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Roche Applied Science

LightCycler® 480 Genotyping Master

Version October 2005

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

Cat. No. 04 707 524 001

Kit for 384 reactions
(20 µl each)

Store the kit at −15 to −25°C

Table of Contents

PROTOCOL

1. What this Product Does

Number of Tests

The kit is designed for 384 reactions with a final reaction volume of 20 µl each.

Kit Contents

Vial/Cap	Label	Contents/Function
1 yellow cap	LightCycler® 480 Genotyping Master; 5× conc.	• 4 × 384 µl LightCycler® 480 Genotyping Master ready-to-use hot-start PCR reaction mix. • 5× conc. Master • Contains a modified Taq DNA Polymerase, reaction buffer, dNTP mix (with dUTP instead of dTTP), and 15 mM $MgCl_2$
2 colorless cap	LightCycler® 480 Genotyping Master; H_2O, PCR-grade	• 4 vials H_2O, PCR-grade, 1 ml each • to adjust the final reaction volume
3 blue cap	LightCycler® 480 Genotyping Master; $MgCl_2$ solution	• 1 vial, 1 ml • Contains a 25 mM $MgCl_2$ solution to adjust $MgCl_2$ conc., if necessary

Storage and Stability

Store the kit at −15 to −25°C through the expiration date printed on the label.

- The kit is shipped on dry ice.
- Once the kit is opened, store the kit components as described in the following table:

Vial	Label	Storage
1	LightCycler® 480 Genotyping Master; 5× conc.	• Store at −15 to −25°C. • **Avoid repeated freezing and thawing!**
2	LightCycler® 480 Genotyping Master; Water, PCR-grade	• Store at −15 to −25°C
3	LightCycler® 480 Genotyping Master; $MgCl_2$ solution	• Store at −15 to −25°C

Additional Equipment and Reagents Required

Additional reagents and equipment required to perform PCR with the LightCycler® 480 Genotyping Master using the LightCycler® 480 System include:

- LightCycler® 480 Instrument, 384-well*
- LightCycler® 480 Multiwell Plate 384* and LightCycler® 480 Sealing Foil*
- Standard swing-bucket centrifuge containing a rotor for multiwell plates with suitable adaptors.
- LightCycler® Uracil-DNA Glycosylase* (optional ‡)
- Nuclease-free, aerosol-resistant pipette tips
- Pipettes with disposable, positive-displacement tips
- Sterile 1.5 ml reaction tubes for preparing master mixes and dilutions

‡ For prevention of carry-over contamination; see Related Procedures section for details.

** available from Roche Applied Science*

Application

LightCycler® 480 Genotyping Master is designed for research studies on the LightCycler® 480 System. The LightCycler® 480 Genotyping Master is a ready-to-use hot-start reaction mix designed specifically for genotyping (SNP analysis with melting curves). It is optimized for the use with HybProbe probes but can also be used with SimpleProbe probes.

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.

In principle, the LightCycler® 480 Genotyping Master can be used to amplify and detect any DNA 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 size should not exceed 700 bp in length. For optimal results, select a product length of 100 – 500 bp for monoplex and ≤350 bp for multiplex assays.

⚠ The performance of the kit described in this Instruction Manual is warranted only when it is used with the LightCycler® 480 System.

Assay Time

Variable, depending on the number of cycles, annealing time and melting curve program. For example, a LightCycler® 480 PCR run will last about 50 min if the program specifies 10 min pre-incubation, followed by 40 cycles amplification (each with 5 s denaturation, 5 s annealing, and 5 s elongation), and a monocolor melting curve analysis (see Results).

2. How to Use this Product

2.1 Before You Begin

Sample Material

- 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 use either:

- the MagNA Pure LC Instrument* or the MagNA Pure Compact Instrument* and the 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 Applied Science Biochemicals catalog or home page, www.roche-applied-science.com.

- Use up to 50 ng complex genomic DNA or 10^0 – 10^8 copies plasmid DNA.

If you are using a non-purified cDNA sample from reverse transcription, especially if it contains high background concentrations of RNA and oligonucleotides, you can improve your results by using 2 µl (or less) of that sample in the reaction.

