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

LightCycler® DNA Master HybProbe

Ready-to-use reaction mix for PCR using the LightCycler System

Cat. No. 12 015 102 001
Kit for 96 reactions

Cat. No. 12 158 825 001
Kit for 480 reactions

Store the kit at −15°C to −25°C

Instruction Manual

Version December 2004

Roche

1. Preface

1.1 Table of contents

1.2 Kit Content

Vial	Label	Content and Use
1 red cap	LightCycler DNA Master HybProbe, 10× conc.	• 3× 64 µl (96 reactions) or 15× 64 µl (480 reactions) • ready-to-use reaction mix for PCR • contains Taq DNA polymerase, reaction buffer, dNTP mix (with dUTP instead of dTTP), and 10 mM $MgCl_2$
2 blue cap	$MgCl_2$ stock solution, 25 mM	• 1× 1 ml (96 reactions) or 2× 1 ml (480 reactions) • to adjust $MgCl_2$ concentration
3 colorless cap	H_2O, PCR grade	• 2× 1 ml (96 reactions) or 7× 1 ml (480 reactions) • to adjust the final reaction volume

1.3 Storage and Stability

The complete kit is stable through the expiration date printed on the label (12 months from date of manufacture) if stored properly at -15 to -25°C.

- The kit is shipped on dry ice.
- Store kit components as follows:

Vial	Label	Storage
1 green cap	LightCycler DNA Master HybProbe, 10×	• Store at −15 to −25°C. • After thawing, store at +2 to +8°C for a maximum of 4 weeks. • **Avoid repeated freezing and thawing!**
2 blue cap	$MgCl_2$ stock solution, 25 mM	• Store at −15 to −25°C.
3 colorless cap	H_2O, PCR grade	• Store at −15 to −25°C.

2. Product Overview

Product Description

LightCycler DNA Master HybProbe is a ready-to-use PCR reaction mix. This product is specifically designed for performing PCR in 20 µl glass capillaries using the LightCycler System and the HybProbe detection format.

LightCycler DNA Master HybProbe provides convenience, excellent performance and reproducibility, as well as minimizing the risk of contamination. All you have to supply is template DNA, primers, HybProbe pairs, and additional $MgCl_2$ (if necessary).

Use of HybProbe Probes for Single and Multiple Color Detection

HybProbe probes are two different short oligonucleotides that bind to an internal sequence of the amplified fragment during the annealing phase of the amplification cycle. One probe is labeled at the 5´-end with a LightCycler Red fluorophore (LightCycler Red 610*, 640, 670*, or 705); it is also 3´-phosphorylated, so it cannot be extended. The other probe is labeled at the 3´-end with fluorescein. When hybridized to the template DNA, the two probes are close enough to allow fluorescence resonance energy transfer (FRET) between the two fluorophores.

During FRET, fluorescein (the donor fluorophore) is excited by the light source of the LightCycler Instrument. Fluorescein transfers part of this excitation energy to the LightCycler Red dye (the acceptor fluorophore). Then, the LightCycler Red dye emits fluorescence, which is measured by the LightCycler Instrument.

HybProbe pairs that contain different LightCycler Red labels can be used separately (for single color detection experiments) or combined (for dual or multiple color detection experiments). Color compensation is not necessary for single color detection experiments. However, if you are using HybProbe probes to perform dual or multiple color experiments in a single capillary, you must also use a color compensation file. The color compensation may be applied either during or after a run on the LightCycler Instrument.

Notes:

- See the LightCycler Operator's Manual and the pack insert of the LightCycler Color Compensation Set for more information on the generation and use of a color compensation file.
- LightCycler Red 610 and LightCycler Red 670 can be used on a LightCycler 2.0 Instrument only.
- * These LightCycler Red fluorophores can only be used on a LightCycler 2.0 Instrument.

Application

The *LightCycler DNA Master HybProbe* is designed for research studies. When used with the LightCycler System, suitable primers and HybProbe probes, this kit allows very sensitive detection and quantification of defined DNA sequences.

