

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



## LightCycler® 480 RNA Master Hydrolysis Probes

 **Version: 8**

Content version: August 2015

Easy-to-use Reaction Mix for One-Step RT-PCR using the LightCycler® 480 or LightCycler® 96 Real Time PCR Systems.

Cat. No. 04 991 885 001

1 kit

5 × 100 reactions of 20 µl final volume each

Store the kit at –15 to –25°C

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# 1. General Information

## 1.1. Contents

Vial	Cap	Label	Function	Content
1	red	Master, 2.7x conc.	Contains Tth DNA Polymerase, reaction buffer and dNTP mix (with dUTP instead of dTTP).	5 vials, 740 µl each
2	colorless, grey cap label	Activator, 50 mM	To adjust Mn (OAc) <sub>2</sub> concentration.	1 ml
3	colorless	Water, PCR grade	To adjust the final reaction volume.	5 vials, 1 ml each
4	yellow	Enhancer, 20x conc.	To improve the amplification of difficult (e.g., GC-rich) targets.	500 µl

## 1.2. Storage and Stability

### Storage Conditions (Product)

The kit is shipped on dry ice.

Store the kit at –15 to –25°C until the expiration date printed on the label.

Once the kit is opened, store the kit components as described in the following table:

Vial	Cap	Label	Storage
1	red	Master, 2.7x conc.	Store at –15 to –25°C. <b>⚠️ Avoid repeated freezing and thawing!</b>
2	colorless, grey cap label	Activator, 50 mM	Store at –15 to –25°C.
3	colorless	Water, PCR grade	
4	yellow	Enhancer, 20x conc.	

## 1.3. Additional Equipment and Reagents Required

Refer to the list below for additional reagents and equipment required to perform PCR reactions with the LightCycler® 480 RNA Master Hydrolysis Probes using the LightCycler® 480 Instrument or LightCycler® 96 Instrument:

- LightCycler® 480 Instrument\* and LightCycler® 480 Multiwell Plate 384\* or LightCycler® 480 Multiwell Plate 96\* or
- LightCycler® 96 Instrument and LightCycler® 8-Tube Strips (white)\* or LightCycler® 480 Multiwell Plates 96 (white)\*
- Standard swinging-bucket centrifuge containing a rotor for multiwell plates with suitable adaptors.
- Uracil-DNA Glycosylase, heat-labile\* (optional <sup>1)</sup>)
- Nuclease free, aerosol-resistant pipette tips
- Pipettes with disposable, positive-displacement tips
- Sterile reaction tubes for preparing master mixes and dilutions

**i** <sup>1)</sup> For prevention of carryover contamination please refer to the corresponding chapter.

## 1.4. Application

The LightCycler® 480 RNA Master Hydrolysis Probes is designed for research studies. When combined with the LightCycler® 480 or LightCycler® 96 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 hydrolysis probe are supplied).

## 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 PCR in terms of purity, concentration, and absence of PCR inhibitors. For reproducible isolation of nucleic acids use:

- Either a MagNA Pure System together with a dedicated nucleic acid isolation kit (for automated isolation)
- or a High Pure nucleic acid isolation kit (for manual isolation).

For details, please refer to the Roche Life Science catalogue or the website: [lifescience.roche.com](https://lifescience.roche.com).

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

**i** 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.

#### 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, colorless cap).

##### DNA Contamination Control

To test the template RNA for contamination with residual genomic DNA, perform PCR in combination with LightCycler® 480 Probe Master or FastStart Essential DNA Probes Master (for LightCycler® 96 Instrument). Because in this experimental setup the reverse transcription step is omitted, any PCR product generated is an indication for DNA contamination of the RNA template preparation.

##### Primers

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

##### Probe

Suitable concentrations of hydrolysis probe range from 0.05 to 0.25 µM (final concentration in reaction). The recommended starting concentration is 0.25 µM.

**i** The optimal probe concentration is the lowest concentration that results in the lowest C<sub>q</sub> and an adequate fluorescence for a given target concentration.

**⚠** For a digestible hybridization complex to form correctly, the hydrolysis probe must anneal to the target before primer extension. The T<sub>m</sub> of the probe should be only slightly higher than the T<sub>m</sub> of the PCR primer, so the hybridization complex is stable. Furthermore, the probe sequence must account for mismatches in the DNA template, since these will also affect the annealing temperature.

## General Considerations

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 be used with heat-labile Uracil DNA Glycosylase to prevent carryover contamination during PCR.

In principle, the LightCycler® 480 RNA Master Hydrolysis Probes 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® 480 or LightCycler® 96 System and design specific PCR primers and hydrolysis probe for each target. See the LightCycler® 480 or LightCycler® 96 Operator's Manual for general recommendations.

