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LightCycler® 480 Probes Master

Version: 10

Content version: September 2016

Ready-to-use hot start reaction mix for PCR using the LightCycler® 480 System

Cat. No. 04 707 494 001	5 x 1 ml 5 x 100 reactions of 20 µl final volume each
Cat. No. 04 887 301 001	10 x 5 ml 10 x 500 reactions of 20 µl final volume each
Cat. No. 04 902 343 001	1 x 50 ml 5,000 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 / Bottle	Cap	Label	Function/Description	Catalog Number	Content
1	red	LightCycler® 480 Probes Master, 2x conc.	▪ Ready-to-use hot start PCR mix ▪ Contains FastStart Taq DNA Polymerase, reaction buffer, dNTP mix (with dUTP instead of dTTP), and 6.4 mM $MgCl_2$	04 707 494 001	5 vials, 1 ml each
				04 887 301 001	10 vials, 5 ml each
				04 902 343 001	1 vial, 50 ml
2	colorless	LightCycler® 480 Probes Master, Water, PCR Grade	To adjust the final reaction volume	04 707 494 001	5 vials, 1 ml each
				04 887 301 001	2 vials, 25 ml each
				04 902 343 001	2 vials, 25 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	Master, 2x conc.	Store at −15 to −25°C. ⚠ ***Avoid repeated freezing and thawing!*** After first thawing, the master may be stored for up to 4 weeks at +2 to +8°C.
2	colorless	Water, PCR Grade	Store at −15 to −25°C.

1.3. Additional Equipment and Reagents Required

Additional reagents and equipment required to perform PCR with the LightCycler® 480 Probes Master include:
- LightCycler® 480 Instrument II*
- LightCycler® 480 Multiwell Plate 384* or LightCycler® 480 Multiwell Plate 96*
- LightCycler® 8-Tube Strip Adapter Plate*
- LightCycler® 8-Tube Strips*
- Standard swinging-bucket centrifuge containing a rotor for multiwell plates with suitable adaptors
- LightCycler® Uracil-DNA Glycosylase* (optional[1])
- Nuclease free, aerosol-resistant pipette tips
- Pipettes with disposable, positive-displacement tips
- Sterile 1.5 ml reaction tubes for preparing master mixes and dilutions

[1] For prevention of carryover contamination, please refer to the corresponding chapter.

1.4. Application

LightCycler® 480 Probes Master is designed for research studies on the LightCycler® 480 System. The LightCycler® 480 Probes Master is a ready-to-use hot start reaction mix designed specifically for detecting DNA targets with hydrolysis probes during LightCycler® 480 System PCR. However, it may be used in other types of PCR on the LightCycler® 480 System. For best results, use this master mix with LightCycler® 480 Multiwell Plates.
The kit can also help prevent carryover contamination during PCR (when used with LightCycler® Uracil-DNA Glycosylase*) or to perform the second step of a two-step RT-PCR.

1.5. Preparation Time

Assay Time

The assay time is variable, depending on the number of cycles and the annealing time. For example, for a cycling program of 40 cycles and an annealing time of 20 seconds, the LightCycler® 480 PCR run will require about 45 minutes, including 5 minutes pre-incubation time.

2. How to Use this Product

2.1. Before you Begin

Sample Materials

- Use any template DNA (*e.g.,* genomic or plasmid DNA, cDNA) suitable for PCR, as long as it is sufficiently pure, concentrated, and free of PCR inhibitors.

⚠ ***For reproducible isolation of nucleic acids, we recommend:***

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

For details, see the Roche Life Science homepage, www.lifescience.roche.com.

- Use up to 500 ng complex genomic DNA or up to 10^{10} copies plasmid DNA for a reaction volume of 20 µl. For larger volumes, the amount of template can be increased equivalently.

