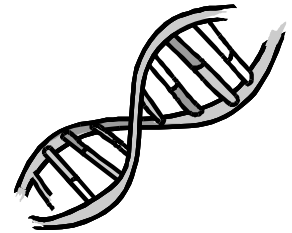




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# MutaREAL<sup>®</sup> ApoE

*real time* PCR Kit



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*real time* PCR test for the investigation of e2-, e3- and e4- allele variants of the apolipoprotein E – gene (ApoE) using the capillary system of the LightCycler<sup>®</sup> from Roche.

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KF290732 (32 Measurements)

KF290796 (96 Measurements)

For *in vitro* Diagnostic use only



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## 1. INTENDED USE

The **MutaREAL® ApoE** *real time* PCR kit is a molecularbiological test kit to be used for analysis of the e2-, e3- and e4- allele-variants in the apolipoproteine E - gene (by means of LightCycler®, Roche).

## 2. INTRODUCTION

Apolipoprotein E (built by astrocytes and microglia) exists in three different isoforms and functions as regulator in the lipid metabolism. Due to its involvement in the lipid transport of injured neurones, Apolipoprotein E (ApoE) is associated with Morbus Alzheimer - one of the most causes of dementia (leading to loss of orientation and judgment capacity). Only phenotype ApoE4 (homozygously) is responsible for the plaques development in the brain of Alzheimer patients. Therefore, the ApoE-genotype can be used as prediction factor for individual Morbus Alzheimer risk. Additionally the isoforms functions in plasma are differentially associated to type III-hyperlipoproteinemia (e2) and LDL-cholesterol (e4).

The three allele-variants are characterized by two base pair replacements (C>T) leading to an exchange from cystein to arginin at the positions 112 and 158 in the amino acid sequence of the apolipoprotein E.

## 3. TEST PRINCIPLE

For analysis of mutation ApoE\*112 and \*158 the potentially mutated region of ApoE gene is amplified by PCR (in a capillary by LightCycler®) using genomic DNA. Amplification products are analyzed afterwards in a melting-point curve analysis with mutation-specific hybridization probes. The melting-point curve analysis allows a clear identification of wildtype, heterozygous or homozygous genotypes.

For PCR are primer used which flank the region around the potential mutation place and produce an amplicon of 258 bp.

In addition, the standard PCR contains for each polymorphism two sequence-specific oligonucleotides (Hybridization Probes) which bind between both primers in proximity to the target DNA. The PCR step follows a genotype discrimination through melting-point curve analysis. One of the two Hybridization Probes binds exactly within the range of mutation site.

For ApoE\*112 and \*158 the Hybridization Probes are designed in this way that their sequence fits exactly onto the **CGC** – sequence. Therefore, in the melting-point curve analysis the mutation peak arises earlier in presence of **TGC**-sequence because of the introduced mismatch (= sequences are not 100 % homologous). In case of heterozygote genotype two peaks are generated – one with lower temperature (TGC) and one with higher temperature (CGC).

## 4. KIT COMPONENTS

Each kit contains enough reagents to perform 32 respectively 96 tests. Each kit also contains a package insert.

Color	Label	KF290732 (32 reactions)	KF290796 (96 reactions)
blue A blue B	enzyme enzyme buffer	5 µl 65 µl	3 x 5 µl 3 x 65 µl
yellow A yellow B	detection Mix *112/*158 DMSO	490 µl 80 µl	3 x 490 µl 3 x 80 µl
red A red B	control template ApoE*112 control template ApoE*158	10 µl 10 µl	30 µl 30 µl
green	negative control	100 µl	300 µl

## 5. REQUIRED INSTRUMENTS AND MATERIALS

*Required materials - provided:*

- PCR reagents
- Instruction manual

*Required materials - not provided:*

- LightCycler<sup>®</sup> instrument (Roche)
- LightCycler<sup>®</sup> capillaries (20 µl, Roche)
- LightCycler<sup>®</sup> capillary centrifuge (Roche)
- LightCycler<sup>®</sup> cooling block (Roche)
- DNA extraction kits for isolation of genomic DNA (ca. 10 ng/µl)
- pipettes (0.5 – 200 µl)
- sterile filter tips for micro pipettes
- sterile reaction containers
- table centrifuge
- laboratory gloves (powder free)

## 6. STORAGE AND HANDLING

- All reagents (except DMSO, yellow B) should be stored at -20°C.
- Store DMSO at room temperature.
- All reagents can be used until the expiration date printed on the labels.
- Avoid freezing and thawing more than twice. Thaw the individual components at 4°C.
- Ready to use enzyme mix (blue A + B) can be stored for three months at -20°C.
- The detection mix (yellow) should be stored in the dark.

