

HFE S65C MUTATION

ORDERING INFORMATIONS

REF: GEN-019-25 RDM Code: 2257822/R
 Tests: 25 Reactions: 31
 REF: GEN-019-50 RDM Code: 2142454/R
 Tests: 50 Reactions: 62
 CND Code: W0106010105
 Manufacturer: BioMol Laboratories s.r.l.

CONTENTS OF THE KIT

The kit consists of: reagents for Real-Time PCR amplification
 *reagents for the extraction of genomic DNA are not supplied in the kit

For in vitro diagnostic use



PRODUCT CHARACTERISTICS

Detection of S65C mutation (c.193 A>T; AGT>TGT; Ser65Cys) of the HFE gene by Real-Time PCR technique. Kit optimized for Real-Time PCR instrumentation Biorad CFX96 Dx, Biorad Opus Dx and Agilent AriaDx.

SCIENTIFIC BACKGROUND

Hemochromatosis is a set of hereditary diseases characterized by the development of a progressive accumulation of iron in the body. Five genetically distinct forms of hemochromatosis are recognized, with varying frequency and severity.

§ Feder J. N., Gnirke A., Thomas W., et al. A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis. *Nature genetics*. 1996; 13 (4):399-408.

§ Lian J, Xu L, Huang Y, Le Y, Jiang D, Yang X, Xu W, Huang X, Dong C, Ye M, Zhou J, Duan S. Meta-analyses of HFE variants in coronary heart disease. *Gene*. 2013 Sep 15; 527(1):167-73. doi: 10.1016/j.gene.2013.06.034.

§ Hanson E. H., Imperatore G., Burke W. HFE Gene and Hereditary Hemochromatosis: A HuGE Review. *American Journal of Epidemiology*. 2001; 154(3):193-206. doi: 10.1093/aje/k154.3.193.

§ Ogoyma-Aworet L, Rabes JP, de Mazancourt P. A Simple RFLP-Based Method for HFE Gene Multiplex Amplification and Determination of Hereditary Hemochromatosis-Causing Mutation C282Y and H63D Variant with Highly Sensitive Determination of Contamination. *Biomed Res Int*. 2020 Dec 28; 2020:9396318. doi: 10.1155/2020/9396318. eCollection 2020. PMID: 33457423

CLINICAL SIGNIFICANCE

Type 1 hemochromatosis: it is an autosomal recessive disease with incomplete penetrance that causes an excessive accumulation of iron in the tissues, responsible for chronic liver disease, fibrosis, cirrhosis and an increase in cardiovascular phenomena such as coronary heart disease (CHD). The most common form is caused by a homozygous C282Y (G>A, rs1800562) mutation in exon 4 of the HFE gene. In exon 2 of the HFE gene, however, there is a further variant, H63D (C>G, rs1799945) widely studied together with the C282Y variant in cases of iron overload, as metagenetic analyzes have shown that this allele is heterozygous with the C282Y allele might in some populations increase the risk of coronary heart disease. Adjacent to the H63 residue, the S65C variant (rs1800730) was also identified, which in the form of the C282Y/S65C genotype can produce a mild HH phenotype.

Type 2 hemochromatosis: rarer than the previous one, it is distinguished in two forms, both transmitted in an autosomal recessive way: type 2a due to mutations in the hemojuvelin gene and type 2b caused by mutations in the hepcidin gene (HAMP).

Type 3 hemochromatosis: determined by mutations in the transferrin receptor gene (TFR2);

Type 4 hemochromatosis: due to mutations in the ferroportin gene (SLC40A1 or FPN1), transmitted in an autosomal dominant manner.

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DESCRIPTION	LABEL	VOLUME		STORAGE
		GEN-019-25	GEN-019-50	
Mix oligonucleotides and probes	Mix HFE S65C 10X	1 x 85 µl	1 x 170 µl	-20°C
Mix buffer and Taq polymerase enzyme	Mix Real-Time PCR 2X	1 x 425 µl	1 x 850 µl	-20°C
Deionized H ₂ O	Deionized H ₂ O	2 x 1 ml	2 x 1 ml	-20°C
Genomic DNA or recombinant DNA	Control + 1	1 x 22µl	1 x 22 µl	-20°C
Genomic DNA or recombinant DNA	Control + 2	1 x 22µl	1 x 22µl	-20°C
Genomic DNA or recombinant DNA	Control + 3	1 x 22µl	1 x 22µl	-20°C

TECHNICAL CHARACTERISTICS

COD. GEN-019-25 / COD. GEN-019-50

STABILITY	18 months
REAGENTS STATUS	Ready to use
BIOLOGICAL MATRIX	Genomic DNA extracted from whole blood, tissue, cells
POSITIVE CONTROL	Recombinant DNA for at least 3 analytical sessions
VALIDATED INSTRUMENTS	Biorad CFX96 Dx, Biorad Opus Dx e Agilent AriaDx
TECHNOLOGY	Real-time PCR; oligonucleotides and specific probes; 2 FAM/HEX fluorescence channels
RUNNING TIME	85 min
THERMAL CYCLING PROFILE	1 cycle at 95 °C (10 min); 45 cycles at 95 °C (15 sec) + 60 °C (60 sec)
ANALYTICAL SPECIFICITY	Absence of non-specific pairings of oligonucleotides and probes; absence of cross-reactivity
ANALYTICAL SENSITIVITY : LIMIT OF DETECTION (LOD)	≥ 0,016 ng of DNA
ANALYTICAL SENSITIVITY : LIMIT OF BLANK (LOB)	0% NCN
REPRODUCIBILITY	99,9%
DIAGNOSTIC SPECIFICITY / DIAGNOSTIC SENSITIVITY	100%/98%