For general laboratory use.
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LightCycler® RNA Master HybProbe

Version February 2006

Easy-to-use Reaction Mix for One-Step RT-PCR using the LightCycler® System

Cat. No. 03 018 954 001 Kit for 96 reactions

Store the kit at −15 to −25°C

Table of Contents

PROTOCOL

1. What this Product Does

Number of Tests The kit is designed for 96 reactions with a final reaction volume of 20 µl each.

Kit Contents

Vial/Cap	Label	Contents / Function
1 red cap	LightCycler® RNA Master HybProbe, 2.7× conc.	• 3× 250 µl • contains Tth DNA Polymerase, reaction buffer, dNTP mix (with dUTP instead of dTTP)
2 colorless cap	$Mn(OAc)_2$ stock solution, 50 mM	• 1 ml • to adjust $Mn(OAc)_2$ concentration
3 colorless cap	H_2O, PCR grade	• 2× 1 ml • to adjust the reaction volume

Storage and Stability

The complete kit is stable through the expiration date printed on the label if stored properly at −15 to −25°C.

- The kit is shipped on dry ice.
- Once the kit is opened, store the kit components as described in the following table:

Vial	Label	Storage
1 red cap	LightCycler® RNA Master HybProbe, 2.7× conc.	• Store at −15 to −25°C. • **Avoid repeated freezing and thawing!**
2 colorless cap	$Mn(OAc)_2$ stock solution, 50 mM	Store at −15 to −25°C.
3 colorless cap	H_2O, PCR grade	

Additional Equipment and Reagents Required

Refer to the list below for additional reagents and equipment required to perform RT-PCR reactions with the LightCycler® RNA Master HybProbe using the LightCycler® System:

- LightCycler® System* (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

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

or

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

⚠ If you use a LightCycler® Instrument version below 2.0, you need in addition the LightCycler® Carousel Centrifuge 2.0 Bucket 2.1*. To adapt the LightCycler® 2.0 Carousel to the former LightCycler® Carousel Centrifuge, you need the LightCycler® Carousel Centrifuge 2.0 Rotor Set*.

- LightCycler® Color Compensation Set*# (optional)
- Uracil-DNA N-Glycosylase, heat-labile*‡ (optional)
- Nuclease-free, aerosol-resistant pipette tips
- Pipettes with disposable, positive-displacement tips
- Sterile reaction (Eppendorf) tubes for preparing master mixes and dilutions

If you want to perform color compensation when using LightCycler® Red 640 and 705-labeled HybProbe pairs in dual color experiments in the same capillary. See section Related Procedures for details.

‡ for prevention of carry-over contamination; see section Related Procedures for details.

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

Application

The LightCycler® RNA Master HybProbe is designed for research studies. When combined with the LightCycler® System, this kit uses a hot start RT-PCR protocol to provide very sensitive detection and quantification of defined RNA sequences (if suitable PCR primers and HybProbe probes are supplied).

The kit is especially suitable for difficult RNA populations since the elevated incubation temperature during the reverse transcription step will help to overcome secondary structures. The hot start feature will minimize mispriming during the initial phase of the reaction, and therefore overall sensitivity of RT-PCR is increased. It can also be used to genotype single nucleotide polymorphisms (SNPs) and analyze mutations. Further, it can be used with heat-labile Uracil DNA Glycosylase to prevent carry-over contamination during PCR.

In principle, the LightCycler® RNA Master HybProbe can be used for the amplification and detection of every RNA target. However, you would need to adapt each amplification protocol to the reaction conditions of the LightCycler® System and design specific PCR primers and HybProbe probes for each target. See the LightCycler® Operator's Manual for general recommendations.

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

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

2. How to Use this Product

2.1 Before You Begin

Sample Material

Use any template RNA (*e.g.,* total RNA or mRNA) suitable for RT-PCR in terms of purity, concentration, and absence of inhibitors.

⚠ Use up to 500 ng total RNA or 100 ng mRNA. Higher concentrations might result in inhibition of the reaction.

