For life science research only. Not for use in diagnostic procedures.

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LightCycler® 480 SYBR Green I Master

Version: 13
Content version: August 2015

Easy-to-use hot start reaction mix for PCR using the LightCycler® 480 System

Cat. No. 04 707 516 001	5 x 1 ml 2x conc. 5 x 100 reactions of 20 µl final reaction volume
Cat. No. 04 887 352 001	10 x 5 ml 2x conc. 10 x 500 reactions of 20 µl final reaction volume

Store the kit at −15 to −25°C

1. General Information

1.1. Contents

Vial / Bottle	Cap	Label	Function	Catalog Number	Content
1	green	LightCycler® 480 SYBR Green I Master, 2x conc.	▪ Ready-to-use hot start PCR mix ▪ Contains FastStart Taq DNA Polymerase, reaction buffer, dNTP mix (with dUTP instead of dTTP), SYBR Green I dye, and $MgCl_2$	04 707 516 001	5 vials, 1 ml each
				04 887 352 001	10 vials, 5 ml each
2	colorless	Water, PCR grade	▪ To adjust the final reaction volume	04 707 516 001	5 vials, 1 ml each
				04 887 352 001	2 vials, 25 ml each

1.2. Storage and Stability

Storage Conditions (Product)

The kit is shipped on dry ice.

Store the kit at −15 to −25°C until 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
1	green	Master, 2x conc.	Store at −15 to −25°C. After first thawing the master may be stored for up to 4 weeks at +2 to +8°C. ⚠ ***Keep vial 1 away from light!*** ⚠ ***Avoid repeated freezing and thawing!***
2	colorless	Water, PCR grade	Store at −15 to −25°C.

Storage Conditions (Working Solution)

The complete PCR mix (that is LightCycler® 480 SYBR Green Master supplemented with primers and template) is stable for up to 24 h at room temperature.

⚠ ***Keep the PCR mix away from light!***

1.3. Additional Equipment and Reagents Required

Additional reagents and equipment required to perform reactions with the LightCycler® 480 SYBR Green I Master using the LightCycler® 480 System include:

- LightCycler® 480 Instrument I * or LightCycler® 480 Instrument II*
- LightCycler® 480 Multiwell Plate 384* or LightCycler® 480 Multiwell Plate 96*
- Standard swinging-bucket centrifuge containing a rotor for multiwell plates with suitable adaptors
- LightCycler® Uracil-DNA Glycosylase* (optional [1])
- Nuclease free, aerosol-resistant pipette tips
- Pipettes with disposable, positive-displacement tips
- Sterile reaction (Eppendorf) tubes for preparing master mixes and dilutions

ⓘ [1] *For prevention of carryover contamination please refer to the corresponding chapter.*

1.4. Application

LightCycler® 480 SYBR Green I Master is designed for research studies. When used with the LightCycler® 480 System, this kit is ideally suited for hot start PCR applications. In combination with the LightCycler® 480 System and suitable PCR primers, this kit allows very sensitive detection and quantification of defined DNA sequences. The kit can also be used to perform two-step RT-PCR. It can also be used with heat-labile Uracil-DNA Glycosylase to prevent carryover contamination during PCR.
In principle, the kit can be used for the amplification and detection of any DNA or cDNA target.

The LightCycler® 480 SYBR Green I Master is a one-component hot start reaction mix for PCR. It contains FastStart Taq DNA Polymerase and DNA double-strand-specific SYBR Green I dye for PCR product detection and characterization. Since the mix is provided as an easy-to-use all-in-one master reagent, reaction setup only requires the addition of template DNA and primers. The mix can be used with different types of DNA (e.g., genomic DNA or cDNA), and is suited for high-throughput applications in 96- or 384-well plates on the LightCycler® 480 Instrument.

2. How to Use this Product

2.1. Before you Begin

Sample Materials

- Use any template DNA (*e.g.,* genomic or plasmid DNA, cDNA) suitable for PCR in terms of purity, concentration, and absence of inhibitors. For reproducible isolation of nucleic acids use:
- Either a MagNA Pure System together with a dedicated nucleic acid isolation kit (for automated isolation)
- or a High Pure nucleic acid isolation kit (for manual isolation).