It can also be used to genotype single nucleotide polymorphisms (SNPs), analyze mutations and perform two-step RT-PCR. Further, it can be used with heat-labile Uracil DNA Glycosylase to prevent carry-over during PCR.

In principle, the *LightCycler DNA Master HybProbe* can be used for the amplification and detection of any DNA or cDNA target. To do this, you would need to adapt your detection protocol to the reaction conditions of the LightCycler System and design specific PCR primers and HybProbe probes for each target. Refer to the LightCycler Operator's Manual for general recommendations.

Notes:

- 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 guaranteed only when it is used with the LightCycler System.

Number of Tests	The kit is designed for 96 or 480 reactions (depending on pack size), with a final reaction volume of 20 µl each.
Quality Control	The *LightCycler DNA Master HybProbe* is function tested using the LightCycler System.

3. Procedures and Material Required

3.1 Before You Begin

Additional Reagents and Equipment Required

Refer to the list below for additional reagents and equipment required for PCR with the LightCycler System:

- LightCycler System* (incl. LightCycler 2.0 Instrument, LightCycler 1.5 Instrument, or an instrument version below)
- LightCycler Capillaries*
- Standard benchtop microcentrifuge containing a rotor for 2.0 ml reaction tubes

Note: The LightCycler System provides adapters that allow LightCycler Capillaries to be centrifuged in a standard microcentifuge rotor.

or

- LightCycler Carousel Centrifuge* (optional)
  or
- LightCycler Carousel Centrifuge 2.0* for use with the LightCycler 2.0 Carousel (optional)
  Note: If you use a LightCycler Instrument version below 2.0, you need in addition the LightCycler Carousel Centrifuge 2.0 Bucket 2.1 when working with LightCycler Carousel Centrifuge 2.0*. To adapt the LightCycler 2.0 Carousel to the former LightCycler Carousel Centrifuge, you need the LightCycler Carousel Centrifuge 2.0 Rotor Set*.

- PCR template (genomic DNA or cDNA)
- PCR primers
- HybProbe probes
- LightCycler Color Compensation Set (optional)
- Uracil-DNA Glycosylase, heat-labile* (optional; for prevention of carry-over contamination)

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

Assay Time/ Hands-On Time

Procedure	Time
Optional: dilution of template DNA	5 min
PCR Set-up	15 min
LightCycler PCR run	25 min
Total assay time	**45 min**

Sample Material

Use any template DNA (*e.g.,* genomic or plasmid DNA, cDNA) that is suitable for PCR in terms of purity, concentration, and absence of inhibitors.

For reproducible isolation of nucleic acids use:

- either the MagNA Pure LC Instrument (Cat. No. 2 236 931) and a dedicated MagNA Pure LC reagent kit (for automated isolation)
- or a High Pure nucleic acid isolation kit (for manual isolation).

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

Primers

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

Note for melting curve assays: If amplification curves show the "hook effect" *(i.e.*, after an exponential rise, the fluorescence signal reaches a maximum, then significantly drops in the later cycles), try using asymmetric PCR. To perform asymmetric PCR, use a higher concentration (0.5 - 1 μM) of the forward primer (*i.e.*, the one priming the strand that binds the probes) and a lower concentration of the reverse primer (titrate down from 0.5 to 0.2 μM). This favors synthesis of the strand that binds the HybProbe probes and will improve the subsequent melting curve analysis.

HybProbe Probes

Use the HybProbe probes at a final concentration of 0.2 μM each. In some cases it might be advantageous to double the concentration of the LightCycler Red-labeled probe to 0.4 μM.

Note: See the LightCycler Operator's Manual for detailed information on designing the HybProbe pairs and labeling them with various dyes. In addition, LightCycler Probe Design Software can help you design HybProbe pairs.

$MgCl_2$

To ensure specific and efficient amplification with the LightCycler System, you must optimize the $MgCl_2$ concentration for each target. The LightCycler DNA Master HybProbe contains a $MgCl_2$ concentration of 1 mM (final concentration).The optimum concentration for PCR with the LightCycler System may vary from 1 to 5 mM.

