For general laboratory use. Not for use in diagnostic procedures. FOR *IN VITRO* USE ONLY.

LightCycler® 480 High Resolution Melting Master

Version July 2009

Easy-to-use Reaction Mix (2× conc.) for PCR and high resolution melting using the LightCycler® 480 Real-Time PCR System

Cat. No. 04 909 631 001 Kit for 5 × 100 reactions (20 µl)

Store the kit at –15 to –25°C

⚠ **Keep the Master Mix (vial 1) away from light.**

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Table of Contents

PROTOCOL

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 green	Master Mix, 2× conc.	• 5 × 1 ml • contains FastStart Taq DNA Polymerase, reaction buffer, dNTP mix (with dUTP instead of dTTP), and High Resolution Melting Dye
2 blue	$MgCl_2$, 25 mM	• 2 × 1 ml • to adjust $MgCl_2$ concentration.
3 colorless	H_2O, PCR-grade	• 5 × 1 ml • to adjust the final reaction volume.

Storage and Stability

The kit is shipped on dry ice.

- If 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	Label	Storage
1 green	Master Mix, 2× conc.	• Store at −15 to −25°C. • **Avoid repeated freezing and thawing!** • After first thawing the Master Mix may be stored for up to 4 weeks at +2 to +8°C. • **Keep away from light!**
2 blue	$MgCl_2$, 25 mM	Store at −15 to −25°C.
3 colorless	H_2O, PCR grade	

Additional Equipment and Reagents Required

Refer to the list below for additional reagents and equipment required to perform PCR reactions with the LightCycler® 480 High Resolution Melting Master 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.
- LightCycler®Uracil-DNA Glycosylase* (optional)

  For prevention of carry-over contamination; see "Related Procedures" on page 11 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 High Resolution Melting Master is designed for research studies in combination with the LightCycler® 480 Real-Time PCR System. The LightCycler® 480 High Resolution Melting Master is a ready-to-use 2× conc. hot-start reaction mix designed for amplification and detection of a specific DNA sequence (if suitable PCR primers are provided) followed by high resolution melting curve analysis for detection of sequence variants among several samples. A separate 25 mM $MgCl_2$ stock solution, supplied with the Master, allows you to easily optimize the Mg^{2+} concentration.

In principle, the LightCycler®480 High Resolution Melting Master can be used for specific amplification of any DNA or cDNA target. However, you would need to adapt each amplification protocol to the reaction conditions of the LightCycler® 480 System and design specific PCR primers for each target. The included LightCycler® 480 High Resolution Melting Dye enables detection of double-stranded DNA by fluorescence, monitoring formation of amplicon during PCR cycling, and melting curve analysis utilizing the high resolution data acquisition capabilities of the LightCycler® 480 Instrument. Samples with variations in DNA sequence are distinguished by discrepancies in melting curve shape. Particularly, heterozygous DNA variants forming mismatched heteroduplices, can be distinguished clearly from homozygotes because of their different melting behavior.

⚠ For best results, use the LightCycler® 480 High Resolution Melting Master together with LightCycler® 480 Multiwell Plates only.

⚠ The shorter the amplicon, the better is the differentiation of samples carrying a sequence variation. Best results are achieved with amplicons up to 300 bp. For amplicons longer than 500 bp the sensitivity of variant detection will decrease.

⚠ 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.

- The LightCycler® 480 High Resolution Melting Master is compatible with additives (*e.g.,* DMSO) that enhance amplification of GC-rich sequences.
- For more information and general recommendations see "Additional Information on this Product" on page 16 or refer to the LightCycler® 480 Gene Scanning Software Manual.

Assay Time

Variable, depending on the number of cycles and the annealing time.

For example, if the cycling program specifies 45 cycles and an annealing time of 10 seconds, a LightCycler® 480 PCR run for gene scanning will last about 75 minutes including 10 minutes pre-incubation time and 15 min high resolution melting.

2. How to Use this Product

2.1 Before You Begin

Sample Material

- Use any template DNA (*e.g.,* genomic DNA or cDNA) suitable for PCR in terms of purity, concentration, and absence of PCR inhibitors.
- Use 5 - 30 ng genomic DNA per 20-µl reaction. Use the same amount of template in each reaction.

⚠ Use the same extraction procedure to prepare all samples to be analyzed via high resolution melting. This eliminates any subtle differences that might be introduced by the reagent components in the final elution buffers of different extraction procedures.
For reproducible isolation of nucleic acids use:

- either the MagNA Pure LC or MagNA Pure Compact Instrument and a dedicated 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

⚠ Resuspend all DNA samples in the same buffer, quantify them using spectrophotometry, and adjust them to the same concentration with the resuspension buffer.