**i** *LightCycler® 480 RNA Master Hydrolysis Probes can be used for simultaneous amplification of 3 - 4 target RNAs. For best results it might be necessary to optimize the individual primer and probe concentrations as well as the Activator [Mn(OAc)<sub>2</sub>] concentration.*

**⚠** *The amplicon size should not exceed 500 bp in length. For optimal results, select a product length less than 100 bp.*

**⚠** *The performance of the kit described in this Instruction Manual is warranted only when it is used with the LightCycler® 480 or LightCycler® 96 Real-Time PCR Systems.*

## Activator [Mn(OAc)<sub>2</sub>]

To ensure specific and efficient amplification use Mn(OAc)<sub>2</sub> (Activator, vial 2) at a final concentration of 3.25 mM. Titration of Activator is not required, except for multiplex assays where optimization of Mn(OAc)<sub>2</sub> concentration might be necessary for best results.

**i** *Addition of 0.1 µl Activator stock solution to a final reaction volume of 20 µl results in an increase of Mn(OAc)<sub>2</sub> concentration of 0.25 mM.*

## 2.2. Protocols

The following procedure is optimized for use with the LightCycler® 480 or LightCycler® 96 System.

**⚠ Program the LightCycler® 480 or LightCycler® 96 Instrument before preparing the reaction mixes.**

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

A protocol for use with LightCycler® 480 or LightCycler® 96 System that uses LightCycler® 480 RNA Master Hydrolysis Probes contains the following programs:

- **Reverse Transcription** of template RNA
- **Denaturation** of cDNA/RNA hybrid
- **Amplification** of the cDNA
- **Cooling** the rotor and thermal chamber (only necessary for the LightCycler® 480 Instrument)

For details on how to program the experimental protocol, see the LightCycler® 480 or LightCycler® 96 Operator's Manual.

### Protocol for use with LightCycler® 480 Multiwell Plate 96

The following table shows the PCR parameters that must be programmed for a LightCycler® 480 System PCR run with the LightCycler® 480 RNA Master Hydrolysis Probes using a LightCycler® 480 Multiwell Plate 96.

Setup					
Block Type	Reaction Volume [µl]				
96	10 -100 µl				
Detection Format	Excitation Filter	Emission Filter			
Monor Color Hydrolysis Probe / UPL Probe FAM	465	510			
or for example: 3 color Hydrolysis Probe FAM	465	510			
VIC / HEX / Yellow555	533	580			
Cy 5 / Cy 5.5	618	660			
For new customized detection formats, set for all selected filters in the “Selected Filter Combination List” (under Tools), the following values:					
Melting Factor	1				
Quantification Factor	10				
Integration Time	2				
Programs					
Program Name	Cycles	Analysis Mode			
Reverse Transcription	1	None			
Initial Denaturation	1	None			
Amplification	45 <sup>1)</sup>	Quantification			
Cooling	1	None			
Temperature Targets					
	Target [°C]	Acquisition Mode	Hold [hh:mm:ss]	Ramp Rate [°C/s]	Acquisitions [per °C]
Reverse Transcription	63 <sup>2)</sup>	None	00:03:00 <sup>3)</sup>	4.4	–
Initial Denaturation	95	None	00:00:30	4.4	–
Amplification	95	None	00:00:10 - 00:00:15	4.4	–
	60 <sup>4)</sup>	None	00:00:30 - 00:00:60	2.2 <sup>5)</sup>	–
	72	Single	00:00:01	4.4	–
Cooling	40	None	00:00:10	2.2 <sup>5)</sup>	–

## Protocol for use with LightCycler® 480 Multiwell Plate 384

The following table shows the PCR parameters that must be programmed for a LightCycler® 480 System PCR run with the LightCycler® 480 RNA Master Hydrolysis Probes using a LightCycler® 480 Multiwell Plate 384.

Setup					
Block Type			Reaction Volume [μl]		
384			3 -20 μl		
Detection Format		Excitation Filter		Emission Filter	
Monor Color Hydrolysis Probe / UPL Probe FAM		465		510	
or for example: 3 color Hydrolysis Probe FAM		465		510	
VIC / HEX / Yellow555		533		580	
Cy 5 / Cy 5.5		618		660	
For new customized detection formats, set for all selected filters in the “Selected Filter Combination List” (under Tools), the following values:					
Melting Factor		1			
Quantification Factor		10			
Integration Time		2			
Programs					
Program Name		Cycles		Analysis Mode	
Reverse Transcription		1		None	
Initial Denaturation		1		None	
Amplification		45 <sup>1)</sup>		Quantification	
Cooling		1		None	
Temperature Targets					
	Target [°C]	Acquisition Mode	Hold [hh:mm:ss]	Ramp Rate [°C/s]	Acquisitions [per °C]
Reverse Transcription	63 <sup>2)</sup>	None	00:03:00 <sup>3)</sup>	4.8	–
Initial Denaturation	95	None	00:00:30	4.8	–
Amplification	95	None	00:00:10 - 00:00:15	4.8	–
	60 <sup>4)</sup>	None	00:00:30 - 00:00:60	2.5 <sup>5)</sup>	–
	72	Single	00:00:01	4.8	–
Cooling	40	None	00:00:10	2.5 <sup>5)</sup>	–