For details see the Roche Life Science catalog or the website: lifescience.roche.com

- Use up to 50 – 100 ng complex genomic DNA or up to 10^8 copies plasmid DNA for reaction volume of 20 µl. For larger volumes, the amount of template can be increased equivalently.

⚠ ***Using a too high amount of template DNA might reduce the maximum fluorescence signal by outcompeting the SYBR Green I dye.***

If you are using a non-purified cDNA sample from reverse transcription, especially if it contains high background concentrations of RNA and oligonucleotides, you can improve your results by using a maximum of 2 µl of that sample and applying a 10 min pre-incubation at 95°C. This will result in lower crossing point (Cp) values with a decreased standard deviation.

Control Reactions

Always run a negative control with the samples. To prepare negative controls:

- Replace the template DNA with Water, PCR grade (vial 2; this will reveal whether a contamination problem exists)
- In a two-step RT-PCR setup omit addition of reverse transcriptase to the cDNA synthesis reaction (this will indicate whether DNA in RNA samples causes false-positive results)

Primers

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

The optimal primer concentration is the lowest concentration that results in the lowest Cp and an adequate fluorescence for a given target concentration.

Mg^{2+} Concentration

The composition of the LightCycler® 480 SYBR Green I Master is optimized for almost all primer combinations.
You do not need to add additional $MgCl_2$ to the mix to get efficient and specific PCR!

General Considerations

Each amplification protocol needs to be adapted to the reaction conditions of the LightCycler® 480 Instrument and design specific PCR primers for each target. See the LightCycler® 480 Operator's Manual for general recommendations.

⚠ ***The amplicon size should not exceed 750 bp in length. For optimal results, select a product length of 500 bp or less.***

The ready-to-use LightCycler® 480 SYBR Green I Master offers convenience and ease of use because
- no additional pipetting steps to combine enzyme and reaction buffer are necessary,
- the addition of $MgCl_2$ to the reaction mixture is not necessary, thus avoiding time-consuming optimization steps.

⚠ ***The performance of the kit described in this Instruction Manual is warranted only when it is used with the LightCycler® 480 System.***

Two-step RT-PCR

LightCycler® 480 SYBR Green I Master 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® 480 System. Subsequent amplification and online monitoring is performed according to the standard LightCycler® 480 System procedure, using the cDNA as the starting sample material. Transcriptor First Strand cDNA Synthesis Kit* is recommended for reverse transcription of RNA into cDNA. Synthesis of cDNA is performed according to the detailed instructions provided with the kit.

⚠ ***For initial experiments, we recommend running undiluted, 1: 10 diluted, and 1: 100 diluted cDNA template in parallel to determine the optimum template amount. If you use undiluted cDNA as template, we recommend to extend the pre-incubation time to 10 minutes.***

2.2. Protocols

LightCycler® 480 Instrument Protocol

The following procedure is optimized for use with the LightCycler® 480 Instrument.

⚠ ***If the instruments type is not stated, "LightCycler® 480 Instrument" stands for LightCycler® 480 Instrument I and II.***

Program the LightCycler® 480 Instrument before preparing the reaction mixes.

A LightCycler® 480 Instrument protocol that uses LightCycler® 480 SYBR Green I Master contains the following programs:

- **Pre-Incubation** for activation of FastStart Taq DNA polymerase and denaturation of the DNA
- **Amplification** of the target DNA
- **Melting Curve** for PCR product identification
- **Cooling** the multiwell plate

For details on how to program the experimental protocol, see the LightCycler® 480 Operator's Manual.