🔍 If the concentration of template RNA is lower than 10 µg/ml, the addition of unspecific carrier RNA (*e.g.,* MS2 RNA*) is recommended. To avoid loss of template RNA due to adsorption effects, the total RNA concentration of solutions (template plus carrier RNA) should not be lower than 10 µg/ml.

For reproducible isolation of nucleic acids use:

- either the MagNA Pure LC Instrument together with a dedicated MagNA Pure LC reagent kit (for automated isolation)
- or a High Pure nucleic acid isolation kit (for manual isolation).

See Ordering Information for selected products recommended for isolation of template RNA. For further information consult the Roche Applied Science Biochemicals catalog or the website: www.roche-applied-science.com .

Primers

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

⚠ 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 to 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 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.

🔍 See the LightCycler® Operator's Manual and the LightCycler® Online Resource Site (www.lightcycler-online.com) for detailed information on designing HybProbe probes and labeling HybProbe probes with various dyes. In addition, LightCycler® Probe Design Software* can help you design HybProbe pairs.

$Mn(OAc)_2$

To ensure specific and efficient amplification with the LightCycler® System, use $Mn(OAc)_2$ at a final concentration of 3.25 mM.

For most RNA targets tested so far, no titration of $Mn(OAc)_2$ was required. However, if necessary titrate $Mn(OAc)_2$ in a range from 2.5 to 4 mM in steps of 0.25 mM (addition of 0.1 ml 50 mM $Mn(OAc)_2$ stock solution to a final volume of 20 ml results in an increase of $Mn(OAc)_2$concentration of 0.25 mM).

Negative Control

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

DNA Contamination Control

To test the template RNA for contamination with residual genomic DNA, perform PCR in combination with LightCycler® DNA Master HybProbe, LightCycler® FastStart DNA Master HybProbe, or LightCycler® FastStart DNA Master^{PLUS} HybProbe. Because in this experimental setup the reverse transcription step is omitted, any PCR product generated is a signal for DNA contamination of the RNA template preparation.

LightCycler® Protocol

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

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

A LightCycler® protocol that uses the LightCycler® RNA Master HybProbe contains the following programs:

- **Reverse Transcription** of template RNA
- **Denaturation** of cDNA/RNA hybrid
- **Amplification** of the cDNA
- **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.

⚠ [1)] Temperature Transition Rate/Slope is 20°C/sec, except where indicated.

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

The following table shows the PCR parameters that must be programmed for a normal LightCycler® RT-PCR Run with the LightCycler® RNA Master HybProbe**.**

Analysis Mode	Cycles	Segment	Target Temperature [1)]	Hold Time	Acquisition Mode
Reverse Transcription					
None	1		61°C	20 min	none
Denaturation					
None	1		95°C	30 s [4)]	none
Amplification					
Quantification	45	Denaturation	95°C	1–5 s [4)]	none
		Annealing	primer dependent [2)]	10–15 s [5)]	single
		Extension	72°C [3)]	product [bp] /25 s [5)]	none
Melting Curve (optional)					
Melting Curves	1	Denaturation	95°C	0 s	none
		Annealing	HybProbe T_m - 5°C	30 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 temperature transition rate/slope to 2–5°C/s.

[4)] For GC-rich templates, it might be necessary to increase the denaturation time in program Denaturation up to 2 min and in program Amplification up to 5 s.

[5)] 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	61°C	
LightCycler® Software prior to Version 3.5		
Display Mode	fluorescence channel F2 (for LightCycler® Red 640) or F3 (for LightCycler® Red 705)	
Fluorescence Gains	**Fluorimeter**	**Gain Value**
	Channel 1 (F1)	1
	Channel 2 (F2)	15
	Channel 3 (F3)	30
LightCycler® Software Version 3.5		
Display Mode • during run • for analysis	• fluorescence channel F2 (for LightCycler® Red 640) or F3 (for LightCycler® Red 705) • For quantification analysis divide by Channel F1 for single color experiments; divide by 'Back-F1' for dual color experiments (*e.g.,* F2/Back-F1). For melting curve analysis do not divide by Channel F1 or Back-F1.	
Fluorescence Gains	not required In data created with LightCycler® Software Version 3.5, all fluorescence values are normalized to a fluorescence gain of "1". This produces a different scale on the Y-axis than that obtained with previous LightCycler® software versions. This difference does not affect the crossing points nor any calculated concentrations obtained.	

