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LightCycler® FastStart DNA Master SYBR Green I

Version: 19
Content version: January 2017

Easy-to-use hot start reaction mix for PCR using SYBR Green I with the LightCycler® Carousel-Based System

Cat. No. 03 003 230 001	1 kit 96 reactions of 20 µl final volume each
Cat. No. 12 239 264 001	1 kit 480 reactions of 20 µl final volume each

Store at −15 to −25°C.

1. General Information

1.1. Contents

Vial / Bottle	Cap	Label	Function / Description	Catalog Number	Content
1a	colorless	LightCycler® FastStart DNA Master SYBR Green I, LC FastStart Enzyme	▪ 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 $MgCl_2$.	03 003 230 001	1 vial 1a, 3 vials 1b, for 3 × 64 µl each LightCycler® FastStart DNA Master SYBR Green I, 10x conc.
1b	green	LightCycler® FastStart DNA Master SYBR Green I, LC FastStart Reaction Mix SYBR Green I, 10x conc.		12 239 264 001	5 vials 1a, 15 vials 1b, for 15 × 64 µl each LightCycler® FastStart DNA Master SYBR Green I, 10x conc.
2	blue	LightCycler® FastStart DNA Master SYBR Green I, $MgCl_2$ stock solution, 25 mM	To adjust $MgCl_2$ concentration in the reaction mix.	03 003 230 001	1 vial, 1 ml
				12 239 264 001	2 vials, 1 ml each
3	colorless	LightCycler® FastStart DNA Master SYBR Green I, Water, PCR Grade	To adjust the final reaction volume.	03 003 230 001	2 vials, 1 ml each
				12 239 264 001	7 vials, 1 ml each

1.2. Storage and Stability

Storage Conditions (Product)

The kit is shipped on dry ice.
When stored at −15 to −25°C, the kit is stable through the expiration date printed on the label.
Once the kit is opened, store the kit components as described in the following table:

Vial / Bottle	Cap	Label	Storage
1a	colorless	LC FastStart Enzyme	Store at −15 to −25°C. ⚠ ***Avoid repeated freezing and thawing.*** ⚠ ***Keep Vial 1b protected from light.***
1b	green	LC FastStart Reaction Mix SYBR Green I, 10x conc.	
1 (after addition of 1a to 1b)	green	LC FastStart DNA Master SYBR Green I, 10x conc.	▪ Store at −15 to −25°C for a maximum of 3 months. ▪ After thawing, store at +2 to +8°C for a maximum of 1 week. ⚠ ***Avoid repeated freezing and thawing.*** ⚠ ***Keep Vial 1 protected from light.***
2	blue	$MgCl_2$ stock solution, 25 mM	Store at −15 to −25°C.
3	colorless	Water, PCR Grade	

Additional Equipment and Reagents Required

Standard Laboratory Equipment

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

For PCR

- LightCycler® Carousel-Based System*
- LightCycler® Capillaries*
- Standard benchtop microcentrifuge, containing a rotor for 2.0 ml reaction tubes.

The LightCycler® Carousel-Based System provides 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 Carousel (optional)
- LightCycler® Uracil-DNA Glycosylase* (optional)

For prevention of carryover contamination see section **Prevention of Carryover Contamination***. Use LightCycler® Uracil-DNA Glycosylase in combination with LightCycler® FastStart DNA Masters only.*

1.3. Application

LightCycler® FastStart DNA Master SYBR Green I is ideally 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.
LightCycler® FastStart DNA Master SYBR Green I can also be used with LightCycler® Uracil-DNA Glycosylase to prevent carryover contamination during PCR.

1.4. Preparation Time

Assay Time

Procedure	Assay Time [min]
Optional: dilution of template DNA	5
PCR Setup	15
LightCycler® Carousel-Based System PCR run (including Melting Curve)	45
Total Assay Time	**65**

2. How to Use this Product

2.1. Before you Begin

Sample Materials

- Use any template DNA suitable for PCR in terms of purity, concentration, and absence of PCR inhibitors.
- Use up to 50 ng complex genomic DNA or 10^1 to 10^{10} copies plasmid DNA.

⚠ ***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.

For reproducible isolation of nucleic acids, we recommend:

- Either a MagNA Pure System together with a dedicated reagent kit (for automated isolation),
- or a High Pure Nucleic Acid Isolation Kit (for manual isolation).

Control Reactions

Always run a negative control with the samples. To prepare a negative control, replace the template DNA with Water, PCR Grade (Vial 3).