Protocol for Use with LightCycler® 480 Multiwell Plate 96

The following table shows the PCR parameters that must be programmed for a LightCycler® 480 System PCR run with the LightCycler® 480 SYBR Green I Master using a LightCycler® 480 Multiwell Plate 96:

Setup					
Block Type			Reaction Volume [µl]		
96			10 – 100		
Detection Format		Excitation Filter		Emission Filter	
SYBR Green / HRM Dye		465		510	
Programs					
Program Name		Cycles		Analysis Mode	
Pre-Incubation		1		None	
Amplification		45 [1]		Quantification	
Melting Curve		1		Melting Curve	
Cooling		1		None	
Temperature Targets					
	Target [°C]	Acquisition Mode	Hold [hh:mm:ss]	Ramp Rate [°C/s]	Acquisitions [per °C]
Pre-Incubation	95	None	00:05:00 [2]	4.4	–
Amplification	95	None	00:00:10	4.4	–
	primer dependent [3]	none	00:00:05 - 00:00:20 [4]	2.2 (Target °C ≥ 50°C) 1.5 (Target °C < 50°C) [7]	–
	72	single	00:00:05 - 00:00:30 [4] [5]	4.4	–
Melting Curve [8]	95	None	00:00:05	4.4	–
	65	None	00:01:00	2.2	–
	97	Continuous	–	–	5 – 10 [6]
Cooling	40	None	00:00:10	1.5	–

Protocol for Use with LightCycler® 480 Multiwell Plate 384

The following table shows the PCR parameters that must be programmed for a LightCycler® 480 System PCR run with the LightCycler® 480 SYBR Green I Master using a LightCycler® 480 Multiwell Plate 384:

Setup					
Block Type			Reaction Volume [µl]		
384			3-20		
Detection Format		Excitation Filter		Emission Filter	
SYBR Green / HRM Dye		465		510	
Programs					
Program Name		Cycles		Analysis Mode	
Pre-Incubation		1		None	
Amplification		45 [1]		Quantification	
Melting Curve		1		Melting Curve	
Cooling		1		None	
Temperature Targets					
	Target [°C]	Acquisition Mode	Hold [hh:mm:ss]	Ramp Rate [°C/s]	Acquisitions [per °C]
Pre-Incubation	95	None	00:05:00 [2]	4.8	–
Amplification	95	None	00:00:10	4.8	–
	primer dependent [3]	None	00:00:05 - 00:00:20 [4]	2.5 (Target °C ≥ 50°C) 2.0 (Target °C < 50°C) [7]	–
	72	Single	00:00:05 - 00:00:30 [4] [5]	4.8	–
Melting Curve	95	None	00:00:05	4.8	–
	65	None	00:01:00	2.5	–
	97	Continuous	–	–	5 - 10 [6]
Cooling	40	None	00:00:10	2.0	–

1) 45 cycles are suitable for most assays. If the assay is optimized and has steep amplification curves and early crossing points (even when target concentrations are low), 40 cycles should be sufficient. Reducing the number of cycles will reduce the time required for the assay!
2) If high polymerase activity is required in early cycles, you might improve results by extending the pre-incubation to 10 min. Especially, apply such an extended pre-incubation time when working with unpurified cDNA samples as template. Do not use more than 2 µl unpurified cDNA sample.
3) For initial experiments, set the target temperature (i.e., the primer annealing temperature) 5°C below the calculated primer Tm.
4) For greater precision in target quantification experiments, it can be advantageous (in some cases) to choose longer annealing and extension times for the amplification cycles.
5) Calculate the hold time for the PCR elongation step by dividing the amplicon length over 25 (e.g., a 500 bp amplicon requires 20 s elongation time).
6) The Tm calculated by Tm Calling Analysis might differ approx. 0.5°C when using either the lowest (5) or highest (10) possible value of Aquisitions/°C.
7) For users of LightCycler® 480 Software 1.1: (From LightCycler® 480 Software 1.2 on, the Cooling Ramp Rate can be set to maximum).
   – For the 96-multiwell plate: For target temperatures of 50°C and above, set the Ramp Rate for Cooling to 2.2°C/s. For target temperatures below 50°C, set the Ramp Rate to 1.5°C/s!
   – For the 384-multiwell plate: For target temperatures of 50°C and above, set the Ramp Rate for Cooling to 2.5°C/s. For target temperatures below 50°C, set the Ramp Rate to 2.0°C/s!
8) The Tm calculated by Tm Calling Analysis might differ by up to 2°C when using either the lowest (10 µl) or the highest (100 µl) possible reaction volume.

Preparation of the PCR Mix

Follow the procedure below to prepare one 20 µl standard reaction.