Negative Control

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

- replace the template DNA with PCR-grade water (vial 2; this will reveal whether a contamination problem exists)
- (in a 2-step RT-PCR setup) omit addition of reverse transcriptase to the cDNA synthesis reaction (this will indicate whether DNA in RNA samples cause false-positive results)

Detection Formats

You can use this kit both for the HybProbe and SimpleProbe detection format.

Use a 1:5 dilution of the LightCycler® 480 Genotyping Master to prepare the PCR Mix for HybProbe probes. The $MgCl_2$ concentration in this mix gives optimal results with nearly all primer combinations.

Use a 1:10 dilution of the LightCycler® 480 Genotyping Master to prepare the PCR Mix for SimpleProbe probes. For this type of analysis, it might be helpful to add additional $MgCl_2$ (vial 3). Melting curve results will improve if you dilute the mix even further (*e.g.*, use a 1:20 dilution). If you dilute the mix further, you must also add additional $MgCl_2$ to the reaction (The final concentration has to be 3 mM, *i.e.*, add an extra 1.8 µl $MgCl_2$ from vial 3 if you dilute 1:20).

Primers Suitable concentrations of PCR primers range from 0.1 – 5 μM (final concentration in reaction). The recommended starting concentration is 0.5 μM each. For some applications (for example SimpleProbe format or multiplexing) it is advantagous to run asymmetric PCR. Therefore you have to titrate your primer ratio. The recommended starting ratio is 1:5.

Probes
- A suitable concentration for HybProbe probes is 0.2 μM each (final concentration in reaction).
- A suitable concentration for SimpleProbe probes is 0.2 μM (final concentration in reaction).

$MgCl_2$ The reaction mix in this kit already contains an optimal concentration of $MgCl_2$, which works well for HybProbe analysis with nearly all primer combinations.

If you use SimpleProbe probes, we recommend using a 1:10 dilution of the Master and adjusting the $MgCl_2$ accordingly (for example, add additional $MgCl_2$ to the PCR mix to make the final concentration 3 mM, see Protocol).

LightCycler® 480 Instrument Protocol

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

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

A LightCycler® 480 Instrument protocol that uses the LightCycler® 480 Genotyping Master should contain the following programs:

- **Pre-Incubation** for activation of modified Taq DNA Polymerase and denaturation of the DNA
- **Amplification** of the target DNA
- **Melting Curve** for amplicon analysis
- **Cooling** of the thermal block

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

The following table shows the PCR parameters that must be programmed for a LightCycler® 480 PCR Run with the LightCycler® 480 Genotyping Master.

Setup				
Detection Format		**Block Type**		**Reaction Volume**
• SimpleProbe • Mono Color HybProbe • Multi Color HybProbe		384		3 – 20 µl
Programs				
Program Name		**Cycles**		**Analysis Mode**
Pre-Incubation		1		None
Amplification		45 [1]		Quantification
Melting Curve		1		Melting Curves
Cooling		1		None
Temperature Targets				
Target (°C)	**Acquisition Mode**	**Hold (hh:mm:ss)**	**Ramp Rate (°C/s)**	**Acquisitions (per °C)**
Pre-Incubation				
95	None	00:10:00 [3]	4.8	–
Amplification				
95	None	00:00:05 – 00:00:10	4.8	–
primer dependent [2]	Single	00:00:05 – 00:00:20 [4]	2.5 [6]	–
72	None	00:00:05 – 00:00:20 [4] [5]	4.8	–
Melting Curve (see also next page)				
95	None	00:01:00	4.8	–
40	None	00:00:30 – 00:04:00	2.0 [6] [7]	–
80	Continuous	00:00:00	–	1 – 10 [8]
Cooling				
40	None	00:00:30	2.0 [6]	–

[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) For initial experiments, set the target temperature (*i.e.,* the primer annealing temperature) 5°C below the calculated primer T_m. Annealing usually occurs at 60°C.
3) If you expect your PCR product in early cycles you can reduce the pre-incubation time to 5 min.
4) 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.
5) Calculate the hold time for the PCR elongation step by dividing the amplicon length over 25 (*e.g.,* a 500 bp amplicon requires 20 s elongation time).
6) Use a maximum ramp rate of 2.5°C/s for temperatures above 40°C. For 40°C and below use a slower ramp rate of 2.0°C/s.
7) We recommend starting with 1 min (00:01:00)
8) For HybProbe probes, 1 – 5 acquisitions/°C; for SimpleProbe probes 10 acquisitions/°C.