⚠ Make sure that the resulting PCR amplification plots have CP values of less than 30 cycles, otherwise there is evidence for

- insufficient amount of template DNA
- insufficient quality of template DNA (*e.g.,* DNA degradation or presence of PCR inhibitors
- unspecific amplification artefacts

Note that compared to other PCR reagents, in some cases CP values might be few cycles higher using the LightCycler® 480 High Resolution Melting Master, because it is optimized for highly specific amplification which is of higher importance for gene scanning experiments than sensitivity.

Primers

- For high resolution melting analysis, specific amplification is essential. Therefore, primer concentration should not be too high. Suitable concentrations of PCR primers range from 0.1 to 0.3 µM (final concentration in PCR). The recommended starting concentration is 0.2 µM each.
- Design PCR primers that have annealing temperatures around 60°C and produce short amplicons (100–250 bp). Use a software package like Primer3 (http://frodo.wi.mit.edu) or LightCycler® Probe Design Software 2.0 for designing the primers.

⚠ Only use primers that have been purified by HPLC.

The optimal primer concentration is the lowest concentration that still results in a high rate of amplicon yield with a low CP and adequate fluorescence dynamics for a given target concentration.

$MgCl_2$

Because specific amplification is essential for high resolution melting analysis, determine the optimum concentration of $MgCl_2$ for each new primer pair. For this, run a positive sample with a dilution series of $MgCl_2$ (1.0, 1.5, 2.0, 2.5, 3.0, and 3.5 mM final concentration in PCR) and analyze the PCR products by agarose gel electrophoresis. The lowest $MgCl_2$ concentration resulting in high yield of target PCR product and no unspecific byproducts is the optimum for this assay.

The table below shows the volume of the $MgCl_2$ stock solution (vial 2, blue cap) that you must add to a 20-µl reaction (final PCR volume) to reach the desired final $MgCl_2$ concentration.

To reach a final Mg^{2+} concentration (mM) of:	1.0	1.5	2.0	2.5	3.0	3.5
Add this amount of 25 mM $MgCl_2$ stock solution (µl)	0.8	1.2	1.6	2.0	2.4	2.8

If all concentrations of the $MgCl_2$ dilution series result in insufficient amplification or unspecific byproducts, optimize the annealing temperature in the PCR protocol by setting it to lower or higher temperatures, respectively.

Negative Control

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

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 High Resolution Melting Master contains the following programs:

- **Pre-Incubation** for activation of FastStart Taq DNA Polymerase and denaturation of template DNA
- **Amplification** of target DNA
- **Melting** of the amplicon with high resolution data acquisition
- **Cooling** the rotor and thermal chamber

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

The following table shows the recommended PCR parameters for an initial LightCycler® 480 System PCR run to establish a gene scanning assay with the LightCycler® 480 High Resolution Melting Master.

Setup				
Detection Format		**Block Type**	**Reaction Volume** [1]	
SYBR Green I		'96' or '384'	'10 -100 µl' or '3 -20 µl'	
Programs				
Program Name		**Cycles**	**Analysis Mode**	
Pre-Incubation		1	None	
Amplification		45 [2]	Quantification	
High Resolution Melting		1	Melting Curve	
Cooling		1	None	
Temperature Targets				
Target (°C)	**Acquisition Mode**	**Hold (hh:mm:ss)**	**Ramp Rate (°C/s) (96-well / 384-well)**	**Acquisitions (per °C)**
Pre-Incubation				
95	None	00:10:00	4.4 / 4.8	-
Amplification				
95	None	00:00:10	4.4 / 4.8	-
primer dependent [3]	None	00:00:15	2.2 / 2.5	-
72	Single	00:00:10 - 00:00:25 [4]	4.4 / 4.8	-

High Resolution Melting				
95	None	00:01:00	4.4 / 4.8	-
40 [5]	None	00:01:00	2.2 / 2.5	-
65 [6]	None	00:00:01	1 / 1	-
95 [6]	Continuous	-	-	25
Cooling				
40	None	00:00:10	2.2 / 2.5	-

In case you do not know the melting temperatures of your PCR primers exactly, it is recommended to apply a touchdown PCR protocol covering a range of annealing temperature from 65 to 53°C. Modify the Temperature Targets of the Amplification program as shown in the table below:

Target (°C)	Acq. Mode	Hold (hh:mm:ss)	Ramp Rate (°C/s) (96-well / 384-well)	Sec Target (°C)	Step Size (°C)	Step Delay (cycles)
Amplification						
95	None	00:00:10	4.4 / 4.8	-		
primer dependent [3]	None	00:00:15	2.2 / 2.5	53	0.5	1
72	Single	00:00:10 - 00:00:25 [4]	4.4 / 4.8	-		

[1] It is recommended to use a reaction volume of 10 to 20 µl for both multiwell plate types (96- or 384-well).

