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

LightCycler® FastStart DNA Master^{PLUS} SYBR Green I

Version October 2007

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

Cat. No. 03 515 869 001	Kit for 96 reactions (20 µl)
Cat. No. 03 515 885 001	Kit for 480 reactions (20 µl)
Cat. No. 03 752 186 001	Kit for 1920 reactions (20 µl) or 384 reactions (100 µl)

Store the kit at –15 to –25°C

www.roche-applied-science.com

Table of Contents

1. **What this Product Does** 3
Number of Tests 3
Kit Contents 3
Storage and Stability 4
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 Procedure 6
LightCycler® Carousel-Based System Protocol 6
Fluorescence and Run Setup Parameters 8
Preparation of the Master Mix 9
Preparation of the PCR Mix 9
2.3 Related Procedures 11
Prevention of Carry-Over Contamination 11
Two-step RT-PCR 11
3. **Results** 12
Quantification Analysis 12
Melting Curve Analysis 13
4. **Troubleshooting** 14
5. **Additional Information on this Product** 18
How this Product Works 18
Test Principle 19
References 20
Product Citations 20
Quality Control 20
6. **Supplementary Information** 21
6.1 Conventions 21
6.1.1 Text Conventions 21
6.1.2 Symbols 21
6.2 Changes to Previous Version 21
6.3 Ordering Information 22
6.4 Disclaimer of License 24
6.5 Trademarks 24

PROTOCOL

PROTOCOL

1. What this Product Does

Number of Tests

The kit is designed for:

- Cat. No. 03 515 869 001: 96 reactions with a reaction volume of 20 µl each
- Cat. No. 03 515 885 001: 480 reactions with a reaction volume of 20 µl each
- Cat. No. 03 752 186 001: 1920 reactions with a reaction volume of 20 µl each, or 384 reactions with a reaction volume of 100 µl each

Kit Contents

<table>
<tr><th>Vial/Cap</th><th>Label</th><th>Contents / Function
a) Cat. No. 03 515 869 001
b) Cat. No. 03 515 885 001
c) Cat. No. 03 752 186 001</th></tr>
<tr><td>1a
white cap</td><td>Enzyme</td><td rowspan="2">a) 1 × vial 1a, 3 × vial 1b
for 3 × 128 µl Master Mix (5× conc.)
b) 5 × vial 1a, 15 × vial 1b
for 15 × 128 µl Master Mix (5x conc.)
c) 4 × vial 1a, 12 × vial 1b
for 12 × 640 µl Master Mix (5× conc.)
•Ready-to-use hot-start PCR Master (after pipetting 14 µl [a, b] or 70 µl [c] from vial 1a into one vial 1b).
•Contains FastStart Taq DNA Polymerase, reaction buffer, $MgCl_2$, SYBR Green I dye, and dNTP mix (with dUTP instead of dTTP).</td></tr>
<tr><td>1b
green cap</td><td>Reaction Mix</td></tr>
<tr><td>2
colorless cap</td><td>Water, PCR grade</td><td>a) 2 vials, 1 ml each
b) 7 vials, 1 ml each
c) 2 vials, 25 ml each
•To adjust the final reaction volume.</td></tr>
</table>

Storage and Stability

The kit is shipped on dry ice.

- Store the kit at −15 to −25°C through the expiration date printed on the label.
- Once the kit is opened, store the kit components as described in the following table:

Vial	Label	Storage
1a white cap	Enzyme	• Store at −15 to −25°C. • **Avoid repeated freezing and thawing!** • **Protect vial 1b from light!**
1b green cap	Reaction Mix	
1 green cap (after addition of 1a to 1b)	Master Mix	• The prepared Master Mix can be aliquoted and stored at −15 to −25°C for a maximum of 3 months or at +2 to −8°C for a maximum of one week, respectively. • **Avoid repeated freezing and thawing!** • **Protect vial 1 from light!**
2 colorless cap	Water, PCR grade	• Store at −15 to −25°C.

Additional Equipment and Reagents Required

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

- LightCycler® Carousel-Based System*
- LightCycler® Capillaries*

  LightCycler® Capillaries (100 μl) can only be used with the LightCycler® 2.0 Instrument.

- Standard benchtop microcentrifuge containing a rotor for 2 ml reaction tubes.

  The LightCycler® Carousel-Based System provides adapters that allow LightCycler® Capillaries to be centrifuged in a standard microcentrifuge rotor.

or

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

  To adapt the LightCycler® 2.0 Sample Carousel to the former LC Carousel Centrifuge, you need the LC Carousel Centrifuge 2.0 Rotor Set*.

