LightMix® in-vitro diagnostics kit

ACE I/D

Cat.-No.: 40-0637-64

Detection of the Insertion / Deletion DNA variation in the *ACE* gene

for use with the

Roche Diagnostics LightCycler® Instruments

SimpleProbe® format

Reagents for 64 reactions

Upon arrival:

Store Premixed PCR reagents and Controls protected from light at room temperature (do not freeze)

Store FastStart DNA Master HybProbe reagents frozen (-20°C) (if included)

CE IVD

Manual Version V131211

Table of Contents

1. Product Information

1.1 Contents: LightMix® Kit ACE I/D

Lyophilized premixed PCR reagents

⚠ **Store at room temperature in the dark**

	Cap color	Label	Description content	Reaction / Tube status	Total
1 x	Red	PSR	Parameter Specific Reagents (PSR) containing premixed and lyophilized primers and probes for 64 reactions. <0,01pg unlabeled oligonucleotides (ACE I/D primers); <0,01pg SimpleProbe 519 labeled ACE INS / DEL probe	64 reactions lyophilized	64 rxs

Standards (Control DNA)

⚠ **Store at room temperature**

	Cap color	Label	Description content	Reaction Tube status	Total
1 x	Yellow	HT	Positive Control INS / DEL Heterozygous ACE <0,01pg plasmid target (synthetic) [about 10E4 genome equivalents]	40 reactions / lyophilized	40 rxs
1 x	Yellow	DEL	Genotyping Standard Deletion <0,01pg plasmid target (synthetic) [about 10E4 genome equivalents]	40 reactions / lyophilized	40 rxs
1 x	Yellow	INS	Genotyping Standard Insertion <0,01pg plasmid target (synthetic) [about 10E4 genome equivalents]	40 reactions / lyophilized	40 rxs

Polymerase Mix: LightCycler® FastStart DNA Master HybProbe

⚠ **Store at -20℃ upon arrival**

FastStart DNA Master HybProbe is included in kits supplied directly by TIB MOLBIOL for customers in Central Europe only(1).
The FastStart DNA Master HybProbe is not included in ACE kits supplied through Roche Diagnostics or its local distributor.

	Cap color	Label	Description content	Reaction Tube storage	Total
1 x	Red	1a	LightCycler® FastStart Enzyme	64 reactions / frozen	64 rxs
1 x	White	1b	LightCycler® FastStart Reaction Mix HybProbe	64 reactions / frozen	64 rxs
1 x	Color-less	Water	H_2O PCR grade	frozen	
1 x	Blue	$MgCl_2$	$MgCl_2$, 25 mM	frozen	

1) FastStart enzyme is shipped by TIB MOLBIOL at ambient temperature.

1.2 Intended Use

This kit allows the detection of the Angiotensin I converting enzyme (ACE) insertion / deletion polymorphism (ACE I/D) in genomic human DNA obtained from a nucleic acid extract made from peripheral blood.

The deletion allele (D) is associated with a number of disease risks, in particular myocardial infarction (see 3.1 Medical Background).

This product is intended to help the clinician to analyze the genetic background for patients with an increased ACE serum level which do not respond to ACE inhibitors, patients who had a myocardial infarct, individuals with a left ventricular hypertrophy, individuals with the Angiotensin Receptor II A166C mutation.

The kit is not intended to be the only basis for therapy decision. The patient´s mutation status should be considered alongside other disease factors.

Note: The performance of the assay can be guaranteed only when used with LightCycler® Instruments (see 1.3.2 for details).

1.3 Specifications

The *LightMix® Kit ACE I/D* is an *in-vitro* diagnostic test and allows the detection of the ACE I/D polymorphism as demonstrated with reference samples.

1.3.1 Clinical Samples

The test requires 2 µl of purified genomic DNA in aqueous solution extracted from clinical specimen, containing from 5 to 100 ng/µl of DNA (10 ng – 200 ng total amount), as determined by UV spectrophotometry (1 OD = 50 µg DNA/ml).

1.3.2 Instruments, Software and Productivity

One kit contains reagents for 64 reactions performed in a 10 µl volume.
Each run requires including one standard and one negative control.
The table below summarizes some features of the kit :

LightCycler® Instrument	Software Version (or higher)	Run Time (approx.)	Max Samples per run (2)	Maximum Productivity of the kit (3)	Minimum Productivity of the kit (4)
LC 1.2	4.10 (1)	60 min	30 + 2 ctrl.	58	20
LC 1.5	4.10 (1)	60 min	30 + 2 ctrl.	58	20
LC 2.0	4.05	60 min	30 + 2 ctrl.	58	20
LC480 (96 wells)	1.5	100 min	94 + 2 ctrl.	60	20
LC480 (384 wells)	1.5	100 min	382(5) + 2 ctrl.	60	20
z480 (open channel)	1.5	100 min	94 + 2 ctrl.	60	20
LC96	1.6 (6)	100 min	94 + 2 ctrl.	60	20
Nano	1.0 (6)	60 min	30 + 2 ctrl.	60	21

1 Running the test with the LightCycler® 1.2 or 1.5 Instruments with the software version 3.5 yields comparable results. Instruction for programming, data analysis and interpretation of results are not described in this manual. **Upgrade to version 4.10 or higher when possible**. LightCycler® software 3.5.3 does not contain the automatic genotyping module; equivalent results can be obtained by trained personnel which must analyze each sample manually.
2 Each run must include one heterozygous control and one No-Target Control (NTC) for a total of 2 control reactions.
3 The first run of the kit requires to include 4 controls to teach the genotyping module (not LC Nano, and LC96). The maximum number of samples that can be processed is reduced accordingly. Depending on local regulations, all 4 genotyping controls may have to be included in each run, reducing the total number of patient's samples that can be analyzed.
4 Calculated considering one single clinical sample analyzed in each run.
5 It requires using more than one kit.
6 Nano LightCycler® software 1.0 and LC96 software 1.6 do not contain the automatic genotyping module, therefore it is not necessary to add two Genotyping Standards; equivalent results can be obtained by trained personnel which must analyze each sample manually.

1.4 Storage and Stability

Note the **different storage conditions** for reagents and polymerase mix !

Storage Conditions

Reagents and Controls:
Store the lyophilized reagents (PSR and Standards) protected from light and at room temperature (18℃ / 25℃).
Do not freeze these dried reagents. Expiration date is printed on the kit label.

Polymerase mix :
Store the LightCycler® FastStart DNA Master HybProbe at -15℃ to -25℃.
See expiration date on the polymerase tube label.

Shipping:
Products are shipped at ambient temperature. Transport stability of reagents and enzyme components have been tested under shipping conditions.