## 7. REFERENCES AND CAUTION MEASURES

- Use only for *in vitro* diagnostics.
- The MutaREAL<sup>®</sup> - kit is adapted for use with the LightCycler<sup>®</sup> analysis system (Roche).
- This test must be performed by trained personnel: The rules of the Good Laboratory Practice (GLP) must be adhered to.
- Be careful while handling human samples, which is potentially infectious material.
- Carefully mix the components before use by pipetting, do not vortex!
- Perform the work in the LightCycler<sup>®</sup> cooling block and work quickly.
- Cool all stock solutions during work and protect the detection-mix against light.
- Always freeze the kit components in an upright position.

## 8. TEST PROCEDURE

Before you start working clean the devices to be used and the work surfaces area. Thaw the components of the kit at 4°C. Leave detection mix (yellow) in the dark. Put the necessary number of capillaries in the precooled LightCycler<sup>®</sup> Cooling block. Do not forget the additional 2 capillaries for controls (red A, B and green). Prepare the DNA samples before use by mixing.

### Enzyme Mix

Thaw blue vials at 4°C and centrifuge shortly. Transfer enzyme buffer (blue B) with steril filter tip to enzyme (blue A) and mix carefully by pipetting. This solution is now the *ready to use* enzyme mix. It is stable for tree months at -20°C, but should not be thawed more than two times.

### Mastermix

The table shows the composition for one reaction (20 µl). For the investigation of several samples the respective volumes are multiplied by the number of samples (inclusive controls) and given to a sterile vial (Master Mix). Thaw and carefully mix the kit components before use by pipetting. The solutions should be added in the same sequence as shown in the table below.

Component	Volume per 20 µl- reaction
Detection Mix (yellow A)	14 µl
DMSO (yellow B)	2 µl
Enzyme Mix (blue A + B)	2 µl

Carefully mix the finished master-mix (until no more streaks are seen) and submit **18 µl** into each capillary.

## Samples

Put 2  $\mu$ l sample DNA in each capillary, starting with position 4. The first three capillaries are designated for the controls: put 2  $\mu$ l for each of the two positive controls (red A, B) and negative control (green A) in the capillary. Cap the filled capillary with the covers. Afterwards transfer the capillaries into the LightCycler<sup>®</sup> Carousel. Centrifuge the samples down in the LightCycler<sup>®</sup> Carousel centrifuge. When using a table centrifuge place the capillary with the holder of the Cooling block in the centrifuge and centrifuge down with 3.000 rpm for 15 seconds.

## Protocol

Insert the LightCycler<sup>®</sup> Carousel with the samples and activate the following program:

Experimental Protocol							
Program:				Type:	None	Cycles	1
Segment Number	Temperature Target (°C)	Hold Time (sec)	Slope (C'/sec)	2° Target Temp (°C)	Stepsize (°C)	Step Delay (Cycles)	Acquisition Mode
1	95	600	20	0	0	0	None
Program:				Type:	Quantification	Cycles	45
Segment Number	Temperature Target (°C)	Hold Time (sec)	Slope (C'/sec)	2° Target Temp (°C)	Stepsize (°C)	Step Delay (Cycles)	Acquisition Mode
1	95	10	20	0	0	0	None
2	60	10	20	0	0	0	Single
3	72	11	20	0	0	0	None
Program:				Type:	Melting Curves	Cycles	1
Segment Number	Temperature Target (°C)	Hold Time (sec)	Slope (C'/sec)	2° Target Temp (°C)	Stepsize (°C)	Step Delay (Cycles)	Acquisition Mode
1	95	20	20	0	0	0	None
2	40	20	20	0	0	0	None
3	80	0	0.2	0	0	0	Continuous
Program:				Type:	None	Cycles	1
Segment Number	Temperature Target (°C)	Hold Time (sec)	Slope (C'/sec)	2° Target Temp (°C)	Stepsize (°C)	Step Delay (Cycles)	Acquisition Mode
1	40	30	20	0	0	0	None

Select a suitable Color Compensation file for this Protocol. It should not be older than six months.