[2] 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!

[3] Annealing temperature is the parameter that most influences specificity and robustness of amplification. For initial experiments set the target temperature (*i.e.,* the primer annealing temperature) 2°C below the calculated primer T_m. The amount of specific product, the presence/absence of undesirable side product, and the presence/absence of dimer product in these experiments will dictate the best way to optimize this parameter. If the reaction produces undesirable product, increase the annealing temperature. If amplification is not robust, decrease the annealing temperature and/or increase the duration of the annealing step.

[4] Calculate the exact elongation time required for your specific target by dividing the amplicon length by 25 (*e.g.,* a 500 bp amplicon requires 20 s elongation time).

[5] This pre-hold temperature ensures that all PCR products have re-associated and encourages heteroduplex formation.

[6] Actual melting conditions depend upon the amplicon. For initial experiments set a wide melting interval, *e.g.,* from 60 to 95°C. Once you have determined where the product will melt, reduce the melting interval to approximately 25°C. Ensure that the melt program starts at least 10°C before and ends at least 10°C after the expected T_m value.

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 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 20× conc. solution of the PCR primers.

❸ 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:

Component	Volume	Final Concentration
Master Mix, 2× conc. (vial 1, green cap)	10.0 µl	1× conc.
Primer mix, 20× conc. [4 µM]	1.0 µl	0.2 µM (each primer)
$MgCl_2$, 25 mM (vial 1, blue cap) [1)]	X µl	
Water, PCR-grade (vial 3, colorless cap)	ad 15.0 µl	–
Total volume	**15 µl**	

[1)] Volume of $MgCl_2$ stock solution to be added is assay specific. See "$MgCl_2$" on page 7.

To prepare the 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 15 µl RT-PCR mix into each well of the LightCycler® 480 Multiwell Plate.
• Add 5 µl of concentration-adjusted DNA 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 1500 × *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

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.

Proceed as described below to prevent carry-over contamination using LightCycler® Uracil-DNA Glycosylase:

❶	Per 20 µl final reaction volume, add 0.5 µl LightCycler® Uracil-DNA Glycosylase (1 U) to the PCR mix.
❷	Add DNA template and incubate the reaction mixture for 10 min at 40°C to destroy any contaminating template.
❸	Inactivate heat-labile UNG by performing the 10 min pre-incubation step at 95°C.

3. Results

The following results were obtained using the LightCycler® 480 High Resolution Melting Master with primers and DNA standards from the LightCycler® 480 Control Kit*. The primer concentration was reduced to half the concentration recommended in the pack insert for the probe-based assay, $MgCl_2$ concentration was 1.5 mM.

The DNA standards represent samples of different genotypes regarding the single nucleotide polymorphism (SNP) C/T in the CyP2C9 gene.

After PCR, the amplicons (144 bp) were analyzed by high resolution melting curve analysis and data were evaluated using the LightCycler® 480 Gene Scanning Software.

DNA samples were spiked with 1/7 amount of wildtype DNA standard to enhance differentiation of the different homozygotes (wildtype and mutation). Depending on the sequence variation, different homozygotes are sometimes difficult to differentiate by melting curve shape. After spiking low amounts (1/10 – 1/5) of wildtype DNA to the samples, the homozygote mutants can be more easily differentiated from wildtype and are still clearly different from the heterozygote mutant samples.

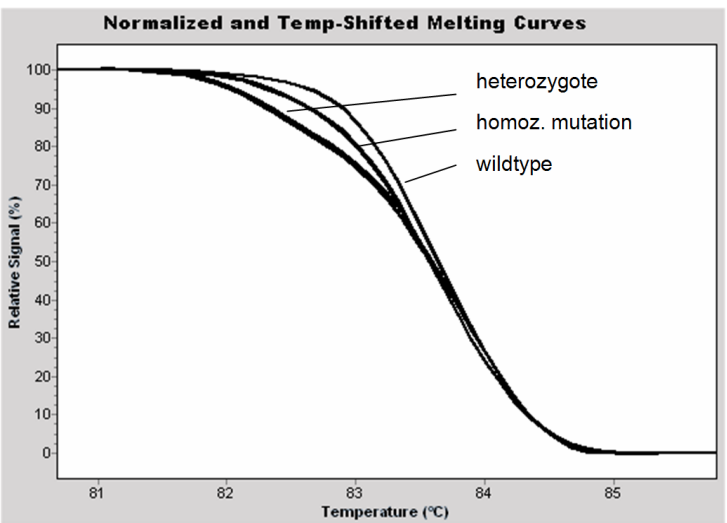

Fig. 1: Normalized, temp-shifted melting curves from CyP2C2 amplicons carrying a sequence variation, evaluated using LightCycler® 480 Gene Scanning Software. Sequence variants can be distinguished by different shape of melting curves.