- LightCycler® Uracil-DNA Glycosylase* (optional)

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

- Nuclease-free, aerosol-resistant pipette tips
- Pipettes with disposable, positive-displacement tips
- Sterile reaction tubes for preparing master mixes and dilutions

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

Application

LightCycler® FastStart DNA Master^{PLUS} SYBR Green I is designed for quantitative PCR applications using the LightCycler® Carousel-Based System. The kit is ideally suited for hot-start PCR applications. In combination with the LightCycler® Carousel-Based System and suitable PCR primers, this kit allows very sensitive detection and quantification of defined DNA sequences. Various sources of DNA (cDNA, genomic DNA, plasmid DNA, etc.) can be used. The kit can also be used to perform two-step RT-PCR in combination with a reverse transcription kit for cDNA synthesis*. The LightCycler® FastStart DNA Master^{PLUS} SYBR Green I can be used in conjunction with heat-labile 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 less than 700 bp.

LightCycler® FastStart DNA Master^{PLUS} SYBR Green I offers convenience and ease of use because adaption of $MgCl_2$ in the reaction mixture is not necessary, thus avoiding time-consuming optimization steps. The new buffer formulation results in increased PCR robustness.

⚠ 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 for 20 µl reactions	Time for 100 µl reactions
PCR Set-up	15 min	15 min
LightCycler® Carousel-Based System PCR run	45 min	90 min
Total assay time	60 min	**105 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 PCR inhibitors. For reproducible isolation of nucleic acids use:

- either the MagNA Pure LC Instrument and a dedicated MagNA Pure LC reagent kit (for automated isolation)

or

- a High Pure nucleic acid isolation kit (for manual isolation).

For details see the Roche Applied Science catalogue or the website: www.roche-applied-science.com

- Use up to 50 ng complex genomic DNA or 10^1 - 10^{10} copies plasmid DNA per 20 µl reaction.
  - ⚠ Using a too high amount of template DNA might reduce signal dynamics by absorption of 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 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 2, colorless cap).

Primers

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

$MgCl_2$

Due to the optimized Reaction Mix of the LightCycler® FastStart DNA Master^{PLUS} SYBR Green I, for almost all primer combinations the PCR is efficient and specific without any $MgCl_2$ adaption. Addition of $MgCl_2$ to the mix is not required. In very rare cases, especially when using short primers with unusual low G/C content or cDNA templates with very high nucleotide and oligonucleotide concentrations (from reverse transcription), addition of $MgCl_2$ (not included in the kit) can be advantageous in a final concentration of up to 1.0 mM (titrate in 0.25 mM steps.)

2.2 Procedure

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 LightCycler® FastStart DNA Master^{PLUS} SYBR Green I contains the following programs:

- **Pre-Incubation** for 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.

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

The following table shows the PCR parameters typical for a LightCycler® Carousel-Based System PCR run with the LightCycler® FastStart DNA Master^{PLUS} 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]	20 µl: 0 - 10 s [5] 100 µl: 30 - 45 s	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	60 s	none
		Melting	95°C Ramp Rate= 0.1°C/sec [1]	0 s	continuous
			Cooling		
None	1		40°C	30 s	none

[1] Ramp Rate is 20°C/sec, except were indicated.
[2] For initial experiments, set the target temperature (*i.e.,* the primer annealing temperature) 5°C below the calculated primer Tm. Calculate the primer Tm 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 ramp rate to 2 - 5°C.
[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 to choose longer extension times for the amplification cycles.

Fluorescence and Run Setup Parameters

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.x		
Default channel	Fluorescence channel 530	
Fluorescence Gains	not required	
"Max. Seek Pos"	Enter the number of consecutive capillaries in the carousel for measurement.	
"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" or "100 µl" dependent on the capillaries used. ⚠ Only for the LightCycler® 2.0 Instrument.	

Preparation of the Master Mix

1. Thaw one vial of "Reaction Mix" (vial 1b, green cap).
2. Briefly centrifuge one vial "Enzyme" (vial 1a, white cap) and the thawed vial of "Reaction Mix" (from step1).
3. Cat. Nos. 03 515 869 001, 03 515 885 001: Pipet 14 µl from vial 1a into vial 1b.
   Cat. No. 03 752 186 001: Pipet 70 µl from vial 1a into vial 1b.
   Each vial 1a contains enough enzyme solution for three vials 1b.
4. Mix gently by pipetting up and down.
   ⚠ Do not vortex.
5. Re-label vial 1b (green cap) with the new labels (vial 1: Master Mix) 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 cool and away from light!

Preparation of the PCR Mix

Proceed as described below to prepare the PCR Mix.

