

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



LightCycler® 480 High Resolution Melting Master

 **Version: 07**

Content version: September 2016

Easy-to-use Reaction Mix (2x conc.) for PCR and high resolution melting using the LightCycler® 480, LightCycler® 96 or LightCycler® Nano Real-Time PCR Systems.

Cat. No. 04 909 631 001 5 x 1 ml
5 x 100 reactions of 20 µl final volume each

Store the kit at –15 to –25°C.

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1. General Information

1.1. Contents

Vial / Bottle	Cap	Label	Function/Description	Content
1	green	LightCycler® 480 High Resolution Melting Master Mix, 2x conc.	Contains FastStart Taq DNA Polymerase, reaction buffer, dNTP mix (with dUTP instead of dTTP), and High Resolution Melting Dye.	5 vials, 1 ml each
2	blue	LightCycler® 480 High Resolution Melting Master, MgCl ₂ , 25 mM	To adjust MgCl ₂ concentration.	2 vials, 1 ml each
3	colorless	LightCycler® 480 High Resolution Melting Master, Water, PCR Grade	To adjust the final reaction volume.	5 vials, 1 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	green	Master Mix, 2x conc.	Store at –15 to –25°C. ⚠ Avoid repeated freezing and thawing! After first thawing, the master can be stored for up to 4 weeks at +2 to +8°C. ⚠ Keep away from light!
2	blue	MgCl ₂ 25 mM	
3	colorless	Water, PCR Grade	Store at –15 to –25°C.

1.3. Additional Equipment and Reagents Required

Refer to the list below for additional reagents and equipment required to perform PCR reactions with the LightCycler® 480 High Resolution Melting Master:

- LightCycler® 480 Instrument II*, LightCycler® 96 Instrument* or LightCycler® Nano Instrument
- LightCycler® 480 Multiwell Plate 384* or LightCycler® 480 Multiwell Plate 96*
- LightCycler® 8-Tube Strips*
- ⓘ *Only LightCycler® 8-Tube Strips (clear) can be used on the LightCycler® Nano Instrument.*
- ⓘ *LightCycler® 8-Tube Strip Adapter Plate* for LightCycler® 480 Instrument only*
- LightCycler® Uracil-DNA Glycosylase* (optional)
- ⓘ *For details about prevention of carryover contamination, see the “Prevention of Carryover Contamination” section.*
- Standard swinging-bucket centrifuge containing a rotor for multiwell plates with suitable adaptors
- Nuclease free, aerosol-resistant pipette tips
- Pipettes with disposable, positive-displacement tips
- Sterile reaction tubes for preparing master mixes and dilutions

1.4. Application

The LightCycler® 480 High Resolution Melting Master is designed for life science research studies in combination with the LightCycler® 480, LightCycler® 96 or LightCycler® Nano Real-Time PCR System. The LightCycler® 480 High Resolution Melting Master is a ready-to-use 2x 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 MgCl₂ stock solution, supplied with the Master, allows you to easily optimize the Mg²⁺ concentration.

1.5. Preparation Time

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 System PCR run for gene scanning will last about 75 minutes including 10 minutes pre-incubation time and 15 minutes high resolution melting. The same run will last about 80 minutes on the LightCycler® 96 System, including 10 minutes high resolution melting.

2. How to Use this Product

2.1. Before you Begin

Sample Materials

- 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 to 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, we recommend:

Either a MagNA Pure System together with a dedicated reagent 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.

⚠ **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 Cq 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).
- Nonspecific amplification artifacts.

Note that compared to other PCR reagents, in some cases Cq values might be a 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.

⚠ **As a precondition for valid HRM results, all Cq values of a given run should be in a range of 3 Cq (e.g., between 25 and 27).**

Control Reactions

Always run a negative control with the samples. To prepare a negative control, replace the template DNA with Water, PCR Grade (Vial 3, colorless cap).

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 to 250 bp).

Use a dedicated software package for designing the primers.

⚠ **Only use primers that have been purified by HPLC.**

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

Mg²⁺ Concentration

Because specific amplification is essential for high resolution melting analysis, determine the optimum concentration of MgCl₂ for each new primer pair. For this, run a positive sample with a dilution series of MgCl₂ (1.0, 1.5, 2.0, 2.5, 3.0, and 3.5 mM final concentration in PCR) and analyze the PCR products by melting curve analysis (T_m Calling), or agarose gel electrophoresis. The lowest MgCl₂ concentration resulting in high yield of target PCR product and no nonspecific byproducts is the optimum for this assay.