ⓘ *When using unpurified cDNA from a reverse transcription reaction, especially when it contains high concentrations of RNA and oligonucleotides, you can improve your results by using 2 µl (or less) of that sample in the reaction and performing a 10-minute pre-incubation at +95°C. This will result in lower crossing point (Cp) values with a decreased standard deviation.*

Control Reactions

Always run a negative control with the samples. To prepare a negative control:

- Replace the template DNA with Water, PCR Grade PCR (Vial 2). This will reveal whether a contamination problem exists.
- For a two-step RT-PCR setup, omit the addition of reverse transcriptase to the cDNA synthesis reaction; this will indicate whether DNA in RNA samples causes false-positive results.

Primers

Use PCR primers at a final concentration of 0.3 to 1 µM. The recommended starting concentration is 0.5 µM each.

⚠ ***Optimize the primer concentration before optimizing the probe concentration.***

ⓘ *The optimal primer concentration is the lowest concentration that results in the lowest Cp and an adequate fluorescence for a given target concentration.*

Probe

Use hydrolysis probes at a final concentration of 0.05 to 0.2 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 Tm of the probe should be only slightly higher than the Tm of the PCR primer, so the hybridization complex is stable. The probe sequence must also take into account mismatches in the DNA template, as these will also affect the annealing temperature.***

Mg^{2+} Concentration

The composition of the reaction mix in this kit already contains an optimal concentration of $MgCl_2$ which works with nearly all primer combinations.

ⓘ *You do not need to adjust the $MgCl_2$ concentration to amplify different sequences.*

General Considerations

Two-Step RT-PCR

The LightCycler® 480 Probes Master can also be used to perform the second step of a two-step RT-PCR. In two-step RT-PCR, the first step, reverse transcription of RNA into cDNA, is performed outside the LightCycler® 480 System. Subsequent amplification and online monitoring is performed according to the LightCycler® 480 System standard procedure, using cDNA as starting sample material.

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

- Transcriptor Reverse Transcriptase*
- Transcriptor First Strand cDNA Synthesis Kit*

Synthesis of cDNA is performed according to the detailed instructions provided with the cDNA synthesis reagent.

⚠ ***For initial experiments, we recommend running undiluted, 1:10 diluted, and 1:100 diluted cDNA template in parallel to determine the optimum template amount. If you use undiluted cDNA as template, we recommend to extend the pre-incubation time to 10 minutes.***

In principle, the LightCycler® 480 PCR Master Probes can be used to amplify and detect any DNA or cDNA target. However, you would need to adapt your detection protocol to the reaction conditions of the LightCycler® 480 Instrument, and design specific PCR primers and probes for each target. See the LightCycler® 480 Operator's Manual for general recommendations.

⚠ ***The amplicon should not excceed 1,000 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 warranted only when it is used with the LightCycler® 480 System.***

Other assay formats may also be adapted to real-time PCR on the LightCycler® 480 Instrument. For example, probe formats that may be adapted to the LightCycler® 480 Instrument in addition to hydrolysis probes include FRET hybridization probes, Molecular Beacons, and Scorpions. However, any fluorescent dyes used in a LightCycler® 480 analysis must be compatible with the optical unit of the LightCycler® 480 Instrument.

2.2. Protocols

LightCycler® 480 Instrument 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 Instument protocol that uses the LightCycler® 480 Probes Master should contain the following programs:

- **Pre-Incubation** for activation of FastStart Taq DNA Polymerase and denaturation of the DNA.
- **Amplification** of the target DNA.
- **Cooling** of the thermal block.

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

A) Protocol for use with the 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 Probes Master using a LightCycler® 480 Multiwell Plate 96.

