For general laboratory use.

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LightCycler® RNA Master HybProbe

Version: 13

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

Cat. No. 03 018 954 001 1 kit
96 reactions of 20 µl final volume each

Store the kit at −15 to −25°C.

1. General Information

1.1. Contents

Vial / Bottle	Cap	Label	Function / Description	Content
1	red	LightCycler® RNA Master HybProbe, LC RNA Master HybProbe, 2.7x conc.	Contains Tth DNA Polymerase, reaction buffer, and dNTP mix (with dUTP instead of dTTP).	3 vials, 250 µl each
2	colorless	LightCycler® RNA Master HybProbe, $Mn(OAc)_2$ stock solution, 50 mM	To adjust the $Mn(OAc)_2$ concentration.	1 vial, 1 ml
3	colorless	LightCycler® RNA Master HybProbe, Water, PCR Grade	To adjust the reaction volume.	2 vials, 1 ml each

1.2. Storage and Stability

Storage Conditions (Product)

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

Vial / Bottle	Cap	Label	Storage
1	red	LC RNA Master HybProbe, 2.7x conc.	Store at −15 to −25°C. ⚠ ***Avoid repeated freezing and thawing.***
2	colorless	$Mn(OAc)_2$ stock solution, 50 mM	Store at −15 to −25°C.
3	colorless	Water, PCR Grade	

1.3. Additional Equipment and Reagents Required

Standard Laboratory Equipment

- Nuclease-free, aerosol-resistant pipette tips
- Sterile reaction tubes for preparing master mixes and dilutions

For RT-PCR

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

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

or

- LC Carousel Centrifuge 2.0* for use with the LightCycler® 2.0 Sample Carousel (20 µl; optional)
- Uracil-DNA Glycosylase, heat-labile* (optional)

ⓘ *For prevention of carryover contamination; see section* **Prevention of Carryover Contamination**.

- LightCycler® Color Compensation Set* (optional)

ⓘ *If you want to perform color compensation when using LightCycler® Red 640 and Cy5.5-labeled HybProbe pairs in dual-color experiments in the same capillary, see section* **Color Compensation**.

1.4. Application

The LightCycler® RNA Amplification Kit HybProbe is designed specifically for the HybProbe detection format using the LightCycler® Carousel-Based System. The kit provides reagents, including RT-PCR enzyme mix, reaction mix, $MgCl_2$, and Water, PCR Grade for very sensitive detection and quantification of defined RNA sequences using the LightCycler® System (suitable RT-PCR primers and HybProbe probes must be supplied).
The kit is especially suitable for difficult RNA populations, as 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.
In addition, the kit can be used with Uracil DNA Glycosylase, heat-labile to prevent carryover contamination during PCR.

1.5. Preparation Time

Assay Time

Procedure	Assay Time [min]
RT-PCR Setup	15
Reverse Transcription	20
LightCycler® Carousel-Based System PCR run (incl. Melting Curve)	25
Total Assay Time	**60**

2. How to Use this Product

2.1. Before you Begin

Sample Materials

- 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.

⚠ ***Using a too high amount of RNA may result in inhibition of the reaction.***

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

For reproducible isolation of nucleic acids, we recommend:
- Either a MagNA Pure System together with a dedicated reagent kit (for automated isolation),
- or a High Pure Nucleic Acid Isolation Kit (for manual isolation).

Control Reactions

Negative Control

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

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*. As the reverse transcription step is omitted, any PCR product generated is a signal for DNA contamination of the RNA template preparation.

Primers

Suitable concentrations of RT-PCR primers range from 0.2 to 1 µM (final concentration). 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.

Probe

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 red-fluorophore-labeled probe to 0.4 µM.

For more information about HybProbe probes, see the LightCycler® Operator's Manual and the section **Test Principle**.
In addition, you can use the LightCycler® Probe Design Software* to help you design HybProbe pairs (free to download at www.lifescience.roche.com).

General Considerations

$Mn(OAc)_2$

To ensure specific and efficient amplification with the LightCycler® Carousel-Based System, use $Mn(OAc)_2$ at a final concentration of 3.25 mM. For most RNA targets tested, 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 µl 50 mM $Mn(OAc)_2$ stock solution to a final volume of 20 µl, results in an increase of $Mn(OAc)_2$ concentration of 0.25 mM). The volume of water in the RT-PCR reaction must be reduced, accordingly.

