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

Version 17

Content version: September 2011

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 1,920 reactions (20 µl), or 384 reactions (100 µl)

Store the kit at –15 to –25°C

Keep LightCycler® FastStart DNA Master^{PLUS} 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:

- 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: 1,920 reactions, with a reaction volume of 20 µl each, or 384 reactions, with a reaction volume of 100 µl each

Kit Contents

Vial/Cap	Label	Contents / Function a) Cat. No. 03 515 869 001 b) Cat. No. 03 515 885 001 c) Cat. No. 03 752 186 001
1a white cap	LightCycler® FastStart Enzyme	a) 1 vial 1a and 3 vials 1b for 3 vials, 128 µl each LightCycler® FastStart DNA Master^{PLUS} SYBR Green I (5× conc.)
1b green cap	LightCycler® FastStart DNA Master^{PLUS} Reaction Mix	b) 5 vials 1a and 15 vials 1b for 15 vials, 128 µl each LightCycler® FastStart DNA Master^{PLUS} SYBR Green I (5× conc.) c) 4 vials 1a and 12 vials 1b for 12 vials, 640 µl each LightCycler® FastStart DNA Master^{PLUS} SYBR Green I (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).
2 colorless cap	H_2O, PCR grade	a) 2 vials, 1 ml each b) 7 vials, 1 ml each c) 2 vials, 25 ml each ▪To adjust the final reaction volume.

Storage and Stability

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

⚠ Keep the LightCycler® FastStart Master^{PLUS} 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 white cap	LightCycler® FastStart Enzyme	▪ Store at −15 to −25°C. ▪ **Avoid repeated freezing and thawing!** ▪ **Protect vial 1b from light!**
1b green cap	LightCycler® FastStart DNA Master^{PLUS} Reaction Mix	
1 green cap (after addition of 1a to 1b)	LightCycler® FastStart DNA Master^{PLUS} SYBR Green I Master Mix, 5× conc.	▪ The prepared Master Mix can be aliquoted and stored at −15 to −25°C for a maximum of three months, or at +2 to +8°C for a maximum of one week. ▪ **Avoid repeated freezing and thawing!** ▪ **Protect vial 1 away from light!**
2 colorless cap	H_2O, PCR grade	▪ Store at −15 to −25°C.

Additional Equipment and Reagents Required

Additional reagents and equipment required to perform PCR reactions with the LightCycler® FastStart DNA Master^{PLUS} 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*

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

- Standard benchtop microcentrifuge, containing a rotor for 2.0 ml reaction tubes

The LightCycler® Carousel-Based System includes Centrifuge Adapters that enable LightCycler® Capillaries to be centrifuged in a standard microcentrifuge rotor.

or

- LC Carousel Centrifuge 2.0* for use with the LightCycler® 2.0 Sample Carousels (20 µl or 100 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 tubes (Eppendorf) for preparing master mixes and dilutions
- LightCycler® Uracil-DNA Glycosylase* (optional ‡)

‡ 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^{PLUS} SYBR Green I is designed for quantitative PCR applications using the LightCycler® Carousel-Based System. The kit is suited for hot start PCR applications. In combination with the LightCycler® Carousel-Based System and suitable PCR primers, this kit enables very sensitive detection and quantification of defined DNA sequences.

Furthermore, the kit can be used to perform two-step RT-PCR, in combination with a reverse transcription kit for cDNA synthesis.

In principle, the LightCycler® FastStart DNA Master^{PLUS} SYBR Green I can be used for the amplification and detection of any DNA or cDNA target.

The LightCycler® FastStart DNA Master^{PLUS} SYBR Green I offers convenience and ease of use because optimization of $MgCl_2$ in the reaction mixture is not necessary, thus avoiding time-consuming optimization steps. The buffer formulation results in increased PCR robustness. You just need to design specific PCR primers for each target. Refer to the LightCycler® System Operator's Manual for general recommendations.

LightCycler® FastStart DNA Master^{PLUS} SYBR Green I can 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® Carousel-Based System.