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Parameter	Setting
LightCycler® Software Version 4.0	
Default Channel • during run • for analysis	• Depending on the LightCycler® Red dye used for labeling the HybProbe probe, choose Channel 610, 640, 670, or 705. • Depending on the LightCycler® Red dye used for labeling the HybProbe probe, choose Channel 610, 640, 670, or 705. For quantification analysis divide by channel 530 for single color experiments; divide by 'Back 530' for dual color experiments (*e.g.,* 640/Back 530). For automated T_m Calling analysis do not divide by channel 530 or "Back 530". Channel 610 and 670 are available on a LightCycler® 2.0 Instrument only.
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. ⚠ For the "6 Ch." instrument type only.

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.

❶ 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.

❷ • Thaw the solutions and, for maximal recovery of contents, briefly spin vials in a microcentrifuge before opening.
• Mix carefully by pipetting up and down and store on ice.

⚠ A reversible precipitate may form in the LightCycler® RT-PCR Reaction Mix HybProbe (vial 2, red cap) during storage. If a precipitate is visible, place the RT-PCR Reaction Mix at room temperature and mix gently from time to time until the precipitate is completely dissolved. This does not influence the performance in RT-PCR.

❸ Prepare a 10× conc. solution of PCR primers and a 10x conc. solution of HybProbe probes.

❹ 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:

Component	Volume	Final conc.
H_2O, PCR-grade (vial 3, colorless cap)	6.2 µl	–
$Mn(OAc)_2$ stock solution, 50 mM (vial 2, colorless cap)	1.3 µl	3.25 mM
Primer mix, 10× conc. [1)]	2.0 µl	0.2 to 1.0 µM each (recommended conc. is 0.5 µM)
HybProbe mix, 10× conc. [2)]	2.0 µl	0.2 to 0.4 µM each
LightCycler® RNA Master HybProbe (vial 1, red cap)	7.5 µl	1×
Total volume	**19 µl**	

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

- Mix gently by pipetting up and down. Do not vortex.
- Pipet 19 µl PCR mix into each LightCycler® Capillary.
- Add 1 µl RNA template.
- Seal each capillary with a stopper.

- Place the adapters (containing the capillaries) into a standard benchtop microcentrifuge.

  ⚠ Place the centrifuge adapters in a balanced arrangement within the centrifuge.
- Centrifuge at 700× *g* for 5 s (3000 rpm in a standard benchtop microcentrifuge).
- Alternatively, use the LightCycler® Carousel Centrifuge for spinning the capillaries.

❺ Transfer the capillaries into the sample carousel of the LightCycler® Instrument.

❻ Cycle the samples as described in section "LightCycler® Protocol".

[1)] Due to possible primer/primer interactions generated during storage it might be necessary to preheat the PCR primer mix for 1 min at 95°C before starting the reaction to achieve optimum sensitivity.

[2)] If you want to perform dual color detection using LightCycler® Red 640- and Red 705-labeled HybProbe pairs simultaneously in one capillary, either use two separated HybProbe mixes (then you will have to add 2 µl each from both of the two mixes) or combine both HybProbe pair preparations in one mix. (You will then have to add 2 µl only from this combined HybProbe mix).

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.

- 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 on a LightCycler® 2.0 Instrument. All multicolor assays must use a specific color compensation protocol. You must prepare a new color compensation object for each set of parameters.
- For more information on the generation and use of a color compensation file, see the LightCycler® Operator's Manual, the LightCycler® Online Resource Site (www.lightcycler-online.com), or the pack inserts of the LightCycler® Color Compensation Set and LightCycler® Multiplex DNA Master HybProbe.

Prevention of Carry-Over Contamination

Heat-labile 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 reaction mixes of all LightCycler® reagent kits) into amplification products, then pretreating later PCR mixtures with UNG. If a dUTP-containing contaminant is present in the later PCRs, it will be cleaved by a combination of the UNG and the high temperatures of the initial denaturation step; it will not serve as a PCR template.