Primers

Suitable concentrations of PCR primers range from 0.2 to 1 µM (final concentration). The recommended starting concentration is 0.5 µM each.

Mg^{2+} Concentration

To ensure specific and efficient amplification with the LightCycler® Carousel-Based 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 optimal concentration for PCR with the LightCycler® Carousel-Based System may vary from 1 to 5 mM.
The table below gives the volumes of the $MgCl_2$ stock solution (Vial 2) that you must add to a 20 µl reaction (final PCR volume), to increase the $MgCl_2$ concentration to the indicated values.

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

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

General Considerations

Two-Step RT-PCR

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® Carousel-Based System. Subsequent amplification and online monitoring is performed according to the standard LightCycler® Carousel-Based System procedure, using the cDNA as the starting sample material. One of the following reagents is required for reverse transcription of RNA into cDNA:

- Transcriptor Reverse Transcriptase*
- Transcriptor First Strand cDNA Synthesis Kit*

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.***

2.2. Protocols

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 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 protocol, see the LightCycler® Operator´s Manual.

⚠ ***Set all other protocol 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 SYBR Green I.

LightCycler® Software Version 4.1				
Programs				
Setup		Setting		
Default Channel		Fluorescence Channel 530		
Seek Temperature		30°C		
Max Seek Pos.		Enter the total number of sample positions the instrument should look for.		
Instrument Type		“6 Ch.” for LightCycler® 2.0 Instrument or “3 Ch.” for LightCycler® 1.5 Instrument		
Capillary Size		Select “20 µl” as the capillary size for the experiment. (available only for LightCycler® 2.0 Instrument (6 channels))		
Programs				
Program Name		Cycles	Analysis Mode	
Pre-Incubation		1	None	
Amplification		45	Quantification	
Melting Curve		1	Melting Curve	
Cooling		1	None	
Temperature Targets				
	Target [°C]	Hold [hh:mm:ss]	Ramp Rate[1] [°C/s]	Acquisition Mode [per °C]
Pre-Incubation	95	00:10:00[2]	20	None
Amplification	95	00:00:10	20	None
	primer dependent[3]	00:00:00 – 00:00:10[4]	20	None
	72[5]	00:00:05 – 00:00:30[6,7]	20	Single
Melting Curve	95	00:00:00	20	None
	65	00:00:15	20	None
	95	00:00:00	0.1[1]	Continuous
Cooling	40	00:00:30	20	None

(1) Temperature Transition Rate/Slope/Ramp Rate is 20°C/second, except where indicated.
(2) A 10 minute pre-incubation time is recommended. However, depending on the individual assay, the pre-incubation time can be reduced to 5 minutes 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 time to 15 minutes.
(3) 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:
Tm = 2°C (A + T) + 4°C (G + C).
(4) For typical primers, choose an incubation time of 0 to 10 seconds for the annealing step. To increase the specificity of primer binding, use an incubation time of <5 seconds.
(5) If the primer annealing temperature is low (<+55°C), reduce the ramp rate to 2 to 5°C/second.
(6) For greater precision in target quantification experiments, it can be advantageous (in some cases) to choose longer extension times for the amplification cycles.
(7) Calculate the hold time for the PCR elongation step by dividing the amplicon length over 25 (*e.g.*, a 500 bp amplicon requires 20 seconds elongation time).

Preparation of the Master Mix

Prepare the 10x conc. Master Mix as described below.

1. Thaw one vial of Reaction Mix (Vial 1b) and protect 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 until the precipitate is completely dissolved. This treatment does not influence the performance in PCR.***

2. Briefly centrifuge one vial Enzyme (Vial 1a) and the thawed vial of Reaction Mix (from Step 1); place the vials on ice.

3. Pipette 10 µl from Vial 1a into Vial 1b.

   ℹ *Each Vial 1a contains enough enzyme solution for three vials of Reaction Mix (Vial 1b).*

4. Mix gently by pipetting up and down.

   ⚠ ***Do not vortex.***

5. Re-label Vial 1b with the new label (Vial 1: LC FastStart DNA Master SYBR Green I, 10x conc.) provided with the kit.

   ⚠ ***Always protect the Master Mix from light.***

6. 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 for a 20 µl standard reaction.

⚠ ***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 into pre-cooled centrifuge adapters or into a LightCycler® Sample Carousel in a pre-cooled LC Carousel Centrifuge Bucket.