Choosing the right temperature and time for the melting curve analysis

If you expect more than one peak, you can improve melting curve results by making a multistep melting program that includes each of the expected or calculated annealing temperatures (from high to low) as a separate step in the program (*i.e.*, perform step 2 below repeatedly, at different annealing temperatures, each with its own hold time). After all different annealing steps, program one final annealing step (step 3 below) and a reheating step (step 4 below).

❶	Denature your double-stranded PCR product at 95°C.
❷	Set the annealing temperature for the melting curve (*e.g.*, 40°C) to at least 5°C below the annealing temperature chosen for the amplification cycles and program a hold at that temperature. The hold time will depend on the parameter analyzed; typical values range from 15 s to 30 s.
❸	After all annealing steps, include a final annealing step (to 40°C from 30 s to 4 min).
❹	After the final annealing step, raise the temperature to 80°C.

Preparation of the PCR Mix

Thaw the LightCycler® 480 Genotyping Master (vial 1), water (vial 2) and, if necessary $MgCl_2$ (vial 3).
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 and store on ice. Avoid foam formation.

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

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 mentioned below:

Component	Volume HybProbe probes	Volume Simple Probe probes
Water, PCR-grade (vial 2)	9 µl	9.8 µl
Primer probe mix [1], 10× conc.	2 µl	2 µl
LightCycler® 480 Genotyping Master 5× conc. (vial 1)	4 µl	2 µl
$MgCl_2$ solution (vial 3)	-	1.2 µl
Total volume	**15 µl**	**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.

❹
- Mix carefully and avoid foam formation. Do not vortex.
- Pipet 15 µl PCR mix into each LightCycler® 480 Multiwell Plate.
- Add 5 µl of the DNA template
- Seal Multiwell Plate with LightCycler® 480 Sealing Foil.

❺
- Place the Multiwell Plate in a 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 well plates with suitable adaptors.

❻ Load the Multiwell Plate into the LightCycler® 480 Instrument.

❼
- Start the PCR program described above.

⚠ If you use a reaction volume <20 µl, it may be necessary to reoptimize the reaction and cycle conditions.

Color Compensation

For more information on generating and using a color compensation file, see the LightCycler® 480 Operator's Manual or the LightCycler® 480 Online Resource Site: www.roche-applied-science.com/lightcycler480

Prevention of Carry-Over 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 the initial denaturation step; it will not serve as a PCR template.

⚠ Use only LightCycler® Uracil-DNA Glycosylase* in combination with the LightCycler® 480 Genotyping Master.

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

The use of UNG might influence the melting temperature (T_m) in melting curve analysis.

Two-step RT-PCR

LightCycler® 480 Genotyping Master can also be used to perform 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 standard LightCycler® 480 System procedure, using cDNA as starting sample material.

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

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

Synthesis 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.

3. Results

Quantification Analysis

The following amplification and melting curves were obtained by using the LightCycler® 480 Genotyping Master in combination with a set of primers and HybProbe probes, specific for a fragment of 207 bp of the ApoB gene. The fluorescence values versus cycle numbers are displayed.

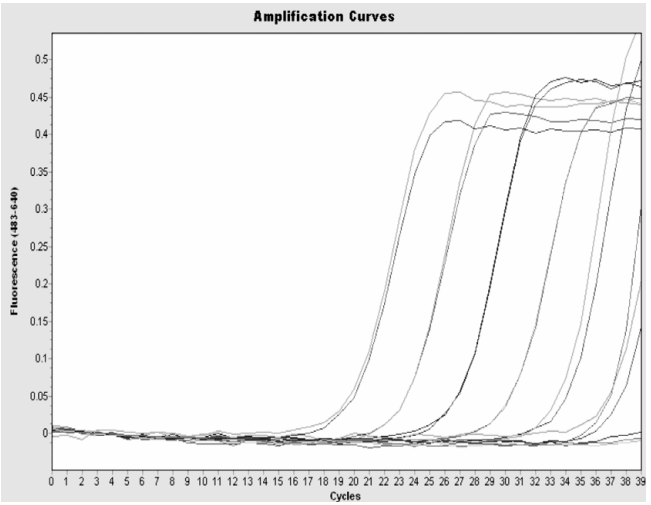

Fig 1: Serial tenfold dilutions of wild type and mutant plasmid DNA (10^6 – 1 copies and negative control) were amplified using the LightCycler® 480 Genotyping Master. As a negative control, template DNA was replaced by PCR-grade water.