Proceed as described in the table below to prevent carry-over contamination using heat-labile UNG:

❶ Add 1 µl heat-labile UNG to the master mix per 20 µl final reaction volume.
❷ Add template RNA and incubate the completed reaction mixture for 5 min at room temperature.
❸ Destroy any contaminating template and inactivate the UNG enzyme by performing the reverse transcription step at 55°C. ⚠ Do not perform an additional inactivation step at higher temperatures (55°C) since the reverse transcriptase would be inactivated.

- When performing an additional melting curve analysis, the use of UNG lowers the respective melting temperature (T_m) by approx. 1°C.

3. Results

Introduction

The following amplification curves were obtained using the LightCycler® RNA Master HybProbe in combination with the LightCycler® Control Kit RNA targeting *in vitro* transcribed cytokine RNA template. The single color detection protocol was performed using LightCycler® Red 640 as acceptor fluorophore. Displayed are the results in channel F2 and F3, with and without color compensation. Equivalent results will be obtained using single color detection with LightCycler® Red 705 as acceptor fluorophore or dual color detection with LC Red 640- and LC Red 705-labeled HybProbe pairs simultaneously.

The fluorescence values versus cycle number are displayed. 100 copies of the cytokine RNA can be reproducibly detected by amplification in the LightCycler® Instrument using the HybProbe detection format.

Fig. 1: Serially diluted samples containing 10^1 to 10^6 copies of cytokine RNA template from the LightCycler® Control Kit RNA were amplified using the LightCycler® RNA Master HybProbe in a LightCycler® Instrument. As a negative control, template RNA was replaced by PCR-grade water. LightCycler® Red 640 was used as acceptor fluorophore. Fig 1a and 1b display results in detection channel F2 without and with color compensation. Fig 1c and 1d display results in detection channel F3 without and with color compensation. Quantification analysis was done using LightCycler® Software 3.5 applying arithmetic background subtraction.

