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

LightCycler® 480 RNA Master Hydrolysis Probes

Version 5.0

Content version: October 2010

Easy-to-use Reaction Mix (2.7× conc.) for One-Step RT-PCR using the LightCycler® 480 Real-Time PCR System

Cat. No. 04 991 885 001 Kit for 5 × 100 reactions (20 µl)

Store the kit at –15 to –25°C

www.roche-applied-science.com

1. What this Product Does

Number of Tests The kit is designed for 5 × 100 reactions with a reaction volume of 20 µl each.

Kit Contents

Vial/Cap	Label	Contents / Function
1 red	LightCycler® 480 RNA Master Hydrolysis Probes, 2.7× conc.	• 5 × 740 µl each • Contains Tth DNA Polymerase, reaction buffer and dNTP mix (with dUTP instead of dTTP).
2 colorless, grey cap label	Activator, 50 mM	• 1 ml • To adjust $Mn(OAc)_2$ concentration.
3 colorless	H_2O, PCR-grade	• 5 × 1 ml • To adjust the final reaction volume.
4 yellow	Enhancer, 20× conc.	• 500 µl • To improve the amplification of difficult (*e.g.,* GC-rich), targets

Storage and Stability

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	Label	Storage
1 red	LightCycler® 480 RNA Master Hydrolysis Probes, 2.7 × conc.	• Store at −15 to −25°C. • **Avoid repeated freezing and thawing!**
2 colorless, grey cap label	Activator, 50 mM	• Store at −15 to −25°C.
3 colorless	H_2O, PCR grade	
4 yellow	Enhancer, 20× conc.	

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:

- LightCycler® 480 Instrument I* or LightCycler® 480 Instrument II*
- LightCycler® 480 Multiwell Plate 384* or LightCycler® 480 Multiwell Plate 96*
- Standard swing-bucket centrifuge containing a rotor for multiwell plates with suitable adaptors.
- Uracil-DNA Glycosylase, heat-labile* (optional)

  For prevention of carry-over contamination; see "Related Procedures" on page 10 for details.
- Nuclease-free, aerosol-resistant pipette tips
- Pipettes with disposable, positive-displacement tips
- Sterile reaction tubes for preparing master mixes and dilutions

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

Application

The LightCycler® 480 RNA Master Hydrolysis Probes is designed for research studies. When combined with the LightCycler® 480 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).

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 carry-over 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 System and design specific PCR primers and hydrolysis probe for each target. See the LightCycler® 480 Operator's Manual for general recommendations.

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 [$Mn(OAc)_2$] concentration.

⚠ The amplicon size should not exceed 500 kb 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 Real-Time PCR 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 PCR in terms of purity, concentration, and absence of PCR inhibitors. For reproducible isolation of nucleic acids use:

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

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

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.

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.

The optimal probe concentration is the lowest concentration that results in the lowest Cp 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_m of the probe should be only slightly higher than the T_m 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.

Activator [$Mn(OAc)_2$]

To ensure specific and efficient amplification use $Mn(OAc)_2$ (Activator, vial 2) at a final concentration of 3.25 mM. Titration of Activator is not required, except for multiplex assays where optimization of $Mn(OAc)_2$ concentration might be necessary for best results.

Addition of 0.1 µl Activator stock solution to a final reaction volume of 20 µl 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® 480 Probe Master. 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.

2.2 Procedure

LightCycler® 480 System Protocol

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

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

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

A LightCycler® 480 Instrument protocol that uses LightCycler® 480 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

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

A) 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				
Detection Format		**Block Type**	**Reaction Volume**	
Mono Color Hydrolysis Probes or Multi Color Hydrolysis Probes		96	10 -100 µl	
Programs				
Program Name		**Cycles**	**Analysis Mode**	
Reverse Transcription		1	None	
Denaturation		1	None	
Amplification		45 [1)]	Quantification	
Cooling		1	None	
Temperature Targets				
Target (°C)	**Acquisition Mode**	**Hold (hh:mm:ss)**	**Ramp Rate (°C/s)**	**Acquisitions (per °C)**
Reverse Transcription				
63 [2)]	None	00:03:00 [3)]	4.4	-
Denaturation				
95	None	00:00:30	4.4	-

Amplification				
95	None	00:00:10 - 00:00:15	4.4	-
60 [4]	None	00:00:30 - 00:00:60	2.2 [5]	-
72	Single	00:00:01	4.4	-
Cooling				
40	None	00:00:10	2.2 [5]	-