2. Prepare a 10x conc. solution of the PCR primers.

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

3. 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:

Reagent	Volume [µl]	Final conc.
Water, PCR Grade (Vial 3)	x	–
$MgCl_2$ stock solution, 25 mM (Vial 2)	y	Use concentration that is optimal for the target.
PCR Primer Mix, 10x conc.	2	0.2 to 1.0 µM each (recommended conc. is 0.5 µM)
LightCycler® FastStart DNA Master SYBR Green I, 10x conc. (Vial 1)	2	1x
Total Volume	18	

   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 plus 1 additional reaction.

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

5. 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 seconds (3,000 rpm in a standard benchtop microcentrifuge).
   – Alternatively, use the LC Carousel Centrifuge for spinning the capillaries.

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

7. Cycle the samples as described above.

2.3. Other Parameters

Prevention of Carryover Contamination

Uracil-DNA Glycosylase (UNG) is suitable for preventing carryover contamination in PCR. This carryover prevention technique involves incorporating deoxyuridine triphosphate (dUTP, a component of the reaction mixes of all LightCycler® System 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 carryover contamination with LightCycler® Uracil-DNA Glyocsylase* prior to beginning real-time PCR.***

Proceed as described in the Instructions for Use and/or in the steps shown below to prevent carryover contamination.

1. Add 0.5 U LightCycler® Uracil-DNA Glycosylase to the master mix per 20 µl final reaction volume.

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2. Add template DNA and incubate the completed reaction mixture for 10 minutes at +40°C.

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3. Destroy any contaminating template and inactivate the UNG enzyme by performing the initial denaturation step for 10 minutes 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 (Tm) by approximately 1°C.

3. Results

Quantification Analysis

The following amplification curves were obtained using the LightCycler® FastStart DNA Master SYBR Green I, in combination with the LightCycler® Control Kit DNA, targeting human β-globin gene. The fluorescence values versus cycle number are displayed. Thirty picograms (approx. 10 genome equivalents) of human genomic DNA can be reproducibly detected by amplifying in the LightCycler® Carousel-Based System and using the SYBR Green I detection format. Three picograms (approx. 1 haploid genome equivalent) are sporadically detected, due to statistical fluctuations.

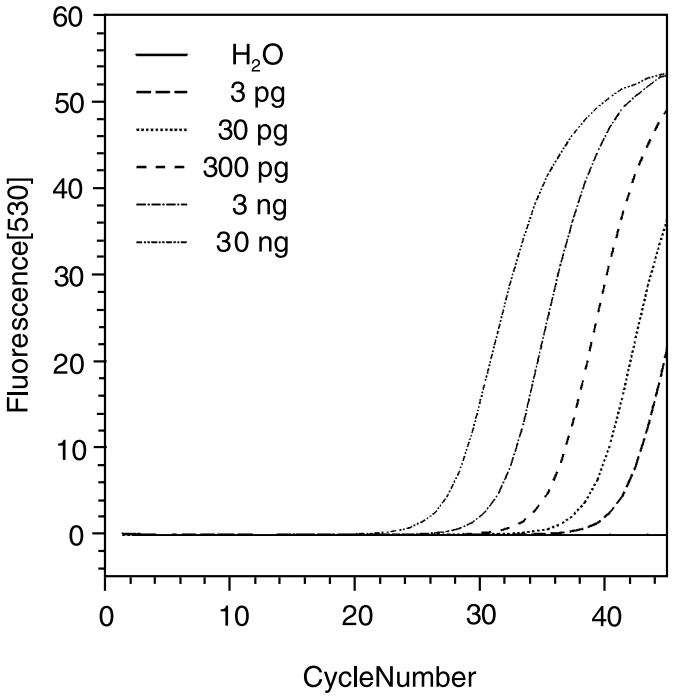

Fig. 1: Serially diluted samples containing 30 ng (far left), 3 ng, 300 pg, 30 pg, or 3 pg (far right) human genomic DNA as starting template were amplified using the LightCycler® FastStart DNA Master SYBR Green I. As a negative control, template DNA was replaced by Water, PCR Grade (flat line).

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 30 ng, 3 ng, 300 pg, 30 pg, or 3 pg human genomic DNA.