⚠ ***Do not touch the surface of the LightCycler® 480 Multiwell Plate and Multiwell Sealing Foil when handling them. Always wear gloves during handling.***

1. Thaw one vial of "LightCycler® 480 SYBR Green I Master" (vial 1, green cap) and Water, PCR grade.
   ⚠ ***Keep the Master mix away from light.***

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

3. In a 1.5 ml reaction tube on ice, prepare the PCR Mix for one 20µl reaction by adding the following components in the order mentioned below:

Reagent	Volume
Water, PCR grade (vial 2, colorless cap)	3 µl
PCR Primer, 10x conc.	2 µl
Master Mix, 2x conc. (vial 1, green cap)	10 µl
Total volume	**15 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 + sufficient additional reactions.

4. Mix carefully by pipetting up and down. Do not vortex.
   Pipet 15 µl PCR mix into each well of the LightCycler® 480 Multiwell Plate.
   Add 5 µl of the DNA template.
   Seal the Multiwell Plate with LightCycler® 480 Multiwell Sealing Foil.

5. Place the Multiwell Plate in the centrifuge and balance it with a suitable counterweight (e.g., another Multiwell Plate).
   Centrifuge at 1,500 × g for 2 min (3,000 rpm in a standard swinging-bucket centrifuge containing a rotor for multiwell plates with suitable adaptors).

6. Load the Multiwell Plate into the the LightCycler® 480 Instrument.

7. Start the PCR program described above.
   ⚠ ***If you use reaction volumes different from 20 µl, be sure to adapt the right volume in the running protocol. As a starting condition, we recommend to use the same hold times as for the 20 µl volume***

2.3. Other Parameters

Prevention of Carryover Contamination

Uracil-DNA N-Glycosylase (UNG) is suitable for preventing carryover contamination in PCR. This carryover prevention technique involves incorporating deoxyuridine triphosphate (dUTP, a component of the Master Mix in this kit) into amplification products, then pretreating later PCR mixtures with UNG. If a dUTP-containing contaminant is present in the later PCRs, it will be cleaved by a combination of the UNG and the high temperatures of the initial denaturation step; it will not serve as a PCR template.

⚠ ***Use only LightCycler® Uracil-DNA Glycosylase* in combination with the LightCycler® 480 SYBR Green I Master.***

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

The use of UNG might influence the melting temperature (Tm) in melting curve analysis.

3. Results

Quantification Analysis

The following amplification curves were obtained using the LightCycler® 480 SYBR Green I Master in combination with the LightCycler® h-G6PDH Housekeeping Gene Set, targeting human glucose-6-phosphate dehydrogenase (G6PDH) mRNA. The fluorescence values versus cycle number are displayed.