B) 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				
Detection Format		**Block Type**	**Reaction Volume**	
Mono Color Hydrolysis Probes or Multi Color Hydrolysis Probes		384	3 -20 µl	
Programs				
Program Name		**Cycles**	**Analysis Mode**	
Reverse Transcription		1	None	
Denaturation		1	None	
Amplification		45 [1]	Quantification	
Cooling		1	None	
Temperature Targets				
Target (°C)	**Acquisition Mode**	**Hold (hh:mm:ss)**	**Ramp Rate (°C/s)**	**Acquisitions (per °C)**
Reverse Transcription				
63 [2]	None	00:03:00 [3]	4.8	-
Denaturation				
95	None	00:00:30	4.8	-
Amplification				
95	None	00:00:10 - 00:00:15	4.8	-
60 [4]	None	00:00:30 - 00:00:60	2.5 [5]	-
72	Single	00:00:01	4.8	-
Cooling				
40	None	00:00:10	2.5 [5]	-

[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 suffcient 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!

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 when handling it.

❶ • 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.

❷ Prepare a 10× conc. solution that contains PCR primers and hydrolysis probe.

❸ 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, 10× conc. [1]	2.0 µl	0.5 µM/0.25 µM
Activator	1.3 µl	3.25 mM $Mn(OAc)_2$
LightCycler® 480 RNA Master Hydrolysis Probes, 2.7× conc. (vial 1, red cap)	7.4 µl	1× conc.
Enhancer, 20× conc. (optional)	(1.0 µl)	1× conc.
Total volume	**18 µl**	

[1] 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.

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 + one additional reaction.

❹	• Mix carefully by pipetting up and down. Do not vortex. • Pipet 18 µl RT-PCR mix into each well of the LightCycler® 480 Multiwell Plate. • Add 2µl of the RNA template. • Seal Multiwell Plate with LightCycler® 480 Sealing Foil.
❺	• Place the Multiwell Plate in the centrifuge and balance it with a suitable counterweight (*e.g.*, another Multiwell Plate). • Centrifuge for 2 min at 1,500 × *g* in a standard swing-bucket centrifuge, containing a rotor for multiwell plates with suitable adaptors.
❻	Load the Multiwell Plate into the LightCycler® 480 Instrument.
❼	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 Related Procedures

Color Compensation

For information on generating and using a color compensation file, see the LightCycler® 480 Operator's Manual or the LightCycler® 480 Online Resource Site (www.lightcycler480.com).

Prevention of Carry-Over Contamination

Uracil-DNA Glycosylase (UNG) is suitable for preventing carry-over 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 hydrolized 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 carry-over contamination using heat-labile UNG:

❶	Per 20 µl final reaction volume, add 1 µl heat-labile UNG to the RT-PCR mix.
❷	Add RNA template and incubate the reaction mixture for 5 min at RT to destroy any contaminating template.
❸	Inactivate heat-labile UNG by performing the reverse transcription step at 61-63°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 β-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).

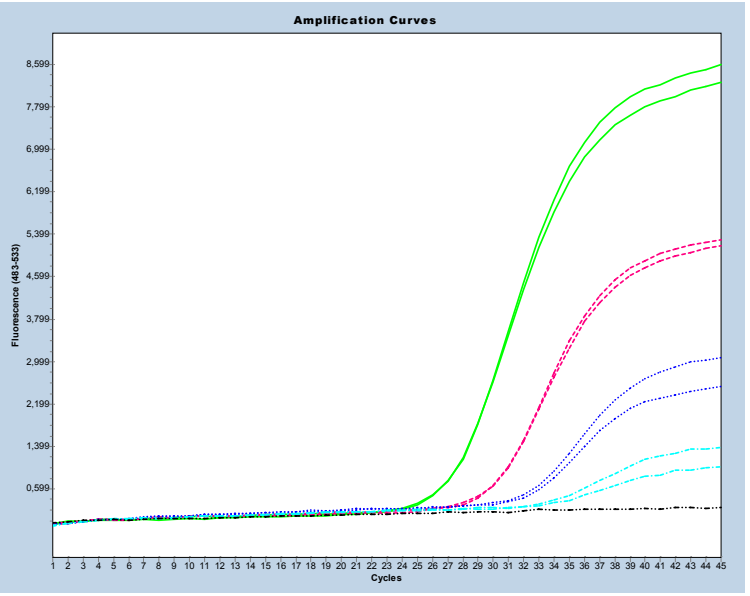

Fig. 1: Amplification of **β**-actin transcript from total HeLa cell RNA (100 pg [—], 10 pg [--], 1 pg [...], 0.1 pg [-.-], negative control [-..-]). RNA samples were reverse transcribed in two-fold replicates for 3 min at 63°C. cDNA was amplified in the LightCycler® 480 Instrument according to the protocol given under "LightCycler® 480 System Protocol" on page 7. **β**-actin specific PCR products were detected with probe #11 from the Universal ProbeLibrary and analyzed in the AbsQuant module of LightCycler® 480 Software.