The table below shows the volume of the $MgCl_2$ stock solution (vial 2, blue cap) that you must add to a 20 μl reaction (final PCR volume) to increase the $MgCl_2$ concentration.

To 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

Negative Control

Always run a negative control with the samples. To prepare a negative control, replace the template DNA with PCR-grade water (vial 3, colorless cap).

Experimental Protocol

Program the LightCycler Experimental Protocol before preparing the reaction mixes.

Normally, a LightCycler protocol that uses the *LightCycler DNA Master HybProbe* contains the following parts:

- **Denaturation of the template DNA**
- **Amplification of the target DNA**
- **Melting curve for amplicon analysis (*Optional; only needed for SNP or mutation detection*)**
- **Cooling the rotor and thermal chamber**

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

The following table shows the PCR parameters that must be programmed for a normal LightCycler PCR Run with LightCycler DNA Master HybProbe.

Notes:

- [1)] Temperature Transition Rate/Slope is 20°C/sec, except where indicated.
- Set all protocol parameters not listed in the table to '0'.

Analysis Mode	Cycles	Segment	Target Temperature[1)]	Hold Time	Acquisition Mode
Denaturation					
None	1		95°C	30 s	none
Amplification					
Quantification	45	Denaturation	95°C	0 s	none
		Annealing	primer dependent [2)]	5-15 s [4)]	single
		Extension	72°C [3)]	≥ Product [bp]/25 s [4)]	none
Melting Curve (optional)					
Melting Curves	1	Denaturation	95°C	0 s	none
		Annealing	Probes T_m –5°C	30–60 s	none
		Melting	95°C Slope = 0.1°C/sec	0 s	continuous
Cooling					
none	1		40°C	30 s	none

[2)] For initial experiments, set the target temperature (*i.e.*, the primer annealing temperature) 5°C below the calculated primer T_m.

[3)] If the primer annealing temperature is low (< 55 °C), reduce the transition rate/slope to 2–5 °C/s.

[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.

Fluorescence and Run Setup Parameters

Parameter	Setting
All LightCycler Software Versions	
Seek Temperature	30°C
LightCycler Software prior to Version 3.5	
Display Mode	• fluorescence channel F2 (if using LightCycler Red 640 label) • fluorescence channel F3 (if using LightCycler Red 705 label)
Fluorescence Gains	**Fluorimeter** / **Gain Value** Channel 1 (F 1) / 1 Channel 2 (F 2) / 15 Channel 3 (F 3) / 30
LightCycler Software Version 3.5	
Display Mode • during run • for analysis	• fluorescence channel F2 or F3 • Divide by Channel F1 for single color experiments; Divide by 'Back-F1' for dual or multiple color experiments (*e.g.*, 640/Back-F1).
Fluorescence Gains	not required ***Note*:** In data created with LightCycler Software Version 3.5, all fluorescence values are normalized to a fluorescence gain of "1". This produces a different scale on the Y-axis than that obtained with previous LightCycler software versions. This difference does not affect the crossing points nor any calculated concentrations obtained.
LightCycler Software Version 4.0	
Default Channel • during run • for analysis	• Depending on the LightCycler Red dye used for labeling theHyb-Probe probe, choose Channel 610, 640, 670, or 705. • Divide by Channel 530 for single color experiments; Divide by 'Back 530' for dual or multiple color experiments (*e.g.*, 640/Back 530). ***Note*:** Channel 610 and 670 are only available on a LightCycler 2.0 Instrument.
Fluorescence Gains	not required
"Max. Seek Pos"	Enter the number of sample positions the instrument should look for.
"Instrument Type"	• "6 Ch.": for LightCycler 2.0 Instrument (selected by default) • "3 Ch.": for LightCycler 1.5 Instrument and instrument versions below.
"Capillary Size"	Select "20 µl" as the capillary size for the experiment. ***Note:*** For the "6 Ch." instrument type only.

