MutaGEL ACE

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1. Intended Use

Code: MG0001

The kit "MutaGEL® ACE" allows to detect the insertion / deletion (**I** / **D**) DNA polymorphism in the intron 16 of the ACE gene coding for the human angiotensin converting enzyme. The insertion itself corresponds to an *alu* repetitive sequence.

2. Introduction

The angiotensin converting enzyme (ACE) is the most important regulator of the renin/ angiotensin/ aldosteron system (RAAS). The enzyme converts angiotensin 1 into the physiological active form angiotensin 2. Latter one unfolds its vasoconstrictive effect directly into the organs (heart, kidney, vessels). The insertion/ deletion polymorphism in the ACE gene is associated immediately at the circulating ACE level. Therefore the **I** / **D** - polymorphism influences the risk for cardiovascular complications.

3. Test Principle

The kit **MutaGEL® ACE** contains a set of primers which amplify the region of the gene where the insertion or deletion occurs. This leads to the following amplification pattern:

- amplicon with 490 bp length when the alu sequence is inserted (allele **I**)
- amplicon with 190 bp length when the alu sequence is deleted (allele **D**)

After PCR, the amplificates are subsequently detected by gelelectrophoretic methods.

4. Materials Supplied (24 determinations)

▪ Primer ACE	1 x 55 µl (**green**)	solution of oligonucleotides specific for the ACE gene.
▪ PCR master mix	1 x 275 µl (**violet**)	ready to use PCR reagents (hot start TAQ enzyme, $MgCl_2$, dNTP, buffer)
▪ ACE positive control	1 x 20 µl (**red**)	aqueous solution of human DNA pool with genotype ACE I/D.
▪ (H2O) deionized	1 x 300 µl (white)	PCR water

5. Material required but not Supplied

Reagents and Instruments:

- DNA extraction kit (e. g. BLOOD MINIPREP, Code: KBR3005)
- Reagents for gelelectrophoresis
- Mineral oil (for thermal cyclers without heated lid)
- Thermal cycler
- Pipettes (0,5 - 200 µl) and sterile pipette tips
- Sterile micro tubes suitable for the thermal cycler in use
- Instruments for gel electrophoresis

6. Storage and Stability

Storage at < -18°C. The reagents are stable in the unopened micro tubes until the expiration date indicated. Don't thaw out the content of the "ACE positive control DNA" more than twice. If necessary, make suitable aliquots.

Before use: Spin tubes briefly before opening (contents may become dispersed during shipment).

Warnings and Precautions

For in vitro diagnostic use.

- Specimens and controls should be handled as if potentially infectious.
- Don't use the kit after its expiration date.
- Set up (if possible) three separate working areas:
  1) DNA isolation
  2) Preparing amplification
  3) Detection of the amplification products
- Use different tips and wear separate coats and gloves in each area
- Use sterile plugged tips for pipetting or use special PCR pipettes for aerosol free pipetting.
- Routinely decontaminate your pipettes and the laboratory benches.
- Avoid aerosols.
- The kit allows performance of 24 amplifications.

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Procedure

The complete procedure is divided in three steps:

1) Sample preparation
2) Amplification with primers specific for the ACE gene
3) Analysis of genotype by gelelectrophoretical detection of the amplified DNA

8. Sample preparation

- Extract total genomic DNA (e.g. from 200 µl whole blood) using a commercial extraction kit after the manufacturer´s instruction.
- Start immediately with the amplification procedure or store the extracted DNA at < -18°C.

9. Amplification

- Every set of amplifications should include a positive and a negative control.
- Prepare for each sample, positive control, and negative control the following Master-Mix (multiply the volumes necessary for each reaction with the number **N** of reactions, and add one volume).

Reagents for PCR	Total volume for each reaction: 25 µl	Volume in the Master-Mix
Primer ACE	2 µl	2 µl x (N+1)
PCR master mix	10 µl	10 µl x (N+1)
PCR water	10 µl	10 µl x (N+1)

- aliquot 22 µl of the master mix in sterile micro tubes suitable for the thermal cycler
- samples: add 3 µl of the extracted DNA to the master mix (about 20 ng/µl).
- positive control: add 3 µl of the “ACE positive control DNA” to the master mix
- negative control: add 3 µl of PCR water to the master mix
- if necessary overlay the Mix with 60 µl of mineral oil
- transfer the micro tubes into the thermal cycler
- perform the following cycles:

Initial Hold:	94°C for 12 minutes
30 cycles:	**94°C** for 60 seconds / **57°C** for 60 seconds / **72°C** for 90 seconds
Final Hold:	72°C for 5 minutes, 4°C follow up

10. Detection of the amplified DNA

- Carry out gel electrophoresis in **1,5 %** agarose (or polyacrylamide 20%) for about **75 Vh** (e. g. 50 min at 90 volt) in 1x TBE-buffer: mix **15 - 20 µl** of each digestion mix with **4 µl** loading buffer (e.g. KAN01070) and load the gel. The length of the amplified DNA fragments can be equalized with a suitable molecular weight standard (e.g. KBR311005).
- The separated DNA is colored by ethidiumbromide or SybrGreen (5 µg/ml) for 5 min and visualised under UV-light (312 nm).
- The amplification produces following amplificates with fragment-lengths corresponding to the present genotype:

GENOTYPE	Length of the amplified DNA (in base pairs)
I/I	490 bp
I/D	490 190 bp
D/D	190 bp

- **The ACE positive control DNA has the genotype I / D.**
- In any case the negative controls must be negative for any amplification product.

12. Restrictions

The PCR results for positive control in DNA fragments of indicated length and for the samples at least one of both amplification products must be detactable. If this is not the case, the sample must be tested a second time or the complete analysis must be repeated with freshly isolated DNA. If there are no positive control DNA fragments present, the amplification was incorrect and the chosen PCR conditions have to been proven/ corrected.

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