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# KIT FOR THE DETECTION OF R3500Q POLYMORPHISM IN THE PROMOTER OF THE GENE APOLIPROTEIN B (APOB)

## AMPLI-SET-ApoB Cat. n.1.355

Apo-lipoproteins, as protein components of plasmatic lipoproteins, play a main function in the lipid metabolism. Apo-lipoprotein B-100 (apoB-100) is the main protein component of low density lipoproteins, lipoproteins which carry almost two third of all the plasmatic cholesterol. ApoB -100 is necessary for the construction, secretion and metabolism of the lipoproteins . Particularly, the high affinity interaction between LDL and the receptor of LDL ( located on the surface of almost all human cells) occurs through apoB-100 , that is the physiological ligand responsible of the regulation of plasmatic LDL-cholesterol (LDL-C) levels.
The gene encoding for apoB-100 (protein of 4563 aa) is located on the short arm of chromosome 2 and is made of 28 introns and 28 exons. Many puntiform mutation have been reported in the putative binding domain of apoB-100 and the LDL receptor .The first mutation reported , the more frequent, is the substitution of a base, from CGG to CAG, at the nucleotide 10708 in exon 26. This mutation leads to an amino acid substitution , in the codon 3500, Arginin in Glutamin (R3500Q) that changes the structure of the binding domain of apob-10 decreasing the affinity to the LDL receptor (3-9% of normal state). The mutation R3500Q is responsible of "family defective apoliproteinB-100", an autosomal disorder related to high plasmatic concentration of total cholesterol and LDL-C. The mutation R3500Q has a frequency between 1:500 and 1:700 in Caucasian population.
The detection of the mutation G10708A is performed with amplification with specific primers for the exon 26 of the apoB gene .The yield is a fragment of 125 bp cut by the enzyme *MspI*. The presence of mutation is confirmed by the loss of a restriction site. **The yield of PCR of the normal allele 10708G produce , after enzymatic digestion, two fragments of 101 bp and 24 bp, whereas the mutant allele 10708A isn't cut and remains a fragment of 125 bp.**

**Principle of method:** A) extraction of genomic DNA B) amplification C) enzymatic digestion D)detection on agarose gel.
**Applicability:** On extracted and purified genomic DNA from whole blood samples.
**Tests:** 45

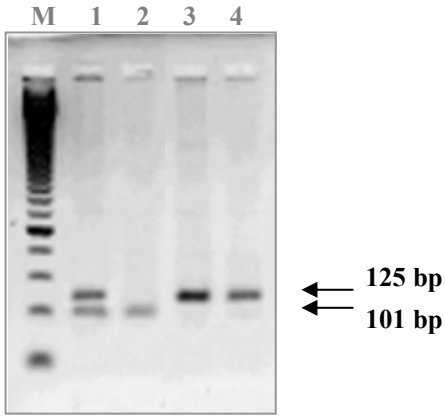
## REAGENTS AND STORAGE

| AMPLIFICATION | |
|---|---|
| Mix PCR APO-B | -20°C |
| Msp I enzyme (20U/µl) | -20°C |
| Digestion buffer 10X | -20°C |
| $H_2O$ RNase/DNase-free | -20°C |
| Taq Polymerase (5U/µl) | -20°C |
| DNA normal control | -20°C |

**Stability:** over 12 months if correctly stored.

## ANALYSIS OF RESULTS

The yield of amplification is a fragment of 125bp. The next restriction section made by the *MspI* enzyme can be done the following results:




M) Marker 50 bp ladder
1) HETEROZIGOUS subject for R3500Q
2) NORMAL subject (DNA normal control)
3) HOMOZIGOTE subject for R3500Q
4) Undigested PCR product

**References:**
Brown MS Sciences 1986 232:34
Knott TJ et al. Nature 1986 323:734
Blackhart BD et al. J Biol Chem 1986 261:15364
Innerarity TL et al. PNAS 1987 84:6919-6923
Tybjaerg-Hansen A et al. Atherosclerosis 1992 96:91-107
Fisher E et al. Clinical Chemistry 1999 45:1026-1038
Vrablik M et al. Physiol Res 2001 50:337-343