

HLA-G 14 bp INS/DEL POLYMORPHISM

ORDERING INFORMATIONS

REF: HLA-001-25 RDM Code: 2256387/R
 Tests: 25 Reactions: 31
 REF: HLA-001-50 RDM Code: 1694059/R
 Tests: 50 Reactions: 62
 CND Code: W106010499
 Manufacturer: BioMol Laboratories s.r.l.

CONTENTS OF THE KIT

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

For in vitro diagnostic use



PRODUCT CHARACTERISTICS

Detection of Ins/Del 14 bp polymorphism of the HLA-G gene (rs16375) by Real-Time PCR technique. Kit optimized for Real-Time PCR instrumentation Biorad CFX96 Dx, Biorad Opus Dx and Agilent AriaDx.

SCIENTIFIC BACKGROUND

Human leukocyte antigen G (HLA-G) is a member of the HLA class I family. The HLA-G gene is located in chromosomal region 6p21.3 and its exon/intron structure resembles that of other classical class I genes (HLA-A, HLA-B or HLA-C), composed of seven introns and eight exons that encode the heavy chain of the molecule. Under physiological conditions HLA-G is highly expressed in fetal cells at the maternal-fetal interface, thymus, pancreas, cornea, nail matrix and erythroblasts during hematopoiesis. The membrane-bound or soluble HLA-G protein strongly binds its receptors on immune cells, inhibits the functions of these effectors, and causes immune inhibition.

§ *Recent Advances in Our Understanding of HLA-G Biology: Lessons from a Wide Spectrum of Human Diseases.* J Immunol Res. 2016;2016:4326495. doi: 10.1155/2016/4326495. Epub 2016 Aug 29. Review

§ *The HLA-G 14bp polymorphism and recurrent implantation failure: a meta-analysis.* J Assist Reprod Genet. 2017 Nov;34(11):1559-1565.

§ *HLA-G 3' untranslated region polymorphic sites associated with increased HLA-G production are more frequent in patients exhibiting differentiated thyroid tumours.* Clin Endocrinol (Oxf). 2017 Apr;86(4):597-605.

§ *The impact of HLA-G 3' UTR variants and sHLA-G on risk and clinical correlates of schizophrenia.* Hum Immunol 2016 Dec;77(12):1166-1171.

CLINICAL SIGNIFICANCE

HLA-G protein can be expressed de novo at high levels in several pathological conditions, including solid and hematologic tumors and during microbial or viral infections, leading to impaired immune response against tumor cells or pathogens, respectively. On the other hand, loss of HLA-G-mediated control of immune responses can lead to the onset of autoimmune/inflammatory diseases, caused by uncontrolled activation of immune effector cells. HLA-G also has an important role in human pregnancy as the different isoforms of HLA-G are expressed by trophoblast cells at the maternal-fetal interface. HLA-G expressed and released by trophoblast cells can interact with cellular receptors expressed by immune (T cells, NK cells, macrophages and dendritic cells) and non-immune cells (endothelial cells) present in the decidua, activating inhibitory or activating signals. It has been demonstrated that low levels of expression of this soluble protein do not seem to trigger the process of immunological tolerance necessary for the survival of the embryo. The most polymorphic regions of the gene are in the 5'UTR and 3'UTR regulatory regions which may contribute to the regulation of HLA-G expression. The 14-bp insertion/deletion polymorphism (rs16375) in the 3'UTR region of exon 8 correlated with mRNA stability and the amount of HLA-G protein produced. The allele with a 14bp insertion was associated with lower HLA-G expression levels than the allele with the 14bp deletion.

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DESCRIPTION	LABEL	VOLUME		STORAGE
		HLA-001-25	HLA-001-50	
Mix oligonucleotides and probes	Mix Ins/Del 14 bp HLA-G 10 X	1 x 85 µl	1 x 170 µl	-20°C
Mix buffer and Taq polymerase enzyme	Mix Real-Time PCR 5X	1 x 170 µl	1 x 340 µl	-20°C
Deionized H ₂ O	Deionized H ₂ O	1 x 1 ml	1 x 1 ml	-20°C
Genomic DNA or recombinant DNA	Control + Del/Del	1 x 22 µl	1 x 22 µl	-20°C
Genomic DNA or recombinant DNA	Control + Ins/Del	1 x 22 µl	1 x 22 µl	-20°C
Genomic DNA or recombinant DNA	Control + Ins/Ins	1 x 22 µl	1 x 22 µl	-20°C

TECHNICAL CHARACTERISTICS

COD. HLA-001-25 / COD. HLA-001-50

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
TECHNOLOGY	Real-time PCR; oligonucleotides and specific probes; 2 fluorescence channels HEX/JOE and FAM
VALIDATED INSTRUMENTS	Biorad CFX96 Dx, Biorad Opus Dx e Agilent AriaDx
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%