Color Compensation (optional)

If performing a multiple color detection experiment in a single capillary (using at least two acceptor HybProbe pairs with different labels), you can activate or deactivate an additional color compensation file during the LightCycler Instrument run. Alternatively, a stored color compensation file can be used for data analysis after the run on the LightCycler Instrument.

***Note**:* See the LightCycler Operator's Manual and the pack insert of the LightCycler Color Compensation Set for more information on the generation and use of a color compensation file.

Preparation of the PCR Mix and Starting the Run

Prepare each 20 µl standard reaction as described below.

Note: The protocol is designed for a final reaction volume of 20 µl. For volumes < 20 µl, the reaction and cycle conditions must be optimized.

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

Step	Action
1	Depending on the total number of reactions, place the required number of LightCycler Capillaries in precooled centrifuge adapters or in a LightCycler Sample Carousel in a precooled LightCycler Centrifuge Bucket.
2	Prepare a 10× conc. solution of the PCR primers and a 10x conc. solution of HybProbe pairs.
3	Thaw the LightCycler DNA Master HybProbe, 10× conc. (vial 1, green cap) and $MgCl_2$ stock solution, 25 mM (vial 2, blue cap), mix gently, and store on ice.
4	In a 1.5 ml reaction tube on ice, prepare the Master Mix for one reaction by adding the following components in the order mentioned below, then mixing gently by pipetting up and down:

Component	Volume	Final conc.
H_2O, PCR grade (vial 3, colorless cap)	x µl	
$MgCl_2$ stock solution (vial 2, blue cap)	y µl	Use concentration that is optimal for the target.
PCR Primer Mix, 10x conc.	2 µl	0.2–1.0 µM each (recommended conc. is 0.5 µM)
HybProbe probe mix, 10×	2 µl	0.2-0.4 µM each
LightCycler DNA Master HybProbe (vial 1, green cap)	2 µl	1x
Final volume	18 µl	

Note: To prepare the Master 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.

5	• Pipet 18 µl Master Mix into the precooled LightCycler Capillary. • Add 2 µl of the DNA template. ***Note:*** Use up to 500 ng complex genomic DNA or 10^1 – 10^{10} copies plasmid DNA.
6	• Seal each capillary with a stopper and place the adapters, containing the capillaries, into a standard benchtop microcentrifuge. • Centrifuge at 700 × *g* for 5 s (3000 rpm in a standard benchtop microcentrifuge). **Note**: Place the centrifuge adapters in a balanced arrangement within the centrifuge. Alternatively, centrifuge the capillaries within the sample carousel in the LightCycler Carousel Centrifuge.
7	Place the LightCycler Sample Carousel with capillaries into the LightCycler Instrument, then start your protocol.

3.3 Related Procedures

Color Compensation

If using acceptor HybProbe probes that contain different LightCycler Red labels in the same capillary, you must compensate for the crosstalk between individual channels by using a (previously generated) color compensation file.

You can activate a previously stored color compensation file during the LightCycler Instrument run or use it for data analysis after the run.

Refer to the LightCycler Operator's Manual and to the pack insert of the LightCycler Color Compensation Set or LightCycler Multiplex DNA Master HybProbe for more information on the generation and use of a color compensation file.

Notes:

- Although the optical filters of each detection channel of the LightCycler Instrument are optimized for the different emission maxima of the fluorescent dyes, some residual crosstalk between the channels will occur unless you correct for it with a color compensation file.
- Color Compensation is instrument-specific. Therefore, a color compensation file must be generated for each LightCycler Instrument.
- No universal color compensation set is available for 6-channel applications. All multicolor assays must use a specific Color Compensation Protocol. You must prepare a new color compensation file for each set of parameters (and each time you receive a new batch of oligonucleotides).

Hot Start

If the reaction components are thoroughly mixed prior to the initial heat denaturation step, nonspecific annealing and primer elongation events may occur. Conventional manual hot start or wax techniques cannot be used with the LightCycler Instrument due to the small capillaries. However, you can run PCR applications that use the HybProbe detection format in "Hot Start" mode by using the *LightCycler FastStart DNA Master HybProbe* or *LightCycler FastStart DNA Master*^{PLUS} *HybProbe*, which contain a chemically modified, inactive form of Taq DNA polymerase, FastStart Taq DNA polymerase, which is activated by heat.