- 1) 45 cycles are suitable for most assays. If the assay is optimized and has steep amplification curves and early crossing points (even when target concentrations are low), 40 cycles should be sufficient. Reducing the number of cycles will reduce the time required for the assay!
- 2) 63°C are the recommended annealing temperature. For some targets it might be necessary to reduce the annealing temperature to 61°C.
- 3) 3 min incubation time are sufficient for most targets. For some targets the time required for reverse transcription might even be reduced to 1 min.
- 4) For initial experiments, set the target temperature (*i.e.*, the primer annealing temperature) to 60°C. Further optimization may be required depending on the melting temperatures of primers and hydrolysis probe.
- 5) For users of LightCycler® 480 Software 1.1:
  - When using the 96-multiwell plate: For target temperatures below 50°C, set the Ramp Rate to 1.5°C/s !
  - When using the 384-multiwell plate: For target temperatures below 50°C, set the Ramp Rate to 2.0°C/s !

## Color Compensation

For information on generating and using a color compensation file, see the LightCycler® 480 Operator's Manual or [www.lightcycler480.com](http://www.lightcycler480.com).

## 2. How to Use this Product

### Protocol for use with LightCycler® 96 Instrument

The following table shows the PCR parameters that must be programmed for a LightCycler® 96 System PCR run with the LightCycler® 480 RNA Master Hydrolysis Probes using a LightCycler® 480 Multiwell Plate 96 or LightCycler® 8-Tube Strips.

Run Editor				
Detection Format			Reaction Volume (µl)	
Selected Dyes used in your assays. For example: Dyes1: FAM			5 - 50	
Programs				
	Temp. [°C]	Ramp [°C/s]	Duration [s]	Acquisition Mode
Reverse Transcription	63 <sup>1)</sup>	4.4	180 <sup>2)</sup>	None
Initial Denaturation	95	4.4	30	None
Three-Step Amplification	No. of Cycles: 45 <sup>3)</sup>			
	95	4.4	10	None
	60 primer dependent <sup>4)</sup>	2.2	30	None
	72	4.4	1	Single

- 1) 63°C are the recommended annealing temperature. For some targets it might be necessary to reduce the annealing temperature to 61°C.
- 2) 3 min incubation time are sufficient for most targets. For some targets the time required for reverse transcription might even be reduced to 1 min.
- 3) 45 cycles are suitable for most assays. If the assay is optimized and has steep amplification curves and early crossing points (even when target concentrations are low), 40 cycles should be sufficient. Reducing the number of cycles will reduce the time required for the assay!
- 4) For initial experiments, set the target temperature (i.e., the primer annealing temperature) to 60°C. Further optimization may be required depending on the melting temperatures of primers and hydrolysis probe.

### Color Compensation

For the LightCycler® 96 Instrument no color compensation or calibration runs are necessary.

## Preparation of the RT-PCR Mix

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

**⚠ Do not touch the surface of the the LightCycler® 480 Multiwell Plate or LightCycler® 8-Tube Strips when handling it.**

- 1 Thaw the solutions and, to ensure recovery of all the contents, briefly spin vials in a microcentrifuge before opening.  
Mix carefully by pipetting up and down and store on ice.

- 2 Prepare a 10x conc. solution that contains PCR primers and hydrolysis probe.

- 3 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 listed below:

Component	Volume	Final Concentration
Water, PCR grade (vial 3, colorless cap)	7.3 µl (6.3 µl)	–
Primer/Probe, 10x conc. <sup>1)</sup>	2.0 µl	0.5 µM/0.25 µM
Activator	1.3 µl	3.25 mM Mn(OAc) <sub>2</sub>
LightCycler® 480 RNA Master Hydrolysis Probes, 2.7x conc. (vial 1, red cap)	7.4 µl	1x conc.
Enhancer, 20x conc. (optional)	(1.0 µl)	1x conc.
Total volume	18 µl	

**i** <sup>1)</sup> Due to possible primer/primer interactions that occur during storage it may be necessary to preheat the PCR primer-probe mix for 1 min at 95°C before starting the reaction. This extra step will ensure optimum sensitivity.

**i** To prepare the RT-PCR mix for more than one reaction, multiply the amount in the “Volume” column above by the number of reactions to be run and one additional reaction.