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

1. 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.
2. Prepare a 10× conc. solution of the PCR primers.

❸ In a 1.5 ml reaction tube on ice, prepare the PCR Mix per 20 µl or 100 µl reaction by adding the following components in the order mentioned below, then mix gently up and down:

Component	Volume for a 20 µl reaction	Volume for a 100 µl reaction
Water, PCR-grade (vial 2, colorless cap)	9 µl	–
PCR Primer 10× conc.	2 µl	10 µl
Master Mix, 5× conc. (vial 1, green cap)	4 µl	20 µl
Total volume	**15 µl**	**30 µl**

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

❹ Mix carefully by pipetting up and down. Do not vortex.
- **For a 20 µl reaction:** Pipet 15 µl PCR mix into each precooled LightCycler® Capillary and add 5 µl of the DNA template.
- **For a 100 µl reaction:** Pipet 30 µl PCR mix into each precooled LightCycler® Capillary and add 70 µl of the DNA template.

❺ Seal each capillary with a stopper.
- If a LC Carousel Centrifuge is available spin the capillaries in the LC Carousel Centrifuge.
- Alternatively, place the capillaries in cooled adapters in a standard benchtop microcentrifuge, centrifuge at 700 × *g* (3,000 rpm) for 5 s, and transfer the capillaries to the LightCycler® Sample Carousel.

⚠ Place the centrifuge adapters in a balanced arrangement within the centrifuge.
- Place the LightCycler® Sample Carousel in the LightCycler® 2.0 Instrument and start the programmed protocol.

2.3 Related Procedures

Prevention of Carry-Over Contamination

Uracil-DNA Glycosylase (UNG) is suitable for preventing carry-over contamination in PCR. This technique relies on the incorporation of deoxyuridine triphosphate (dUTP) into amplification products, and the pretreatment of all successive PCR mixtures with UNG. If there are such amplicons in the PCR mixture, UNG cleaves the DNA at any site where a deoxyuridylate residue has been incorporated. Subsequently, the resulting abasic sites are hydrolized by high temperatures during the initial denaturation step, and cannot serve as PCR templates. Normal DNA contains thymidine, but no uridine, and is therefore not affected by this procedure.

⚠ Use only LightCycler® Uracil-DNA Glycosylase* in combination with LightCycler® FastStart DNA Master^{PLUS} SYBR Green I. Proceed as described in the package insert.

The use of UNG might lower the melting temperature (T_m) in melting curve analysis by up to 1°C.

Two-step RT-PCR

LightCycler® FastStart DNA Master^{PLUS} 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 PCR 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 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 5 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.

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

3. Results

Quantification Analysis

The following amplification and melting curves were obtained using the LightCycler® FastStart DNA Master^PLUS SYBR Green I in combination with the LightCycler® Control Kit DNA targeting human β-globin gene. The fluorescence values versus cycle number are displayed.

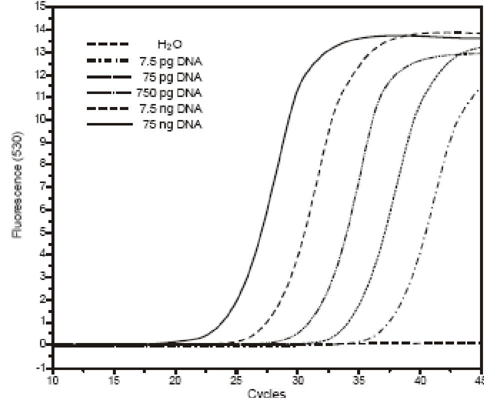

Fig. 1: Serially diluted samples containing 75 ng, 7.5 ng, 750 pg, 75 pg, or 7.5 pg human genomic DNA as starting template were amplified using the LightCycler® FastStart DNA Master^PLUS SYBR Green I. As a negative control, template DNA was replaced by PCR-grade water. Arithmetic background subtraction was applied and the fluorescence channel was set to 530.

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 75 ng, 7.5 ng, 750 pg, 75 pg, or 7.5 pg human genomic DNA.

