CERTIFIED COMPANY
UNI EN ISO 9001, UNI EN ISO 13485

MAJOR HISTOCOMPATIBILITY COMPLEX, CLASS I, G (HLA-G)
Variant 3'UTR 14bp del/ins

AMPLI Set HLA G Cat. n.1.505

HLA-G (major histocompatibility complex class I, G) is a protein coding gene.
HLA-G gene is on chromosome 6 (6p21.3) and the gene most polymorphic regions are in the regulation region le region 5' (5'UTR) and in the untranslated region 3' (3'UTR); those regions may contribute to expression HLA-G regulation.
The diseases associated with HLA-G are vaginal cancer, serious pre-eclampsia and couple's infertility. As fetus genotype depends on both parents, in order to evaluate the embryo potential protein sHLA-G production the testing of HLA-G gene variants is performed on both partners.
The HLA-G molecules, membrane (HLA-G1, G2, G3 e G4) and soluble (sHLA-G1 by proteolytic cleavage, sHLA-G5 by alternative splicing, sHLA-G6 e G7), have tolerogenic functions to innate and adaptative cell response. On maternal-fetal interface, the HLA-G molecule is one of the responsible factors for the immunologic tolerance establishing promoting embryo implantation.
The majority of embryos doesn't implant (> 70%) and only a minority (about 14%) will lead to a near term pregnancy. Nowadays the embryo screening is based mainly on morphologic criteria and cell division.
Scientific articles have been showing the importance of some molecules in embryo developing regulation before the implantation and to the implanting itself. A potential marker is sHLA-G protein (HLA-G soluble). sHLA-G was found in cultures' supernatant of human embryos obtained by IVF; a recent study shows that the presence of this protein is a mandatory prerequisite, but not sufficient, for the implanting process and therefore pregnancy. A clinic pregnancy is possible only if sHLA-G is in the embryo supernatant culture when the transferring is taking place. In addition a poor maternal sHLA-G expression, has been associated with pre-eclampsia, miscarriage and IVF failures. HLA-G seems to have a protective role in the organ transplantation (avoiding allegenic rejection) and in the autoimmune diseases (inhibiting the immune response against self antigene). Recently the HLA-G molecule expression was observed in some tumors, where it may have an important role in the "immune-editing" and "immune-escape". The insertion/deletion of 14 bp (rs16375) in 3'UTR of eson 8 has been correlated to mRNA stability and to protein HLA-G quantity; the insertion (+14 bp) makes mRNA more unstable, consequently it gives rise to a smaller production of HLA-G and lower levels of sHLA-G.
This kit allows the identification of the insertion/deletion of 14bp in 3' UTR of exon 8 of HLA-G gene with bi-PASA (bidirectional PCR Allele Specific Amplification) and revelation on agarose gel 3% or capillary electrophoresis. As result of the amplification the homozygous ins/ins will show two fragments of 436 and 304 bp; homozygous del/del will show two fragments of 436 and 158 bp; heterozygous ins/del will produce three fragments of 436, 304 and 158 bp.

Assay principle: A) genomic DNA estraction B) amplification C) agarose gel revelation/capillary electrophoresis.
Applicability: genomic DNA from peripheral blood or mouth swab.
Test: 25

REAGENTS AND STORAGE

AMPLIFICATION	
PCR mix HLA G ins/del	-20°C
H_2O sterile	
Taq Polymerase (5U/µl)	-20°C
Heterozygous control ins/del	-20°C

Stability: over 12 months if correctly stored.

RESULTS' INTERPRETATION

Eterozigote Ins/Del	Omozigote Ins/Ins	Omozigote Del/Del
436 bp	436 bp	436 bp
304 bp	304 bp	
158 bp		158 bp

Reference:
Eskandari-Nasab E. Cancer Biomark.; 13, 4: 253-9 (2013)
Piancatelli D. Transplant Proc. 2009 May;41(4):1187-8.