2. Additional Devices and Reagents

2.1 Required

LightCycler® 2.0 Instrument	**Roche Diagnostics**
LightCycler® 2.0 Instrument	Cat.-No. 12 011 468 001
LightCycler® Software Version 4.05 or	Discontinued
LightCycler® Software Version 4.10 or higher	Cat.-No. 04 779 584 001
LightCycler® Capillaries (20 µl)	Cat.-No. 11 909 339 001
Or	
LightCycler® 480 Instruments	**Roche Diagnostics**
LightCycler® 480 Instrument (model I)	Discontinued
LightCycler® 480 II Instrument	Cat.-No. 05 015 278 001
Cobas z 480 Analyzer	Cat.-No. 05 200 881 001
LightCycler® Software Version 1.5 or higher	Cat.-No. 04 994 884 001
LightCycler® 480 Multiwell Plate 96 white or	Cat.-No. 04 729 692 001
LightCycler® 480 Multiwell Plate 384 white	Cat.-No. 04 729 749 001
Or	
LightCycler® 96 Instrument	**Roche Diagnostics**
LightCycler® 96 Instrument	05 815 916 001
LightCycler® Software Version 1.0 or higher	Included with Instrument
LightCycler® 480 Multiwell Plate 96 white	Cat.-No. 04 729 692 001
LightCycler® 8 tube strips (white)	Cat.-No. 06 612 601 001
Or	
LightCycler® Nano Instrument	**Roche Diagnostics**
LightCycler® Nano Instrument	Cat.-No. 06 407 773 001
LightCycler® Software Version 1.0 or higher	Included with Instrument
LightCycler® Nano tubes	Cat.-No. 06 327 672 001
Or	
LightCycler® 1.x Instruments	**Roche Diagnostics**
LightCycler® 1.2 and 1.5 Instruments	Discontinued
LightCycler® Software Version 4.10	Cat.-No. 04 779 584 001
LightCycler® Capillaries (20 µl)	Cat.-No. 11 909 339 001

2.2 Optional

Instruments:	**Roche Diagnostics**
LC Carousel Centrifuge 2.0 (230 Volt)	Cat.-No. 03 709 582 001
Capping Tool	Cat.-No. 03 357 317 001

2.3 Sample Preparation

Manual Sample Preparation:	**Roche Diagnostics**
High Pure PCR Template Preparation Kit	Cat.-No. 11 796 828 001
Nuclease-free PCR grade water	any supplier
Ethanol p.a.	any supplier
Isopropanol p.a.	any supplier
Automatic Sample Preparation:	**Roche Diagnostics**
MagNA Pure Instrument	Discontinued
MagNA Pure LC DNA Isolation Kit I	Cat.-No. 03 003 990 001
MagNA Pure 2.0 Instrument	Cat.-No. 05 197 686 001
MagNA Pure LC DNA Isolation Kit I	Cat.-No. 03 003 990 001
MagNA Pure Compact Instrument	Cat.-No. 03 731 146 001
MagNA Pure Compact Nucleic Acid Isolation Kit I	Cat.-No. 03 730 964 001
MagNA Pure 96 Instrument	Cat.-No. 05 195 322 001
MagNA Pure 96 DNA and Viral NA Small Volume Kit	Cat.-No. 05 467 497 001

3. Background Information

3.1 Medical Background

The dipeptidyl carboxypeptidase Angiotensin I converting enzyme (EC 3.4.15.1) (ACE) converts angiotensin I into angiotensin II, which is a strong vasopressor. ACE can also inactivate bradykinin, a potent vasodilator. The enzyme plays a key role in the renin-angiotensin system for blood pressure regulation.

ACE is a key drug target for treatment of high blood pressure. ACE inhibitors lead to to systematic dilation of the arteries and veins and a decrease in arterial blood pressure and diminishes angiotensin II-mediated aldosterone secretion from the adrenal cortex, leading to a decrease in water and sodium reabsorption and a reduction in extracellular volume

The gene is located on chromosome 17q23 (GeneID: 1636). Rigat et al.[1] described in 1990 an ACE I/D insertion / deletion polymorphism in intron 16, differing in a 287 bp Alu repeat element, accounting for some of the variations in the serum ACE activity. The ACE levels are higher in individuals homozygous for the D allele and lowest for individuals with the homozygous I allele.

Since then, the ACE D allele has been associated with a number of disease risks, including myocardial infarction in low-risk patients [2], left ventricular hypertrophy [3], as well as progressive diabetic nephropathy [4]; for review see Sayed-Tabatabaei et al., 2006 [5]. Other authors have studied the impact on cancer [6] and there are continously new studies on the ACE I/D polymorphism [7].

The literature reports a synergistic effect with the Angiotensin II receptor 1 (AT2R1) A1166C mutation.

The frequency of the DD alllele is in the range of 33%, ID heterozygotes about 45%, and the II alllele about 20% in the Caucasian population.

PCR based methods have been published already 1992 by Rigat et al.[8] and FRET based Real-Time-PCR testing has been described already 2001 [9].

3.2 Methodology and Assay Principle

Using PCR methodology, a 480 bp (I) or 193 bp (D) fragment of the ACE gene is amplified with specific primers. The fragment is detected with a mutation-specific detection probe internally labeled with SimpleProbe® 519 reagent.

SimpleProbe® probes are fluorescent only when bound to complement DNA.

The probe binds to a part of the amplified fragment spanning the mutation site. Any mismatch covered by the probe destabilizes the hybrid. During the melting curve analysis the temperature is slowly increased. The probe melts off at a specific melting temperatures causing the fluorescence to decrease.

In this product the probe matches the sequence of the deleted genotype and the presence of the insertion will result in a reduced Tm.

Reading of the genotype results is based on the melting temperatures compared to the supplied standards. If permitted by the instrument software reading of the genotype results can be achieved by the automated genotyping (instrument-dependent: software module 'Melt Curve Genotyping').

Automated genotyping results must be reviewed by eye for deviating curves and intermediate melting point temperatures. In case that automated typing fails to report consistent genotype results, the genotype must be deducted from the melting temperatures following the criteria described in chapter 7.

The supplied control DNA standards allow a comparison with clinical samples.

3.3 Performance Characteristics

Analytical Specificity
The specificity to the target gene and the suitability of the PCR amplification employed in the present test for the detection of the mutation site was demonstrated by direct sequencing of the amplicon spanning variable region.

Analytical Sensitivity
Detection of serial dilutions of several heterozygous human genomic DNAs has revealed that limit of detection for the present kit is 500 copies (3.0 ng).

Diagnostic Specificity and Sensitivity
A total number of 62 different genomic DNA samples from individuals of Caucasian origin were analyzed in parallel with a previously established kit.

Study results: Results for both analytical methods were in 100% concordance.
In particular, 22 samples were homozygous for the genotype D/D, 36 samples was heterozygous (genotype D/I) and 4 samples were homozygous for the genotype I/I.

4. Precautions and Warnings

Handling Requirements

The present product is an *in-vitro* diagnostic device and therefore must be used by qualified personnel only.

General precautions for the handling of generic laboratory materials are required.

The laboratory work-flow must conform to standard practices. Due to the risk of contamination, PCR preparation and PCR amplification must be performed in physically separated areas.

Do not mix reagents from different lots.

Do not use the reagents after the expiration date.
Use the manual version which is delivered with the kit (see kit label).

Laboratory Procedures

All materials of human origin and related waste must be considered potentially infectious. Thoroughly clean and treat all work surfaces with disinfectants approved by local authorities.

Do not eat, drink or smoke in the laboratory working area.

Do not pipet by mouth.

Wear disposable protective gloves, laboratory coats and adequate eye protection during the handling of samples and set components.

Avoid microbial or nuclease contamination of the reagents while pipetting the aliquots. The use of disposable sterile tips is essential.

Thoroughly wash your hands after handling the samples and the sets components.

Sample Preparation

Regarding proper handling and disposal refer to the safety instructions enclosed in the package insert of the product employed (see chapter 2.3).

Amplification and Detection

Before using this product, please read the LightCycler® Operator's Manual .

Save a sample file to identify each position for correct sample identification.

Check LightCycler® Instrument settings and make sure that they match those reported in the following section "PCR protocol" specific for your Instrument.

Do not touch the capillary surface or plate cover without gloves.

Please refer to all the operative and safety instructions of the LightCycler® Instrument.