- 4 Mix carefully by pipetting up and down. Do not vortex.  
Pipet 18 µl RT-PCR mix into each reaction vessel.  
Add 2 µl of the RNA template.  
Seal Multiwell Plate with LightCycler® 480 Sealing Foil or LightCycler® 8-tube strips with the corresponding lid.
- 5 Place the Multiwell Plate in a standard swinging-bucket centrifuge with a suitable adapter and balance it with a suitable counterweight (e.g., another Multiwell Plate)  
Or  
Place the 8-tube strips into a standard multiwell plate 96 and balance them in the centrifuge.
- 6 Load the reaction vessels into the LightCycler® 480 or LightCycler® 96 Instrument.
- 7 Start the PCR program described above.

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

### 2.3. Other Parameters

#### Prevention of Carryover Contamination

Uracil-DNA Glycosylase (UNG) is suitable for preventing carryover contamination in PCR. This technique relies on the incorporation of deoxyuridine triphosphate (dUTP) into amplification products, and the pretreatment of all successive PCR mixtures with UNG. If there are such amplicons in the PCR mixture, UNG cleaves the DNA at any site where a deoxyuridylate residue has been incorporated. Subsequently, the resulting abasic sites are hydrolyzed by high temperatures during the initial denaturation step, and cannot serve as PCR templates. Normal DNA contains thymidine, but no uridine, and is therefore not affected by this procedure.

**⚠ For RT-PCR use Uracil-DNA Glycosylase, heat-labile\* only. Do not use the standard Uracil-DNA Glycosylase from *E. coli* : Due to its higher thermostability, UNG from *E. coli* would destroy the newly synthesized cDNA strand.**

Proceed as described below to prevent carryover contamination using heat-labile UNG:

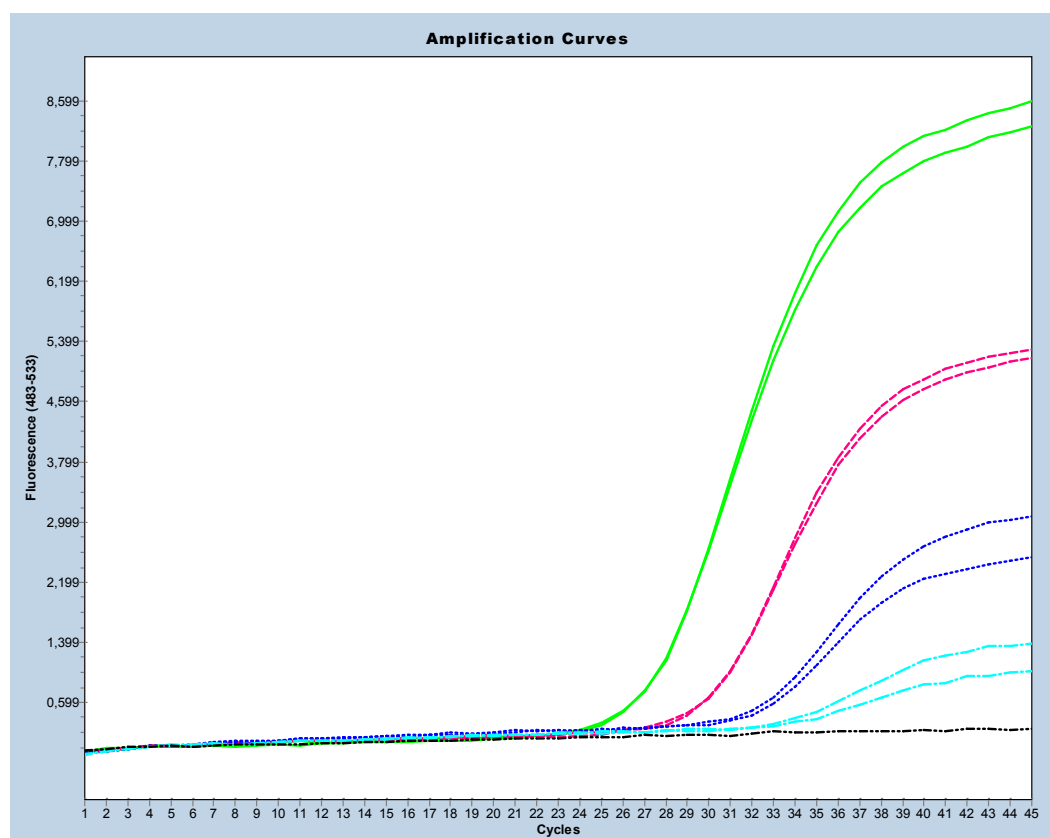
- 1 For a 20 µl final reaction volume, add 1 µl heat-labile UNG to the RT-PCR mix
- 2 Add RNA template and incubate the reaction mixture for 5 min at +15 to +25°C to destroy any contaminating template.
- 3 Inactivate heat-labile UNG by performing the reverse transcription step at +61 to +63°C.