Prevention of Carry-Over Contamination

Uracil DNA N-Glycosylase (UNG) is suitable for preventing carry-over contamination in PCR. This carry-over prevention technique involves incorporating deoxyuridine triphosphate (dUTP, a component of the 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.

Notes:

- Since your target DNA template contains thymidine rather than uridine, it is not affected by this procedure.
- Refer to the pack insert of Uracil-DNA Glycosylase, heat-labile* for details on application.

Two-step RT-PCR

The *LightCycler DNA Master HybProbe* can also be used to perform two-step RT-PCR.

In two-step RT-PCR, the reverse transcription of RNA into cDNA is separated from the other reaction steps and is performed outside the LightCycler System. Subsequent amplification and online monitoring is performed according to the standard LightCycler System procedure, using the cDNA as the starting sample material.

One of the following reagents is required for reverse transcription of RNA into cDNA (see Ordering Information for details):

- 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.

Note: Do not use more than 8 µl of undiluted cDNA template per 20 µl final reaction volume because greater amounts may inhibit the PCR. For initial experiments, we recommend running undiluted, 1:10 diluted, and 1:100 diluted cDNA template in parallel to determine the optimum template amount.

4. Results

Introduction

The following amplification curves were obtained by performing the procedure for single color detection and using LightCycler Red 640 as acceptor fluorophore. Displayed are the results in channel 2 and 3, with and without color compensation.
Equivalent results (according to the table above) will be obtained using single color detection with LightCycler Red 705 as acceptor fluorophore or dual color detection with LightCyclerRed 640 and LightCycler Red 705 simultaneously.
The fluorescence values versus cycle number are displayed. 30 pg (approx. 10 genome equivalents) of human genomic DNA can be reproducibly detected by amplifying in the LightCycler Instrument and using the HybProbe detection format. Three picograms (approx. 1 genome equivalent) are sporadically detected due to statistical fluctuations.

