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HLA-G siRNA (h): sc-42920

BACKGROUND

Major histocompatibility complex (MHC), human leukocyte antigen (HLA) molecules are cell-surface receptors that bind foreign peptides and present them to T lymphocytes. MHC class I molecules consist of two polypeptide chains, an α or heavy chain, and a non-covalently associated protein, β -2-Microglobulin. Cytotoxic T lymphocytes bind antigenic peptides presented by MHC class I molecules. Antigens that bind to MHC class I molecules are typically 8-10 residues in length and are stabilized in a peptide binding groove. MHC class II molecules are encoded by polymorphic MHC genes and consist of a non-covalent complex of an α and β chain. Helper T lymphocytes bind antigenic peptides presented by MHC class II molecules. MHC class II molecules bind 13-18 amino acid antigenic peptides. Accumulating in endosomal/lysosomal compartments and on the surface of B cells, HLA-DM and -DO molecules regulate binding of exogenous peptides to class II molecules (HLA-DR) by sustaining a conformation that favors peptide exchange. The differential structural properties of MHC class I and class II molecules account for their respective roles in activating different populations of T lymphocytes.

REFERENCES

1. Fournel, S., et al. 2000. Comparative reactivity of different HLA-G monoclonal antibodies to soluble HLA-G molecules. *Tissue Antigens* 55: 510-518.
2. Lozano, J.M., et al. 2002. Monocytes and T lymphocytes in HIV-1-positive patients express HLA-G molecule. *AIDS* 16: 347-351.
3. Pangault, C., et al. 2002. Lung macrophages and dendritic cells express HLA-G molecules in pulmonary diseases. *Hum. Immunol.* 63: 83-90.
4. Fuzzi, B., et al. 2002. HLA-G expression in early embryos is a fundamental prerequisite for the obtainment of pregnancy. *Eur. J. Immunol.* 32: 311-315.
5. Boyson, J.E., et al. 2002. Disulfide bond-mediated dimerization of HLA-G on the cell surface. *Proc. Natl. Acad. Sci. USA* 99: 16180-16185.

CHROMOSOMAL LOCATION

Genetic locus: HLA-G (human) mapping to 6p22.1.

PRODUCT

HLA-G siRNA (h) is a pool of 2 target-specific 19-25 nt siRNAs designed to knock down gene expression. Each vial contains 3.3 nmol of lyophilized siRNA, sufficient for a 10 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see HLA-G shRNA Plasmid (h): sc-42920-SH and HLA-G shRNA (h) Lentiviral Particles: sc-42920-V as alternate gene silencing products.

For independent verification of HLA-G (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-42920A and sc-42920B.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.

STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at -20° C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at -20° C, avoid contact with RNAses and repeated freeze thaw cycles.

Resuspend lyophilized siRNA duplex in 330 μ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μ l of RNase-free water makes a 10 μ M solution in a 10 μ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

HLA-G siRNA (h) is recommended for the inhibition of HLA-G expression in human cells.

SUPPORT REAGENTS

For optimal siRNA transfection efficiency, Santa Cruz Biotechnology's siRNA Transfection Reagent: sc-29528 (0.3 ml), siRNA Transfection Medium: sc-36868 (20 ml) and siRNA Dilution Buffer: sc-29527 (1.5 ml) are recommended. Control siRNAs or Fluorescein Conjugated Control siRNAs are available as 10 μ M in 66 μ l. Each contain a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA. Fluorescein Conjugated Control siRNAs include: sc-36869, sc-44239, sc-44240 and sc-44241. Control siRNAs include: sc-37007, sc-44230, sc-44231, sc-44232, sc-44233, sc-44234, sc-44235, sc-44236, sc-44237 and sc-44238.

GENE EXPRESSION MONITORING

HLA-G (4H84): sc-21799 is recommended as a control antibody for monitoring of HLA-G gene expression knockdown by Western Blotting (starting dilution 1:200, dilution range 1:100-1:1000) or immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500).

To ensure optimal results, the following support reagents are recommended: 1) Western Blotting: use m-IgG κ BP-HRP: sc-516102 or m-IgG κ BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker[™] Molecular Weight Standards: sc-2035, UltraCruz[®] Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgG κ BP-FITC: sc-516140 or m-IgG κ BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz[®] Mounting Medium: sc-24941 or UltraCruz[®] Hard-set Mounting Medium: sc-359850.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor HLA-G gene expression knockdown using RT-PCR Primer: HLA-G (h)-PR: sc-42920-PR (20 μ l, 481 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Yao, G.D., et al. 2014. Expression and potential roles of HLA-G in human spermatogenesis and early embryonic development. *PLoS ONE* 9: e92889.

RESEARCH USE

For research use only, not for use in diagnostic procedures.