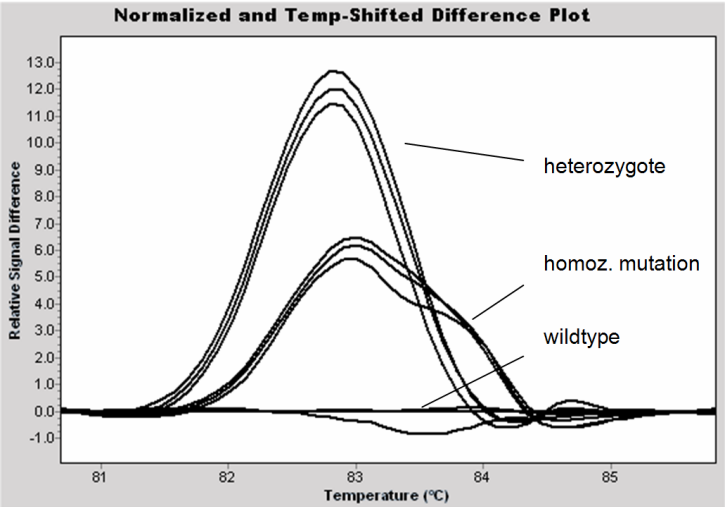

Fig. 2: Deduced Difference Plot from CyP2C2 amplicons carrying a sequence variation, evaluated using LightCycler® 480 Gene Scanning Software. Samples are now grouped in dependence of curve shape relating to their sequence.

4. Troubleshooting

	Possible Cause	Recommendation
Curve shapes in the Difference Plot are variable without recognizable pattern, no reasonable grouping.	Concentration or quality of DNA samples is not consistent	• The lowest signal increase during amplification must not be less than 60% of the highest. The signal levels at the beginning of the melting curve should be as similar as possible. • Assure that amplification curves show CP values < 30 cycles and are in a range of less than 3 cycles for all samples.
	Default setting of sliders in the Normalization screen of the Gene Scanning Software is not suitable.	Set sliders directly before and after the relevant melting domain. Exclude additional melting events of unspecific byproducts.
	Amplicon length is too high.	Best differentiation of sequence variations is expected with amplicons up to 300 bp. For amplicons of more than 600 bp, it may be difficult to reliably detect sequence variations due to the limited resolution.
	All amplicons in the experiment are of identical sequence.	No failure.
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 melting curve program will continue.
	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 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 (*i.e.,* SYBR Green I) 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 initial pre-incubation step (95°C for 10 min). • Make sure denaturation time during amplification is at least 10 s.
	Pipetting errors or omitted reagents.	Check for missing or impaired reagents.

	Possible Cause	Recommendation
	Difficult template, *e.g.,* unusual GC-rich sequence.	• Optimize primer sequences. • Optimize temperatures and times used for the amplification cycles. • Repeat PCR but add increasing amounts of DMSO. (Use up to 10% DMSO in the reaction.)
	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 1500 × *g*) for all reagents to reach the bottom of the microwell and/or to expel air bubbles.
Fluorescence intensity variable or too low.	Low concentration or deterioration of dye in the reaction mixtures because Master Mix was not stored properly.	• Keep Master Mix and complete PCR mix 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 primers and $MgCl_2$. • Optimize protocol.
Negative control sample gives an increasing signal during PCR.	Reagents are contaminated.	• Use contamination-free components. • Perform prevention of carry-over contamination using UNG.

5. Additional Information on this Product

How this Product Works

LightCycler® 480 High Resolution Melting Master is a ready-to-use reaction mix developed for the detection of DNA samples that differ in sequence from others. 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 productsf (1, 2, 3, 4), and LightCycler® 480 High Resolution Melting Dye for monitoring of formation and denaturation of double-stranded DNA.