## 3. Results

### Quantification Analysis

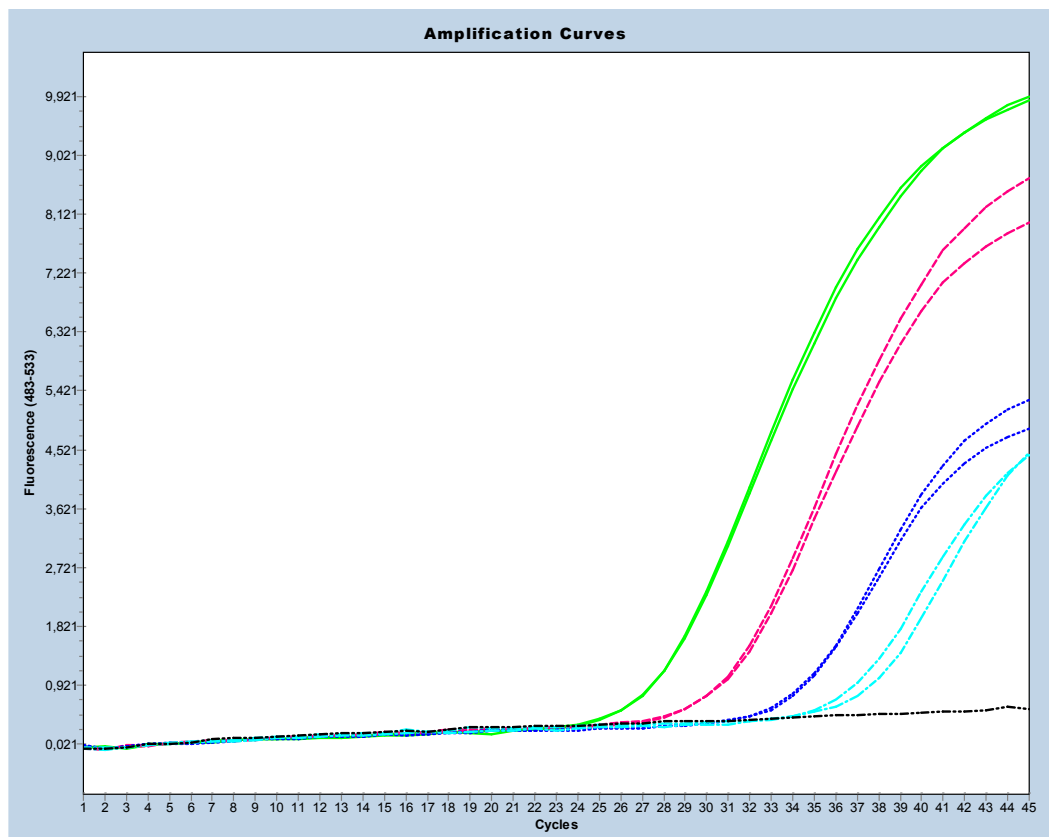
The following amplification curves were obtained using the LightCycler® 480 RNA Master Hydrolysis Probes in combination with

- probe #11 from the Universal ProbeLibrary targeting human  $\beta$ -actin mRNA and dilutions of human (HeLa cells) total RNA (Fig. 1 and 2).
- a hydrolysis probe specific for human ApoE mRNA and dilutions of human total liver RNA (Fig. 3).