Assay Time

Procedure	Time for 20 µl reactions	Time for 100 µl reactions
PCR Setup	15 min	15 min
LightCycler® Carousel-Based System PCR run (incl. Melting Curve)	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 one of the following:
  - one of the MagNA Pure LC Instruments 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 catalogue or home page: www.roche-applied-science.com. See Ordering Information for selected products, recommended for the isolation of template DNA.

- Use up to 50 ng complex genomic DNA or 10^1 to 10^{10} copies plasmid DNA per 20 µl reaction.

⚠ Using a too high amount of template DNA may 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.

Primers

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

$MgCl_2$

Due to the 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 $MgCl_2$ optimization. Addition of $MgCl_2$ to the mix is not required. In very rare cases, especially when using short primers with unusually 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.)

Negative Control

Always run a negative control with the samples. To prepare a negative control, replace the template DNA with H_2O, PCR grade (vial 2, 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® FastStart DNA Master^{PLUS} SYBR Green I, contains the following programs:

- **Pre-Incubation** for activation of the FastStart DNA polymerase and denaturation of the 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® System Operator's Manual.

⚠ Set all other parameters not listed in the table 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® 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] Temperature Transition Rate/Slope/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 prime 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] In assays where the primer annealing temperature is low (< +55°C), reduce the ramp rate to 2 to 5°C/s.

[4] A 10 min pre-incubation time is recommended. However, depending on the individual assay, the pre-incubation time can be reduced to 5 min with no change in performance. In assays where high polymerase activity is required in the early cycles, in some cases, results can be improved by extending the pre-incubation to 15 min.

[5] 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.

[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 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, or 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" or "100 µl", dependent on the capillary size used 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).
❷	Briefly centrifuge one vial “Enzyme” (vial 1a, white cap) and the thawed vial of “Reaction Mix“ (from Step 1), then place the vials back on ice.
❸	Cat. Nos. 03 515 869 001, 03 515 885 001: Pipette 14 µl from vial 1a (white cap) into vial 1b (green cap). Cat. No. 03 752 186 001: Pipette 70 µl from vial 1a (white cap) into vial 1b (green cap). Each vial 1a contains enough enzyme solution 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 label (vial 1: Master Mix) provided with the kit. ⚠ Always keep the Master Mix away from light!
❻	Store on ice, or in the pre-cooled LightCycler® Centrifuge Adapters Cooling Block, until ready to use.

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.

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	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 per 20 µl or 100 µl reaction, by adding the following components in the order mentioned below:

Component	Volume for a 20 µl reaction	Volume for a 100 µl reaction
H_2O, PCR grade (vial 2, colorless cap)	9 µl	—
PCR Primer Mix, 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 z, where z = the number of reactions to be run + one additional reaction.

❹
- Mix gently by pipetting up and down. Do not vortex.
- **For a 20 µl reaction:** Pipette 15 µl PCR mix into each pre-cooled LightCycler® Capillary and add 5 µl of the DNA template.
- **For a 100 µl reaction:** Pipette 30 µl PCR mix into each pre-cooled LightCycler® Capillary and add 70 µl of the DNA template.
- Seal each capillary with a stopper.

❺
- 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.

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

❼ Cycle the samples as described above.

2.3 Related Procedures

Prevention of Carry-Over Contamination

Uracil-DNA 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^{PLUS} SYBR Green I, perform prevention of carry-over contamination with LightCycler® Uracil-DNA Glycosylase*. Proceed as described in the package insert and/or in the table below, to prevent carry-over contamination.

❶	Add 0.5 U LightCycler® Uracil-DNA Glycosylase to the master mix per 20 µl final reaction volume.
❷	Add template DNA and incubate the completed reaction for 10 min at 40°C.
❸	Destroy any contaminating template and inactivate the UNG enzyme, by performing the initial denaturation step for 10 min at 95°C.

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

When performing Melting Curve analysis, the use of UNG may lower the melting temperature (T_m) by approx. 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 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 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® 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.