Setup					
Block Type			Reaction Volume [µl]		
96			10 – 100		
Detection Format		Excitation Filter		Emission Filter	
Mono Color Hydrolysis Probe / UPL Probe:					
FAM		465		510	
or for example:					
3 Color Hydrolysis Probe:					
FAM VIC/Hex/Yellow 555 Cy5/Cy5.5		465 533 618		510 580 660	
For new customized detection formats (hydrolysis probes), 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	
Pre-Incubation		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]
Pre-Incubation	95	None	00:00:05 – 00:10:00[2]	4.4 (or 2.0)[3]	–
Amplification	95	None	00:00:10	4.4 (or 2.0)[3]	–
	primer dependent[4]	None	00:00:15 – 00:50:00[5]	2.2 (Target °C ≥50°C) 1.5 (Target °C <50°C)[6]	–
	72	Single	00:00:01	4.4 (or 2.0)[3]	–
Cooling	40	None	00:00:10	1.5	–

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 PCR Probes Master and hydrolysis probes using a LightCycler® 480 Multiwell Plate 384.

Setup					
Block Type			Reaction Volume [µl]		
384			3 – 20		
Detection Format	Excitation Filter		Emission Filter		
Mono Color Hydrolysis Probe / UPL Probe:					
FAM	465		510		
or for example:					
3 Color Hydrolysis Probe:					
FAM VIC/Hex/Yellow 555 Cy5/Cy5.5	465 533 618		510 580 660		
For new customized detection formats (hydrolysis probes), 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		
Pre-Incubation	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]
Pre-Incubation	95	None	00:00:05 – 00:10:00[2]	4.8	–
Amplification	95	None	00:00:10	4.8	–
	primer dependent[4]	None	00:00:15 – 00:30:00[5]	2.5 (Target °C ≥50°C) 2.0 (Target °C <50°C)[6]	–
	72	Single	00:00:01	4.8	–
Cooling	40	None	00:00:10	2.0	–

[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] If high polymerase activity is required in early cycles, you can sometimes improve results by extending the pre-incubation to 10 minutes. This is especially recommended for higher reaction volumes and when working with unpurified cDNA samples as template. Do not use more than 2 µl unpurified cDNA sample.

[3] A ramp rate of 2.0°C/s is recommended for reaction volumes of 50 µl or higher.

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

[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. This is especially recommended for higher reaction volumes.

[6] For users of LightCycler® 480 Software 1.1: (From LightCycler® 480 Software 1.2 on, the Cooling Ramp Rate can be set to maximum.)
– For the 96-multiwell plate: For target temperatures of 50°C and above, set the Ramp Rate for Cooling to 2.2°C/s. For target temperatures below 50°C, set the Ramp Rate to 1.5°C/s.
– For the 384-multiwell plate: For target temperatures of 50°C and above, set the Ramp Rate for Cooling to 2.5°C/s. For target temperatures below 50°C, set the Ramp Rate to 2.0°C/s.

Preparation of the 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 and Multiwell Sealing Foil when handling them. Always wear gloves during handling.***

1. Thaw the LightCycler® 480 Probes Master (Vial 1) and Water (Vial 2) 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 PCR mix for one 20 µl reaction by adding the following components in the order listed below:

Reagent	Volume
Water, PCR Grade (Vial 2)	3 µl
Primer-probe mix[1], 10x conc.	2 µl
LightCycler® 480 Probes Master (2x conc.) (Vial 1)	10 µl
Total volume	**15 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 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 + two additional reactions.*

4. Mix carefully by pipetting up and down. Do not vortex.
   – Pipette 15 µl PCR mix into each well of the LightCycler® 480 Multiwell Plate.
   – Add 5 µl of the DNA template.
   – Seal Multiwell Plate with the LightCycler® 480 Sealing Foil*.

5. Place the Multiwell Plate in the centrifuge and balance it with a suitable counterweight (*e.g.,* another Multiwell Plate).
   – Centrifuge for 2 min at 1500 × *g* in a standard swinging-bucket centrifuge, containing a rotor for multiwell plates with suitable adaptors.

6. Load the Multiwell Plate into the LightCycler® 480 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.***

Color Compensation

For information on generating and using a color compensation file, see the LightCycler® 480 Operator's Manual.

