DETECTION OF N144H MUTATION IN THE FERROPORTIN GENE (SLC11A3)

AMPLI-set-EMO N144H Cat. n. 1.322

Hemochromatosis is an inherited disorder with an estimated prevalence of up to 1in 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 N144H in the SLC11A3 gene changing an aspartic acid in histidine. The detection of the mutation is carried out using the amplification of a fragment of 310 bp with specific primers followed by restriction section due to Apo I enzyme. The mutation causes the loss of a Apo I site, so the normal allele produces two fragments of 230 bp and 80 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
Apo I Enzyme (10U//µl)	-20°C
Digestion BUFFER 10X	-20°C
BSA 100X	-20°C
Wild type control	-20°C

Stability: over 12 months if correctly stored.

ANALYSIS OF RESULTS

1 **Absence of mutation normal homozygous**	2 **Presence of mutation mutant heterozygous**	3 **Presence of mutation Mutant homozygous**
Presence of 2 band	Presence of 3 bands	Presence of 1 band
230 bp **80 bp**	**310 bp** **230 bp** **80 bp**	**310 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).*