Melting Curve Analysis

Specificity of the amplified PCR product was assessed by performing a melting curve analysis. The resulting melting curves allow discrimination between wild type, mutant and heterocygote products. The specific ApoB wild type product melts at a higher temperature than the mutant product. The melting curves display the specific amplification of the ApoB sequence, when starting from 10^6, 10^5, 10^4, 10^3, 10^2, 10^1, 10^0 copies of DNA.

Smaller reaction volumes may result in melting temperature variations.

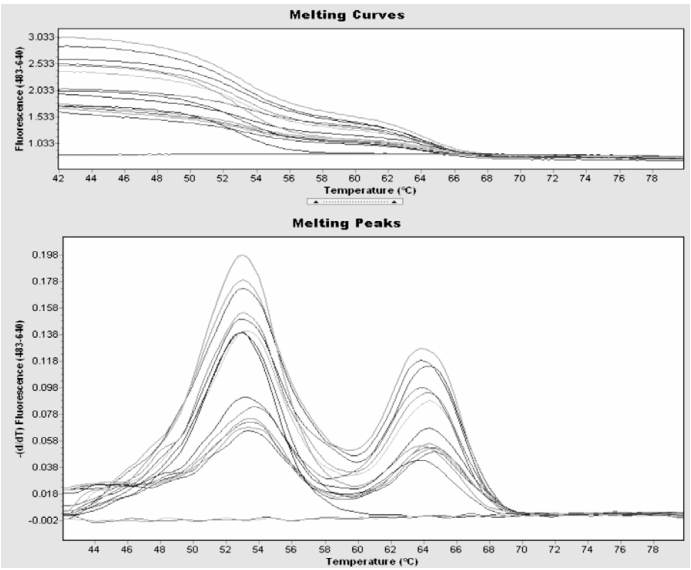

Fig. 2: Melting curve analysis of amplified samples containing 10^6, 10^5, 10^4, 10^3, 10^2, 10^1, 10^0 copies of DNA as starting template. As a negative control, template DNA was replaced by PCR-grade water.

	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 start automatically.
	The number of cycles is too high.	Reduce the number of cycles in the program *Amplification*.
Log-linear phase of amplification just starts as the amplification program finishes.	Starting amount of nucleic acid is very low.	• Improve PCR conditions (*e.g.,* primer and probe design). • Use more starting DNA template. • Repeat the run.
	The number of cycles is too low.	Increase the number of cycles in the cycle program.
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. • Do not use more than 5 µl of DNA per 20 µl PCR reaction mixture.
	Modified Taq DNA polymerase is not sufficiently activated.	• Make sure PCR includes an initial 5 – 10 min pre-incubation at 95°C. • Make sure denaturation time during cycles is at least 10 s.
	Pipetting errors or omitted reagents.	Check for missing or defective reagents.
	Scale of axes on graph are unsuitable for analysis.	Change the values for the x- and y-axis: right click on the chart and select Chart Preferences from the context menu. In the Chart Preference window, reset the maximum and/or minimum axis values.
	Measurements do not occur.	Check the temperatures in the experimental protocol. For probe detection formats, choose "Single" as the acquisition mode at the end of the annealing phase.
	Amplicon is too long.	Do not design primers that produce amplicons $>$400 bp in multiplex and $>$700 bp in monoplex assays. Amplification efficiency is reduced with amplicons of this size. Optimal results are obtained for amplicons $\leq$350 bp.

