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

Roche Applied Science

LightCycler® FastStart DNA Master SYBR Green I

Version September 2005

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

Cat. No. 03 003 230 001 Kit for 96 reactions
Cat. No. 12 239 264 001 Kit for 480 reactions

Store the kit at −15 to −25°C

⚠ Keep LightCycler® FastStart Reaction Mix SYBR Green I (vial 1b, 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 (depending on pack size) with a final reaction volume of 20 µl each.

Kit Contents

Vial/Cap	Label	Contents/Function a) Cat. No. 03 003 230 001 (96 reactions) b) Cat. No. 12 239 264 001 (480 reactions)
1a colorless cap	LightCycler® Fast-Start Enzyme	a) 1 × vial 1a, 3 × vial 1b for 3 × 64 µl LightCycler® FastStart DNA Master SYBR Green I (10× conc.) b) 5 × vial 1a, 15 × vial 1b for 15 × 64 µl LightCycler® FastStart DNA Master SYBR Green I (10× conc.) • Ready-to-use hot-start PCR reaction mix (after pipetting 10 µl from vial 1a into one vial 1b). • Contains FastStart Taq DNA Polymerase, reaction buffer, dNTP mix (with dUTP instead of dTTP), SYBR Green I dye, and 10 mM $MgCl_2$
1b green cap	LightCycler® Fast-Start Reaction Mix SYBR Green I, 10×conc.	
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® FastStart Reaction Mix SYBR Green I (vial 1b, 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
1a colorless cap	LightCycler® Fast-Start Enzyme	• Store at −15 to−25°C. • **Avoid repeated freezing and thawing!**
1b green cap	LightCycler® Fast-Start Reaction Mix SYBR Green I, 10× conc.	• Keep vial 1b away from light

Vial	Label	Storage
1 green cap (after addition of 1a to 1b)	LightCycler® FastStart DNA Master SYBR Green I	• Store at −15 to−25°C for a maximum of three months. • After thawing store at +2 to +8°C for a maximum of 1 week. • **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

Refer to the list below for additional reagents and equipment required to perform reactions with the LightCycler® FastStart DNA Master SYBR Green I using the LightCycler® System:

- 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 Carousel (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 Carousel to the former LC Carousel Centrifuge, you need the LC Carousel Centrifuge 2.0 Rotor Set*.

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

‡ For prevention of carry-over contamination; see section Related Procedures for details. Use LightCycler® Uracil-DNA Glycosylase in combination with LightCycler® FastStart DNA Masters only.

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

Application

LightCycler® FastStart DNA Master SYBR Green I is designed for research studies. When used with the LightCycler® 2.0 System, this kit is ideally suited for hot-start PCR applications. In combination with the LightCycler® 2.0 System and suitable 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® FastStart DNA Master SYBR Green I can be used for the amplification and detection of any DNA or cDNA target. However, you would need to adapt your detection protocol to the reaction conditions of the LightCycler® Instrument and design specific PCR primers for each target. Refer to the LightCycler® Operator's Manual for general recommendations.

LightCycler® FastStart DNA Master SYBR Green I can also be used with LightCycler® Uracil-DNA Glycosylase 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
LightCycler® PCR run (incl. Melting Curve)	45 min
Total assay time	**65 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® FastStart 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® protocol that uses LightCycler® FastStart DNA Master SYBR Green I contains the the following programs:

- **Pre-Incubation** activation of FastStart DNA polymerase and denaturation of the 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® PCR Run with the LightCycler® FastStart DNA Master SYBR Green I.

Analysis Mode	Cycles	Segment	Target Temperature [1)]	Hold Time	Acquisition Mode
Pre-Incubation					
None	1		95°C	10 min [4)]	none
Amplification					
Quantification	45	Denaturation	95°C	10 s	none
		Annealing	primer dependent [2)]	0 – 10 s [5)]	none
		Extension	72°C [3)]	= (amplicon [bp]/25) s [6)]	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)] If high polymerase activity is required in early cycles, you can sometimes improve results by extending the pre-incubation to 15 min.

[5)] 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.

[6)] 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 instr ument versions below	
"Capillary Size"	Select "20 µl" as the capillary size for the experiment. ⚠ For the "6 Ch." instrument type only.	

Preparation of the Master Mix

❶ Thaw one vial of "Reaction Mix" (vial 1b, green cap) and shield it from light.

⚠ A reversible precipitate may form in the LightCycler® FastStart Reaction Mix SYBR Green I (vial 1b) during storage. If a precipitate is visible, place the Reaction Mix at 37°C and mix gently from time to time until the precipitate is completely dissolved. Recentrifuge to collect the reagent at the bottom of the tube, then put the vial back on ice. This treatment does not influence the performance in PCR.

❷ Briefly centrifuge one vial "Enzyme" (vial 1a, colorless cap) and the thawed vial of "Reaction Mix" (from Step 1).

❸ Pipet 10 µl from vial 1a (colorless cap) into vial 1b (green cap).

🔍 Each vial 1a contains enough enzyme for three vials of Reaction Mix (vial 1b).

❹ Mix gently by pipetting up and down.

⚠ Do not vortex.

❺ Re-label vial 1b (green cap) with the new labels (vial 1: LightCycler® FastStart DNA Master SYBR Green I) that are provided with the kit. Place one on the top of the cap and one on the side of the vial.