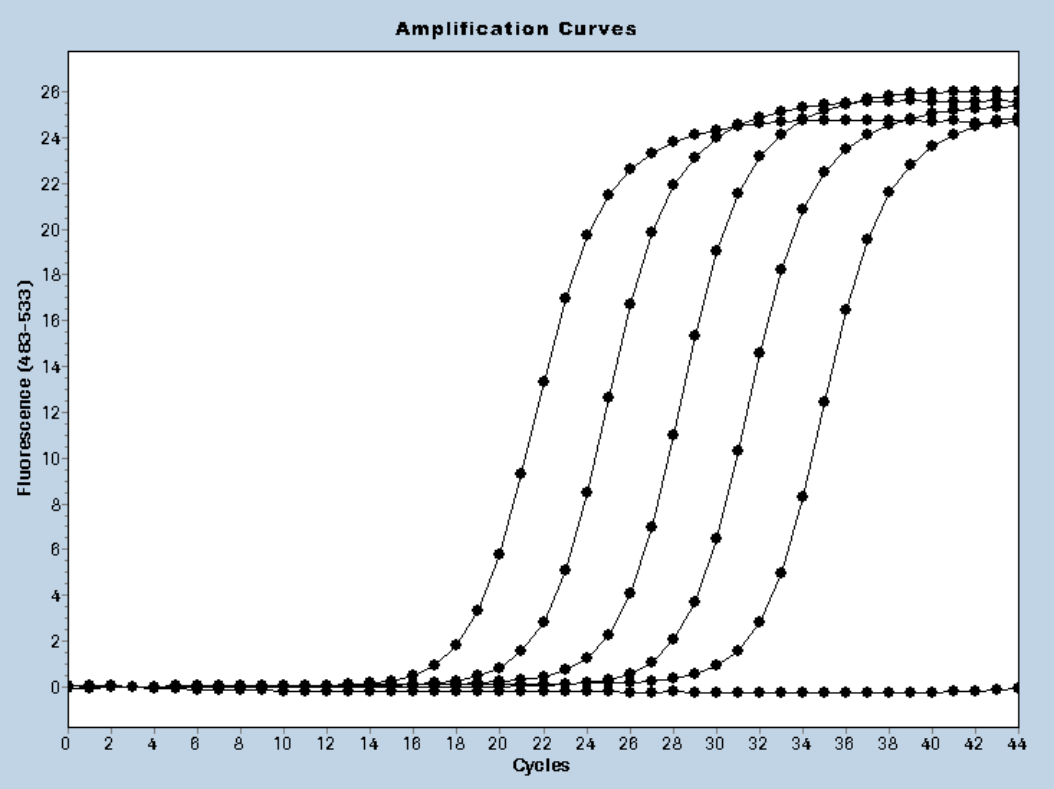

Fig. 1: Serially diluted samples containing cDNA derived from 5 × 10^5 [far left], 5 × 10^4, 5 × 10^3, 5 × 10^2, and 50 copies [far right] of in vitro transcript as starting template were amplified using the LightCycler® 480 SYBR Green I Master. As a negative control, template cDNA was replaced by Water, PCR grade [flat line].

Melting Curve Analysis

Specificity of the amplified PCR product was assessed by performing a melting curve analysis on the LightCycler® 480 Instrument. The resulting melting curves allow discrimination between primer-dimers and specific product. The specific product melts at a higher temperature than the primer-dimers (no primer dimers visible for the G6PDH example shown below). The melting curves display the specific amplification of the G6PDH RNA when starting from cDNA derived from 5 × 10^5, 5 × 10^4, 5 × 10^3, 5 × 10^2, and 50 copies of in vitro transcript.

Smaller reaction volumes may result in melting temperature variations.