2.3. Other Parameters

Prevention of Carryover Contamination

Uracil DNA N-Glycosylase (UNG) can help prevent carryover contamination in PCR. The prevention technique involves incorporating deoxyuridine triphosphate (dUTP, a component of the Master Mix in this kit) into amplification products, then pretreating later PCR mixtures with UNG. If a dUTP-containing contaminant is present in the later PCRs, it will be cleaved by a combination of the UNG and the high temperatures of thepre-incubation step; it will not serve as a PCR template.

Since your target DNA template contains thymidine rather than uridine, it is not affected by this procedure.

To ensure optimum results in carryover prevention reactions with the LightCycler® 480 Probes Master, always use LightCycler® Uracil-DNA Glycosylase*. Follow the instructions in the Instructions for Use for the enzyme.

3. Results

The following amplification curves were obtained by using the LightCycler® 480 Probes Master. The fluorescence values versus cycle number are displayed.

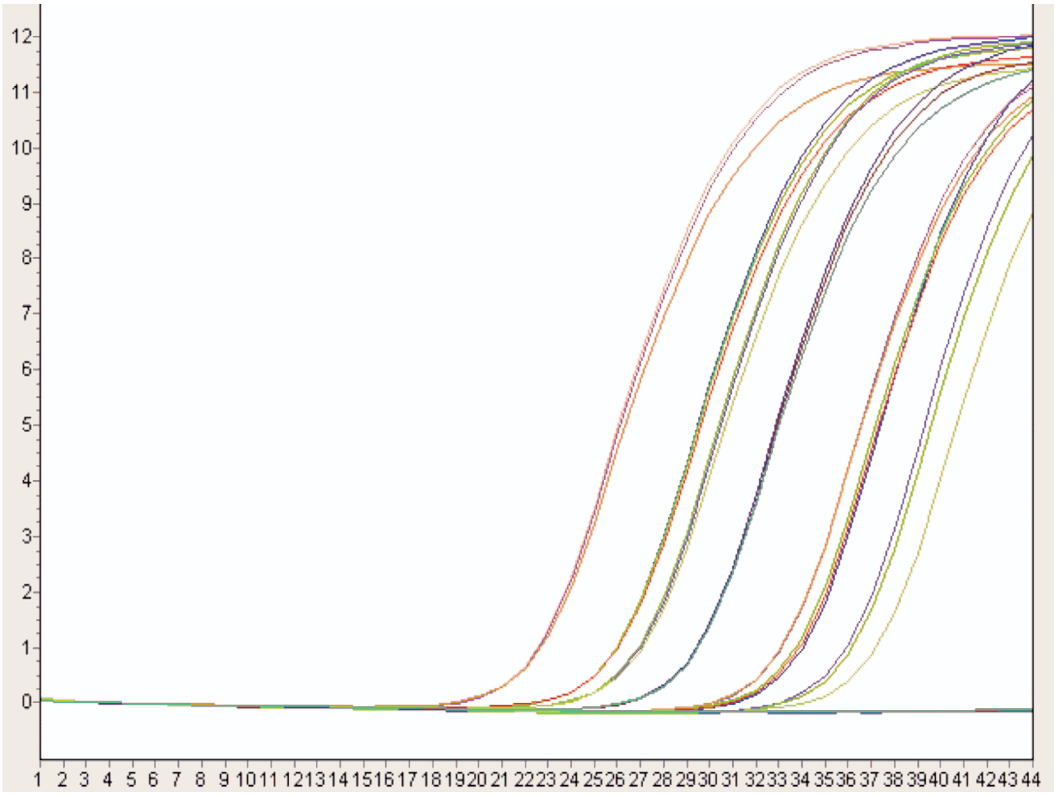

Fig. 1: Amplification curves were obtained from serial dilutions (triplicates) of 1 (far right), 10, 50 100, 1,000, 5,000, 10,000, and 100,000 (far left) copies Cytochrome P450 2C9 transcript per well. A specific set of primers and a FAM/TAMRA-**l**abeled hydrolysis probe that recognizes a 196-bp fragment of the Cytochrome P450 2C9 gene was used.