	Cause	Recommendation
No amplification detectable.	Difficult template, *e.g.*, GC-rich sequence.	• Optimize temperatures and times in the amplification cycles. • Optimize primer/probe sequences. • Repeat PCR under same conditions and add increasing amounts of DMSO, up to 10% final concentration. ⚠ Melting peaks will be lower with increasing DMSO concentrations.
	Unsuitable HybProbe probes	Check sequence and location of the HybProbe probes.
Fluorescence intensity is too low.	Low concentration or deterioration of dyes in the reaction mixtures due to unsuitable storage conditions.	• Store the dye containing reagents at −15 to −25°C, protected from light. • Avoid repeated freezing and thawing. • After thawing, store the Genotyping Master at +2 to +8°C for a maximum of 1 week. • Low Hybridization Probe signals can be improved by using a two times higher concentration of the LC Red labeled probe than of the fluorescein-labeled probe.
	Reaction conditions are not optimized, leading to poor PCR efficiency.	• Primer concentration should be between 0.2 and 1.0 µM. HybProbe concentration should be between 0.2 and 0.4 µM. • Check annealing temperature of primers and probes. • Check experimental protocol. • Always run a positive control along with your samples. • Titrate $MgCl_2$ concentration.
	Mutation analysis using HybProbe probes: The melting temperature of the mismatch strand-Hybridization probe hybrid is lower than the annealing temperature. Thus, the HybProbe probes cannot bind and create no signal.	• This will not affect amplification efficiency. • Ensure that the melting curve will start at a temperature below the annealing temperature used for PCR. Then you will get a clear signal after melting-curve analysis and will be able to interpret the data.
Fluorescence intensity varies.	Skin oils on the surface of the sealing foil.	Always wear gloves when handling the Multiwell Plate and the sealing foil.
	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.

	Cause	Recommendation
Negative control samples give a positive signal.	Contamination	• Exchange of all critical solutions. • Pipet on a clean bench. • Use heat-labile UNG to eliminate carry-over contamination.
No precise melting peak can be identified.	• HybProbe probes are inhomogeneous, and/or contain secondary structures. • Pseudogenes lead to multiple PCR products.	• Redesign HybProbe probes. • Check PCR products on an agarose gel. • Melting Peaks can be optimized by performing an asymmetric PCR by favoring the amplification of the DNA strand that the HybProbe probes bind to.
	Primer-dimers have out competed specific PCR product for available primers.	• Keep all samples at +2 to +8°C until the run is started. • Keep the time between preparing the reaction mixture and starting the run as short as possible. • Increase starting amount of DNA template. • Increase annealing temperature in order to enhance stringency.
	Annealing temperature not adapted	• Lower the annealing temperature used for melting curve analysis by an additional 3°C and/or increase the hold time (up to 4 minutes). • If you expected more than one melting peak, include each of the different annealing temperatures (from high to low) as a separate step in the program, each with its own hold time (from 30 s to 4 min). After all steps, program a "cool down" step (to 40°C) and another hold step (for 2 min), followed by a reheating step (to 80°C).
Melting temperature of a product varies from experiment to experiment.	Variations in reaction mixture (*e.g.,* salt concentration).	• Check purity of template solution. • Reduce variations in parameters such as heat-labile UNG, primer preparation, program settings and $MgCl_2$.
One peak of the same height occurs in all samples.	Contamination in all samples.	Use fresh solutions.
High Background	Very low fluorescence signals, therefore the background seems relatively high.	Follow general optimization strategies for the LightCycler® PCR.
	Hybridization Probe concentration is too high.	Hybridization Probe concentration should be in range of 0.2-0.4 μM.
	Insufficient quality of HybProbe probes.	Prepare a new pair of HybProbe probes.

	Cause	Recommendation
Amplification curve decreases after reaching the plateau in late cycles.	"Hook effect": competition between binding of the HybProbe probes pair and re-annealing of the PCR product.	This does not affect interpretation of the results. It can be avoided by performing an asymmetric PCR by favoring the amplification of the DNA strand that the HybProbe probes bind to.
Fluorescence values in real-time quantification drop below initial background fluorescence in late cycles. This might cause wrong CP-calls in the range of decreasing fluorescence.	High DNA /amplicon concentrations influence buffer conditions, reflected by a decrease of the fluorescence in the Fluos channel 530.	• This does not affect interpretation of general results. • Dilution of your template decreases the effect. • Repeat the assay while using Pyrophosphatase*. Add 0.05 units/µl of Pyrophosphatase (final concentration) to each master mix before starting the PCR.

5. Additional Information on this Product

How this Product Works

The LightCycler® 480 Genotyping Master is a ready-to-use reaction mix designed for the HybProbe detection format and SimpleProbe detection format using the LightCycler® 480 System. It is used to perform hot-start PCR in LightCycler® 480 Multiwell Plates.

Hot-start PCR has been shown to significantly improve the specificity and sensitivity of PCR (1, 2, 3, 4) by minimizing the formation of nonspecific amplification products at the beginning of the reaction.