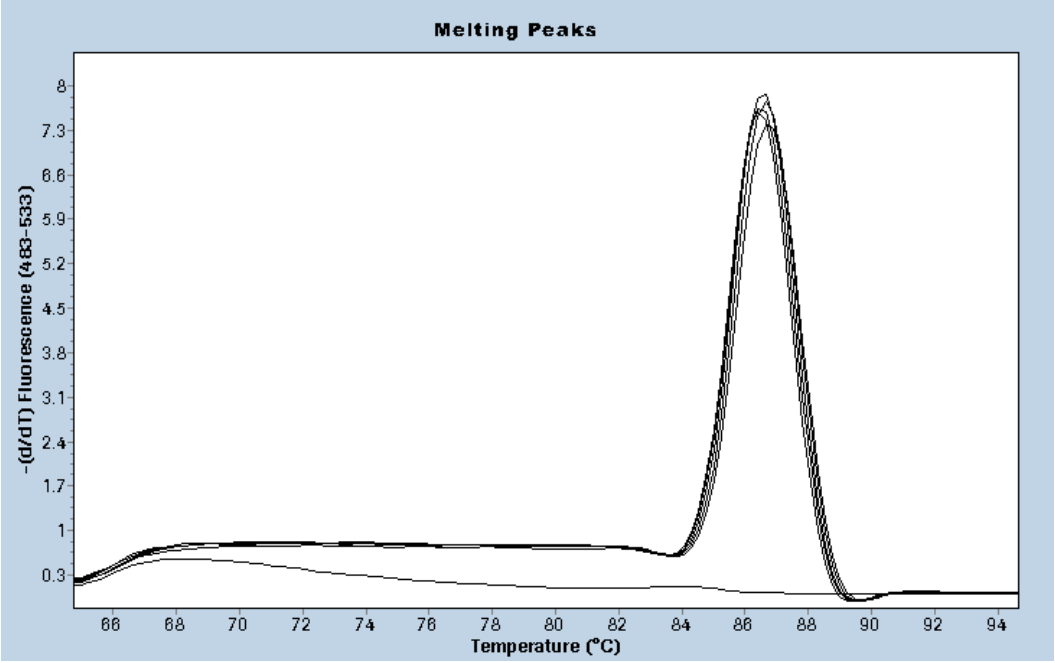

Fig. 2: Melting curve analysis of amplified samples with cDNA derived from 5 × 10^5, 5 × 10^4, 5 × 10^3, 5 × 10^2, and 50 copies of in vitro transcript as starting template. As a negative control, template DNA was replaced by Water, PCR grade.

4. Troubleshooting

Observation	Possible cause	Recommendation
Amplification curves reach plateau phase before cycling is complete.	Starting amount of nucleic acid is very high.	Stop the cycling program by clicking 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 program *Amplification*.
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., primer design).
		Use more starting DNA template.
		Repeat the run.
	The number of cycles is too low.	Increase the number of cycles in the cycle program.
No amplification occurs.	Wrong Detection Format	When programming the experiment, select *SYBR Green I / HRM Dye* as Detection Format.
	FastStart Taq DNA polymerase is not fully activated.	Make sure PCR included a pre-incubation step at 95°C for 5 - 10 min.
		Make sure denaturation time during cycles is 10 s.
	Pipetting errors or omitted reagents.	Check for missing reagents.
		Check for missing or defective dye.
	Scale of axes on graph are unsuitable for analysis.	Change the values for the x- and y-axis: right-click on the chart and select *Chart Preferences* from the context menu. Change the maximum and/or minimum axis values appropriately.
	Measurements do not occur.	Check the temperature targets of the experimental protocol. For SYBR Green I detection format, choose *Single* as the acquisition mode at the end of the elongation phase.
	Amplicon length is >900 bp.	Do not use amplicons >900 bp. Optimal results are obtained with amplicons of 500 bp or less.
	Impure sample material inhibits reaction.	Do not use more than 5 µl of DNA per 20 µl PCR reaction mixture.
		Repurify the nucleic acids to ensure removal of inhibitory agents.
Fluorescence intensity is too low.	Deterioration of dye in reaction mixtures; dyes not stored properly.	Store the Master Mix at −15 to −25°C, and keep it away from light.
		Avoid repeated freezing and thawing.
	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.	Skin oils on the surface of the multiwell sealing foil.	Always wear gloves when handling the multiwell plate and the sealing foil.
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 – 100 ng of complex genomic DNA in a 20 µl reaction.
		Instead of SYBR Green I use a sequence-specific probe-based detection format (e.g., hydrolysis probes) which allows analysis of up to 500 ng DNA (for a 20 µl reaction volume).
	Dye bleached.	Make sure the Master Mix is kept away from light. Avoid repeated freezing and thawing.

Observation	Possible cause	Recommendation
Negative control samples give a positive signal.	Contamination, or presence of primer-dimers.	Remake all critical solutions.
		Pipet reagents on a clean bench.
		Use heat-labile UNG to eliminate carryover contamination.
Double melting peak appears for one product.	Two products of different length or GC-content are 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 ▪ using a probe-based detection format 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 heat-labile UNG, primer preparation, and program settings.
	Different intensity of lamp (due to aging or exchange of lamp).	Run positive control.
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 thoroughly.
	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 high acquisition/°C rate (>10 acquisitions/°C).
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.	Use fresh solutions.
High standard deviation of crossing point (Cp) values.	Impure, heterogenous DNA template	Increase pre-incubation time to 10 min.
		Use a maximum of 2 µl unpurified cDNA sample.

5. Additional Information on this Product

5.1. Test Principle

LightCycler® 480 SYBR Green I Master is a ready-to-use reaction mix designed specifically for applying the SYBR Green I detection format on the LightCycler® 480 Instrument. It is used to perform hot start PCR in 96- or 384-multiwell plates. Hot start PCR has been shown to significantly improve the specificity and sensitivity of PCR (Chou Q, et al, 1992) and (Kellogg DE, et al, 1994) 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 non-specifically. The enzyme is completely activated (by removal of blocking groups) in a single pre-incubation step (95°C, 5 – 10 minutes) before cycling begins. Activation does not require the extra handling steps typical of other hot start techniques.