FastStart Taq DNA Polymerase is a chemically modified form of thermostable recombinant Taq DNA polymerase that shows no activity at temperatures up to 75°C. The enzyme is active only at high temperatures, where primers do not bind nonspecifically. The enzyme is activated (by removal of blocking groups) during a single pre-incubation step (95°C, 5 – 10 minutes) before cycling begins.

LightCycler® 480 High Resolution Melting Dye is a new fluorescent dye that enables detection of sequence variations by different melting curve shape, especially if a sample is heterozygous for a particular mutation. This feature is not shared with other dyes traditionally used in real-time PCR (*e.g.,* SYBR Green I or ethidium bromide). LightCycler® 480 High Resolution Melting Dye is not inhibitoric to amplification enzymes. Thus, high concentrations of the dye do not affect the PCR. These high concentrations completely saturate the dsDNA in the sample. dsDNA remains dye-saturated during the subsequent melting experiment. Under these conditions, even single nucleotide exchanges result in subtle, but reproducibly detectable changes in melting curve shape. (5, 6).

Test Principle

"Gene scanning" or "mutation scanning" techniques detect the presence of sequence variations in target-gene derived PCR amplicons. The method is based on high resolution melting, a novel, closed-tube post-PCR method enabling genomic researchers to analyze genetic variations in PCR amplicons prior to or as alternative to sequencing. High resolution melting provides high specificity, sensitivity and convenience at significantly higher speed and much lower cost than other established (*e.g.,* gel-based) methods.

In a LightCycler® 480 System gene scanning experiment, sample DNA is first amplified via real-time PCR in the presence of LightCycler® 480 High Resolution Melting Dye. After PCR, the successive melting experiment can be performed on the same LightCycler® 480 Instrument, and analyzed with the LightCycler® 480 Gene Scanning Software to identify sequence variants. Thus, the entire experiment can be done on the LightCycler® 480 Instrument without opening the reaction vessels and without additional handling steps after the PCR setup.

References

1 Chou, Q et al. (1992). Prevention of pre-PCR mis-priming and primer dimerization improves low-copy-number amplifications. *Nuc. Acids Res.* **20**, 1717-1723.

2 Kellogg, DE et al. (1994). TaqStart Antibody: "hot start" PCR facilitated by a neutralizing monoclonal antibody directed against Taq DNA polymerase. *Biotechniques* **16**, 1134-1137.

3 Birch, DE (1996). Simplified hot start PCR. *Nature* **381**, 445-446.

4 PCR Applications Manual, Roche Diagnostics (2006). 3rd edition, 66-73.

5 Wittwer, CT et al. (2003). High-Resolution Genotyping by Amplicon Melting Analysis Using LCGreen. *Clini. Chem.* **49**, 853–860

6 Herrmann, MG et al (2006). Amplicon DNA Melting Analysis for Mutation Scanning and Genotyping: Cross-Platform Comparison of Instruments and Dyes. *Clin. Chem.* **53**, 494-503.

Quality Control

The LightCycler® 480 High Resolution Melting Master 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 changes

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 90 8001
	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	05 015 219 001
	LightCycler® 480 Thermal Block Cycler Unit (384-well) Silver	384-well thermal block cycler unit, including block cycler cover, storage box and loading device	05 015 197 001
	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 µl (10 × 500 reactions, 20 µl each)	04 707 516 001 04 887 352 001
	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 µl 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
	LightCycler® RNA Master Hydrolysis Probe	1 kit (5 × 100 reactions)	04 991 885 001
Universal Probe-Library	Universal ProbeLibrary Set, Human	Library of 90 pre-validated detection probe	04 683 633 001
	Universal ProbeLibrary Set, Mouse	Library of 90 pre-validated detection probe	04 683 641 001
	Universal ProbeLibrary Set, Rat	Library of 90 pre-validated detection probe	04 683 641 001
	Universal ProbeLibrary Set 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

The purchase price of this product includes a limited, non-transferable license under U.S. Patent Nos. 5,994,056 and 6,171,785 and corresponding patent claims outside the United States, owned by Roche Molecular Systems, Inc. and F. Hoffmann-La Roche Ltd ("Roche"), and under U.S. Patent No. 6,569,627 and corresponding patent claims outside the United States, licensed from Idaho Technology Inc., for using only this amount of the product for dsDNA-binding dye processes covered by said patent solely for the purchaser's own internal research and development activities. This product is also a Licensed Dye Binding Kit for use with service sublicenses available from Applied Biosystems.

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

Parts of the Software used for the LightCycler® 480 System are licensed from Idaho Technology Inc., Salt Lake City, UT, USA.

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(i) commercially make, have made or sell reagents and/or kits, or

(ii) buy or use reagents and/or kits provided by a third party

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