The polymerase in this master is a 5´-3´-exo-minus, N-terminal deletion of thermostable recombinant Taq DNA polymerase and furthermore is chemically modified. It shows no activity up to 75°C because of the heat-labile blocking groups on some of the amino acid residues of the enzyme. Therefore, there is no elongation during non-specific primer binding. The modified enzyme is "activated" by removing the blocking groups at a high temperature (*i.e.*, preincubation at 95°C for a maximum of 10 minutes).

The LightCycler® 480 Genotyping Master provides convenience, excellent performance, reproducibility and minimal contamination risk. All you have to supply is template DNA, PCR primers, HybProbe or SimpleProbe probes, and additional $MgCl_2$ (if necessary).

Test Principle

HybProbe probes consist of two different oligonucleotides that hybridize to an internal sequence of the amplified fragment during the annealing phase of PCR cycles. One probe is labeled at the 5´-end with a suitable acceptor fluorophore (LightCycler® Red 610, LightCycler® Red 640, or Cy5), and to avoid extension, modified at the 3´-end by phosphorylation. The second probe is labeled at the 3´-end with the donor dye fluorescein (Fluos). Only after hybridization, the two probes are in close proximity, resulting in fluorescence resonance energy transfer (FRET) between the two fluorophores. During FRET, fluorescein, the donor fluorophore, is excited by the light source of the LightCycler® 480 Instrument, and part of the excitation energy is transferred to LightCycler® Red, the acceptor fluorophore. The emitted fluorescence of the acceptor fluorophore is measured. LightCycler® Red 610, LightCycler® Red 640, or Cy5-labeled HybProbe probes can be used separately or in combination, therefore allowing single- or multiple-color detection. Refer to the LightCycler® 480 Operator`s Manual for further information.

The SimpleProbe format uses only one oligonucleotide probe. This single probe is designed to specifically hybridize to a target sequence that contains the SNP of interest. Once hybridized, the SimpleProbe probe emits much more fluorescence than it does when it is not hybridized. As a result, fluorescent signal changes are based solely on the hybridization status of the probe. SimpleProbe assays can distinguish between wild type, mutant and heterozygous samples.

References

1 Chou, Q. et al. (1992). Prevention of pre-PCR mis-priming and primer dimerization improves low-copy-number amplifications. *Nucleic Acids Research* **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, D.E. (1996). Simplified hot start PCR. *Nature* **381**, 445-446.
4 PCR Manual, Roche Diagnostics (1999) 2nd edition (1999) 2, 52-58.

Quality Control

The LightCycler® 480 Genotyping Master is function tested using the LightCycler® 480 System.

6. Supplementary Information

6.1 Conventions

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.

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 for:

- The LightCycler® 480 System: www.roche-applied-science.com/lightcycler480
- The MagNA Pure System family for automated nucleic acid isolation: http://www.magnapure.com
- DNA & RNA preparation - Versatile Tools for Nucleic Acid Purification: http://www.roche-applied-science.com/napure

	Product	Pack Size	Cat. No.
Instrument and Accessories	LightCycler® 480 Instrument, 384-well	1 instrument with data workstation and accessories	04 545 885 001
	LightCycler® 480 Multiwell Plate 384	50 plates and sealing foils	04 729 749 001
	LightCycler® 480 Sealing Foil	50 foils	04 729 757 001
LightCycler® 480 Kits for PCR	LightCycler® 480 Probes Master	1 kit (5 × 100 reactions of 20 µl each)	04 707 494 001
	LightCycler® 480 DNA SYBR Green I Master	1 kit (5 × 100 reactions of 20 µl each)	04 707 516 001
	LightCycler® 480 Control Kit	3 runs	04 710 924 001
Associated Kits and Reagents	LightCycler® Uracil-DNA Glycosylase	100 U (50 µl)	03 539 806 001
	Transcriptor Reverse Transcriptase	250 U 500 U 2000 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
Labeling Reagents	SimpleProbe 519 Labeling Reagent	100 µmol	04 687 132 001
	LightCycler® Fluorescein CPG	1 g 5 columns	03 138 178 001 03 113 906 001
	LightCycler® Red 610-N-hydroxy-succinimide ester	1 vial	03 561 488 001
	LightCycler® Red 640-N-hydroxy-succinimide ester	1 vial	12 015 161 001

6.4 Disclaimer of License

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