Color Compensation

If using acceptor HybProbe probes that contain different red fluorophore 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® Carousel-Based System 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® Carousel-Based System 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 or the Instructions for Use of the LightCycler® Color Compensation Set.

2.2. Protocols

LightCycler® Carousel-Based System Protocol

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

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

A LightCycler® Carousel-Based System protocol that uses the LightCycler® RNA Master HybProbe contains the following programs:

- **Reverse Transcription** of template RNA
- **Pre-Incubation** for denaturation of cDNA/RNA hybrid
- **Amplification** of the cDNA
- **Melting Curve** for amplicon analysis (optional: only required for SNP or mutation detection)
- **Cooling** the rotor and the thermal chamber

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

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

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

Programs	
Setup	Setting
Default Channel	**During the run**: Depending on the red fluorophore dye used for labeling the HybProbe probe, choose channel 610, 640, 670, or 705. **For analysis**: Divide by channel 530 for single-color experiments; divide by "Back 530" for dual-color experiments (e.g., 640/Back 530). For automated Tm Calling analysis, do not divide by channel 530 or "Back 530". ⓘ *Channel 610 and 670 are available on a LightCycler® 2.0 Instrument only.*
Seek Temperature	61°C
Max Seek Pos.	Enter the total number of sample positions the instrument should look for.
Instrument Type	"6 Ch." for LightCycler® 2.0 Instrument or "3 Ch." for LightCycler® 1.5 Instrument
Capillary Size	Select "20 µl" as the capillary size for the experiment. Available only for the LightCycler® 2.0 Instrument (6 channels).

Programs		
Program Name	Cycles	Analysis Mode
Reverse Transcription	1	None
Pre-Incubation	1	None
Amplification	45	Quantification
Temperature Gradient or Melting Curve	1	Color Compensation or Melting Curve
Cooling	1	None

Temperature Targets				
	Target [°C]	Hold [hh:mm:ss]	Ramp Rate[1] [°C/s]	Acquisition Mode [per °C]
Reverse Transcription	61	00:20:00[2]	20	None
Pre-Incubation	95	00:00:30[3]	20	None
Amplification	95	00:00:01 – 00:00:05[3]	20	None
	primer dependent[4]	00:00:10 – 00:00:15[5]	20	Single
	72[6]	00:00:05 – 00:00:30 [5,7]	20	None
Melting Curve	95	00:00:00	20	None
	HybProbe Tm – 5°C	00:00:30	20	None
	95	00:00:00	0.1	Continuous
Cooling	40	00:00:30	20	None

[1] Temperature Transition Rate/Slope/Ramp Rate is 20°C/second, except where indicated.

[2] When amplifying GC-rich templates, or templates with a high degree of secondary structures, it is recommended to extend the reverse transcription incubation time to 30 minutes or longer.

[3] When amplifying GC-rich templates or templates with a high degree of secondary structures, it is recommended to increase the initial denaturation incubation time up to 2 minutes and the denaturation time in program “Amplification” up to 5 seconds.

[4] For initial experiments, set the target temperature (i.e., the primer annealing temperature) 5°C below the calculated primer Tm. Calculate the primer Tm according to the following formula, based on the nucleotide content of the primer:
Tm = 2°C (A + T) + 4°C (G + C).

[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.

[6] If the primer annealing temperature is low (< +55°C), reduce the ramp rate to 2 to 5°C/second.

[7] Calculate the hold time for the PCR elongation step by dividing the amplicon length over 25 (e.g., a 500 bp amplicon requires 20 seconds elongation time).

Preparation of the RT-PCR Mix

Proceed as described below for a 20 µl standard reaction.

⚠ ***Do not touch the surface of the capillaries. Always wear gloves when handling the capillaries.***

1. Depending on the total number of reactions, place the required number of LightCycler® Capillaries into pre-cooled centrifuge adapters, or into a LightCycler® Sample Carousel in a pre-cooled LC Carousel Centrifuge Bucket.