Generation of PCR products can be detected by measurement of the SYBR Green I fluorescence signal. SYBR Green I intercalates into the DNA helix (5). In solution, the unbound dye exhibits very little fluorescence; however, fluorescence (wavelength, 530 nm) is greatly enhanced upon DNA-binding. Therefore, during PCR, the increase in SYBR Green I fluorescence is directly proportional to the amount of double-stranded DNA generated. Since SYBR Green I dye is very stable (only 6% of the activity is lost during 30 amplification cycles) and the LightCycler® 480 Instrument's 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® 480 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 97°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 non-specific products are present, they will be shown as additional melting peaks. Checking the Tm of a PCR product can thus be compared with analyzing a PCR product by length in gel electrophoresis.

5.2. References

- Chou Q, Russell M, Birch DE, Raymond J, Bloch W (1992) Prevention of pre-PCR mis-priming and primer dimerization improves low-copy-number amplifications Nucleic Acids Research 7 1717-1723
- Kellogg DE, Rybalkin I, Chen S, Mukhamedova N, Vlasik T, Siebert PD, Chenchika A (1994) TaqStart antibody : hot start PCR facilitated by a neutralizing monoclonal antibody directed against Taq DNA polymerase BioTechniques 6 1134-1137

5.3. Quality Control

The LightCycler® 480 SYBR Green I Master is function tested using the LightCycler® 480 Instrument.

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	
i	*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® 480 Block Kit 96 Silver	1 block kit, for 96-well PCR multiwell plates	05 015 219 001
LightCycler® 480 Block Kit 384 Silver	1 block kit, for 384-well PCR multiwell plates	05 015 197 001
LightCycler® 480 LIMS Interface Module	1 software package	05 066 310 001
Accessories software		
LightCycler® 480 Gene Scanning Software	1 software package	05 103 908 001
LightCycler® 480 Software, Version 1.5	1 software package	04 994 884 001
Consumables		
LightCycler® 480 Multiwell Plate 96, white	5 × 10 plates, with sealing foils	04 729 692 001
LightCycler® 480 Multiwell Plate 384, white	5 x 10 plates, with sealing foils	04 729 749 001
LightCycler® 480 Multiwell Plate 96, clear	5 × 10 plates, with sealing foils	05 102 413 001
LightCycler® 480 Multiwell Plate 384, clear	5 x 10 plates, with sealing foils	05 102 430 001
LightCycler® 480 Sealing Foil	50 foils	04 729 757 001
Instruments		
LightCycler® 480 Instrument II	1 instrument, 96-well version	05 015 278 001
	1 instrument, 384-well version	05 015 243 001
Reagents , kits		
LightCycler® Uracil-DNA Glycosylase	50 µl, 100 U (2 U/µl)	03 539 806 001
Transcriptor Reverse Transcriptase	250 U, for up to 25 reactions	03 531 317 001
	500 U, for up to 50 reactions	03 531 295 001
	2,000 U (4 x 500 U), for up to 200 reactions	03 531 287 001
Transcriptor First Strand cDNA Synthesis Kit	1 kit, for up to 50 reactions, including 10 control reactions	04 379 012 001
	1 kit, for up to 100 reactions	04 896 866 001
	1 kit, for up to 200 reactions	04 897 030 001

6.4. Trademarks

LIGHTCYCLER, FASTSTART, MAGNA PURE, MAGNA LYSER and HIGH PURE are trademarks of Roche.
SYBR is a trademark of Life Technologies Corporation.
Other brands or product names are trademarks of their respective holders.

6.5. License Disclaimer

For patent license limitations for individual products please refer to: http://technical-support.roche.com.

6.6. Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

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.
Please also contact us if you have suggestions for enhancing Roche product performance or using our products in new or specialized ways. Such customer information has repeatedly proven invaluable to the research community worldwide.

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support** Site.

Visit **lifescience.roche.com**, to download or request copies of the following **Materials**:
- Instructions for Use
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- Certificates of Analysis
- Information Material

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