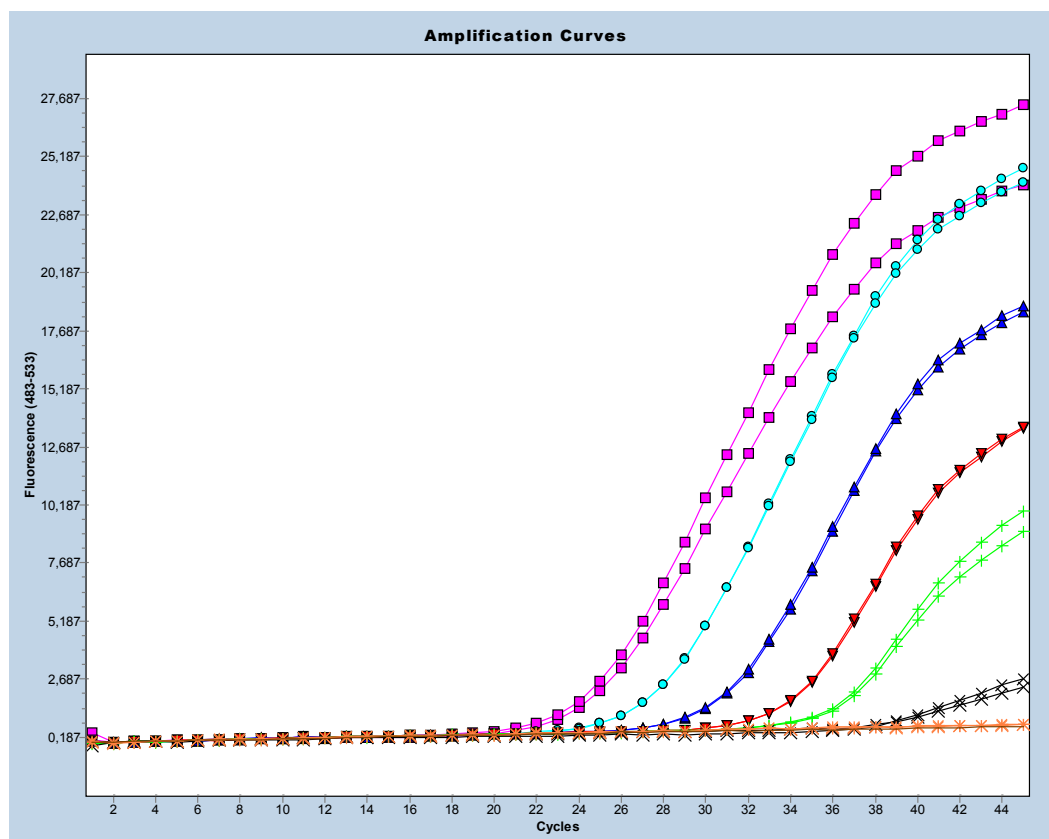


**Fig. 1:** Amplification of  $\beta$ -actin transcript from total HeLa cell RNA (100 pg [far left], 10 pg, 1 pg, 0.1 pg [far right], negative control [flat line]). RNA samples were reverse transcribed in duplicates for 3 min at 63°C. cDNA was amplified in the LightCycler® 480 Instrument according to the protocol given under “Protocol for use with LightCycler® 480 System”.  $\beta$ -actin specific PCR products were detected with probe #11 from the Universal ProbeLibrary and analyzed in the AbsQuant module of LightCycler® 480 Software.

### 3. Results



**Fig. 2:** Amplification of  $\beta$ -actin transcript from total HeLa cell RNA (100 pg [far left], 10 pg, 1 pg, 0.1 pg [far right], negative control [flat line]) **in the presence of Enhancer**. RNA samples were reverse transcribed in duplicates for 3 min at 63°C. cDNA was amplified in the LightCycler® 480 Instrument according to the protocol given under "Protocol for use with LightCycler® 480 System".  $\beta$ -actin specific PCR products were detected with probe #11 from the Universal ProbeLibrary and analyzed in the AbsQuant module of LightCycler® 480 Software.



**Fig. 3:** Amplification of ApoE transcript (76% GC content) from total human liver RNA (100 ng [far left], 10 ng, 1 ng, 0.1 ng, 10 pg, 1 pg [far right], negative control [flat line]). RNA was reverse transcribed for 3 min at 63°C. cDNA was amplified in the LightCycler® 480 Instrument according to the protocol given under “Protocol for Use with the LightCycler® 480 System”. ApoE-specific PCR products were detected with a FAM/TAMRA-labeled hydrolysis probe and analyzed in the AbsQuant module of LightCycler® 480 Software.

## 4. Troubleshooting

Observation	Possible cause	Recommendation
<b>Fluorescence intensity varies.</b>	Some of the reagent is still in the upper part of the microwell, or an air bubble is trapped in microwell.	Repeat centrifugation, but allow sufficient centrifugation time (e.g., 2 min at $1,500 \times g$ ) for all reagent to reach the bottom of the microwell and/or to expel air bubbles.
	Skin oils or dirt on the surface of the microwell.	Always wear gloves when handling the Multiwell Plate.
<b>Fluorescence intensity is very low.</b>	Low concentration or deterioration of dyes in the reaction mixtures because dye was not stored properly.	Keep dye-labeled reagents away from light. Store the reagents at $-15$ to $-25^{\circ}\text{C}$ and avoid repeated freezing and thawing.
	Poor PCR efficiency (reaction conditions not optimized).	Primer concentration should be in the range of $0.2$ to $1.0 \mu\text{M}$ , probe concentration should be in the range of $0.05$ to $0.25 \mu\text{M}$ .
		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 $500 \text{ ng}$ total RNA or $100 \text{ ng}$ mRNA.
		To improve dynamic range or to raise the fluorescence signal when using low RNA concentrations, add Enhancer ( $1 \mu\text{l}$ per $20 \mu\text{l}$ reaction).
	Chosen imaging time is too low.	Choose adequate Roche Detection Format in combination with “dynamic” detection mode or Increase imaging time when using “manual” detection mode. For details see LightCycler® 480 Operator's Manual.
	Inhibitory effects of the sample material due to insufficient purification.	Do not use more than $8\text{--}10 \mu\text{l}$ of total RNA per $20 \mu\text{l}$ RT-PCR reaction mixture. Repurify the nucleic acids to ensure removal of inhibitory agents.
	Unsuitable probe.	Check sequence and location of the hydrolysis probe. Check PCR product on an agarose gel.
	Unsuitable primers.	Check primer design (quality). Check 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.
<b>Negative control sample gives a positive signal.</b>	Contamination.	Remake all critical solutions.
		Pipet reagents on a clean bench.
		Use heat-labile UNG to eliminate carryover contamination.
	Pipetting errors or omitted reagents.	Check for missing reagents. Check for missing or defective dye.
	Scale of axes on graph are unsuitable for analysis.	Change the values for the x- and the y-axis by double-clicking on the maximum and/or minimum values, then changing to suitable values.
	Amplicon length is $>500 \text{ bp}$ .	Do not use amplicons $>500 \text{ bp}$ . Optimal results are obtained with amplicons of $100 \text{ bp}$ or less.
	Impure sample material inhibits reaction.	Dilute sample $1:10$ and repeat the analysis. Repurify the nucleic acids to ensure removal of inhibitory agents.
	Difficult template (e.g., unusual GC-rich sequence).	Repeat RT-PCR under same conditions and add Enhancer. If performance is still not satisfying, optimize annealing temperature in combination with Enhancer.

