



*Immunoassay Kits Beyond The Ordinary*

## **MutaGEL<sup>®</sup> ACE**

To detect the insertion / deletion (I/D) DNA polymorphism in the intron 16 of the ACE gene coding for the human angiotensin converting enzyme.

For Research Use Only. Not For Use In Diagnostic Procedures.

Catalog Number:	30-MG0001
Size:	24 Determinations
Version:	01/2005 – ALPCO 02/20/06

### **ALPCO Diagnostics**

26 Keewaydin Drive Unit G • Salem, NH 03079

Phone: (800) 592-5726 • Fax: (603) 898-6854

[www.alpco.com](http://www.alpco.com) • Email: [web@alpco.com](mailto:web@alpco.com)

This protocol is for reference purposes only.  
DO NOT use this copy to run your assay;  
use the protocol included with the kit ONLY.

**1. Intended Use****Code: 30-MG0001**

The kit "MutaGEL<sup>®</sup> 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<sup>®</sup> 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 amplicates are subsequently detected by gelelectrophoretic methods.

**4. Materials Supplied (24 determinations)**

- |                                |            |  |
|--------------------------------|------------|--|
| ▪ primers ACE                  | 1 x 55 µl  | solution of oligonucleotides specific for the ACE gene.                            |
| ▪ PCR master mix               | 1 x 275 µl | ready to use PCR reagents (hot start TAQ enzyme, MgCl <sub>2</sub> , dNTP, buffer) |
| ▪ ACE positive control         | 1 x 15 µl  | aqueous solution of human DNA pool with genotype ACE I/D.                          |
| ▪ (H <sub>2</sub> O) deionized | 1 x 300 µl | PCR water, also for negative control   |

**5. Material required but not Supplied**

Reagents:

- DNA extraction kit (f.e.: HELIX FAST BLOOD DNA, Code: KDH8010 or BLOOD MINIPREP, Code: KBR3005)
- Reagents for gelelectrophoresis
- Mineral oil (for thermal cyclers without heated lid)

Instruments:

- Thermal cycler
- Pipettes (0,5 - 1000 µl) and sterile pipette tips
- Sterile micro tubes suitable for the thermal cycler in use
- apparatuses for gel electrophoresis

**6. Storage and Stability**

Store 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 RESEARCH use only.*

- 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 (e.g. DNA Cleaner; Code KDDC001).
- Avoid aerosols.
- The kit allows to perform 24 amplifications (20 samples and 4 controls).
- The PCR process is owned by Hoffman-La Roche Ltd and is covered by European patents n. 201.184 and 200.362. Use of products to perform PCR requires to obtain a license from Hoffman-La Roche Ltd. .

**This protocol is for reference purposes only.  
DO NOT use this copy to run your assay;  
use the protocol included with the kit ONLY.**

**Procedure**

The complete procedure is divided in three steps:

- 1) Sample preparation
- 2) Amplification with primers specific for the ACE gene
- 3) Detection of the amplified DNA

**8. Sample preparation**

- Extract total genomic DNA from 200 µl of 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: 45 µl	Volume in the Master-Mix
primers 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:

<b>1 cycle:</b>	94°C for 5 minutes
<b>30 cycles:</b>	94°C for 60 seconds / 57°C for 60 seconds / 72°C for 90 seconds
<b>1 cycle:</b>	72°C for 5 minutes

**10. Detection of the amplified DNA**

- Carry out a gel electrophoresis (2 % agarose or polyacrylamide 10 %) with at least 10 µl of both the amplified and the digested DNA in order to obtain a complete separation of the different fragments.
- The use of pre-cast polyacrylamide gels (Code: KAN10112), 5x TBE running buffer (Code: KAN10060), 6x loading buffer (Code: KAN01070) and molecular weight marker pBS/ *MspI* (KDGC015001) is recommended.
- The amplification produces following fragments:

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.