2. How to Use this Product






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 nonspecific byproducts, optimize the annealing temperature in the PCR protocol by setting it to lower or higher temperatures, respectively.

General Considerations



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, LightCycler® 96 or LightCycler® Nano 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 the formation of amplicon during PCR cycling, and melting curve analysis utilizing the high resolution data acquisition capabilities of the LightCycler® 480, LightCycler® 96 or LightCycler® Nano Instrument. Samples with variations in DNA sequence are distinguished by discrepancies in melting curve shape. Particularly, heterozygous DNA variants forming mismatched heteroduplexes, 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 (white) 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 for Use is warranted only when it is used with the LightCycler® 480, LightCycler® 96 or LightCycler® Nano 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, see chapter “Additional Information on this Product” or refer to the LightCycler® 480, LightCycler® 96 or LightCycler® Nano Software Manual.*

2.2. Protocols

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 thermal block

-  *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					
Block Type			Reaction Volume [μl] ⁽¹⁾		
96 (384)			10 – 100 (3 – 20)		
Detection Format		Excitation Filter		Emission Filter	
Sybr Green I / HRM Dye		465		510	
Programs					
Program Name		Cycles		Analysis Mode	
Pre-Incubation		1		None	
Amplification		45 ⁽²⁾		Quantification	
Melting Curve		1		Melting Curve	
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	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)	–
Melting Curve	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)	–

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

Temperature Targets							
	Target [°C]	Acquisition Mode	Hold [hh:mm:ss]	Ramp Rate [°C/s]	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)	–	–	–

LightCycler® 96 System Protocol

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

⚠ Program the LightCycler® 96 System before preparing the reaction mixes.

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

The following table shows the recommended PCR parameters for an initial LightCycler® 96 System PCR run to establish a gene scanning assay with the LightCycler® 480 High Resolution Melting Master.

Run Editor				
Detection Format			Reaction Volume (µl) ⁽¹⁾	
ResoLight			10 – 50	
Programs				
	Temp. [°C]	Ramp [°C/s]	Duration [s]	Acquisition Mode
Pre-Incubation	95	4.4	600	None
3-Step Amplification	No. of Cycles: 45 ⁽²⁾			
	95	4.4	10	None
	primer dependent ⁽³⁾	2.2	15	None
	72	4.4	10 – 25 ⁽⁴⁾	Single
High Resolution Melting	95	4.4	60	None
	40 ⁽⁵⁾	2.2	60	None
	65 ⁽⁶⁾	2.2	1	None
	97 ⁽⁶⁾	–	1	Continuous 15 Readings / °C

LightCycler® Nano System Protocol

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

⚠ Program the LightCycler® Nano System before preparing the reaction mixes.

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

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

Run Settings					
Optics Settings			Reaction Volume (μl)		
Intercalating Dye, Normal Quality and Optimize Melt Acquisition			20		
Programs					
	Temp. [°C]	Ramp [°C/s]	Duration [s]	Acquire	Cycles
Pre-Incubation	95	5	600	–	–
3-Step Amplification	95	5	10	No	–
	primer dependent ⁽³⁾	4	25	No	–
	72	5	15 ⁽⁴⁾	Yes	45 ⁽²⁾
Pre-melt Hold	95	5	60	–	–
Pre-melt Hold	40 ⁽⁵⁾	4	60	–	–
High Resolution Melting	65 ⁽⁶⁾	4	1	–	–
	95 ⁽⁶⁾	0.05	1	–	–

- ⁽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). To compensate for the missing overshoot/equilibration times of the LightCycler® Nano Instrument, add 10 s to the calculated 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, for example, 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 LightCycler® 480 Multiwell Plate when handling the plate.

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

-
- 2 Prepare a 20x conc. solution of the PCR primers.
-

- 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:

Component	Volume	Final conc.
Master Mix, 2x conc. (Vial 1, green cap)	10.0 µl	1x conc.
Primer mix, 20x conc. (4 µM)	1.0 µl	0.2 µM (each primer)
MgCl ₂ , 25 mM (Vial 2, blue cap) ⁽¹⁾	X µl	
Water, PCR Grade (Vial 3, colorless cap)	adjust to 15.0 µl	
Total volume	15 µl	

⁽¹⁾ Volume of MgCl₂ stock solution to be added is assay specific. For more details, see “MgCl₂ concentration” section.

i 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.