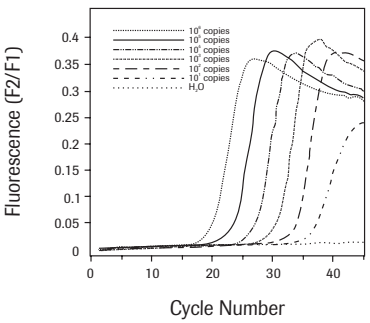

Fig. 1a: Channel F2 (F2/F1) without color compensation

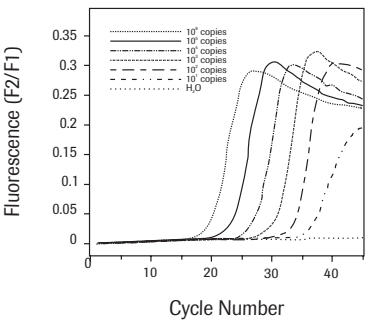

Fig. 1b: Channel F2 (F2/F1) with color compensation

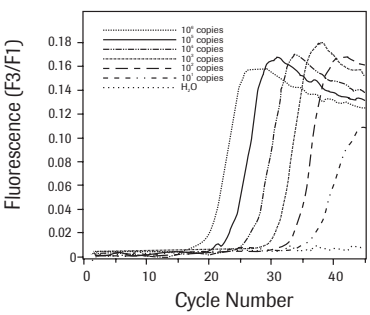

Fig. 1c: Channel 3 (F2/F1) without color compensation

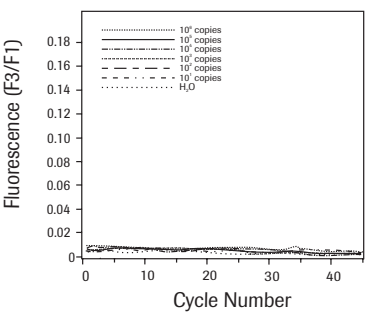

Fig. 1d: Channel F3 (F2/F1) with color compensation

	Possible cause	Recommendation
Amplification reaches plateau phase before the program is finalized.	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 cycle program.
Log-linear phase of amplification just starts when the amplification program finishes.	Very low starting amounts of nucleic acid.	• Increase number of cycles by 10 in the corresponding cycle program. • Improve PCR conditions (*e.g.*, primer and probe concentration or design). • Use higher amount of starting material. • Repeat the run.
No amplification occurs.	Wrong channel has been chosen to detect amplification online.	Check the channel chosen in the programming screen and change. (The data obtained up to this point will be saved.)
	Pipetting errors or omitted reagents.	• Check for missing reagents. • Check for missing or defective dye.
	Measurements do not occur	Check the cycle programs. For HybProbe detection format, choose “single” as acquisition mode at the end of the annealing phase.
	Amplicon length is >750 bp	Do not use amplicons >750 bp. Amplification efficiency is reduced with amplicons of this size. Optimal results are obtained for amplicons up to 500 bp.
	Inhibitory effects of the sample material due to insufficient purification.	• Do not use more than 8-10 µl of RNA per 20 µl RT-PCR reaction mixture. • Repurify the nucleic acids to ensure removal of inhibitory agents.
	Unsuitable HybProbe probes.	• Check sequence and location of the HybProbe probes. • Check PCR product on a agarose gel.
	Unsuitable primers.	• Check primer design (quality). • Check PCR product on a gel.
	RNA degradation due to unproper storage or isolation.	• Check RNA quality on a gel. • Check RNA with an established primer pair if available.
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. The background fluorescence at measuring temperature should not exceed 20 for HybProbe probes. ⚠ Avoid bleaching of dyes by using an extra sample for this procedure.

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	Possible cause	Recommendation
Fluorescence intensity is too low.	Low concentration or deterioration of dyes in the reaction mixtures due to unsuitable storage conditions.	• Store the dye containing reagents at −15 to −25°C, protected from light. • Avoid repeated freezing and thawing. • Low HybProbe signals can be improved by using a two times higher concentration of the LC Red-labeled probe than of the fluorescein-labeled probe.
	Chosen gains are too low.	Optimize gain setting using the Real Time Fluorimeter function. Change the gain settings in the cycle programs appropriately and repeat the run.
	Poor PCR efficiency due to non-optimized reaction conditions.	• Primer concentration should be in the range of 0.2 to 1.0 µM, probe concentration should be in the range of 0.2 to 0.4 µM. • Check annealing temperature of primers and probes. • Check experimental protocol. • Always run a positive control along with your samples. • Increase amount of RNA template up to 500 ng total RNA or 100 ng mRNA. • For most RNA targets tested so far no titration of $Mn(OAc)_2$ has been required. However, if necessary a titration of $Mn(OAc)_2$ in a range from 2.5 to 4 mM in steps of 0.25 mM might be considered (0.1 µl $Mn(OAc)_2$ stock solution, 50 mM correspond to 0.25 mM $Mn(OAc)_2$ in a final volume of 20 µl).
	Poor PCR efficiency due to high GC content or high degree of secondary structures of the RNA.	Extend the incubation time for Reverse Transcription to 30 min, and for denaturation during cycling to 5 s.
	Poor PCR efficiency due to unsuitable primers or probes.	• Check PCR product on agarose gel. • Redesign primer, probes.
Fluorescence intensity varies	Pipetting errors	When using HybProbe probes and single color detection, pipetting errors can be diminished by interpreting the results in the F2/F1 or F3/F1 (640/530 or 705/530) mode.
	Prepared PCR mix is still in the upper vessel of the capillary. Air bubble is trapped in the capillary tip.	Repeat centrifugation step.
	Skin oils on the surface of the capillary tip	Always wear gloves when handling the capillaries.
Negative control samples are positive.	Contamination	• Exchange all critical solutions. • Pipet reagents on a clean bench. • Close lid of the negative control reaction tube immediately after pipetting. • Use heat-labile UNG for prevention of carry-over contamination.
High background	Very low fluorescence signals, therefore the background seems relatively high.	Follow general optimization strategies for LightCycler® PCR.
	HybProbe probe concentration is too high.	HybProbe probe concentration should be in the range of 0.2 to 0.4 mM.
	Insufficient quality of HybProbe probes.	Prepare a new pair of HybProbe probes.
	Gain settings are too high.	• Reduce value of gain setting. • Use the Real Time Fluorimeter option to optimize the gain settings.
Amplification curve decreases after reaching the plateau in late cycles.	"Hook effect": competition between binding of the Hybridization probes and reannealing of the PCR product.	This does not affect interpretation of the results. It can be avoided by performing an asymmetric PCR by favoring the amplification of the DNA strand that the HybProbe probes bind to.

