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CE

DETECTION OF A77D MUTATION IN THE FERROPORTIN GENE (SLC11A3)

AMPLI-set-EMO A77D Cat. n. 1.321

Hemochromatosis is an inherited disorder with an estimated prevalence of up to 1 in 100 individuals in northen European population. Many patients (80%) present an autosomal-recessive pattern. The mutation is located in the HFE gene.
Recently two mutations (A77D and N144H) inherited as autosomal dominant trait in the gene encoding for Ferroportin have been described. Ferroportin (SLC11A3) is a transmembrane iron export protein.
The kit allows the detection of the mutation A77D in the SLC11A3 gene changing an alanine in aspartic acid. The detection of the mutation is carried out using the amplification with specific primers followed by restriction section due to MboII enzyme.The mutation creates a new MboII site, therefore the PCR fragment (125 bp) containing the mutation is cleaved into two fragments (94 and 31 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.
Numbers of Tests: 45.

REAGENTS AND STORAGE

AMPLIFICATION and DIGESTION	
PCR mix	-20°C
Water DNase-RNase free	-20°C
Taq Polymerase (5U/µl)	-20°C
Mbo II Enzyme (10U//µl)	-20°C
Digestion BUFFER 10X	-20°C
Positive control heterozigous	-20°C

Stability: over 12 months if correctly stored.

ANALYSIS OF RESULTS

The amplification yield is of 127 bp .The mutation A77D adds a restriction site for the MboII enzyme, obtaining two fragments respectively of 94 bp and 33 bp.Il prodotto di amplificazione e' un frammento di 127 bp. La mutazione A77D provoca l'inserzione di un sito di restrizione per l'enzima MboII in modo da ottenere due frammenti rispettivamente di 94bp e 33 bp.

1 **Absence of mutation normal homozygous**	2 **Presence of mutation mutant heterozygous**	3 **Presence of mutation Mutant homozygous**
Presence of 1 band	Presence of 3 bands	Presence of 2 band
127 bp	**127 bp** **94 bp** **33 bp**	**94 bp** **33 bp**

References:
Robert E. Fleming et al. *J. Clin. Invest. 108:521-522 (2001).*
Njajou OT et al. *Nat. Genet. 28 (2001).*
Montosi G et al. *J. Clin. Invest. 108: 619-623 (2001).*