2. 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® RNA Master HybProbe (Vial 1) during storage. If a precipitate is visible, place the vial at +15 to +25°C and mix gently until the precipitate is completely dissolved. This does not influence the performance in RT-PCR.***

3. Prepare a 10x conc. solution of RT-PCR primers and a 10x conc. solution of HybProbe probes.

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

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

Reagent	Volume [µl]	Final conc.
Water, PCR Grade (Vial 3)	6.2	–
$Mn(OAc)_2$ stock solution, 50 mM (Vial 2)	1.3	3.25 mM
RT-PCR Primer Mix, 10x conc.[1]	2.0	0.2 to 1 µm (recommended conc. is 0.5 µM)
HybProbe Mix, 10x conc.[2]	2.0	0.2 to 0.4 µM each
LC RNA Master HybProbe (Vial 1)	7.5	1x
Total Volume	19	

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

5. Mix gently by pipetting up and down. Do not vortex.
   – Pipette 19 µl RT-PCR mix into each pre-cooled LightCycler® Capillary.
   – Add 1 µl of the RNA template.
   – Seal each capillary with a stopper.

6. Place the centrifuge adapters containing the capillaries into a standard benchtop microcentrifuge.

   ⚠ ***Place the centrifuge adapters in a balanced arrangement within the centrifuge.***
   – Centrifuge at 700 × *g* for 5 seconds (3,000 rpm in a standard benchtop microcentrifuge).
   – Alternatively, use the LC Carousel Centrifuge for spinning the capillaries.

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

8. Cycle the samples as described above.

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

[2] If you want to peform dual-color detection using LightCycler® Red 640 and Cy5.5-labeled HybProbe pairs simultaneously in one capillary, use either two separated HybProbe mixes (add 2 µl each from both of the mixes) or combine both HybProbe pair preparations in one mix (add 2 µl only from this combined HybProbe mix).

2.3. Other Parameters

Prevention of Carryover Contamination

Uracil-DNA Glycosylase, heat-labile (UNG) is suitable for preventing carryover contamination in PCR. This carryover prevention technique involves incorporating deoxyuridine triphosphate (dUTP, a component of the reaction mixes of all LightCycler® 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 steps below to prevent carryover contamination using heat-labile UNG.

1. Add 1 µl UNG, heat-labile to the master mix per 20 µl final reaction volume.

2. Add template RNA and incubate the completed reaction mixture for 5 minutes at +15 to +25°C.

3. Destroy any contaminating template and inactivate the UNG enzyme by performing the reverse transcription step at +61°C.

   ⚠ ***Do not perform an additional inactivation step at higher temperatures than +61°C since the reverse transcriptase would be inactivated.***

i *When performing an additional Melting Curve analysis, the use of UNG lowers the respective melting temperature (Tm) by approximately 1°C.*

3. Results

Quantification Analysis

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 [640][(1)] and F3 [705][(1)], with and without color compensation. Equivalent results will be obtained using single-color detection with Cy5.5 as acceptor fluorophore, or dual-color detection with LightCycler® Red 640- and Cy5.5-labeled HybProbe pairs simultaneously.
The fluorescence values versus cycle number are displayed. One-hundred copies of the cytokine RNA can be reproducibly detected by amplification in the LightCycler® Carousel-Based System Instrument using the HybProbe detection.

(1) Values in square brackets refer to the LightCycler® Software 4.x; this includes LightCycler® Software 4.0, 4.05, and 4.1.

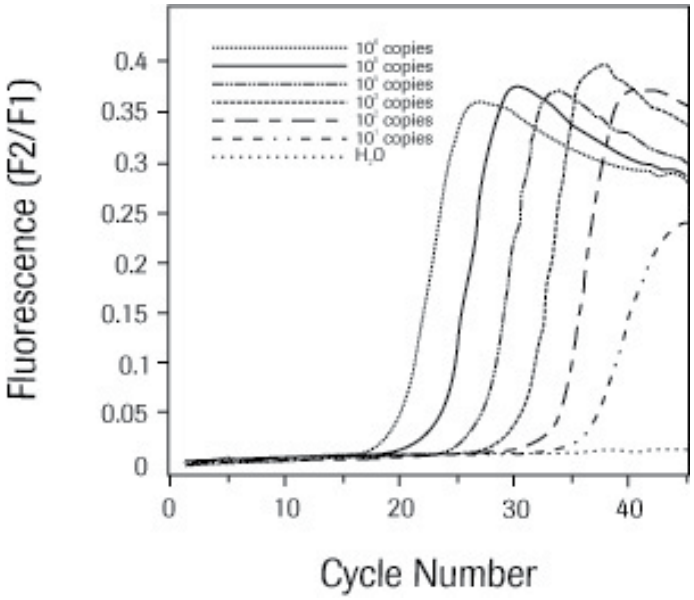


Fig. 1a: Channel F2 [640][(1)] (F2/F1) without color compensation.