4. Troubleshooting

Observation	Possible cause	Recommendation
Amplification curves reach plateau phase before cycling is complete.	Starting amount of nucleic acid is very high.	Stop the cycling program by clicking the *End Program* button. The next cycle program will continue automatically.
	The number of cycles is too high.	Reduce the number of cycles in the cycling program.
Log-linear phase of amplification just starts as the cycling program ends.	The number of cycles is too low.	While cycling is still going on, use the *Add 10 Cycles* button to increase the number of cycles.
		Increase the number of cycles in the cycling program.
		Use more starting material.
		Optimize PCR conditions (primer/probe design, protocol).
No amplification detectable.	Wrong filter combination was used to display amplification on screen.	Select appropriate filter combination for your assay on the analysis screen and start again.
	Wrong detection format was chosen for experimental protocol.	Select appropriate detection format for your assay and start again.
	Impure sample material inhibits reaction.	Try a 1:10 dilution of your sample.
		Purify the nucleic acids from your sample material to ensure removal of inhibitory agents.
	FastStart Taq DNA Polymerase is not sufficiently activated.	Make sure PCR protocol includes an pre-incubation step (95°C for 5 – 10 minutes).
		Make sure denaturation time during amplification is at least 10 seconds.
	Pipetting errors or omitted reagents.	Check for missing or defective reagents.
	Amplicon length is >1 kb.	Do not design primers that produce amplicons >1 kb, as they are inefficiently amplified. Optimal results are obtained with amplicons <500 bp.
	Difficult template, for example, unusual GC-rich sequence.	Optimize temperatures and times used for the amplification cycles.
		Optimize primer/probe sequences.
		Repeat PCR but add increasing amounts of DMSO. Use as much as 10% DMSO in the reaction.
Fluorescence intensity varies.	Some of the reagent is still in the upper part of the microwell, or an air bubble is trapped in the microwell.	Repeat centrifugation, but allow sufficient centrifugation time (*e.g.,* 2 minutes 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.

Observation	Possible cause	Recommendation
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).	Check concentrations of reagents and probes.
		Optimize protocol.
	Chosen imaging time is too low.	Choose adequate Roche Detection Format in combination with “dynamic” detection mode.
		Increase imaging time when using “manual” detection mode. For details, see the LightCycler® 480 Operator’s Manual.
Negative control sample gives a positive signal.	Contamination	Remake all critical solutions.
		Pipette reagents on a clean bench.
		Use UNG to eliminate carryover contamination.
High background.	Fluorescence signals are very low, therefore the background seems relatively high.	Follow general strategies for optimizing PCR runs in the LightCycler® 480 System.
	Probe quality is poor.	Prepare a new probe solution.
High standard deviation of crossing point (Cp) values.	Impure, heterogenous DNA template.	Increase pre-incubation time to 10 minutes.
		Use a maximum of 2 µl unpurified cDNA sample.

5. Additional Information on this Product

5.1. Test Principle

LightCycler® 480 Probes Master is a ready-to-use reaction mix specifically developed for the hydrolysis probe detection format in multiwell plates on the LightCycler® 480 Instrument. It contains FastStart Taq DNA Polymerase for hot start PCR, which significantly improves the specificity and sensitivity of PCR by minimizing the formation of nonspecific amplification products (Chou, Q. et al., 1992, Kellogg, D.E. et al., 1994, and Birch, D.E., 1996).

FastStart Taq DNA Polymerase is a chemically modified form of thermostable recombinant Taq DNA Polymerase that shows no activity up to 75°C. The enzyme is active only at high temperatures, where primers no longer bind nonspecifically. The enzyme is completely activated (by removal of blocking groups) in a single pre-incubation step (95°C, 5 – 10 minutes) before cycling begins. Activation does not require the extra handling steps typical of other hot start techniques.