Handling of Waste Materials

Dispose of the unused reagents and waste materials according to the current laws.

5. Programming

5.1 Color Compensation

No Color Compensation is required for the use of the *LightMix® Kit ACE I/D.* Reading data with 'Color Compensation' activated will not change the readout of the results.

5.2 Capillary Based LightCycler® Instruments

For details see the LightCycler® Operator's Manual.

Programming:
The protocol consists of four program steps (Tab.1):

1. **Denaturation** of sample and activation of the enzyme
2. **Cycling** PCR-amplification of the target DNA
3. **Melting** Identification of PCR amplified DNA sequence
4. **Cooling** of the Instrument

Step:	**1**	**2**			**3**			**4**
Parameter								
Analysis Mode	**None**	**Quantification mode**			**Melting Curves mode**			**None**
Cycles	**1**	**45**			**1**			**1**
Target [℃]	**95**	**95**	**60**	**72**	**95**	**43**	**75**	**40**
Hold [hh:mm:ss]	**00:10:00**	**00:00:05**	**00:00:10**	**00:00:15**	**00:00:20**	**00:00:20**	**00:00:00**	**00:00:30**
Ramp Rate [℃/s]*	**20**	**20**	**20**	**20**	**20**	**20**	**0.2**	**20**
Sec Target [℃]	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Step Size [℃]	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Step Delay [cycles]	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Acquisition Mode	None	None	Single	None	None	None	Cont.	None

* For LightCycler 1.x Instruments using software version 3.5.3 read 'Temperature Transition Rate' [℃/s] instead of Ramp Rate.

Tab. 1: Programming of capillary based Instruments for using *LightMix® Kit ACE I/D*

Note:
While programming maintain default software values: channel = 530, max. samples = 32, seek temperature = 30℃ and capillary size = 20 µl. Do not change the capillary size value to 100 µl.
Store the program and the default values as '**RUN Template'** which can be loaded to start every ACE LightCycler® run.

Just before starting the run, modify max. samples (default = 32) to the number of samples plus controls included in the run to prevent stopping of the instrument due to missing capillaries.

5.3 LightCycler® 480 Instruments

For details see the LightCycler® Operator's Manual.

Detection Format: SimpleProbe
Note:

This kit can be run in combination with LightMix® Kit HFE H63D S65C C282Y CE (cat. 40-0340-32) following the instruction for the Detection Format and Programming described in the HFE kit manual.

Reaction Volume: 10 µl

Programming:

The protocol consists of four program steps (Tab.2):

1. **Denaturation** of sample and activation of the enzyme
2. **Cycling** PCR-amplification of the target DNA
3. **Melting** Identification of PCR amplified DNA sequence
4. **Cooling** of the Instrument

Step:	**1**	**2**			**3**			**4**
Parameter								
Analysis Mode	**None**	**Quantification mode**			**Melting Curves mode**			**None**
Cycles	**1**	**45**			**1**			**1**
Target [°C]	**95**	**95**	**60**	**72**	**95**	**43**	**75**	**40**
Acquisition Mode	**None**	**None**	**Single**	**None**	**None**	**None**	**Cont.**	**None**
Hold [hh:mm:ss]	**00:10:00**	**00:00:05**	**00:00:10**	**00:00:15**	**00:00:30**	**00:02:00**	**00:00:00**	**00:00:30**
Ramp Rate [°C/ s] **96**	**4.4**	**4.4**	**2.2**	**4.4**	**4.4**	**1.5**	**0.29**	**1.5**
Ramp Rate [°C/ s] **384**	**4.6**	**4.6**	**2.4**	**4.6**	**4.6**	**2.0**	**0.29**	**2.0**
Acquisitions [per °C]	**-**	**-**	**-**	**-**	**-**	**-**	**2**	**-**
Sec Target [°C]	**0**	**0**	**0**	**0**	**-**	**-**	**-**	**-**
Step Size [°C]	**0**	**0**	**0**	**0**	**-**	**-**	**-**	**-**
Step Delay [cycles]	**0**	**0**	**0**	**0**	**-**	**-**	**-**	**-**

Tab. 2: Programming of LightCycler® 480 Instruments (96 well and 384 well formats) and cobas z 480 Analyzer for using the LightMix® Kit *ACE I/D*

Note:

a) Store the program and the default values as '**RUN Template'** which can be loaded to start every ACE LightCycler® run.
b) Ensure to program **2 acquisitions per second** instead the default value 5; more acquisitions reduce the slope of the melting curve, increase experiment time and cause kit malfunction.

5.4 LightCycler® 96 Instrument

For details see the LightCycler® Operator's Manual.

Measurement:

Detection Format: 470/514 FAM			**General**
Quant Factor	Melt Factor	Integration Time (S)	Volumes (µl)
10.00	1.20	1.00	10

Profile:

The protocol consists of four program steps (Tab.3):

1. **Preincubation** of sample and activation of the enzyme
2. **Step Amplification** PCR-amplification of the target DNA
3. **Melting** Identification of PCR amplified DNA sequence
4. **Cooling** of the Instrument

Step:	**1**	**2**			**3**			**4**
Parameter								
Cycles	**1**	**45**			**1**			**1**
Ramp [°C/ s]	**4.4**	**4.4**	**2.2**	**4.4**	**4.4**	**1.5**	**0.20**	**1.5**
Duration [s]	**600**	**5**	**10**	**15**	**30**	**120**	**1**	**30**
Target [°C]	**95**	**95**	**60**	**72**	**95**	**43**	**75**	**40**
Mode		Standard	Standard	Standard				
Acquisition Mode	**None**	**None**	**Single**	**None**	**None**	**None**	**Cont.**	**None**
Readings /°C							**5**	

Tab. 3: Programming of LightCycler® 96 Instrument for using the LightMix® Kit *ACE I/D*

Note: Store the program and the default values as **'Experiment file'** which can be loaded to start every ACE LightCycler® run.

5.5 LightCycler® Nano Instrument

For details see the LightCycler® Operator's Manual.

Run Setting / Optical setting

Intercalating Dyes
Normal Quality

Profile:

The protocol consists of four program steps (Tab.4):

1. **Denaturation** of sample and activation of the enzyme
2. **Cycling** PCR-amplification of the target DNA
3. **Denaturation** of amplified PCR product.
4. **Melting** Identification of PCR amplified DNA sequence

Step:	**1**	**2**			**3**	**4**	
Parameter							
Name	**Hold**	**3 Step Amplification**			**Hold**	**Melting Stage**	
						Initial Stage	**Final Stage**
Cycles		**45**					
Temp [℃]	**95**	**95**	**60**	**72**	**95**	**43**	**75**
Ramp (℃/s)	**5**	**5**	**4**	**5**	**5**	**4**	**0.2**
Hold (s)	**600**	**10**	**15**	**20**	**30**	**120**	**1**
Acquire			√				

Tab. 4: Programming of LightCycler® Nano Instrument for using the *LightMix® Kit ACE I/D*

Note:

Store the program and the default values as **'Experiment file'** which can be loaded to start every ACE LightCycler® run.

6. Experimental protocol

Program the Instrument before preparing the solutions (see 5. Programming and read the LightCycler® Operator's Manual for details).
The described performance of the assay can be guaranteed only when used with Roche Diagnostics LightCycler® systems.