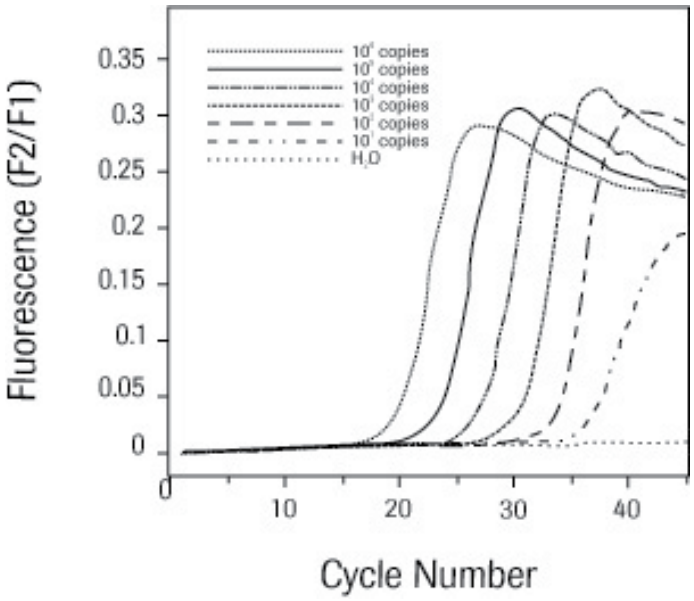


Fig. 1b: Channel F2 [640][(1)] (F2/F1) with color compensation.

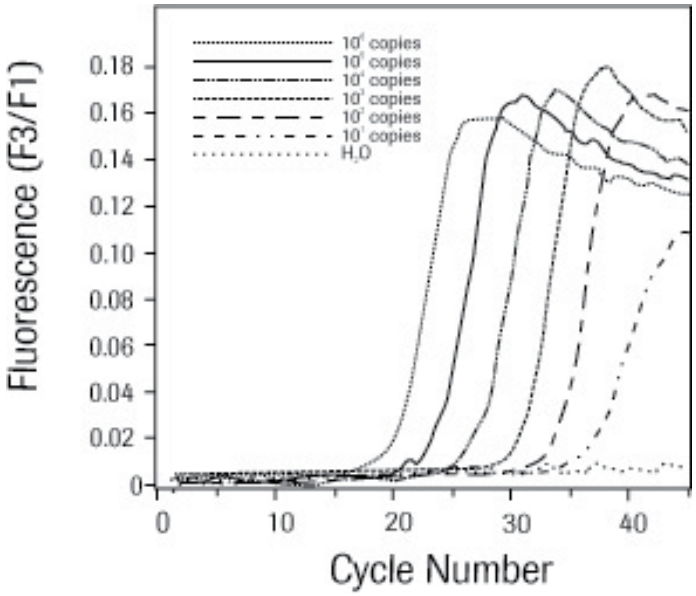


Fig. 1c: Channel F3 [705][(1)] (F2/F1) without color compensation.

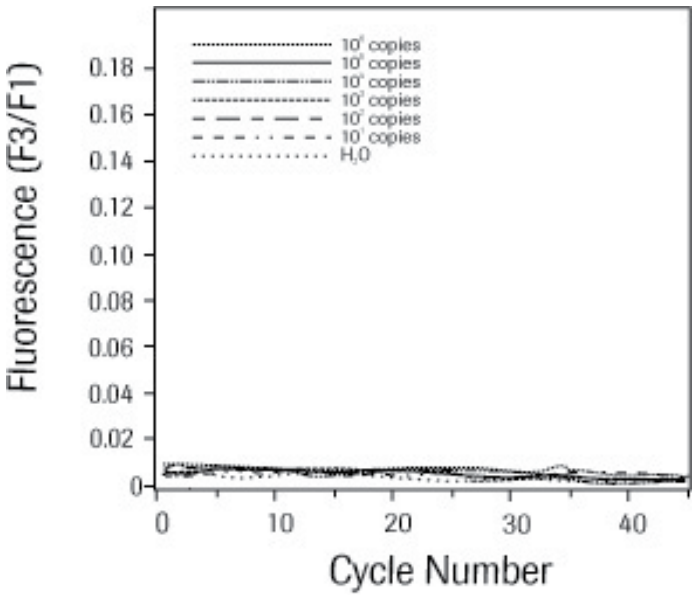


Fig. 1d: Channel F3 [705][(1)] (F2/F1) with color compensation.