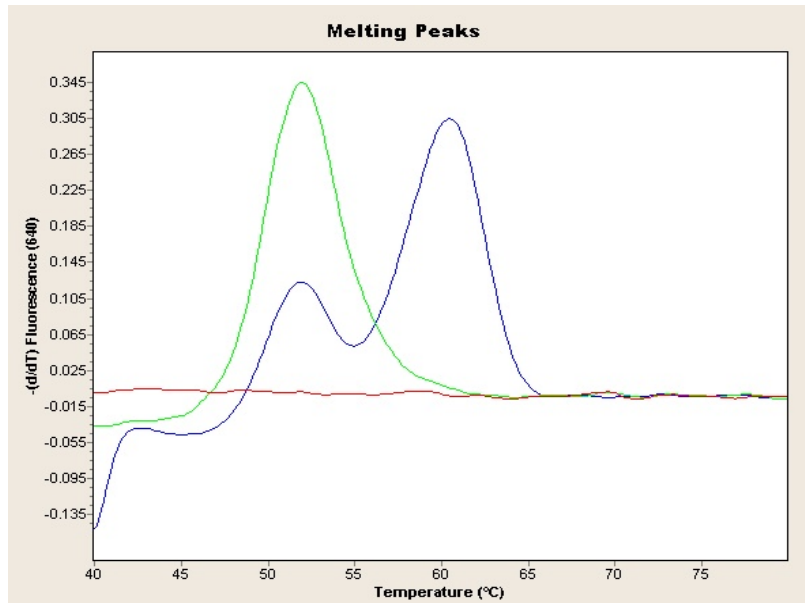
## 9. INTERPRETATION OF RESULTS

The provided positive controls (red A, B) contain templates heterozygous for the corresponding mutations (ApoE\*112 respectively ApoE\*158).

The results of the melting point curve analysis for mutation ApoE\*112 is measured on channel F2 with 640 nm and for mutation ApoE\*158 on channel F3 with 705 nm.

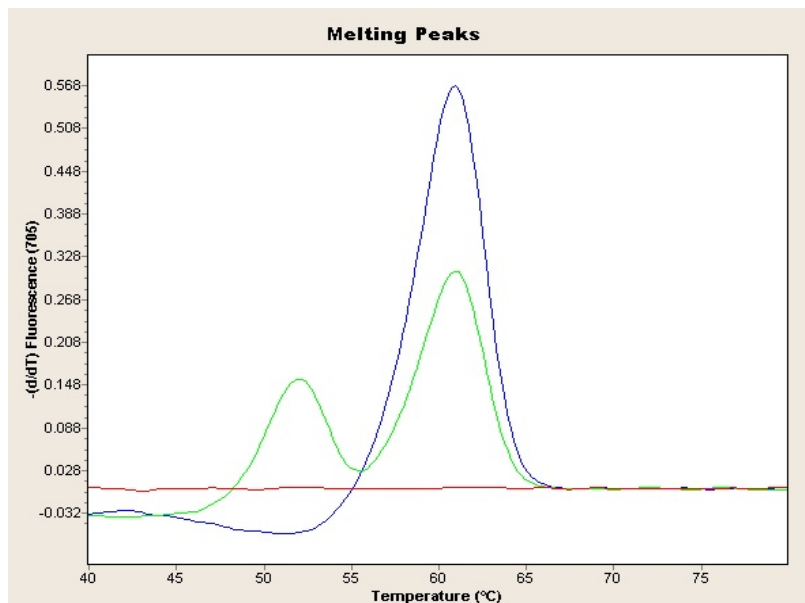
The following diagram shows typical example of heterozygous samples. The temperatures should be in the range of +/-2°C.

**APOE\*112**



temperature TGC: 52,0 °C  
 temperature CGC: 61,0 °C

**APOE\*158**



temperature TGC: 52,0 °C  
 temperature CGC: 60,5 °C

Interpretation of ApoE genotypes:

	*112 TGC	*112 CGC	*158 TGC	*158 CGC
e2/ e2	X	-	X	-
e3/ e3	X	-	-	X
e4/ e4	-	X		X
e2/ e3	X	-	X	X
e2/ e4	X	X	X	X
e3/ e4	X	X	-	X

## 10. TROUBLESHOOTING

**No** fluorescence peak with positive control or the samples at 640 Nm (F2) or 705 Nm (F3):

- Check the Run Protocol of the LightCycler®.  
⇒ Repeat the analysis with corrected Run Protocol.
- The MutaREAL® ApoE (LightCycler® PCR kit) was thawed more than twice and frozen again or was exposed for a longer time to temperatures of more than 4°C.  
⇒ Consider the storage conditions – especially for the enzyme. Repeat analysis with a new MutaREAL® ApoE (LightCycler® PCR kit).
- Poor sample DNA quality.  
⇒ Exactly follow the instruction manual provided with the DNA -extraction Kit.

**Weak** fluorescence peak at 640 Nm (F2) or 705 Nm (F3):

- Mix the components before use by careful pipetting, do not vortex!
- Cool all solutions during work (enzyme at -20°C) and protect detection-mix against light.
- Work only with a Cooling block, which has been precooled at +4 °C.