6.1 Sample Preparation

For preparation of genomic DNA use human peripheral blood (EDTA, citrate). The use of heparin is strongly discouraged since this anticoagulant might interfere with the PCR.
Perform nucleic acid purification using the High Pure PCR Template Preparation Kit or the MagNA Pure LC Instruments using the extraction kit appropriate to the MagNA Pure Instrument used (see 2. Additional Devices and Reagents) as described in the respective protocols.

In the depicted assays (7.5. Reading of the Results) the DNA was manually extracted from 200 µl of blood using the High Pure PCR Template Preparation Kit following the manufacturer's instructions; 100 µl of elution buffer were used for the final elution of the purified DNA from the column.

6.2 Reagents Preparation

6.2.1 Preparation of the LightCycler® FastStart DNA Master

1	Keep LightCycler® FastStart Enzyme **1a** cold.
2	Thaw the LightCycler® FastStart Reaction Mix **1b** by warming up the tube at 30°- 35°C for 3 - 5 minut es.
3	Quickly spin tubes to collect drops.
4	The solution must be free of particles.
5	Add 60 µl of **1b** to the vial **1a**.
6 ⚠	Mix the solution carefully with a pipette. Do not vortex! Avoid the production of bubbles.
7	Spin the tubes to collect drops.
8	Use reagent to prepare the Reaction Mix (6.3).
9	Store left over reagent at 4°C.

6.2.2 Preparation of Parameter-Specific Reagents (PSR)

►	Each **PSR** reagent tube is sufficient for 64 reactions.
1	Spin the premixed **PSR** tube at 10,000 RPM for 1 minute.
2	Check that the pellet is located at the bottom.
3	To each **PSR** tube add **66 µl** of PCR-grade **Water**.
4	Incubate for 20 sec at room temperature.
5	Vortex for 10 sec.
6	Spin the tubes to collect drops.

► Use 1 µl of **PSR** reagent for a 10 µl PCR reaction.

6.2.3 Preparation of Positive Control

►	**HT Positive Control** tube is sufficient for 40 reactions.
1	Spin the tube at 10,000 RPM for 1 minute.
2	Check that the blue pellet is located at the bottom of the tube.
3	Dissolve pellet by adding 80 µl PCR-grade **Water**.
4	Incubate for 20 sec at room temperature.
5	Vortex for 10 sec.
6	Spin the tubes to collect drops.

► Use **2 µl** of **Positive Control** for a 10 µl PCR reaction.
► **Positive Control** must be used in each run.
Please note: Opening the vials may cause contaminations of the work-space (aerosol).

6.2.4 Preparation of Genotyping Standards

The LightCycler® software 4.05 and later (capillary based instruments) and software 1.5 (LightCycler®480 instruments) can be calibrated with reference standards to perform an automated genotyping of unknown clinical samples.

►	If not used, keep the Genotyping Standards lyophilized; dispose reagents when the kit is used up or after reaching the expiration date.
1	Spin the **DEL** and **INS** Genotyping Standard tubes at 10,000 RPM for 1 minute.
2	Check that the blue pellet is located at the bottom of the tube.
3	Dissolve pellet by adding 80 µl PCR-grade **Water**.
4	Incubate for 20 sec at room temperature.
5	Vortex for 10 sec.
6	Spin the tubes to collect drops.

► Use 2 µl of **DEL** and **INS** Genotyping Standard for a 10 µl PCR reaction.
► Both **Genotyping Standards** must be used in the first run of the kit to calibrate the genotyping module.
Please note: Opening the vials may cause contaminations of the work-space (aerosol).

6.3 Preparation of the Reaction Mix

6.3.1 Preparation of 64 LightCycler® Reaction Mix

We recommend preparing 64 reactions to prevent storage of dissolved or activated reagents in varying volumes (6.2). See chapter 6.4 for storage and stability of dilute components. For the preparation of reaction mix for less samples, please go to step 6.3.2 “Reaction mix for single reaction”.

Prepare the reaction mix in the PSR reagent tube (cooled):

Components	**64 reactions**
To the **PSR** tube (red cap) already containing	66.0 µl
Add:	
H_2O, PCR-grade (colorless cap)	343.2 µl
Mg^{2+} solution 25 mM (blue cap)	52.8 µl
LightCycler® FastStart DNA Master HybProbe (red cap), see **6.2.1**	66.0 µl
 Substitute of the “long neck cap” of the **PSR** tube with the red cap from FastStart	
Total Volume	**528.0 µl**

Tab. 5: Volumes of components for preparing 64 reaction mixture

6.3.2 Preparation of the Single LightCycler® Reaction Mix

Prepare the reaction mix by multiplying each volume (Tab. 6) by the number of biological samples to be analyzed plus three reactions (Negative Control, **Positive Control**, one excess) and (optionally) two **Genotyping Standards**.

Prepare the reaction mix in a cooled vial:

Components	Single reaction
H_2O, PCR-grade (colorless cap)	5.2 µl
Mg^{2+} solution 25 mM (blue cap)	0.8 µl
PSR (red cap), see **6.2.2**	1.0 µl
LightCycler® FastStart DNA Master HybProbe (red cap), see **6.2.1**	1.0 µl
Volume of reaction mix	**8.0 µl**

Tab. 6: Volumes of components for preparing a single reaction mixture

Gently pipette up and down the reaction mix
An high percentage of experimental failure are due to a non homogeneous reaction mix!

6.3.3 Capillary / Well Loading Procedure

Each run must include one Negative Control (**NTC**) to demonstrate the absence of contaminations with genomic DNA or ACE PCR product and **Positive Control** to identify run specific melting temperatures. Regulatory agencies or local laboratory rules might require including also the two Genotyping Standards.

Capillary / Well Loading Procedure

▶	Remember to include the controls when setting up the run.
1	Mix gently, spin down and check for the absence of air bubbles in the reaction mix vial.
2	Dispense **8 µl** per capillary/well of reaction mix.
3	**Mandatory:** Add **2 µl** of PCR-grade **H_2O** as **Negative Control** (**NTC**) in position 1 (A1). Add **2 µl** of **HT Positive Control** in position 2 (A2).
	Optional*: Add **2 µl** of **DEL** Genotyping Standard in position 3 (A3). Add **2 µl** of **INS** Genotyping Standard in position 4 (A4).
4	Add **2 µl** of **Sample** in the remaining capillaries / wells.
5 ⚠	Close the capillary/plate and centrifuge. Check that no air bubbles are present.
6	Place the rotor/plate into the LightCycler® Instrument.
7	Capillary-based users only: input number of samples.
8	Start the run.
9	Input experiment's name when instructed.
10	Save sample data in the samples' window.

* See section **6.5** for the Sample loading and Genotyping Standards calibration.

6.4 Storage and Stability of Diluted Components

Reaction Mix
The complete reaction mix containing Parameter-Specific Reagents (**PSR**), LightCycler® FastStart DNA Master HybProbe and $MgCl_2$ can be stored refrigerated (4℃ - 8℃) for 30 days.

Avoid prolonged exposure to light.

Parameter Specific Reagents (PSR)
Once diluted, store PSR refrigerated at 4℃ - 8℃ f or up to 30 days.

Avoid prolonged exposure to light.

LightCycler® FastStart DNA Master HybProbe
The combined FastStart DNA Master HybProbe master mix (1a+1b) can be stored refrigerated (4℃ - 8℃) for 30 days.

Positive Control
The dissolved Positive Control is stable for 30 days when stored refrigerated (4℃ - 8℃).

Genotyping Standards
The dissolved **Genotyping Standard** is stable for 30 days when stored refrigerated (4℃ - 8℃).