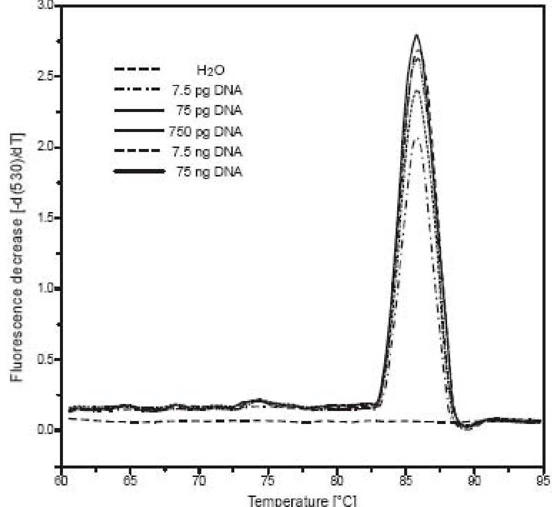

Fig. 2: Melting curve analysis of amplified samples containing 75 ng, 7.5 ng, 750 pg, 75 pg, or 7.5 pg human genomic DNA as starting template. As a negative control, template DNA was replaced by PCR-grade water.

4. Troubleshooting

	Possible Cause	Recommendation
Amplification reaches plateau phase before the program is complete.	Starting amount of nucleic acid is very high.	Stop the program by clicking the **End Program** button. The next cycle program will start automatically.
	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.
	Number of cycles is too low.	• Increase the number of cycles in the protocol. • Use the **+ 10 cycles** button to increase the number of cycles in the program.
No amplification occurs.	Using wrong channel to display amplification on screen.	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 or higher do not require a gain setting.
	Scale of axes on graph are unsuitable for analysis.	Change the values for the x- and the 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.

	Possible Cause	Recommendation
	Impure sample material inhibits reaction.	• Dilute sample 1:10 and repeat the analysis. • Repurify the nucleic acids to ensure removal of inhibitory agents.
	Difficult template (*e.g.,* unusual GC-rich sequence).	Repeat PCR under same conditions and add increasing amounts of DMSO (up to 10 % of the final concentration).
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 or dirt are on the surface of the capillary tip.	Always wear gloves when handling the capillaries.
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.	Low concentration or deterioration of dyes 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® FastStart DNA Master^{PLUS} SYBR Green I at +2 to +8°C for a maximum of 1 week and keep it away from light.
	Reaction conditions are not optimized, leading to poor PCR efficiency.	• Primer concentration should be between 0.2 and 1.0 µM. • Check annealing temperature of primers. • Check experimental protocol. • Always run a positive control along with your samples.

	Possible Cause	Recommendation
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 per 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, hot start antibody, and program settings.

	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. • Increase annealing temperature in order to enhance stringency.
	Quality of primer is poor.	• Purify primer more thouroughly. • Use a hot start method.
	Sequence of primers is 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 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. • 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® FastStart DNA Master^{PLUS} SYBR Green I is a ready-to-use reaction mix for PCR in glass capillaries using the LightCycler® Carousel-Based System. The hot start effect of FastStart Taq DNA Polymerase minimizes formation of nonspecific products and improves sensitivity for the desired target [1, 2, 3, 4].

The FastStart Taq DNA Polymerase is a modified form of thermostable recombinant Taq DNA polymerase. It is enzymatically inactive because of blocking groups in the active site of the enzyme. At high temperatures, when no non-specific primer binding occurs, the blocking groups are removed and the enzyme becomes active. Activation is done before cycling in a pre-incubation step at 95°C for 10 minutes.

The composition of the reaction mix is optimized for a fixed $MgCl_2$ concentration. This kit achieves an efficient amplification with almost all primer combinations, without any sequence specific-adaption.

Amplification can be detected by measurement of the fluorescence signal of SYBR Green I. When bound to double-stranded DNA the SYBR Green I dye will emit a fluorescence signal detectable at 530 nm. This signal increases in proportion to the amount of double-stranded DNA during PCR.

To prove specificity of amplification, cycling has to be followed by melting curve analysis. When the mixture is heated slowly, the two strands of an amplicon separate at a definite temperature and fluorescence suddenly drops. This melting temperature is dependent on GC-content and amplicon length and is characteristic for a particular DNA fragment. Melting curve analysis reveals only one peak at the characteristic melting temperature, when PCR results only in the specific amplicon. Primer dimers and other nonspecific products would cause additional peaks.

The LightCycler® FastStart DNA Master^{PLUS} SYBR Green I provides convenience, high performance, reproducibility, and minimizes contamination risk. Only primers and template DNA have to be added.

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 proportional to the amount of double-stranded DNA generated.

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 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 Chou, Q. *et al* (1992) Prevention of pre-PCR mis-priming and primer dimerization improves low-copy-number amplifications. *Nucleic Acid Res.* **20**, 1717-1723.

2 Kellogg, D.E. *et al* (1994) TaqStart Antibody: hot-start PCR facilitated by a neutralizing monoclonal antibody directed against Taq DNA polymerase. *BioTechniques* **16**, 1134-1137.