-
- 4 Mix carefully by pipetting up and down. Do not vortex.
 - Pipette 15 µl RT-PCR mix into each well of the LightCycler® 480 Multiwell Plate/LightCycler® 8-Tube Strips.
 - Add 5 µl of concentration-adjusted DNA template.
 - Seal Multiwell Plate with a 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 minutes at 1500 × g in a standard swinging-bucket centrifuge containing a rotor for multiwell plates with suitable adaptors.
-

2. How to Use this Product

- 6 Load the Multiwell Plate/LightCycler® 8-Tube Strips into the LightCycler® 480, LightCycler® 96 or LightCycler® Nano Instrument.
-

- 7 Start the PCR program.

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

2.3. Other Parameters

Prevention of Carryover Contamination

Uracil-DNA Glycosylase (UNG) is suitable for preventing carryover contamination in PCR. This technique relies on the incorporation of deoxyuridine triphosphate (dUTP) into amplification products, and the pretreatment of all successive PCR mixtures with UNG. If there are such amplicons in the PCR mixture, UNG cleaves the DNA at any site where a deoxyuridylate residue has been incorporated. Subsequently, the resulting abasic sites are hydrolyzed by high temperatures during the pre-incubation 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 carryover contamination using LightCycler® Uracil-DNA Glycosylase:

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

3. Results

The following results were obtained using the LightCycler® 480 System and 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 Instructions for Use 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 wild type DNA standard to enhance differentiation of the different homozygotes (wild type 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 wild type DNA to the samples, the homozygote mutants can be more easily differentiated from wild type and are still clearly different from the heterozygote mutant samples.

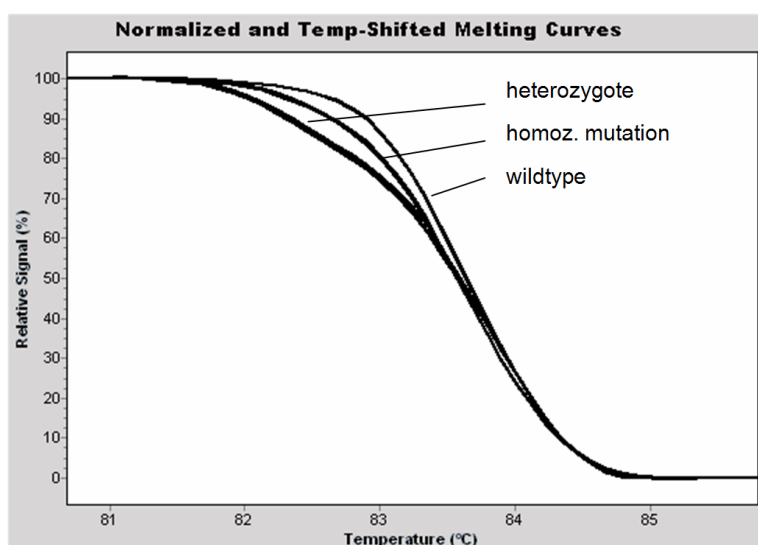


Fig. 1: Normalized, temp-shifted melting curves from CyP2C2 amplicons carrying a sequence variation, evaluated using the LightCycler® 480 Gene Scanning Software. Sequence variants can be distinguished by the different shapes of the melting curves.