6.5 Loading of Controls and Genotyping Standards

Samples in positions 1 to 2 (A1 to A2) must be filled in each run as described in the table below.

⚠ Genotype results are based on melting temperatures.
The use of the automated genotyping module present in the LightCycler® 2.0 and LightCycler® 480 software is optional.

Samples in positions 3 to 4 (plate: A3 to A4) are required for teaching of Genotyping Standards only in the first run of the kit.

Refer to LightCycler® Operator's Manual for details.

6.5.1 Capillary Based Instruments

In "Samples data - Capillary View", input Sample Name as described in the second column.
Select "Analysis Type – Genotyping". Select Channel 530 and deselect all others. From the pull down menu select "Sample Type" and copy the "Genotype" description

Pos	Sample Name	Channel	Target Name	Sample Type	Genotype
1	**NTC**	530	Target 1	Negative Control	
2	**HT**	530	Target 1	Melting Standard	ACE I/D Heterozygous
3	**DEL**	530	Target 1	Melting Standard	ACE DEL
4	**INS**	530	Target 1	Melting Standard	ACE INS

6.5.2 LightCycler® 480 Instruments

In the "Sample Editor" window, in "Step1: Select Workflow" section, select "Melt Geno". Filter combination 465-510. Input the description of **Positive Control** and **Genotyping Standards** as follows:

Pos	Sample Name	Melt Geno Sample Type	Melt Geno Genotype
1	**NTC**	Negative Control	
2	**HT**	Melting Standard	ACE I/D Heterozygous
3	**DEL**	Melting Standard	ACE DEL
4	**INS**	Melting Standard	ACE INS

6.5.3 LightCycler® 96 Instrument

In the “Sample Editor” window input, as described below, the description of **Positive Control** and optionally **Genotyping Standards** in:

Table View

Color	Position	Sample Name	Sample Type	Dye
	A1	**NTC**	Unknown	FAM
	A2	**HT**	Unknown	FAM
	A3	**DEL**	Unknown	FAM
	A4	**INS**	Unknown	FAM

Leave empty all other not described cells.

6.5.4 LightCycler® Nano Instrument

Samples:

Input, as shown below, the description of **Positive Control** and optionally **Genotyping Standards** into the “Samples” window; input name and select Dye into the “Target” window:

Samples:

Color	Name	Note
	NTC	
	HT	
	DEL	
	INS	

Target:

Color	Name	Dye	Reference
	channel 530	FAM	

Well as table

Pos	#	Note	Sample	FAM	Type
A1	1		**NTC**	channel 530	U
A2	2		**HT**	channel 530	U
A3	3		**DEL**	channel 530	U
A4	4		**INS**	channel 530	U

7. Data Analysis and Interpretation

7.1 Limits and Interferences

The present assay is specific for the ACE I/D DNA.
No interferences for this assay are known.

7.2 Calibration

Calibration has to be performed following the procedure described in 6.2.4, 6.3.3, 6.5, 7.3.2 and 7.3.3.

7.3 Quality Control – Acceptance Criteria

In order to perform a reliable genotyping analysis, it is essential that Negative Control **NTC** and **HT** Positive Control are included in each run.

NOTE: The test is performed at an annealing temperature of 60ºC at which the probes will not bind well, yielding low or even no signals in 'quantification'. For this reason, the acceptance criteria are based only on the definition of the melting-curve patterns as described below.

7.3.1 Negative Control

NTC Negative Control (Mandatory - position 1).
Melting-curve analysis of the Negative Control must provide a negative result: no assay-specific melting peaks (see 7.3.2) must be detected.

In case that the **NTC** should report one or more specific peaks (compare signal with sample results to avoid that the software enlarges background noise to window size suggesting the presence of melting peaks), a contamination or a pipetting error has occurred; the session is not valid and the procedure has to be repeated. If the problem sustains, change water and/or reagents and repeat. Feel free to ask for assistance at service@tib-molbiol.de.

In case a peak is detected at an unspecific temperature (see paragraph 7.3.4), the software might incorrectly identify it as positive, causing automatic genotyping impossible (LightCycler® 480 software 1.5 reports : "*Sample NTC in position A1 is a negative control not in the negative group"*).

In this case - to enable the automatic genotyping – change the NTC sample from "Negative Control" to "Unknown" (see 6.5 Sample loading and calibration of Genotyping Standards); alternatively, results must be read from the melting temperatures (see 7.3.4 Samples and 7.6 Interpretation of the Results).

7.3.2 Positive Control DNA

Melting-curve analysis should always show:

HT Positive Control (Mandatory - position 2).
two melting peaks in channel 530 at 54℃± 2,5℃ and 63℃± 2,5℃.
The peaks difference is of 9℃± 1,5℃.
HT is mimicking an **heterozygous** clinical samples. (see **7.5**).

7.3.3 Genotyping Standards DNA

Melting-curve analysis should always show:

DEL Genotyping Standard (Optional - position 3).
One single melting peaks in channel 530 at 63°C± 2,5°C.
DEL is mimicking an homozygous **deleted** clinical sample. (see **7.5**).

INS Genotyping Standard (Optional – position 4).
One single melting peaks in channel 530 at 54°C± 2,5°.C
INS is mimicking an homozygous clinical sample with **insertion**. (see **7.5**).

Before repeating a run consider common errors; check in particular the amplification profile, correct master-mix and $MgCl_2$ concentration used, and keep in mind that also inadequate storage of reagents may cause a failure of the device.

7.3.4 Samples

The result of the present assay must always show one or two melting peaks in channel 530 at 54°C± 2,5°C and/or 63°C± 2,5°C.

⚠ No more than two peaks per sample are expected.
The melting peak profiles must be conformable to the acceptance criteria described in the present chapter. Otherwise, the result is not valid and the whole procedure has to be repeated (sample preparation, amplification and detection). See also **7.6 Interpretation of results**

7.3.5 Abnormal Melting Curves

If an abnormal melting curve persists, it can be due to a defect in the product or can be caused by other variations (mutations) in the probe binding region. In the latter case another method must be used for comparison / verification of the sequence.
Submit the PCR fragment for DNA sequencing to confirm the sequence or identify any unknown mutations. Report deviations to service@tib-molbiol.de
Feel free to send deviant melting samples to the Berlin laboratories to confirm the obtained results and/or identify other mutations by DNA sequencing.

7.4 Saving External Genotyping Standards

⚠ (Not applicable for LC1.x software versions below 4.0, LightCycler®96 and for LightCycler® Nano Instruments).

After the genotyping analysis, if samples 1 to 4 comply with the acceptance criteria (see **7.3 Quality Control – Acceptance Criteria**), save the Genotyping Standards as follows and use External Standard in all successive runs.

7.4.1 Capillary Based Instruments

In the Melting Curve analysis - Genotyping window open the “Standard (Int)” menu and select “Save standards as External”.

7.4.2 LightCycler®480 Instruments

In the Melt Curve Genotyping analysis window open the “Standards (In-run)” menu and select “Save as ext.”

7.5 Reading the Results

Perform data analysis as described in the LightCycler® Operator’s Manuals.

7.5.1 Typical Data for Amplification

The amplification curves do not contain any analytical information (see section **7.3 Quality Control – Acceptance Criteria**), but, nevertheless, an example from LightCycler® 2.0 is depicted below (Fig. 1).
The PCR has been performed with approximately 100 ng of genomic DNA from samples previously sequenced, one Negative Control **NTC**, **Positive Control**, and **Genotyping Standards**.