5. Additional Information on this Product

How this Product Works

The LightCycler® RNA Master HybProbe is an easy-to-use hot start reaction mix, specifically adapted for one-step RT-PCR in 20 µl glass capillaries using the LightCycler® Instruments and HybProbe probes as the detection format. Amplification and on-line monitoring of the template RNA is achieved by a combined procedure on the LightCycler® Instruments. The results are interpreted directly after completing the PCR. The amplicon is detected by fluorescence using target-specific HybProbe probes (not provided by the kit).

The LightCycler® RNA Master HybProbe provides convenience, high performance, reproducibility, and minimizes contamination risk. Only template RNA, PCR primers, HybProbe probes, and $Mn(OAc)_2$, have to be added.

Background Information

The hot start feature of the LightCycler® RNA Amplification Kit HybProbe is achieved by using Tth DNA Polymerase in combination with Aptamers. Tth DNA Polymerase is a thermostable enzyme with RNA-dependent reverse transcriptase activity and DNA-dependent polymerase activity, allowing the combination of RT and PCR in a single-tube reaction. Aptamers are dedicated oligonucleotides that bind in the active center of the polymerase and prevent attachment to nucleic acid targets at temperatures below the optimal reaction temperature of the Tth enzyme. Therefore, no primer elongation occurs during setup of the reaction and the following heating phase prior to the RT step. At higher temperatures, the Aptamers are released from the enzyme, and RT or DNA polymerization can be initiated. In addition, the recommended incubation temperature for reverse transcription with Tth (61°C) is helpful to overcome secondary structures of RNA. This results in highly specific and efficient cDNA synthesis, which leads to highly specific and sensitive PCR. Hot start with Aptamers is highly effective and very convenient because it does not require additional incubation steps, pipetting steps, or an extension of reaction time. The hot start protocol with Aptamers does not interfere with other enzymatic processes, the online detection of amplification products, or subsequent handling steps.

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

LightCycler® Red 610 and LightCycler® Red 670 can be used on a LightCycler® 2.0 Instrument only.

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Quality Control

The LightCycler® RNA Master HybProbe is function tested using the LightCycler® Control Kit RNA.

6. Supplementary Information

6.1 Conventions

Text Conventions

To make information consistent and memorable, the following text conventions are used in this Instruction Manual:

Text Convention	Usage
Numbered stages labeled ①, ②, etc.	Stages in a process that usually occur in the order listed.
Numbered instructions labeled ❶, ❷, etc.	Steps in a procedure that must be performed in the order listed.
Asterisk *	Denotes a product available from Roche Applied Science.

Symbols

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

Symbol	Description
	Information Note: Additional information about the current topic or procedure.
	Important Note: Information critical to the success of the procedure or use of the product.

6.2 Changes to Previous Version

- Information for usage of LightCycler® Software 4.0 added.
- Standard protocol replaces protocol specific for the LightCycler® Control Kit RNA.
- References describing product application added.