Figures 1a–d: Serially diluted samples containing 10^1 (far right) to 10^6 (far left) copies of cytokine RNA template from the LightCycler® Control Kit RNA were amplified using the LightCycler® RNA Master HybProbe in a LightCycler® Carousel-Based System Instrument. As a negative control, template RNA was replaced by Water, PCR Grade (flat line). LightCycler® Red 640 was used as the acceptor fluorophore.
Figures 1a and 1b display results in detection channel F2 [640][(1)] without and with color compensation.
Figures 1c and 1d display results in detection channel F3 [705][(1)] without and with color compensation.
Quantification analysis was performed using LightCycler® Software 3.5, applying arithmetic background subtraction.

4. Troubleshooting

Observation	Possible cause	Recommendation
Amplification reaches plateau phase before the program is complete.	Very high starting amount of nucleic acid.	The program can be finished by clicking on the **End Program** button. The next cycle program will start automatically.
	The number of cycles is too high.	Reduce the number of cycles in the amplification program.
Log-linear phase of amplification just starts as the amplification program finishes.	Very low starting amounts of nucleic acid.	Improve PCR conditions (*e.g.,* primer and probe concentration or design).
		Use higher amount of starting material.
		Repeat the run.
	The number of cycles is too low.	Increase the number of cycles in the amplification program.
		Use the **+10 cycles** button to increase the number of cycles in the amplification program.
	Wrong channel has been chosen to display amplification online.	Change the channel setting on the programming screen. The data obtained up to this point will be saved.
	Pipetting errors or omitted reagents.	Check for missing reagents.
		Check for missing or defective dye.
	Measurements do not occur.	Check the amplification program. For the HybProbe detection format, choose “single” as the 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 to 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 RT-PCR product on an agarose gel.
	Unsuitable RT-PCR primers.	Check primer design (quality).
		Check RT-PCR product on an agarose gel.
	RNA degradation due to improper 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. ⚠ ***Use an extra sample for this procedure so that the dyes in your experimental samples will not be bleached. LightCycler® Software versions 3.5 and higher do not require a gain setting.***

Observation	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 red fluorophore-labeled probe than of the fluorescein-labeled probe.
	Chosen gain settings are too low.	Optimize gain settings using the **Real Time Fluorimeter** function. Then repeat the run using the optimal gain settings in the cycle programs.
	Reaction conditions are not optimized leading to poor PCR efficiency.	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, 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 may be considered (0.1 µl $Mn(OAc)_2$ stock solution, 50 mM corresponds 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 minutes and for denaturation during cycling to 5 seconds.
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.
	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 are positive.	Contamination	Remake all critical solutions.
		Pipette reagents on a clean bench.
		Close lid of the negative control reaction immediately after pipetting.
		Use heat-labile UNG for prevention of carryover contamination.

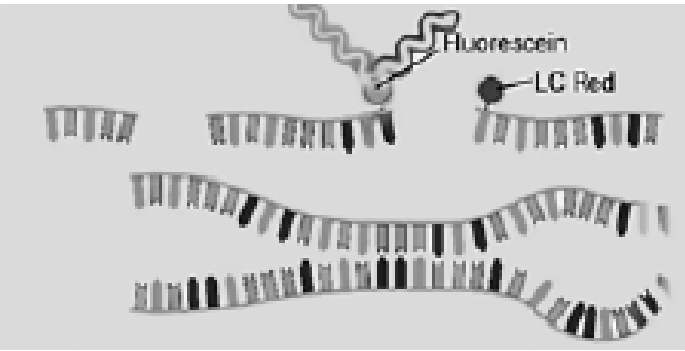
Observation	Possible cause	Recommendation
High background	Very low fluorescence signals, therefore the background seems relatively high.	Follow general optimization strategies for PCR using the LightCycler® Carousel-Based System.
	HybProbe probe concentration is too high.	HybProbe probe concentration should be in the range of 0.2 to 0.4 µM.
	Insufficient quality of HybProbe probes.	Prepare a new pair of HybProbe probes.
	Gain settings are too high.	Reduce value of gain settings.
		Use the **Real Time Fluorimeter** option to optimize the gain settings.
Amplification curve decreases after reaching a plateau in the later cycles.	"Hook effect": competition between binding of the HybProbe probes and reannealing of the PCR product.	This does not affect interpretation of the results. It can be avoided by performing an asymmetric PCR, favoring the amplification of the DNA strand to which the HybProbe probes bind.