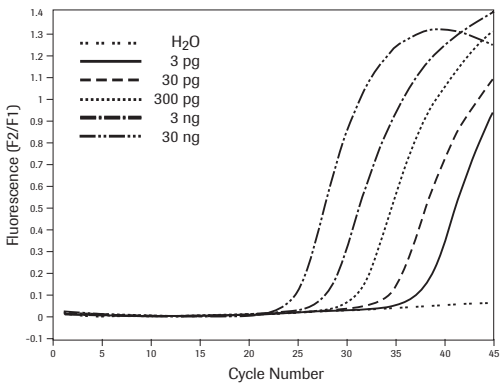

Fig. 1a: Channel 2 (F2/F1) without color compensation

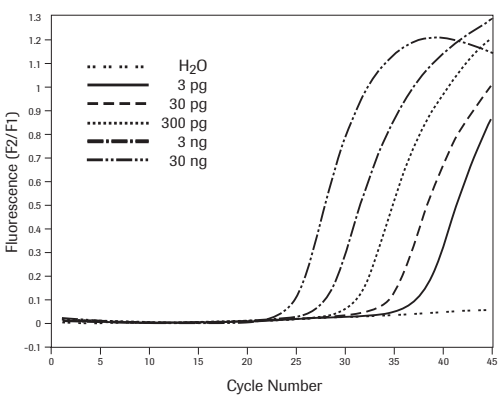

Fig. 1b: Channel 2 (F2/F1) with color compensation

5. Appendix

5.1 Troubleshooting

Problem	Possible cause	Recommendation
Amplification reaches plateau phase before the program is complete.	Starting amount of nucleic acid is very high.	Stop the program by clicking on the **End Program** button. The next cycle program will start automatically.
	The number of cycles is too high.	Reduce the number of cycles in the cycle program.
Log-linear phase of amplification just starts as the amplification program finishes.	Starting amount of nucleic acid is very low.	• Use the **Add 10 Cycles** button to increase number of cycles in the program. • Improve PCR conditions (e.g. $MgCl_2$ concentration, primer and probe design). • Use more starting material. • Repeat the run.
No amplification occurs.	Using wrong channel to display amplification onscreen.	Change the channel setting on the programming screen.(Data obtained up to this point will be saved.)
	Pipetting errors or omitted reagents.	• Check for missing reagents. • Titrate $MgCl_2$ concentration. • Check for missing or defective dye.
	Measurements do not occur at the right time.	Check the cycle programs. For HybProbe detection format, choose “single” as the acquisition mode at the end of the annealing phase.
	Difficult, *e.g.*, GC-rich, templates.	• Repeat PCR under same conditions and add increasing amounts of DMSO (up to 10% of the final concentration). • If performance is still not satisfactory, optimize annealing temperature and $MgCl_2$ concentration, while also titrating the DMSO concentration.
	Amplicon length is >1 kb.	Do not use amplicons >1 kb. Optimal results are obtained with amplicons of 700 bp or less.
	Impure sample material inhibits reaction.	• Do not use more than 8-10 µl of DNA per 20 µl PCR reaction mixture. • Repurify the nucleic acids to ensure removal of inhibitory agents.
	Unsuitable HybProbe pair.	Check sequence and binding site of the HybProbe pair.

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Problem	Possible cause	Recommendation
Flurescence intensity is too high and reaches overflow.	Gain settings are too high.	Gain settings cannot be changed during or after a run, so you must repeat the run. Before repeating the run, use the **Real Time Fluorimeter** option to find suitable gain settings. For the HybProbe detection format, the background fluorescence at measuring temperature should not exceed 20. ***Note***: Use an extra sample for this procedure, so the dyes in your experimental sample will not be bleached.
Fluorescence intensity is too low.	Low concentration or deterioration of dyes in the reaction mixtures; dyes not stored properly.	• Store the dye-containing reagents at −20°C, away from light. • Avoid repeated freezing and thawing. • Improve low HybProbe signals by making the concentration of the LightCycler Red-labeled probe twice as high as the concentration of the fluorescein-labeled probe.
	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.
	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; probe concentration should be between 0.2 and 0.4 μM. • Check annealing temperature of primers and probes. • Check experimental protocol. • Always run a positive control along with your samples.
	Mutation analysis using HybProbe detection format: The melting temperature of the hybrid between the mismatch strand and the HybProbe pair is lower than the annealing temperature. Therefore, the HybProbe pair can't bind and create a signal.	This will not affect amplification efficiency. Ensure that the melting curve starts at a temperature below the annealing temperature used for PCR. Then, you will get a clear signal after melting curve analysis and will be able to interpret the data.

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Problem	Possible cause	Recommendation
Fluorescence intensity varies.	Pipetting errors.	When performing a HybProbe single color detection experiment, you can minimize the effects of pipetting errors by viewing the results in the F2/F1 or F3/F1 mode.
	• Prepared 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.
Negative control samples give a positive signal.	Contamination.	• Remake all critical solutions. • Pipet reagents on a clean bench. • Close lid of the negative control reaction tube immediately after pipetting it. • Use heat-labile UNG to eliminate carry-over contamination.
High background	Fluorescence signals are very low, therefore the background seems relatively high.	Follow general optimization strategies for LightCycler PCR.
	HybProbe concentration is too high.	HybProbe concentration should be between 0.2 and 0.4 μM.
	Quality of HybProbe probes is poor.	Prepare a new HybProbe pair.
	Gain settings are too high.	• Reduce value of gain setting. • Use the **Real Time Fluorimeter** option to optimize the gain settings.
Amplification curve decreases in late cycles after reaching a plateau.	"Hook effect": Competition between binding of the HybProbe pair and re-annealing of the PCR product.	This does not affect interpretation of the results. You can avoid it by performing an asymmetric PCR, which favors amplification of the DNA strand to which the HybProbe pair binds.
Melting peak is very broad and peaks can't be differentiated.	**°C to Average** setting is too high.	Reduce the value of **°C to Average**. Note: Applicable for LightCycler Software 3.0 to 3.5 only.
Melting temperature of a product varies from experiment to experiment.	Variations in reaction mixture.	• Check purity of template solution. • Reduce variations in parameters such as $MgCl_2$, heat-labile UNG, and program settings.
No precise melting peak can be identified.	• HybProbe pairs are not homogeneous and/or contain secondary structure. • Pseudogenes lead to multiple PCR products.	• Redesign HybProbe pairs. • Check PCR products on an agarose gel.