View data for amplification as follows:

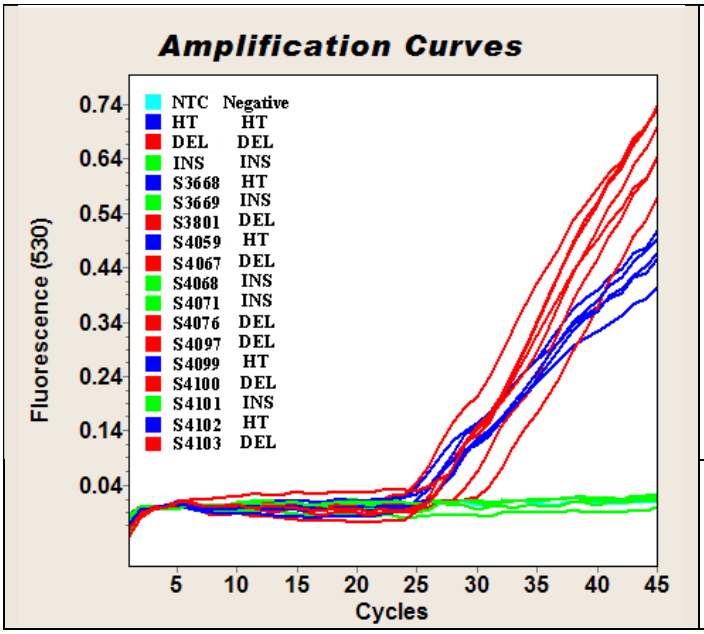
LC 2.0 Instrument (or LC1.x with software versions 4.1):
View ACE I/D amplification in channel 530, “Absolute Quantification” analysis mode.

LC 480 Instruments:
View ACE I/D amplification data in channel 483-533 for use with LightCycler® 480 Instrument and in channel 465-510 for use with LightCycler® 480 II Instrument, “Abs Quant/2nd Derivative Max” analysis mode.

LC 96 Instrument:
View ACE I/D amplification in “Abs Quant” analysis mode.

LC Nano Instrument:
View ACE I/D amplification in “Automatic Quantification” mode.

LC1.x, software versions 3.5:
View ACE I/D amplification in fluorescence channel F1 “Quantification – Second Derivative Maximum” mode.



Data from channel 530.

The **NTC** Negative Control (light blue line) must show no amplification signal.

The **INS** Genotyping Standard (green line) and the **homozygous samples with insertion** (S3669, S4068, S4071,S4101) show **no amplification signal**.

All the remaining biological samples, the **HT** Positive Control (blue line) and **DEL** Genotyping Standard (red line) show an amplification signal.

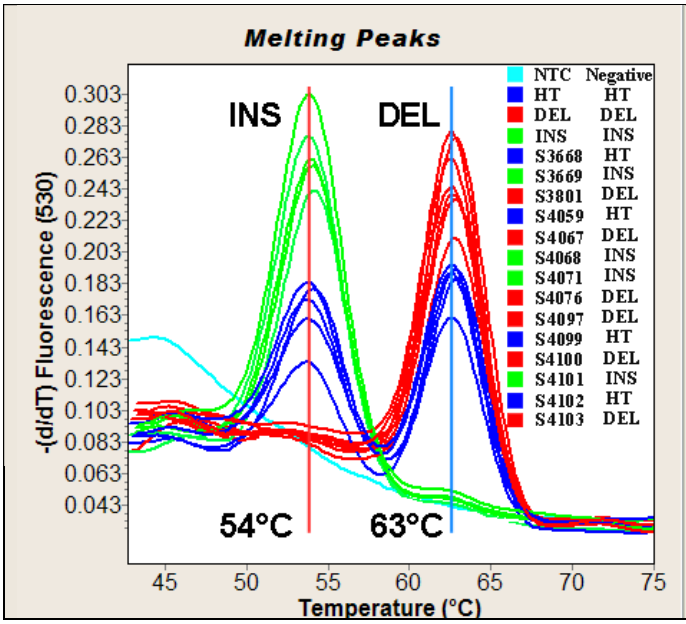
Fig. 1: Amplification plot.
Sample data for ACE I/D

7.5.2 Melting Analysis: Capillary Based Instruments

The melting-curve peaks (Fig. 2) discriminate between deletion, insertion and heterozygous genotypes.

View *ACE I/D* data for Melting as follows:
LC 2.0 Instrument (or LC1.x with software versions 4.1):
View Melting data for ACE I/D in channel 530.
Analysis Type "Melting Curve Analysis – Genotyping" mode.

LC1.x, software version 3.5.3
View Melting data for ACE I/D in channel F1 instead of channel 530.
"Melting Curve" mode.



Channel 530

NTC Negative Control
(light blue line–position 1) no assay-specific melting peaks must be detected.
HT Positive Control (blue line) and heterozygous samples (S3668, S4059, S4099, S4102) show melting peaks at 54℃ and 63℃.
DEL Standard (red line) and homozygous deleted samples (S3801, S4067, S4076, S4097, S4100, S4103) show a melting peak at 63℃.
INS Standard (green line) and homozygous samples with insertion (S3669, S4068, S4071, S4101) show a peak at 54℃.

Fig. 2: Melting sample data for ACE I/D

Interpretation of the Results

Genotype:	**homozygote ACE INS / INS**	**heterozygote ACE I/D**	**homozygote ACE DEL / DEL**
Number of melting peaks	**1**	**2**	**1**
Melting temperature of peaks	**54℃**	**54℃ and 63℃**	**63℃**
Temperature difference between peaks	**---**	**9℃**	**---**
Phenotype	Normal population risk	Normal population risk	Increased risk for myocardial infarction and diabetic nephropathy

Tab. 7: Typical analysis results

Note:
The values of the melting temperatures (Tm) may vary ±2.5℃ between different experiments.
The ΔT between the melting peaks for heterozygous genotypes may vary ±1.5℃.

In case of variations see instructions: **7.3.5 Abnormal Melting Curves.**

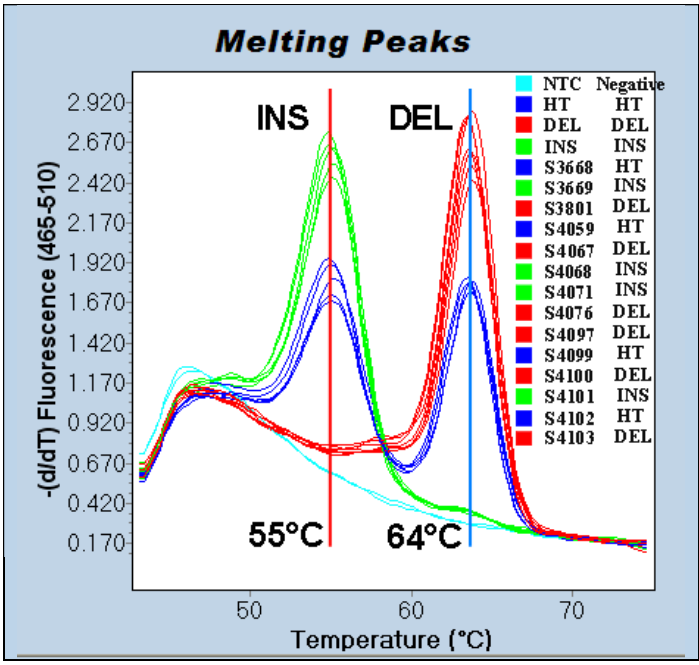
In case of automatic genotype module failure (score <0.6 or res<0.4), switch to manual identification of melting curve (Tm calling) and compare results with table above or use table 11 in chapter **7.6. Interpretation of the Results**.