3 Birch, D.E. *et al* (1996) Simplified hot start PCR. *Nature* **381**, 445-446.

4 PCR Manual, Roche Diagnostics (1999) 2nd edition (1999) **2**, 52-58.

Product Citations

5 Hansen, M.B. *et al* (2005) Gene transcripts as potential diagnostic markers for allergic contact dermatitis. *Contact dermatitis* **53**, 100-6.

6 Wikman, H. *et al* (2005) Caveolins as tumour markers in lung cancer detected by combined use of cDNA and tissue microarrays. *The Journal of Pathology* **203**, 584-593.

7 Mehrle, S. *et al* (2005) SAP and SLAM expression in anti-CD3 activated lymphocytes correlates with cytotoxic activity. *Immunology and Cell Biology* **83**, 33-9.

8 Ellis, R.C. *et al* (2004) Cathepsin B mRNA and protein expression following contusion spinal cord injury in rats. *Journal of Neurochemistry* **88**, 689.

9 Klein, J.D. *et al* (2004) Upregulation of Urea Transporter UT-A2 and Water Channels AQP2 and AQP3 in Mice Lacking Urea Transporter UT-B. J *Am Soc Nephrol* **15**, 1161-7.

10 Paux, E. *et al* (2004) Identification of genes preferentially expressed during wood formation in Eucalyptus. *Plant Molecular Biology* **55**, 263-80.

11 Qiu, X.B. *et al* (2004) Nrdp1-mediated degradation of the gigantic IAP, BRUCE, is a novel pathway for triggering apoptosis. *EMBO J* **23**, 800-10.

12 Soares-Da-Silva, PATR *et al* (2004) Cloning and gene silencing of LAT2, the L-3,4-dihydroxyphenylalanine (L-DOPA) transporter, in pig renal LLC-PK1 epithelial cells. *FASEB J* **18**, 1489-98.

13 Thomas, J *et al* (2004) Active transport of imatinib into and out of cells: implications for drug resistance. *Blood* **104**, 3739-45.

Quality Control

The LightCycler® FastStart DNA Master^{PLUS} SYBR Green I is function tested using the LightCycler® Carousel-Based System.

6. Supplementary Information

6.1 Conventions

6.1.1 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.

6.1.2 Symbols

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

Symbol	Description
(information symbol)	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.

6.2 Changes to Previous Version

- **Page 6, $MgCl_2$**: Switch “final concentration up to 1 mM (titrate in 0.25 mM steps)”.

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® Carousel-Based System family for real-time, online PCR: http://www.lightcycler.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.
Instruments 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
	LightCycler® Capillaries (100 µl)	1 pack (8 boxes, each with 96 capillaries and stoppers	03 337 090 001
	LC Carousel Centrifuge	1 centrifuge plus rotor and bucket	03 709 507 001 (115 V) 03 709 582 001 (230 V)
	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
Software	LightCycler® Software 4.05	1 software package	04 717 392 001

	Product	Pack Size	Cat. No.
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® Fast-Start DNA Master HybProbe	1 kit (96 reactions) 1 kit (480 reactions	03 003 248 001 12 239 272 001
	LightCycler® Fast-Start DNA Master^{PLUS} Hyb-Probe	1 kit (96 reactions) 1 kit (480 reactions) 1 kit (384 reactions,100µl)	03 264 785 001 03 515 567 001 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® Fast-Start DNA Master SYBR Green I	1 kit (96 reactions) 1 kit (480 reactions)	03 003 230 001 12 239 264 001
cDNA Synthesis Kit	Transcriptor Reverse Tran-scriptase	250 U 500 U 2000 U	03 531 317 001 03 531 295 001 03 531 287 001
	Transcriptor First Strand cDNA Syn-thesis 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 Glyco-sylase	100 U (50 ml)	03 539 806 001

6.4 Disclaimer of License

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.

This product is covered in-part by US 5,871,908 or any foreign equivalents, co-exclusively licensed from Evotec OAI AG. The purchase price includes a license to practice the methods covered by US 5,871, 908 by using the product. Purchase of this product, however, does not convey to the purchaser a license or right to

(i) commercially make, have made or sell reagents and/or kits, or

(ii) buy or use reagents and/or kits provided by a third party

used in conjunction with the product or any other thermocycler to practice the methods covered by US 5,871,908 or any foreign equivalents.

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

6.5 Trademarks

LIGHTCYCLER, LC, MAGNA PURE, FASTSTART, HIGH PURE and HYBPROBE 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.

Other brand or product names are trademarks of their respective holders.

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 worldwide 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.

1007.03568385001 ⑪

Diagnostics

Roche Diagnostics GmbH
Roche Applied Science
68298 Mannheim
Germany