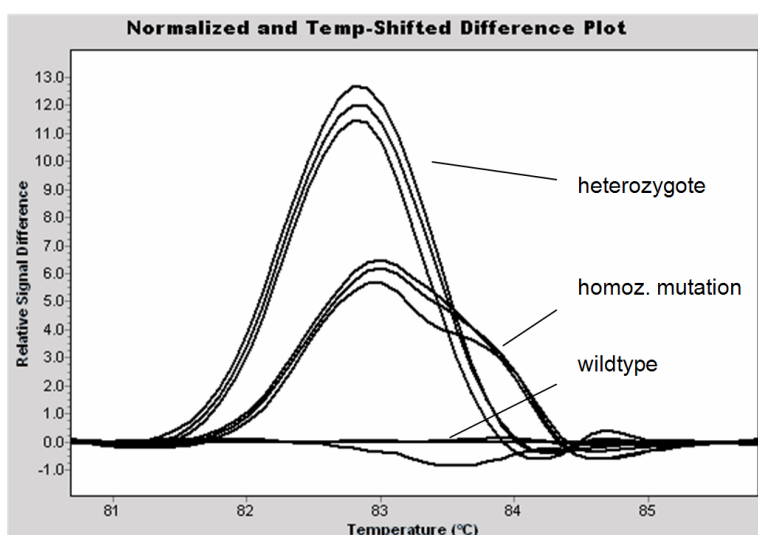


Fig. 2: Deduced Difference Plot from CyP2C2 amplicons carrying a sequence variation, evaluated using the LightCycler® 480 Gene Scanning Software. Samples are now grouped, dependent on the curve shape relating to their sequence.

For example results obtained with the LightCycler® 96 or LightCycler® Nano Systems, please refer to the corresponding User Training Guides.

4. Troubleshooting

Observation	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 Cq 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 nonspecific byproducts.
	Amplicon length is too high.	Best differentiation of sequence variations is expected with amplicons up to 300 bp. For amplicons of more than 500 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 <i>End Program</i> button. The melting curve program will continue (only applies to LightCycler® 480 Instrument).
	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 <i>Add 10 Cycles</i> 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).

Observation	Possible cause	Recommendation
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 (LightCycler® 480 System only).
	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 a pre-incubation step (95°C for 10 minutes).
		Make sure denaturation time during amplification is at least 10 seconds.
	Pipetting errors or omitted reagents.	Check for missing or impaired reagents.
	Difficult template, such as unusual GC-rich sequence.	Optimize primer sequences.
		Optimize temperatures and times used for the amplification cycles.
Fluorescence intensity variable or too low.	Some of the reagent is still in the upper part of the microwell, or an air bubble is trapped in the microwell.	Repeat PCR but add increasing amounts of DMSO. Use up to 10% DMSO in the reaction.
		Repeat centrifugation, but allow sufficient centrifugation time (e.g., 2 minutes at 1500 × g) so that all reagents reach the bottom of the microwell and/or to expel air bubbles.
	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.
Negative control sample gives an increasing signal during PCR.	Poor PCR efficiency (reaction conditions not optimized).	Check concentrations of primers and MgCl ₂ .
		Optimize protocol.
	Reagents are contaminated.	Use contamination-free components.
		Perform prevention of carryover contamination using UNG.

5. Additional Information on this Product

5.1. 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 an 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 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 instrument, and analyzed with the LightCycler® 480, LightCycler® 96 or LightCycler® Nano Software to identify sequence variants. Thus, the entire experiment can be done on the instrument without opening the reaction vessels and without additional handling steps after the PCR setup.

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 products (Chou, Q., et al., 1992, Kellogg, D.E., et al., 1994, Birch, D.E., 1996), 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 inhibitory 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 (Wittwer, C.T., et al., 2003, Herrmann, M.G., et al., 2006).

5.2. References

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5.3. Quality Control

The LightCycler® 480 High Resolution Melting Master is function tested using the Light-Cycler® 480 Real-Time PCR System.

6. Supplementary Information

6.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
 <i>Information Note: Additional information about the current topic or procedure.</i>	
 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.
① ② ③ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

6.2. Changes to previous version

Layout changes.
Editorial changes.

6.3. Ordering Information

Roche offers a large selection of reagents and systems for life science research. For a complete overview of related products and manuals, please visit and bookmark our homepage lifescience.roche.com.

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 Gene Scanning Software	1 software package	05 103 908 001
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
LightCycler® 96 Instrument	1 instrument	05 815 916 001
Reagents , kits		
LightCycler® Uracil-DNA Glycosylase	50 µl, 100 U, (2 U/µl)	03 539 806 001
LightCycler® 480 Control Kit	1 kit, 3 control reactions	04 710 924 001

6.4. Trademarks

FASTSTART, HIGH PURE, LIGHTCYCLER, MAGNA PURE and RESOLIGHT are trademarks of Roche.

SYBR is a trademark of Thermo Fisher Scientific Inc..

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.

Visit **lifescience.roche.com**, to download or request copies of the following **Materials**:

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- Safety Data Sheets
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