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 reporter) 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 (Holland, P. M. et al., 1991). 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. 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 Life Science recommends using BHQ-2 (quenching range 550 –650 nm) for all hydrolysis probe reporter dyes listed above***

5.2. References

- Birch DE, Kolmodin L, Wong J, Zangenberg GA, Zoccoli MA, McKinney N, Young KKY - Simplified hot start PCR (1996) *Nature* **381** (6581), 445-446
- Chou Q, Russell M, Birch DE, Raymond J, Bloch W - Prevention of pre-PCR mis-priming and primer dimerization improves low-copy-number amplifications (1992) *Nucleic Acids Research* 7, 1717-1723
- Holland PM, Abramson RD, Watson R, Gelfand DH - Detection of specific polymerase chain reaction product by utilizing 5´ → 3´ the exonuclease activity of Thermus aquaticus DNA polymerase (1991) *Proc Natl Acad Sci U S A* 16, 7276-7280
- Kellogg DE, Rybalkin I, Chen S, Mukhamedova N, Vlasik T, Siebert PD, Chenchika A - TaqStart antibody : hot start PCR facilitated by a neutralizing monoclonal antibody directed against Taq DNA polymerase (1994) *BioTechniques* **16** (6), 1134-1137

5.3. Quality Control

The LightCycler® 480 Probes Master is function tested using the LightCycler® 480 Instrument, according to the kit protocols.

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® 480 Block Kit 96 Silver	1 block kit	05 015 219 001
LightCycler® 480 Block Kit 384 Silver	1 block kit	05 015 197 001
LightCycler® 8-Tube Strip Adapter Plate	1 piece, adapter plate, The adapter plate can be used multiple times	06 612 598 001
Accessories software		
LightCycler® 480 LIMS Interface Module	1 software package	05 066 310 001
LightCycler® 480 Software, Version 1.5	1 software package	04 994 884 001
Consumables		
LightCycler® 480 Multiwell Plate 96, white	5 x 10 plates	04 729 692 001
LightCycler® 480 Multiwell Plate 384, white	5 x 10 plates	04 729 749 001
LightCycler® 480 Sealing Foil	50 foils	04 729 757 001
LightCycler® 480 Multiwell Plate 96, clear	5 x 10 plates	05 102 413 001
LightCycler® 480 Multiwell Plate 384, clear	5 x 10 plates	05 102 430 001

LightCycler® 8-Tube Strips (clear)	10 x 12 clear strips and caps, Each pack of LightCycler® 8-Tube Strips contains 10 x 12 strips of both tubes and flat caps, in 10 non-sterile plastic bags., Each pack of LightCycler® 8-Tube Strips contains 10 x 12 strips of both tubes and flat caps, in 10 non-sterile plastic bags.	06 327 672 001
LightCycler® 8-Tube Strips (white)	10x 12 white strips and clear caps.	06 612 601 001
Instruments		
LightCycler® 480 Instrument II	1 instrument	05 015 278 001
	1 instrument	05 015 243 001
Reagents , kits		
LightCycler® Uracil-DNA Glycosylase	50 µl, 100 U, (2 U/µl)	03 539 806 001
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	04 683 633 001
Transcriptor Reverse Transcriptase	250 U, 25 reactions of 20 µl final volume	03 531 317 001
	500 U, 50 reactions of 20 µl final volume	03 531 295 001
	2,000 U, 4 x 500 U, 200 reactions of 20 µl final volume	03 531 287 001
Transcriptor First Strand cDNA Synthesis Kit	1 kit, 50 reactions, including 10 control reactions	04 379 012 001
	1 kit, 100 reactions	04 896 866 001
	1 kit, 200 reactions	04 897 030 001

6.4. Trademarks

FASTSTART, HIGH PURE, 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 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.

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

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