5. Additional Information on this Product

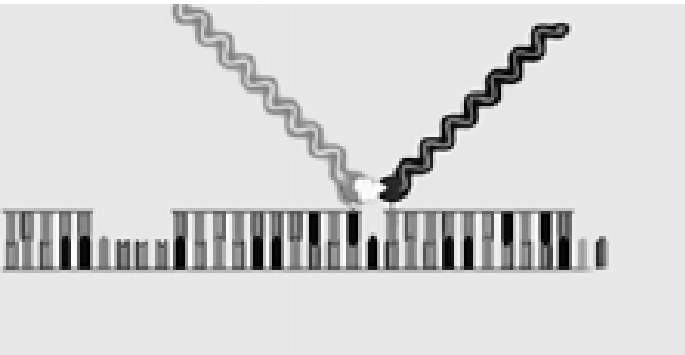
5.1. Test Principle

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 that 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 consist of two different short oligonucleotides that bind to an internal sequence of the amplified fragment during the annealing phase of the amplification cycle.
The basic steps of DNA detection by HybProbe probes during real-time PCR on the LightCycler® Carousel-Based System are:

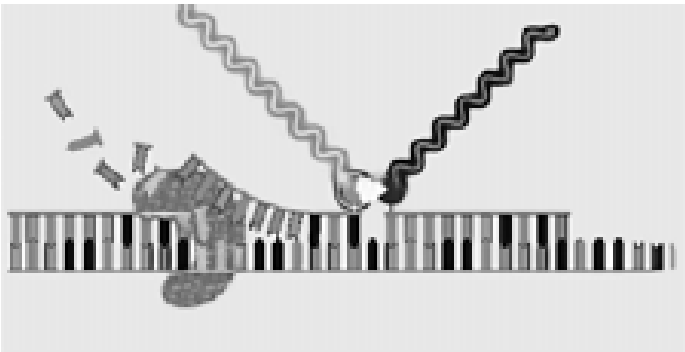
① The donor dye probe has a fluorescein label at its 3′ end and the acceptor dye probe has a red fluorophore label (LightCycler® Red 610*, LightCycler® Red 640*, Cy5, or Cy5.5) at its 5′ end (it is 3′-phosphorylated, so it cannot be extended). Hybridization does not occur during the denaturation phase of PCR. As the distance between the unbound dyes prevents energy transfer, no fluorescence will be detected from the red acceptor dye during this phase.



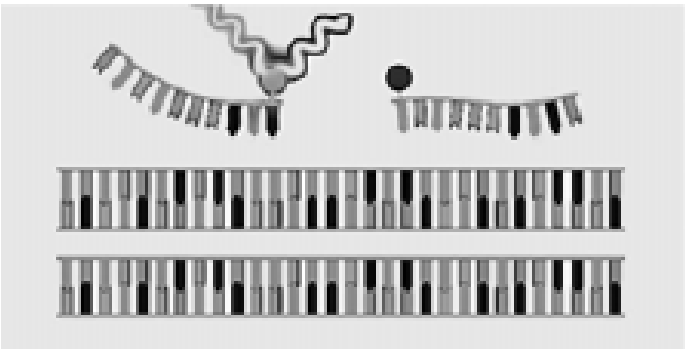
② During the annealing phase, the probes hybridize to the amplified DNA fragment in a head-to-tail arrangement, thereby bringing the two fluorescent dyes close to each other. Fluorescein is excited by the light source of the LightCycler® Carousel-Based System which causes it to emit green fluorescent light. The emitted energy excites the red fluorophore by fluorescence resonance energy transfer (FRET). The red fluorescence emitted by the acceptor dye is measured at the end of each annealing step, when the fluorescence intensity is greatest.