5.2 References

1 F. de Monbrison, D. Raynaud, C. Latour-Fondanaiche, A. Staal, S. Favre, K. Kaisera, F. Peyrona, and S. Picot (2003). Real-time PCR for chloroquine sensitivity assay and for pfmdr1–pfcrt single nucleotide polymorphisms in Plasmodium falciparum. *Journal of Microbiological Methods*, Vol. **54**, pp. 391-401.

2 E. Albanese, X.Y. Yang, and H. Fernandes. Identification of Cytokine SNPs Using LightCycler Hybridization Probes and Melting Curve Analysis. *Biochemica* 2003, No. **2**, pp. 4-5.

3 Y. W. Wong, G. M. Sia and H. P. Too (2002). Quantification of mouse glial cell-line derived neurotrophic factor family receptor alpha 2 alternatively spliced isoforms by real time detection PCR using SYBR Green I. Neuroscience Letters Vol. **320**, pp 141-145.

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5 M. Lareu, J. Puente, B. Sobrino, B. Quintáns, M. Brión and A. Carracedo (2001). The use of the LightCycler for the detection of Y chromosome SNPs. *Forensic Science International*, Vol. **118**, pp 163-168.

5.3 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 Special Interest Sites including:

- The LightCycler System family for real-time, online PCR: *http://www.lightcycler-online.com*
- the MagNA Pure Family for automated nucleic acid isolation: *http://www.magnapure.com*
- manual nucleic acid isolation and purification: *http://www.roche-applied-science.com/napure/*

Product	Pack size	Cat. No.
Instruments and Accessories		
LightCycler 2.0 Instrument	1 instrument plus accessories	03 531 414 201
LightCycler 1.5 Instrument*	1 instrument plus accessories	04 484 495 001
LightCycler Capillaries (20 µl)	1 pack (8× 96 capillaries)	11 909 339 001
LightCycler Carousel Centrifuge	1 centrifuge plus rotor and bucket	03 030 512 001 (115 V) 12 189 682 001 (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
LightCycler Carousel Centrifuge 2.0	1 centrifuge plus rotor and bucket	03 709 507 001 (115 V)/ 03 709 582 001 (230 V)
Software		
LightCycler Probe Design Software 2.0	1 package	04 342 054 001
cDNA Synthesis Reagents		
Transcriptor Reverse Transcriptase	250 U 500 U 2000 U	03 531 317 001 03 531 295 001 03 531 287 001
Transcriptor First Strand cDNA Synthesis Kit	1 kit	04 379 012 001
First Strand cDNA Synthesis Kit for RT-PCR (AMV)	1 kit	11 483 188 001
Associated Reagents and Kits		
LightCycler FastStart DNA Master HybProbe	1 kit (96 reactions) 1 kit (480 reactions)	12 015 099 001 12 158 817 001
LightCycler FastStart DNA Master^{PLUS} HybProbe	1 kit (96 reactions) 1 kit (480 reactions)	03 003 230 001 12 239 264 001
LightCycler FastStart DNA Master^{PLUS} HybProbe, 100 µl Reactions	1 kit (384 reactions)	03 752 168 001
LightCycler Color Compensation Set	Set for 5 calibration runs	12 158 850 001
LightCycler Multicolor Demo Set	20 reactions & 5 color compensation runs	03 624 854 001
LightCycler Control Kit DNA	1 kit (50 reactions)	12 158 833 001
Uracil-DNA Glycosylase, heat-labile	100 U 500 U	11 775 367 001 11 775 375 001

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