## 5. Additional Information on this Product

### 5.1. Test Principle

The LightCycler® 480 RNA Master Hydrolysis Probes is an easy-to-use hot start reaction mix, specifically adapted for one-step RT-PCR in the -LightCycler® 480 and LightCycler® 96 Instrument. The amplicon is detected by fluorescence monitoring using a target-specific hydrolysis probe (not provided by the kit). Amplification and online monitoring of the template RNA is achieved by a combined procedure on the LightCycler® 480 or LightCycler® 96 Instrument. The results are interpreted directly after completing the RT-PCR.

The LightCycler® 480 RNA Master Hydrolysis Probes provides convenience, high performance, reproducibility, and minimizes contamination risk. Only template RNA, PCR primers, hydrolysis probe, and  $\text{Mn}(\text{OAc})_2$ , have to be added.

The hot start feature of the LightCycler® 480 RNA Master Hydrolysis Probes 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 reverse transcription 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 Tth DNA Polymerase. Therefore, no primer elongation occurs during setup of the reaction and the following heating phase prior to the reverse transcription step. At higher temperatures, the Aptamers are released from the enzyme, and reverse transcription or DNA polymerization can be initiated. In addition, the recommended incubation temperature for reverse transcription with Tth DNA Polymerase (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.

Sequence-specific detection of PCR products relies on sequence-specific oligonucleotide probes that are coupled to fluorophores. These probes hybridize to their complementary sequence in target PCR products. Probe chemistries that are suitable for use in the LightCycler® 480 Instrument include single-labeled probes, hybridization probes, and hydrolysis probes. For the LightCycler® 96 Instrument use hydrolysis probes. Hybridization and hydrolysis probe chemistries use the so-called FRET principle. Fluorescence Resonance Energy Transfer (FRET) is based on the transfer of energy from one fluorophore (the donor or reprotor) to another adjacent fluorophore (the acceptor or quencher).

Hydrolysis probe assays can technically be described as homogeneous 5'-nuclease assays, since a single 3'-non-extendable probe, which is cleaved during PCR amplification, is used to detect the accumulation of a specific target DNA sequence. This single probe contains two labels, a fluorescent reporter and a quencher, in close proximity to each other. When the probe is intact, the quencher dye is close enough to the reporter dye to suppress the reporter fluorescent signal (fluorescent quenching takes place via FRET). During PCR, the 5'-nuclease activity of the polymerase cleaves the hydrolysis probe, separating the reporter and quencher. The reporter dye is no longer quenched and emits a fluorescent signal when excited.

The LightCycler® 480 Instrument can detect hydrolysis probes that are labeled with the reporter dyes LightCycler® Red 610, LightCycler® Red 640, LightCycler® Cyan 500, FAM or HEX (or any other dye that matches the emission and detection filters of the LightCycler® 480 Instrument). The LightCycler® 96 Instrument can detect hydrolysis probes that are labeled with the reporter dyes FAM, VIC, HEX, Yellow 555, LightCycler® Red 610, Texas Red and Cy5. These labeled hydrolysis probes can be used separately or in combination, which permits either single- or multicolor detection.

**⚠ For multicolor hydrolysis probe assays it is recommended to use dark quencher dyes (i.e., dye molecules which efficiently quench the fluorescence of a FRET reporter dye without emitting fluorescence themselves). Roche recommends to use BHQ-2 (quenching range 550 – 650 nm) for all hydrolysis probe reporter dyes listed above.**



### 5.2. Quality Control

The LightCycler® 480 RNA Master Hydrolysis Probes is function tested using the LightCycler® 480 Real Time PCR 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	
 <b>Information Note:</b> Additional information about the current topic or procedure.	
 <b>Important Note:</b> 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.
① ② ③ 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](http://lifescience.roche.com).