6.3 Ordering Information

Roche Applied Science offers a large selection of reagents and systems for life science research. For a complete overview of related products and manuals, please visit and bookmark our home page, www.roche-applied-science.com, and our Special Interest Sites including:

- The LightCycler® 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/

Instruments and Accessories

Product	Pack Size	Cat. No.
LightCycler® 2.0 Instrument	1 instrument plus accessories	03 351 414 001
LightCycler® 1.5 Instrument	1 instrument plus accessories	04 484 495 001
LightCycler® Capillaries (20 µl)	1 pack (8 boxes, each with 96 capillaries and stoppers)	11 909 339 001
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)
MagNA Pure LC Instrument	1 instrument plus accessories	12 236 931 001
LightCycler® Software 4.0	1 software package	03 640 012 001
LightCycler® Probe Design Software 2.0	1 software package	04 342 054 001

	Product	Pack Size	Cat. No.
RNA Isolation Kits	MagNA Pure LC RNA Isolation Kit – High Performance	1 kit (192 isolations)	03 542 394 001
	MagNA Pure LC RNA Isolation Kit III (Tissue)	1 kit (192 isolations)	03 330 591 001
	MagNA Pure LC mRNA Isolation Kit I (Blood, Blood Cells)	1 kit (192 isolations)	03 004 015 001
	MagNA Pure LC mRNA Isolation Kit II (Tissue)	1 kit (192 isolations)	03 172 627 001
	High Pure RNA Isolation Kit	1 kit (50 isolations)	11 828 665 001
	High Pure RNA Tissue Kit	1 kit (50 isolations)	12 033 674 001
	High Pure Viral RNA Kit	1 kit (100 isolations)	11 858 882 001
LightCycler® One-Step RT-PCR Kits	LightCycler® RNA Amplification Kit SYBR Green I	1 kit (96 reactions)	12 015 137 001
	LightCycler® RNA Amplification Kit HybProbe	1 kit (96 reactions)	12 015 145 001
	LightCycler® RNA Master SYBR Green I	1 kit (96 reactions)	03 064 760 001
	LightCycler® RNA Master HybProbe	1 kit (96 reactions)	03 018 954 001
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
LightCycler® Reagent Kits for Two-Step RT-PCR	LightCycler® DNA Master HybProbe	1 kit (96 reactions) 1 kit (480 reactions)	12 015 102 001 12 158 825 001
	LightCycler® FastStart DNA Master HybProbe	1 kit (96 reactions) 1 kit (480 reactions)	03 003 248 001 12 239 272 001
	LightCycler® FastStart DNA Master^{PLUS} HybProbe	1 kit (96 reactions) 1 kit (480 reactions)	03 515 575 001 03 515 567 001
	LightCycler® DNA Master SYBR Green I	1 kit (96 reactions) 1 kit (480 reactions)	12 015 099 001 12 158 817 001
	LightCycler® FastStart DNA Master SYBR Green I	1 kit (96 reactions) 1 kit (480 reactions)	03 003 230 001 12 239 264 001
	LightCycler® FastStart DNA Master^{PLUS} SYBR Green I	1 kit (96 reactions) 1 kit (480 reactions)	03 515 869 001 03 515 885 001
Associated Kits and Reagents	LightCycler® Color Compensation Set	1 set (5 reactions)	12 158 850 001
	LightCycler® Multicolor Demo Set	20 reactions & 5 color compensation runs	03 624 854 001
	LightCycler® Control Kit RNA	1 kit (50 control reactions)	12 158 841 001
	Uracil-DNA N-Glycosylase, heat-labile	100 U	11 775 367 001
	RNA, MS2	10 A_{260} U (500 µl)	10 165 948 001

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The purchase price of this product includes a limited, non-transferable license under U.S. Patent Nos. 6,174,670, 6,245,514 and corresponding patent claims outside the United States, licensed from Idaho Technology Inc., to use only this amount of the product for HybProbe assays and related processes described in said patents solely for the research and development activities of the purchaser.

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Further information on purchasing licenses to practice real-time PCR processes may be obtained by contacting the Director of Licensing, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, USA.

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If you have questions or experience problems with this or any Roche Applied Science (RAS) product, please contact our Technical Support staff. Our scientists commit themselves to providing rapid and effective help.

We also want you to contact us if you have suggestions for enhancing RAS product performance or using our products in new or specialized ways. Such customer information has repeatedly proven invaluable to RAS and the world-wide research community.

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

www.roche-applied-science.com/support

To call, write, fax, or email us, visit the Roche Applied Science home page, www.roche-applied-science.com, and select your home country.
Country-specific contact information will be displayed.

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