7.5.3 Melting Analysis: LightCycler® 480 Instruments

The melting-curve peaks (Fig. 3) discriminate between deletion, insertion and heterozygous genotypes.

View data for Melting as follows:

LC 480 Instruments:
For use in LightCycler® 480 Instrument view Factor V G1691A Melting data in channel 483-533.
For use in LightCycler® 480 II Instrument view Factor V G1691A Melting data in channel 465-510.
For use in cobas z 480 Analyzer view Factor V G1691A Melting data in channel 465-510.
"Melt Curve Genotyping" mode.



Channel 530

NTC Negative Control
(light blue line–position 1) no assay-specific melting peaks must be detected
HT Positive Control (blue line) and heterozygous samples (S3668, S4059, S4099, S4102) show melting peaks at 55℃ and 64℃.
DEL Standard (red line) and homozygous deleted samples (S3801, S4067, S4076, S4097, S4100, S4103) show a melting peak at 64℃.
INS Standard (green line) and homozygous samples with insertion (S3669, S4068, S4071, S4101) show a peak at 55℃.

Fig. 3: Melting sample data for ACE I/D.

Interpretation of the Results

Genotype:	homozygote ACE INS / INS	heterozygote ACE I/D	homozygote ACE DEL / DEL
Number of melting peaks	**1**	**2**	**1**
Melting temperature of peaks	**55℃**	**55℃ and 64℃**	**64℃**
Temperature difference between peaks	**---**	**9℃**	**---**
Phenotype	Normal population risk	Normal population risk	Increased risk for myocardial infarction and diabetic nephropathy

Tab. 8: Typical analysis results

Note:

The values of the melting temperatures (Tm) may vary ±2.5℃ between different experiments.
The ΔT between the melting peaks for heterozygous genotypes may vary ±1.5℃.

In case of variations see instructions: **7.3.5 Abnormal Melting Curves.**

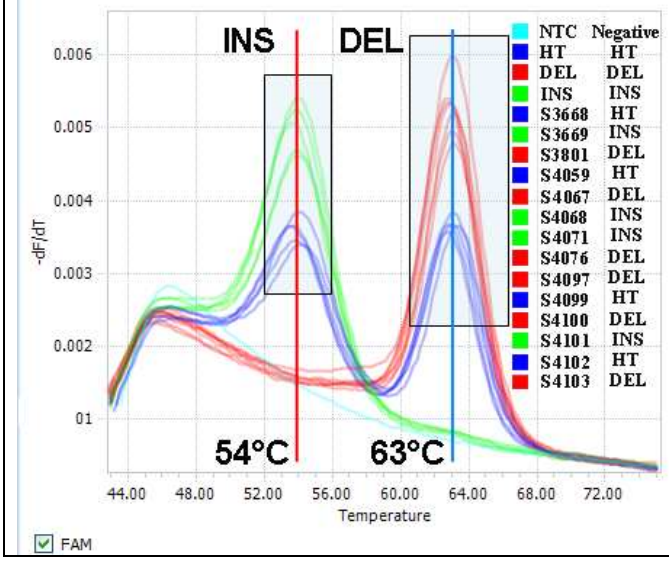
In case of automatic genotype module failure (score <0.6 or res<0.4), switch to manual identification of melting curve (Tm calling) and compare results with table above or use table 11 in chapter **7.6. Interpretation of the Results**.

7.5.4 Melting Analysis: LightCycler® 96 Instrument

The melting-curve peaks (Fig. 4) discriminate between deletion, insertion and heterozygous genotypes.

Add Analysis: **Tm Calling**
View data in: **Melting peak**
Select peaks by using the: **Area marker tool**

Note : The LightCycler® 96 Instrument needs a manual input to mark the region which shall be used for determining the melting peak; if the peak is not contained in the box (marker tool) the software will not report a Tm value.
Visually analyze the melting curve of each patient in comparison with the melting curve of **Positive Control**.
Use also table 9 below or table 11 in chapter **7.6. Interpretation of the Results** for comparison.



Channel 530

NTC Negative Control
(light blue line–position 1) no assay-specific melting peaks must be detected
HT Positive Control (blue line) and heterozygous samples (S3668, S4059, S4099, S4102) show melting peaks at 54°C and 63°C.
DEL Standard (red line) and homozygous deleted samples (S3801, S4067, S4076, S4097, S4100, S4103) show a melting peak at 63°C.
INS Standard (green line) and homozygous samples with insertion (S3669, S4068, S4071, S4101) show a peak at 54°C.

Fig. 4: Melting sample data for ACE I/D.

Interpretation of the Results

Genotype:	**homozygote ACE INS / INS**	**heterozygote ACE I/D**	**homozygote ACE DEL / DEL**
Number of melting peaks	**1**	**2**	**1**
Melting temperature of peaks	**54°C**	**54°C and 63°C**	**63°C**
Temperature difference between peaks	**---**	**9°C**	**---**
Phenotype	Normal population risk	Normal population risk	Increased risk for myocardial infarction and diabetic nephropathy

Tab. 9: Typical analysis results

Note:

The values of the melting temperatures (Tm) may vary ±2.5°C between different experiments.
The ΔT between the melting peaks for heterozygous genotypes may vary ±1.5°C.

In case of variations see instructions: **7.3.5 Abnormal Melting Curves.**

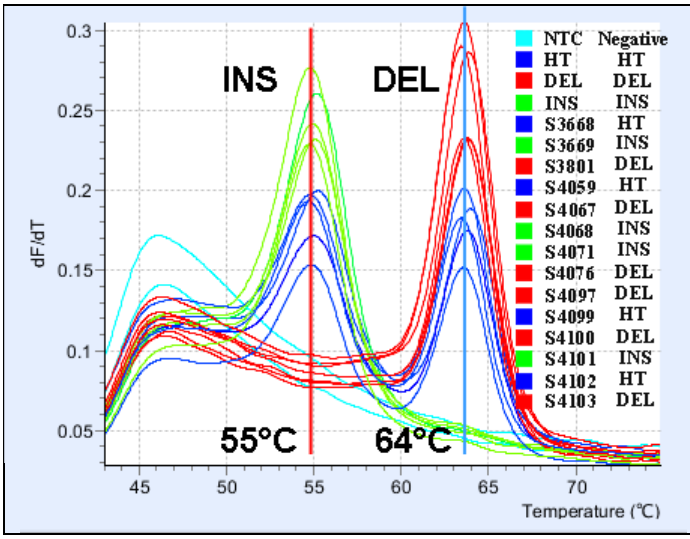
7.5.5 Melting Analysis: LightCycler® Nano Instrument

The melting-curve peaks (Fig. 5) discriminate between deletion, insertion and heterozygous genotypes.

View data for Melting as follows:

Analysis
In window: **Select Analysis**
Select: Tm Calling
In window: **Setting**
Select: Use negative Derivative "Yes"
Select: Noise Reduction Range (℃) = 1
Select: Target: Channel 530
Melt Peaks

Manually compare the melting curve of each patient with the melting curve of Standards and use table 10 below or table 11 in chapter **7.6. Interpretation of the Results** for comparison.



Channel 530

NTC Negative Control
(light blue line–position 1) no assay-specific melting peaks must be detected
HT Positive Control (blue line) and heterozygous samples (S3668, S4059, S4099, S4102) show melting peaks at 55℃ and 64℃.
DEL Standard (red line) and homozygous deleted samples (S3801, S4067, S4076, S4097, S4100, S4103) show a melting peak at 64℃.
INS Standard (green line) and homozygous samples with insertion (S3669, S4068, S4071, S4101) show a peak at 55℃.