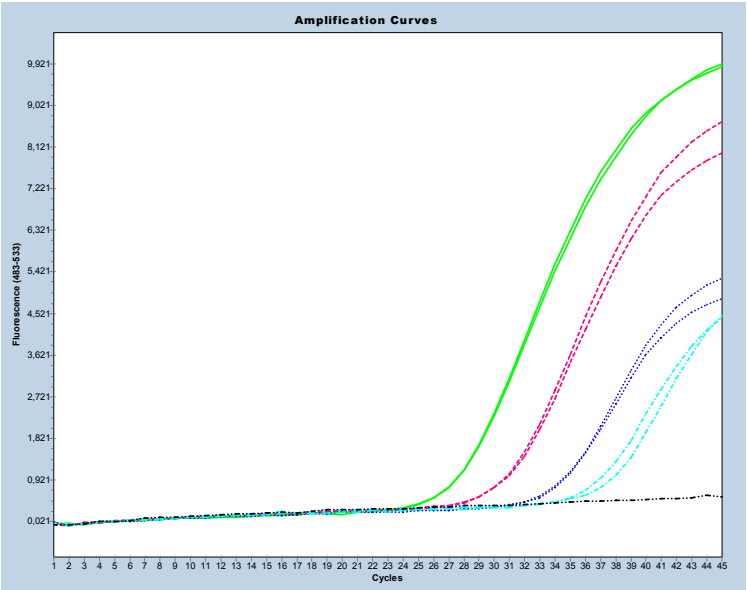

Fig. 2: Amplification of β-actin transcript from total HeLa cell RNA (100 pg [—], 10 pg [--], 1 pg [...], 0.1 pg [-.-], negative control [-..-]) **in the presence of Enhancer**. RNA samples were reverse transcribed in two-fold replicates for 3 min at 63°C. cDNA was amplified in the LightCycler® 480 Instrument according to the protocol given under "LightCycler® 480 System Protocol" on page 7. β-actin specific PCR products were detected with probe #11 from the Universal ProbeLibrary and analyzed in the AbsQuant module of LightCycler® 480 Software.

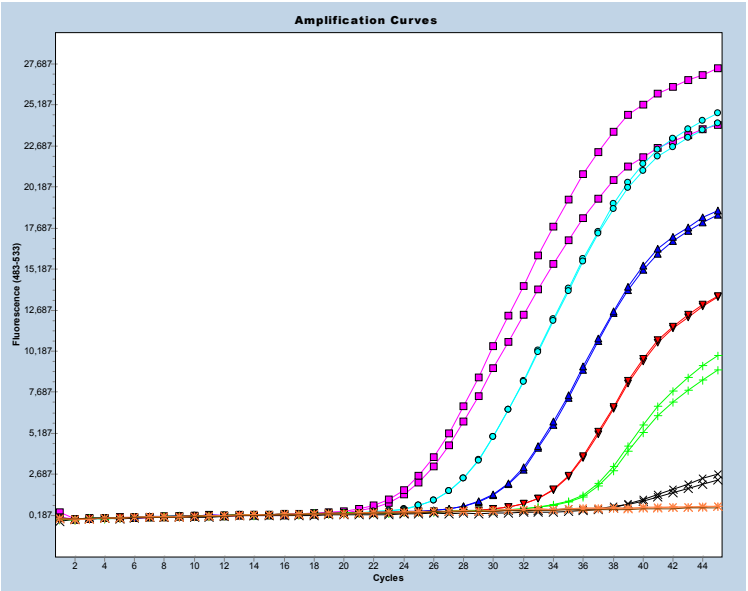

Fig. 3: Amplification of ApoE transcript (76% GC content) from total human liver RNA (100 ng [■], 10 ng [●], 1 ng [▲], 0.1 ng [▼], 10 pg [+], 1 pg [×], negative control [✳]). RNA was reverse transcribed for 3 min at 63°C. cDNA was amplified in the LightCycler® 480 Instrument according to the protocol given under "LightCycler® 480 System Protocol" on page 7. 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

	Possible Cause	Recommendation
Fluorescence intensity varies.	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 × *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.
Fluorescence intensity is very low.	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°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 µM, probe concentration should be in the range of 0.05 to 0.25 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. • To improve dynamic range or to raise the fluorescence signal when using low RNA concentrations, add Enhancer (1 µl per 20-µl 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 8-10 µl of total RNA per 20-µl 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 unproper storage or isolation.	• Check RNA quality on a gel. • Check RNA with an established primer pair if available.
Negative control sample gives a positive signal.	Contamination	• Remake all critical solutions. • Pipet reagents on a clean bench. • Use heat-labile UNG to eliminate carry-over contamination.
	Pipetting errors or omitted reagents.	• Check for missing reagents. • Check for missing or defective dye.