⚠ ***Smaller reaction volumes may result in melting temperature variations.***

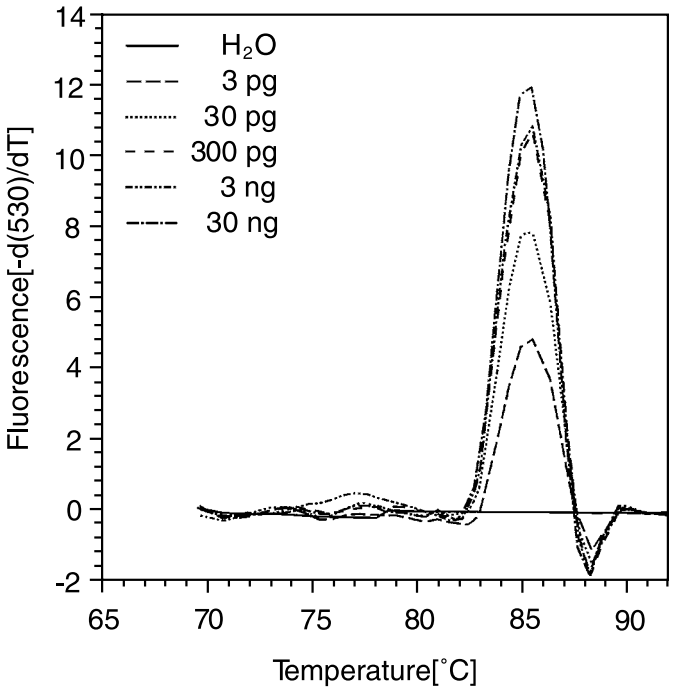

Fig. 2: Melting Curve analysis of amplified samples containing 30 ng (highest peak), 3 ng, 300 pg, 30 pg, or 3 pg (lowest peak) human genomic DNA as starting template. As a negative control, template DNA was replaced by Water, PCR Grade (flat line).

4. Troubleshooting

Observation	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 template.
		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 minutes.
		Ensure that the denaturation time during the amplification cycles is 10 seconds.
	Pipetting errors or omitted reagents.	Check for missing reagents.
		Titrate $MgCl_2$ concentration.
		Check for defective SYBR Green I dye.
	Chosen gain settings are too low.	Optimize gain settings using the Real Time Fluorimeter function. Then repeat the run, using the optimal gain settings in the cycle programs.
	Scale of axes on graph are unsuitable for analysis.	Change the values for the x- and y-axis by double-clicking on the maximum and/or minimum values, then change to more suitable values.
	Measurements do not occur.	Check the amplification program. For SYBR Green I detection format, choose “single” as the acquisition mode at the end of the elongation phase.
	Amplicon length is >1 kb.	Do not use amplicons >1 kb. Optimal results are obtained with amplicons of 700 bp or less.
	Impure sample material inhibits the reaction.	Do not use more than 8 to 10 µl of DNA per 20 µl PCR reaction mixture.
		Dilute sample 1:10 and repeat the analysis.
		Repurify the nucleic acids to ensure removal of inhibitory agents.

Observation	Possible cause	Recommendation
Fluorescence intensity is too high and reaches overflow.	Unsuitable gain settings.	Gain settings cannot be changed during or after a run. Before repeating the run, use the **Real Time Fluorimeter** option to find suitable gain settings. For SYBR Green I, the background fluorescence at measuring temperature should not exceed 10. ⚠ ***Use an extra sample for this procedure, so the dyes in your experimental samples will not be bleached. LightCycler® Software versions 3.5 and higher do not require a gain setting.***
Fluorescence intensity is too low.	Low concentration or deterioration of SYBR Green I 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 protected from light.
		Avoid repeated freezing and thawing.
		After thawing, store the LightCycler® FastStart DNA Master SYBR Green I at +2 to +8°C for a maximum of one week and keep it protected from light.
	Reaction conditions are not optimized, leading to poor PCR efficiency.	Titrate $MgCl_2$ concentration.
		Primer concentration should be between 0.2 and 1.0 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 (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 protected 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.
		Use LightCycler® UNG to eliminate carryover 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 versions prior to version 4.0).

Observation	Possible cause	Recommendation
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 touchdown 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.
Only a primer-dimer peak appears, with no specific PCR product peak; or very high primer-dimer peaks.	Primer-dimers have out-competed amplification of specific PCR product.	Keep all samples at +2 to +8°C until the run is started.
		Keep the time between preparing the reaction mixture and starting the run as short as possible.
		Increase starting amount of DNA template.
		Titrate $MgCl_2$.
		Increase annealing temperature in order to enhance stringency.
	Quality of primers is poor.	Purify primers more thoroughly.
	Sequence of primers is 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. 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 directly 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, it is the reagent of choice when measuring total DNA.
The basic steps of DNA detection by SYBR Green I, during real-time PCR on the LightCycler® Carousel-Based System are:

① At the beginning of amplification, the reaction mixture contains the denatured DNA, the primers, and the 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.