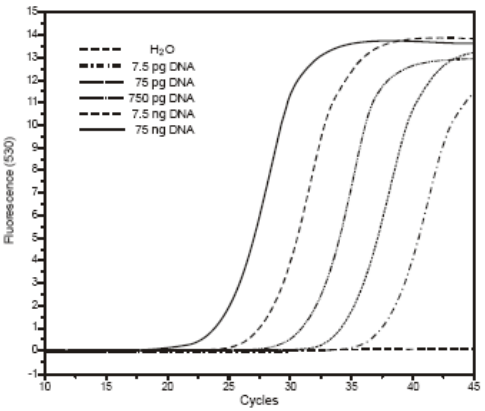

Fig. 1: Serially diluted samples containing 75 ng, 7.5 ng, 750 pg, 75 pg, or 7.5 pg human genomic DNA as starting template were amplified using the LightCycler® FastStart DNA Master^{PLUS} SYBR Green I. As a negative control, template DNA was replaced by PCR-grade water.

Melting Curve Analysis

Specificity of the amplified PCR product was assessed by performing a Melting Curve analysis. The resulting melting curves enable 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 75 ng, 7.5 ng, 750 pg, 75 pg, or 7.5 pg human genomic DNA.

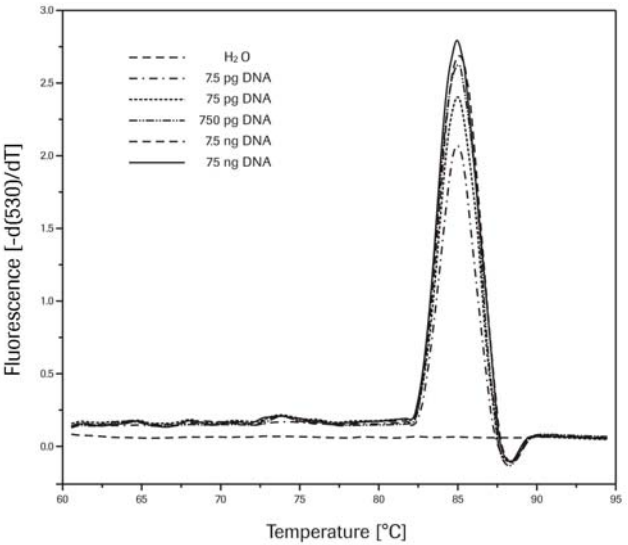

Fig. 2: Melting Curve analysis of amplified samples containing 75 ng, 7.5 ng, 750 pg, 75 pg, or 7.5 pg human genomic DNA as starting template. As a negative control, template DNA was replaced by PCR-grade water.

4. Troubleshooting

	Possible Cause	Recommendation
Amplification reaches plateau phase before the program is complete.	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 as 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 material. ▪ Repeat the run.
	The number of cycles is too low.	▪ Increase the number of cycles in the amplification program. ▪ Use the **+10 cycles** button, to 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 on the programming screen. (The data obtained up to this point will be saved.)
	FastStart Taq DNA polymerase is not fully activated.	▪ Ensure that the PCR programming includes a pre-incubation step at 95°C for 10 min. ▪ Ensure that the denaturation time during the amplification cycles is 10 s.
	Pipetting errors or omitted reagents	▪ Check for missing reagents. ▪ Titrate $MgCl_2$ concentration. ▪ Check for defective SYBR Green I dye.
	Scales of the axes on the 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 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.	▪ 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).

	Possible Cause	Recommendation
Fluorescence intensity is too low.	Low concentration or deterioration of SYBR Green I dye in the reaction mixtures, due to unsuitable storage conditions.	▪ Store the SYBR Green I 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 one 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.
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 SYBR Green I dye, leading to a high background signal. Insufficient amounts of SYBR Green I 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 format of the HybProbe sample (which enables analysis of up to 500 ng DNA), instead of SYBR Green I.
	SYBR Green I dye bleached.	Ensure the reagents containing the SYBR Green I 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 LightCycler® UNG to eliminate carry-over contamination.
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 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.