③ After annealing, an increase in temperature leads to elongation and displacement of the probes.



④ At the end of the elongation step, the PCR product is double-stranded, while the displaced HybProbe probes are back in solution and too far apart to allow FRET to occur.



HybProbe probes that carry different red fluorophore 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. Color compensation may be applied either during or after a run on the LightCycler® Carousel-Based System.

See the LightCycler® Operator's Manual and the Instructions for Use of the LightCycler® Color Compensation Set for more information on the generation and use of a color compensation file, or object.

LightCycler® Red 610 and Cy5 can only be used on a LightCycler® 2.0 Instrument.

How this Product Works

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 detection format of the HybProbe probe on the LightCycler® Carousel-Based System. Amplification and online monitoring of the template RNA is achieved by a combined procedure on the LightCycler® Carousel-Based System Instrument. The results are interpreted directly after completing the PCR and Melting Curve. The amplicon is detected by fluorescence using target-specific HybProbe probes (not supplied with the kit).
The LightCycler® RNA Master HybProbe provides convenience, high performance, reproducibility, and minimizes contamination risk. All you need to supply is the template RNA, RT-PCR primers, and HybProbe probes.

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

⚠ ***The amplicon size should not exceed 750 bp in length. For optimal results, select a product length of 500 bp or less.***
⚠ ***The performance of the kit described in this Instructions for Use is guaranteed only when it is used with the LightCycler® Carousel-Based System.***

5.2. Quality Control

The LightCycler® RNA Master HybProbe is function tested using the LightCycler® Control Kit RNA with the LightCycler® Carousel-Based System.

6. Supplementary Information

6.1. Conventions

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

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

6.2. Changes to previous version

Layout changes.
Editorial changes.

6.3. Ordering Information

Roche offers a large selection of reagents and systems for life science research. For a complete overview of related products and manuals, please visit and bookmark our homepage lifescience.roche.com.

Product	Pack Size	Cat. No.
Accessories general (hardware)		
LightCycler® Software 4.1	1 software package	04 898 915 001
LC Carousel Centrifuge 2.0	1 centrifuge plus rotor and rotor bucket (115 V)	03 709 507 001
	1 centrifuge plus rotor and rotor bucket (230 V)	03 709 582 001
Consumables		
LightCycler® Capillaries (20 µl)	5 x 96 capillaries, containing 5 boxes, each with 96 capillaries and stoppers, 5 boxes, each with 96 capillaries and stoppers	04 929 292 001
Instruments		
LightCycler® 2.0 Instrument	1 instrument	03 531 414 001
Reagents , kits		
LightCycler® DNA Master HybProbe	1 kit, 96 reactions of 20 µl final volume each	12 015 102 001
	1 kit, 480 reactions of 20 µl final volume each	12 158 825 001
LightCycler® RNA Amplification Kit HybProbe	1 kit, 96 reactions of 20 µl final volume each	12 015 145 001
LightCycler® FastStart DNA Master HybProbe	1 kit, 96 reactions of 20 µl final volume each	03 003 248 001
	1 kit, 480 reactions of 20 µl final volume each	12 239 272 001
LightCycler® FastStart DNA Master^{PLUS} HybProbe	1 kit, 96 reactions of 20 µl final volume each	03 515 575 001
	1 kit, 480 reactions of 20 µl final volume each	03 515 567 001
	1 kit, 1,920 reactions of 20 µl or 384 reactions of 100 µl final volume each	03 752 178 001
LightCycler® Color Compensation Set	1 set, 4 vials, 5 calibration runs	12 158 850 001
Uracil-DNA Glycosylase, heat-labile	100 U, 1 U/µl	11 775 367 001
	500 U, 1 U/µl	11 775 375 001
RNA, MS2	500 µl, 10 A260 units	10 165 948 001

6.4. Trademarks

FASTSTART, HIGH PURE, HYBPROBE, LIGHTCYCLER and MAGNA PURE are trademarks of Roche.
All third party product names and trademarks are the property of their respective owners.

6.5. License Disclaimer

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

6.6. Regulatory Disclaimer

For general laboratory use.

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**:
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