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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.

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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.

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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 (Tm) of a particular DNA product (where the DNA is 50% double-stranded and 50% single-stranded). The most important factors that determine the Tm of dsDNA are the length and the GC-content of that fragment. If PCR generated only one amplicon, Melting Curve analysis will show only one melting peak. If primer-dimers or other nonspecific products are present, they will be shown as additional melting peaks. Checking the Tm of a PCR product can thus be compared with analyzing a PCR product by length in gel electrophoresis.

How this Product Works

LightCycler® FastStart DNA Master 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 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 (Chou, Q. et al.,1992; Kellogg, D.E. et al., 1994; Birch, D.E. et al., 1996), by minimizing the formation of nonspecific 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 nonspecifically. 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.
LightCycler® FastStart DNA Master SYBR Green I provides convenience, excellent performance, and reproducibility, as well as minimizing contamination risk. All you need to supply is template DNA, PCR primers, and additional $MgCl_2$ (if necessary).
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 optimize the detection protocol to the reaction conditions of the LightCycler® Carousel-Based System and design specific PCR primers for each target. Refer to the LightCycler® Operator's Manual for general recommendations.

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

5.2. References

- Birch DE, Kolmodin L, Wong J, Zangenberg GA, Zoccoli MA, McKinney N, Young KKY - Simplified hot start PCR (1996) *Nature* **381** (6581), 445-446
- Chou Q, Russell M, Birch DE, Raymond J, Bloch W - Prevention of pre-PCR mis-priming and primer dimerization improves low-copy-number amplifications (1992) *Nucleic Acids Research* 7, 1717-1723
- Kellogg DE, Rybalkin I, Chen S, Mukhamedova N, Vlasik T, Siebert PD, Chenchika A - TaqStart antibody : hot start PCR facilitated by a neutralizing monoclonal antibody directed against Taq DNA polymerase (1994) *BioTechniques* **16** (6), 1134-1137

5.3. Quality Control

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

6. Supplementary Information

6.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

6.2. Changes to previous version

Layout changes.
Editorial changes.

6.3. Ordering Information

Roche 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 homepage lifescience.roche.com.

Product	Pack Size	Cat. No.
Accessories general (hardware)		
LightCycler® Software 4.1	1 software package	04 898 915 001
LC Carousel Centrifuge 2.0	1 centrifuge plus rotor and rotor bucket (115 V)	03 709 507 001
	1 centrifuge plus rotor and rotor bucket (230 V)	03 709 582 001
LC Carousel Centrifuge 2.0 Rotor Set	1 rotor + 2 rotor buckets	03 724 697 001
LC Carousel Centrifuge 2.0 Bucket 2.1	1 rotor bucket for LC Carousel Centrifuge 2.0	03 724 689 001
Consumables		
LightCycler® Capillaries (20 µl)	5 x 96 capillaries, containing 5 boxes, each with 96 capillaries and stoppers, 5 boxes, each with 96 capillaries and stoppers	04 929 292 001
Instruments		
LightCycler® 2.0 Instrument	1 instrument	03 531 414 001
Reagents , kits		
LightCycler® Control Kit DNA	1 kit, 50 reactions with 20 µl final volume each	12 158 833 001
LightCycler® Uracil-DNA Glycosylase	50 µl, 100 U, (2 U/µl)	03 539 806 001
Transcriptor Reverse Transcriptase	250 U, 25 reactions of 20 µl final volume	03 531 317 001
	500 U, 50 reactions of 20 µl final volume	03 531 295 001
	2,000 U, 4 x 500 U, 200 reactions of 20 µl final volume	03 531 287 001
Transcriptor First Strand cDNA Synthesis Kit	1 kit, 50 reactions, including 10 control reactions	04 379 012 001
	1 kit, 100 reactions	04 896 866 001
	1 kit, 200 reactions	04 897 030 001

6.4. Trademarks

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6.5. License Disclaimer

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6.6. Regulatory Disclaimer

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6.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

6.8. Contact and Support

If you have questions or experience problems with this or any Roche product for Life Science, please contact our Technical Support staff. Our scientists are committed to providing rapid and effective help.
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