Possible Cause	Recommendation
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 bp	Do not use amplicons >500 bp. Optimal results are obtained with amplicons of 100 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

How this Product Works

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 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 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 $Mn(OAc)_2$, 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.

Test Principle

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. 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 reproter) 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 (1). 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). 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 Applied Science recommends to use BHQ-2 (quenching range 550 –650 nm) for all hydrolysis probe reporter dyes listed above.

References

1 Holland, P.M. *et al* (1991). Detection of specific polymerase chain reaction product by utilizing the 5´->3´ exonuclease activity of *Thermus aquaticus* DNA polymerase. *Proc. Natl. Acad. Sci. USA*. **88**, 7276-7280.

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

6.1.1 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.

6.1.2 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

- Editorial corrections
- Vial 2: Increase of volume and change of cap color from green to colorless with grey cap label.

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:

- Real-time PCR Systems (LightCycler® Carousel-Based System, LightCycler® 480 System, and Universal ProbeLibrary): www.lightcycler.com
- Automated Sample Preparation (MagNA Lyser Instrument, MagNA Pure Compact System, and MagNA Pure LC System): www.magnapure.com
- Real-Time qPCR Assays with prevalidated UPL-probes:
- http://www.universalprobelibrary.com

	Product	Pack Size	Cat. No.
Instruments	LightCycler® 480 Instrument II, 96-well	1 instrument with control unit and accessories	05 015 278 001
	LightCycler® 480 Instrument II, 384-well	1 instrument with control unit and accessories	05 015 243 001
Software	LightCycler® 480 Software, Version 1.5	1 software package	04 994 884 001
	LightCycler® 480 LIMS Interface Module	1 software package	05 066 310 001
	LightCycler® 480 Gene Scanning Software	1 software package	05 103 908 001
	LightCycler® 480 Multiple Plate Analysis Software	1 software package	05 075 122 001
Accessories	LightCycler® 480 Thermal Block Cycler Unit (96-well) Silver	96-well thermal block cycler unit, including block cycler cover, storage box and loading device	05 015 197 001
	LightCycler® 480 Thermal Block Cycler Unit (384-well) Silver	384-well thermal block cycler unit, including block cycler cover, storage box and loading device	05 015 219 001
	LightCycler® 480 Multiwell Plate 96	50 plates with 50 sealing foils	04 729 692 001
	LightCycler® 480 Multiwell Plate 384	50 plates with 50 sealing foils	04 729 749 001
	LightCycler® 480 Multiwell Plate 96, clear	50 plates with 50 sealing foils	05 102 413 001
	LightCycler® 480 Multiwell Plate 384, clear	50 plates with 50 sealing foils	05 102 430 001
	LightCycler® 480 Sealing Foil	1 × 50 foils	04 729 757 001
	LightCycler® 480 Sealing Foil Applicator		04 706 170 001

	Product	Pack Size	Cat. No.
PCR Reagents	LightCycler® 480 SYBR Green I Master	5 × 1 ml (5 × 100 reactions, 20 µl each) 10 × 5 ml (10 × 500 reactions, 20 µl each)	04 707 516 001 04 887 352 001
	LightCycler® High Resolution Melting Master	1 kit (5 × 100 reactions, 20 µl each)	04 909 631 001
	LightCycler® 480 Probes Master	1 kit (5 × 100 reactions, 20 µl each) 1 kit (10 × 500 reactions, 20 µl each) 1 kit (1 × 5,000 reactions, 20 µ each)	04 707 494 001 04887 301 001 04 902 343 001
	LightCycler® Genotyping Master	1 kit (5 × 100 reactions, 20 µl each)	04 707 524 001
Universal ProbeLibrary	Universal ProbeLibrary Set, Human	Library of 90 pre-validated detection probes	04 683 633 001
	Universal ProbeLibrary Set, Mouse	Library of 90 pre-validated detection probes	04 683 641 001
	Universal ProbeLibrary Set, Rat	Library of 90 pre-validated detection probes	04 683 650 001
	Universal ProbeLibrary Extension Set	Library of 75 pre-validated detection probes (probes #91 to #165)	04 869 877 001
Associated Kits and Reagents	LightCycler® Uracil-DNA Glycosylase	100 U (50 µl)	03 539 806 001
	LightCycler® h-G6PDH Housekeeping Gene Set	1 set (96 reactions)	03 261 883 001
	Transcriptor Reverse Transcriptase	250 U 500 U 2,000 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

6.4 Disclaimer of License

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