	Possible Cause	Recommendation
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 preparations and program settings.
Only a primer-dimer peak appears, with no specific PCR product peak; or very high primer-dimer peaks.	Primer-dimers have outcompeted 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 possible. ▪ Increase starting amount of DNA template. ▪ Increase annealing temperature, in order to enhance stringency.
	Quality of primers are poor.	Purify primer more thoroughly.
	Sequence of primers are inappropriate.	Redesign primers.
Primer-dimer and product peaks are very close together.	Unusually high GC-content of PCR primers.	▪ Redesign primers. ▪ Run melting curve at the lowest ramp 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.,* 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

5.1 How this Product Works

LightCycler® FastStart DNA Master^{PLUS} SYBR Green I is a ready-to-use PCR reaction mix, designed specifically for real-time PCR assays using the SYBR Green I detection format on the LightCycler® Carousel-Based System. It can be used to perform hot start PCR in 20 or 100 µl glass capillaries. Hot start PCR has been shown to significantly improve 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 min) before cycling begins. Activation does not require the extra handling steps typical of other hot start techniques.

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 optimization.

LightCycler® FastStart DNA Master^{PLUS} SYBR Green I provides convenience, high performance and reproducibility, as well as minimizing contamination risk. All you must supply is primers and template DNA.

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 SYBR Green I 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.

As 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.

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 SYBR Green I dye. The unbound SYBR Green I dye molecules weakly fluoresce, producing a minimal background fluorescence signal, which is subtracted during computer analysis.
②	After annealing of the primers, a few SYBR Green I dye molecules can bind to the double strand. DNA binding results in a dramatic increase of the SYBR Green I dye molecules to emit light upon excitation.
③	During elongation, more and more SYBR Green I 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 SYBR Green I 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 demonstrate 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.

5.2 Quality Control

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

5.3 References

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

2 Kellogg, DE. *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, DE. *et al.* (1996). Simplified hot start PCR. *Nature* **381**, 445-446.

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

5.4 Product Citations

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6. Supplementary Information

6.1 Conventions

Text Conventions To make information consistent and memorable, the following text conventions are used in this document:

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 document, 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 document, the following abbreviations are used:

Abbreviation	Meaning
Cp	crossing point
dsDNA	double-stranded DNA
T_m	melting temperature
UNG	Uracil-DNA Glycosylase

6.2 Changes to Previous Version

Update of License Disclaimer

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:

- Real-time PCR Systems (LightCycler® Carousel-Based System, LightCycler® 480 System, LightCycler® 1536 System, RealTime ready qPCR assays and Universal ProbeLibrary): http://www.lightcycler.com
- Automated Sample Preparation (MagNA Lyser Instrument, MagNA Pure Compact System, MagNA Pure LC Systems and MagNA Pure 96 System): http://www.magnapure.com

	Product	Pack Size	Cat. No.
Instruments and Accessories	LightCycler® 2.0 Instrument	1 instrument plus accessories	03 531 414 001
	LightCycler® Capillaries (20 µl)	1 pack (5 boxes, each with 96 capillaries and stoppers)	04 929 292 001
	LightCycler® Software 4.1	1 software package	04 898 915 001
	LC Carousel Centrifuge 2.0	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
	MagNA Pure LC 2.0 Instrument	1 instrument plus accessories	05 197 686 001
	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
Total Nucleic Acid Isolation Kits	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 LC Total Nucleic Acid Isolation Kit – High Performance	1 kit (96 – 288 isolations)	05 323 738 001
	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
RNA Isolation Kits	MagNA Pure LC RNA Isolation Kit – High Performance	1 kit (192 isolations)	03 542 394 001

	Product	Pack Size	Cat. No.
	MagNA Pure LC RNA Isolation Kit III (Tissue)	1 kit (192 isolations)	03 330 591 001
	MagNA Pure LC mRNA HS Kit [1]	1 kit (192 isolations)	03 267 393 001
	MagNA Pure Compact RNA Isolation Kit	1 kit (32 isolations)	04 802 993 001
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® 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 SYBR Green I	1 kit (96 reactions) 1 kit (480 reactions)	03 003 230 001 12 239 264 001
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	LightCycler® Uracil-DNA Glycosylase	100 U (50 µl)	03 539 809 001
	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

[1] the MagNA Pure LC mRNA HS Kit is only available for use with the MagNA Pure LC 1.0 Instrument (Cat. No. 12 236 931 001).

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6.5 Regulatory Disclaimer

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