Identification of EMO

C282Y, H63D and S65C gene mutation in capillary electrophoresis

AMPLI-set- EMO C282Y, H63D, S65C Cat. n. 1.323

Hemochromatosis is a condition that causes increased absorption of dietary iron and a progressive accumulation of iron in the body, often it is an inherited disorder. Unfortunately it is still underappreciated and often accidentally discovered during regular examinations or as a result of the appearance of one of its complications.
It is estimated in Italy and in some regions of Europe and elsewhere that there are 2-5 patients out of 1,000 individuals and 9-15 bearers of 100. Hemochromatosis is therefore the most common hereditary disease in the western world.
In 1996 the hemochromatosis gene (HFE gene) was identified and three mutations (C282Y, H63D, and S65C) have been described. The majority of individuals with hemochromatosis (80-100%) are homozygous for the C282Y mutation, while a small percentage of them was found to be compound heterozygotes for C282Y and H63D mutations. The homozygosity for the H63D mutation is not clearly associated with hemochromatosis. The S65C mutation, however, is present in 1.5% of the European population.
The kit allows the simultaneous analysis of the mutations C282Y, H63D and S65C by PCR (polymerase chain reaction) technique and subsequent reaction of "primer extension". The reaction is carried out by multiplex PCR in which amplifies exon 2 of the gene for mutations H63D and S65C, and the exon 4 for the C282Y mutation. Subsequently the amplified it will be used as a mold for a extension reaction using a specific oligonucleotide (primer extension). This allows the identification of the individual mutations to difference in a single nucleotide base. The amplification products, labeled with different fluorophores for each nucleotide base, can be solved with a tool for capillary electrophoresis equipped with software for analysis of the results.

Principle of method: A) extraction of genomic DNA
B) amplification.
Applicability: On extracted and purified genomic DNA sample

number of tests: 45.

Kit's content and its storage

Amplification and digestion	
I PCR multiplex mix	-20°C
Primer extension mix C282Y, H63D, S65C	-20°C
H_2O sterile	-20°C
Taq Polymerase (5U/µl)	-20°C
Control C282Y eterozigote	-20°C
Control H63D eterozigote	-20°C
Control S65C eterozigote	-20°C

Stability: more than 18 months if properly stored.

Analysis of the results

The reaction of "primer extension" is carried out with oligonucleotides of different length in nucleotide bases in relation to the mutation to be analyzed: 25 bases for the H63D mutation, the mutation S65C to 31 bases and 21 bases for the C282Y mutation. Each dideoxynucleotide triphosphate (ddNTP) is labeled with a different fluorophore (A with dR6G, with dTAMRA C, G and T with dR110 drox). The incorporation of the single dideoxynucleotide triphosphate (ddNTP) labeled and complementary to the base that characterizes the mutation allows the diagnosis for the different mutations analyzed. The resolution of capillary electrophoresis allows, therefore, to identify the difference in length of the nucleotide bases and in the type of built-in base (C or G for the H63D mutation, T or A in the mutation S65C, G or A for the C282Y mutation).

Biografia:
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