Product	Pack Size	Cat. No.
Accessories general ( hardware )		
LightCycler® 480 Block Kit 96 Silver	1 block kit, for 96-well PCR multiwell plates	05 015 219 001
LightCycler® 480 Block Kit 384 Silver	1 block kit, for 384-well PCR multiwell plates	05 015 197 001
LightCycler® 480 LIMS Interface Module	1 software package	05 066 310 001
Accessories software		
LightCycler® 480 Gene Scanning Software	1 software package	05 103 908 001
LightCycler® 480 Software, Version 1.5	1 software package	04 994 884 001
Consumables		
LightCycler® 480 Multiwell Plate 96, white	5 × 10 plates, with sealing foils	04 729 692 001
LightCycler® 480 Multiwell Plate 384, white	5 × 10 plates, with sealing foils	04 729 749 001
LightCycler® 480 Sealing Foil	50 foils	04 729 757 001
LightCycler® 480 Multiwell Plate 96, clear	5 × 10 plates, with sealing foils	05 102 413 001
LightCycler® 480 Multiwell Plate 384, clear	5 × 10 plates, with sealing foils	05 102 430 001
LightCycler® 8-Tube Strips (white)	10 × 12 white strips and clear caps. Each pack contains 10 × 12 strips of both tubes and flat caps, in 10 non-sterile plastic bags.	06 612 601 001
LightCycler® 8-Tube Strips (clear)	10 × 12 clear strips and caps Each pack contains 10 × 12 strips of both tubes and flat caps, in 10 non-sterile plastic bags.	06 327 672 001
Instruments		
LightCycler® 480 Instrument II	1 instrument, 96-well version	05 015 278 001
	1 instrument, 384-well version	05 015 243 001
LightCycler® 96 Instrument	1 instrument	05 815 916 001
Reagents , kits		
Universal ProbeLibrary Extension Set, Probes #91 to #165	1 set, of 75 Universal ProbeLibrary probes, 125 µl, 10 µM, each.	04 869 877 001

Universal ProbeLibrary Set, Human	1 set, of 90 Universal ProbeLibrary probes, 125 µl, 10 µM, each.	04 683 633 001
LightCycler® 480 Probes Master	5 × 1 ml, up to 5 × 100 reactions, 20 µl final volume	04 707 494 001
	10 × 5 ml, up to 10 × 500 reactions, 20 µl final volume	04 887 301 001
	1 × 50 ml, up to 5,000 reactions, 20 µl final volume	04 902 343 001
LightCycler® 480 SYBR Green I Master	5 × 1 ml, 2x conc., approx. 500 reactions of 20 µl final reaction volume	04 707 516 001
	10 × 5 ml, 2x conc., approx. 10 × 500 reactions of 20 µl final reaction volume	04 887 352 001
LightCycler® 480 Genotyping Master	4 × 384 µl, 5x conc., approx. 384 reactions of 20 µl reaction volume each	04 707 524 001
LightCycler® 480 High Resolution Melting Master	5 × 1 ml, 500 reactions, 20 µl each	04 909 631 001
FastStart Essential DNA Probes Master	5 × 1 ml for up to 5 × 100 reactions of 20 µl final volume	06 402 682 001
	10 × 5 ml for up to 10 × 500 reactions of 20 µl final volume	06 924 492 001
FastStart Essential DNA Green Master	5 × 1 ml for up to 5 × 100 reactions of 20 µl final volume	06 402 712 001
	10 × 5 ml for up to 10 × 500 reactions of 20 µl final volume	06 924 204 001
Transcriptor Reverse Transcriptase	250 U, for up to 25 reactions of 50 µl final volume each containing 10 U Transcriptor Reverse Transcriptase	03 531 317 001
	500 U, for up to 50 reactions of 50 µl final volume each containing 10 U Transcriptor Reverse Transcriptase	03 531 295 001
	2,000 U (4 × 500 U), for up to 200 reactions of 50 µl final volume each containing 10 U Transcriptor Reverse Transcriptase	03 531 287 001
Uracil-DNA Glycosylase, heat-labile	100 U	11 775 367 001
	500 U	11 775 375 001
First Strand cDNA Synthesis Kit for RT-PCR (AMV)	1 kit, 30 reactions, including 5 control reactions	11 483 188 001
Transcriptor First Strand cDNA Synthesis Kit	1 kit, for up to 50 reactions, including 10 control reactions	04 379 012 001
	1 kit, for up to 100 reactions	04 896 866 001
	1 kit, for up to 200 reactions	04 897 030 001

## 6. Supplementary Information

### 6.4. Trademarks

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### 6.5. License Disclaimer

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### 6.6. Regulatory Disclaimer

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

### 6.7. Safety Data Sheet

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

### 6.8. Contact and Support

If you have questions or experience problems with this or any Roche product for Life Science, please contact our Technical Support staff. Our scientists are committed to providing rapid and effective help.  
Please also contact us if you have suggestions for enhancing Roche product performance or using our products in new or specialized ways. Such customer information has repeatedly proven invaluable to the research community worldwide.

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- Certificates of Analysis
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