finished 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 amplification program.
Log-linear phase of amplification just starts when the amplification program finishes.	Very low starting amount of nucleic acid.	• Improve PCR conditions (*e.g.,* $MgCl_2$ concentration, primer concentration or design). • Use higher amount of starting template. • Repeat the run.
	The number of cycles is too low.	Increase the number of cycles in the amplification program.
No amplification occurs.	Wrong channel has been chosen to display amplification online.	Change the channel setting in 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 settings 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 change to more suitable values.
	Measurements do not occur.	Check the amplification program. 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 the reaction.	• Do not use more than 8 to 10 µl of DNA per 20 µl PCR reaction mixture. • Repurify the nucleic acids to ensure removal of inhibitory agents.

	Possible cause	Recommendation
Fluorescence intensity is too high and reaches overflow.	Unsuitable gain settings	Gain settings cannot be changed during or after a 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.	Low concentration or deterioration of SYBR Green I in the reaction mixtures, due to unsuitable storage conditions.	• Store the SYBR Green I 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 0.2 and 1.0 mM • Check annealing temperature of primers. • Check experimental protocol. • Always run a positive control along with your samples.
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, leading to a high background signal. Insufficient amounts of dye are 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.	Ensure the reagents containing the dye are stored away from light. Avoid repeated freezing and thawing.
Negative control samples are positive.	Contamination, or presence of primer-dimers.	• Remake all critical solutions. • Pipette 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.

	Possible cause	Recommendation
Melting peak is very broad and peaks can not be differentiated.	°C to Average setting is too high.	Reduce the value of **°C to Average** (only applicable for LightCycler® Software versions prior to version 4.0).
Double melting peak appears for one product.	Two products of different length or GC content have been amplified (*e.g.,* due to pseudogenes or misprim-ing).	• 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 mix-ture (*e.g.,* salt concentra-tion)	• 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 amplification of specific PCR product.	• 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 possi-ble. • Increase starting amount of DNA template. • Titrate $MgCl_2$. • Increase annealing temperature in order to enhance stringency.
	Quality of the primers are poor.	• Purify primers more thoroughly. • Use a hot-start method.
	Sequence of the primers are inappropriate.	Redesign primers.
Primer-dimer and product peaks are very close together.	Unusually high GC-content of the primers	• Redesign primers. • Run melting curve at the 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 versions prior to version 4.0).
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 sam-ples	• Close capillaries during centrifugation step. • Use fresh solutions.

5. Additional Information on this Product

5.1 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® Carousel-Based System. LightCycler® DNA Master SYBR Green I provides convenience, excellent performance and reproducibility, as well as minimizing contamination risk. All you must 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® Carousel-Based 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 in 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 Tm 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 Tm of a PCR product can thus be compared with analyzing a PCR product by length in gel electrophoresis.

5.2 Quality Control

The LightCycler® DNA Master SYBR Green I is function tested using the LightCycler® Carousel-Based System.

5.3 References

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6. Supplementary Information

6.1 Conventions

6.1.1 Text Conventions

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.

6.1.2 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.

6.1.3 Abbreviations

In this Instruction Manual the following abbreviations are used:

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

6.2 Changes to Previous Version

- New Disclaimer on page 1
- Editorial changes and catalogue number changes and additions.

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	03 531 414 001
	LightCycler® 1.5 Instrument	1 instrument plus accessories	04 484 495 001
	LightCycler® Capillaries (20 µl)	1 pack (5 boxes, each with 96 capillaries and stoppers)	04 929 292 001
	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
	MagNA Pure Compact Instrument	1 instrument plus accessories	03 731 146 001
DNA Isolation Kits	MagNA Pure LC DNA Isolation Kit I	1 kit (192 isolations)	03 003 990 001
	MagNA Pure LC DNA Isolation Kit II (Tissue)	1 kit (192 isolations)	03 186 229 001
	MagNA Pure LC DNA Isolation Kit III (Bacteria, Fungi)	1 kit (192 isolations)	03 264 785 001
	MagNA Pure LC DNA Isolation Kit - Large Volume	1 kit (96 - 288 isolations)	03 310 515 001

	Product	Pack Size	Cat. No.
	MagNA Pure LC Total Nucleic Acid Isolation Kit	1 kit (192 isolations)	03 038 505 001
	MagNA Pure LC Total Nucleic Acid Isolation Kit - Large Volume	1 kit (192 isolations)	03 264 793 001
	MagNA Pure Compact Nucleic Acid Isolation Kit I	1 kit (32 isolations)	03 730 964 001
	MagNA Pure Compact Nucleic Acid Isolation Kit I - Large Volume	1 kit (32 isolations)	03 730 972 001
LightCycler® Kits for PCR	LightCycler® DNA Master HybProbe	1 kit (96 reactions) 1 kit (480 reactions)	12 015 102 001 12 158 825 001
	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) 1 kit (384 reactions, 100 µl)	03 515 575 001 03 515 567 001 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) 1 kit (384 reactions, 100 µl)	03 515 869 001 03 515 885 001 03 752 186 001
cDNA Synthesis Reagents	Transcriptor Reverse Transcriptase	250 U 500 U 2,000 U	03 531 317 001 03 531 295 001 03 531 287 001
	Transcriptor First Strand cDNA Synthesis Kit	1 kit (50 reactions, incl. 10 control reactions) 1 kit (100 reactions) 1 kit (200 reactions)	04 379 012 001 04 896 866 001 04 897 030 001
	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 500 U	11 775 367 001 11 775 375 001
	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

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