⚠ Always keep the Master Mix away from light!

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 LightCycler® 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 10× 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:

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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® FastStart 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.

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 (3000 rpm in a standard benchtop microcentrifuge).
- Alternatively, use the LightCycler® Carousel centrifuge for spinning the capillaries.

❻ Transfer the capillaries into the sample carousel of the LightCycler® Instrument.

❼ Cycle the samples as described above.

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.

⚠ If you use the LightCycler® FastStart DNA Master SYBR Green I, perform prevention of carry-over contamination with LightCycler® Uracil-DNA Glyocsylase*. Proceed as described in the package insert.

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® FastStart 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® System. Subsequent amplification and online monitoring is performed according to the standard LightCycler® 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® Fast-Start 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® Instrument and using SYBR Green I as detection format. 3 pg (approx. 1 genome equivalent) are sporadically detected due to statistical fluctuations.

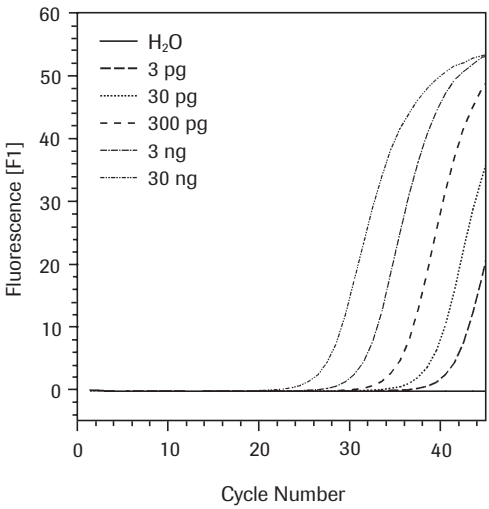

Fig. 1: Serially diluted samples containing 30 ng, 3 ng, 300 pg, 30 pg, or 3 pg human genomic DNA as starting template were amplified using the LightCycler® FastStart DNA Master SYBR Green I. As a negative control, template DNA was replaced by PCR-grade water. Arithmetic background subtraction was applied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

⚠ Smaller reaction volumes may result in melting temperature variations.

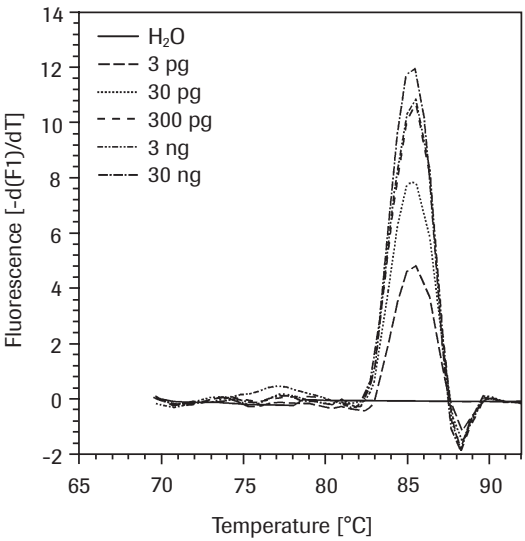

Fig. 2: Melting curve analysis of amplified samples containing 30 ng, 3 ng, 300 pg, 30 pg or 3 pg human genomic DNA as starting template. As a negative control, template DNA was replaced by PCR-grade water.

	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.)
	FastStart Taq DNA polymerase is not fully activated.	• Make sure PCR included a pre-incubation step at 95°C for 10 min. • Make sure denaturation time during cycles is 10 s.
	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. ⚠ LightCycler® software versions 3.5 and higher do not require a gain setting.
	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.

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	Possible cause	Recommendation
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 1 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.
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 LightCycler® 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 HybProbe Probes 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, primer preparation, and program settings.

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	Possible cause	Recommendation
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.
	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.*, concatemers, loops)	• Redesign primers.
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® FastStart DNA Master SYBR Green I is a ready-to-use reaction mix designed specifically for real-time PCR assays using the SYBR Green I detection format on the LightCycler® 2.0 System. It is used to perform hot-start PCR in 20 µl glass capillaries. Hot-start PCR has been shown to significantly improve the specificity and sensitivity of PCR (1–4) by minimizing the formation of non-specific amplification products at the beginning of the reaction.

FastStart Taq DNA Polymerase is a chemically modified form of thermostable recombinant Taq DNA polymerase that shows no activity up to 75°C. The enzyme is active only at high temperatures, where primers no longer bind non-specifically. The enzyme is completely activated (by removal of blocking groups) in a single pre-incubation step (95°C, 10 minutes) before cycling begins. Activation does not require the extra handling steps typical of other hot-start techniques.

The LightCycler® FastStart 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® 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.

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Quality Control

The LightCycler® FastStart 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

- Error in section “Storage and Stability” corrected: LightCycler® FastStart DNA Master SYBR Green I has to be stored at +2 to +8°C after first thawing, not at −15 to −25°C.

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, incl. disposable plastics, accessories, isolation kits, and other related products:
  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® DNA Master SYBR Green I	1 kit (96 reactions) 1 kit (480 reactions)	12 015 099 001 12 158 817 001
	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	LightCycler® Uracil-DNA Glycosylase	100 U (50 ml)	03 539 806 001
	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

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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.

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