Fig. 5: Melting sample data for ACE I/D.

Interpretation of the Results

Genotype:	homozygote ACE INS / INS	heterozygote ACE I/D	homozygote ACE DEL / DEL
Number of melting peaks	**1**	**2**	**1**
Melting temperature of peaks	**55℃**	**55℃ and 64℃**	**64℃**
Temperature difference between peaks	**---**	**9℃**	**---**
Phenotype	Normal population risk	Normal population risk	Increased risk for myocardial infarction and diabetic nephropathy

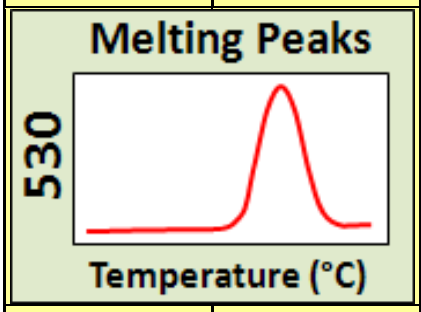
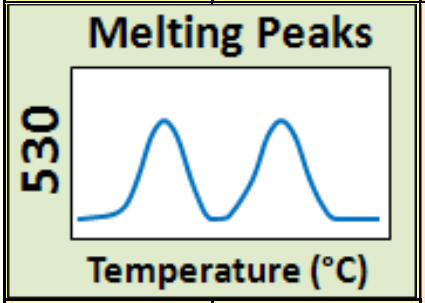
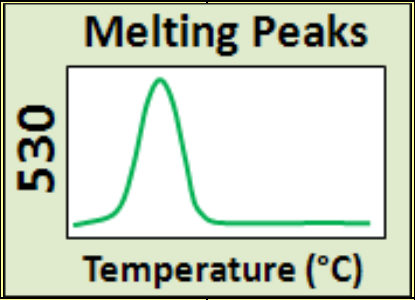
Tab. 10: Typical analysis results

Note:
The values of the melting temperatures (Tm) may vary ±2.5℃ between different experiments.
The ΔT between the melting peaks for heterozygous genotypes may vary ±1.5℃.

In case of variations see instructions: **7.3.5 Abnormal Melting Curves.**

7.6. Interpretation of the Results

In case of automatic genotype module failure, switch to manual identification of melting curve (Tm calling) and compare results

ACE I/D Channel 530 Melting peak(s)		ACE Genotypes	Phenotype
INS	DEL		
		ACE DEL / DEL	Increased risk for myocardial infarction and diabetic nephropathy
-	63-64		
		ACE INS / DEL	Normal population risk
54-55	63-64		
		ACE INS / INS	Normal population risk
54-55	-		
ΔTm 8-10℃			

Tab. 11: Typical analysis results

Note: The values of the melting temperatures (T_M) may vary ±2.5℃ between different runs. The ΔT between the melting peaks for heterozygous genotypes may vary ±1.5℃.

In case of variations see instructions: **7.3.5 Abnormal Melting Curves**

Compare your results with the known allele's frequency.

Notes:

8. Troubleshooting

Instrument specific codes:	Capillary based instruments LightCycler® Nano	LightCycler® 480 instruments LightCycler® 96 instrument
Event	**Possible Reason**	**Solution**
No sample detected	No centrifugation	Centrifuge capillary
All negative PCRs	Incorrect selection of detection channel	Set correct channel before analyzing
	Incorrect amplification protocol	Check instrument program
Inconsistent base-line among various samples	Incorrect pipetting	Ensure homogeneity of mix quantity in each sample
	Non homogenous reaction mix	Pipette reaction mix up and down 10 x with a clean 200µl tip before distribution in reaction vessel
	Multiwell plate badly sealed	Ensure that multiwell plate is properly sealed
Baseline "Saw teeth like"	Bubble in the well	Centrifuge plate before run
	Bubble in the well	Centrifuge plate before run
	Incorrect positioning of capillary in the carousel	Firmly press capillary in the carousel
No signal in the Positive Control	Error while setting the instrument	Check the position settings of the Positive Control
	Incorrect PSR / $MgCl_2$ concentration	Repeat assay
	Positive Control or standard degradation	Use a new aliquot of Positive Control or standard
Positive signal in **NTC** Negative Control	Error while setting the instrument	Check the position settings of the Negative Control
	Dispensing error	Comply with the work sheet when dispensing samples, Negative Controls, Positive Controls and standards
	Dispensing error	Always change tips among samples
	Dispensing error	Avoid spilling the contents of the sample test tube
	Contamination of the PCR- grade water.	Use a new aliquot of PCR- grade water
	Contamination of the reaction mix	Use new aliquots of reagents to prepare the reaction mix
	Contamination of the extraction/preparation area for amplification reactions	Clean surfaces and instruments with aqueous detergents, wash lab coats, replace test tubes and tips in use
	Contamination of the extraction/preparation area for amplification reactions	Add LightCycler® Uracil-DNA Glycosylase (Cat.-No.03 539 806 001) to the reaction mix according to instructions
No signal in samples	Low DNA amount	Check DNA concentration
	Sample inhibition	Dilute sample and repeat PCR, or repeat extraction and PCR, or add Positive Control and repeat
Melting curve outside the expected temperature range	With peaks TM **concordant** with Positive Control: Incorrect reagent concentration	Manually assign results accordingly to Positive Control
	With peaks TM **discordant** with Positive Control: Possible extraction inhibitor	Repeat assay diluting the DNA 1:3
	With peaks TM **discordant** with Positive Control: Possible different mutation	Repeat assay by sequencing and report unexpected variant to service@tib-molbiol.de

9. References

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PCR detection of the insertion/deletion polymorphism of the human angiotensin I converting enzyme gene (DCP1) (dipeptidyl carboxypeptidase 1).
NAR 1992;20:1433

9) Somogyvari F, Szolnoki Z, Marki-Zay J, Fodor L.
Real-time PCR assay with fluorescent hybridization probes for exact and rapid genotyping of the angiotensin-converting enzyme gene insertion/deletion polymorphism.
Clin Chem 2001 Sep;47(9):1728-9

Classification / Reference

Reference	Classification
EDMA	16 01 04 90
CPV	33694000-1
EAN	4260159332452
Roche SAP No.	07161182001

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FastStart Enzyme

FastStart DNA Master HybProbe is included in the package for TIB MOLBIOL customers in Central Europe only.

When the present kit is distributed through Roche Diagnostics or their local distributors, FastStart DNA Master HybProbe is supplied as a separate product:

Roche Diagnostics Cat.-No. 03 003 248 001 kit for 96 reactions
Roche Diagnostics Cat.-No. 12 239 272 001 kit for 480 reactions

Material Safety Data

According to OSHA 29CFR1910.1200, Commonwealth of Australia [NOHSC:1005, 1008 (1999)] and the European Union Directives 67/548/EC and 1999/45/EC any products which not contain more than 1% of a component classified as hazardous or classified as carcinogenic do not require a Material Safety Data Sheet (MSDS).

Product is not hazardous, not toxic, not IATA-restricted. Product is not from human, animal or plant origin. Product contains synthetic oligonucleotide primers and probes.

Version History

Notes in red mark events that require to change laboratory procedures
Notes in blue mark improvements and changes in composition

Version	Event	Date
V131211	Release Version	28/11/2013

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