

HLA-DR α siRNA (m): sc-37114

BACKGROUND

Major histocompatibility complex (MHC) class II molecules destined for presentation to CD4⁺ helper T cells is determined by two key events. These events include the dissociation of class II-associated invariant chain peptides (CLIP) from an antigen binding groove in mhc ii- α/β dimers through the activity of MHC molecules HLA-DM and -DO, and subsequent peptide antigen binding. Accumulating in endosomal/lysosomal compartments and on the surface of B cells, HLA-DM, -DO molecules regulate the dissociation of CLIP and the subsequent binding of exogenous peptides to HLA class II molecules (HLA-DR, -DQ and -DP) by sustaining a conformation that favors peptide exchange. RFLP analysis of HLA-DM genes from rheumatoid arthritis (RA) patients suggests that certain polymorphisms are genetic factors for RA susceptibility. HLA-B belongs to the HLA class I heavy chain paralogs. Class I molecules play a central role in the immune system by presenting peptides derived from the endoplasmic reticulum lumen. HLA-B and -C can form heterodimers consisting of a membrane anchored heavy chain and a light chain (β -2-Microglobulin). Polymorphisms yield hundreds of HLA-B and -C alleles.

REFERENCES

- Horejsi, V., et al. 1986. Characterization of seven new monoclonal antibodies against human DR, DR + DP and DQ1 + DQ3 antigens. *Tissue Antigens* 28: 288-297.
- Horejsi, V., et al. 1986. Monoclonal antibodies against human leucocyte antigens. I. Antibodies against β -2-Microglobulin, immunoglobulin κ light chains, HLA-DR-like antigens, T8 antigen, T1 antigen, a monocyte antigen, and a pan-leucocyte antigen. *Folia Biol.* 32: 12-25.
- Kropshofer, H., et al. 1998. A role for HLA-DO as a co-chaperone of HLA-DM in peptide loading of MHC class II molecules. *EMBO J.* 17: 2971-2981.
- Siegmund, T., et al. 1999. HLA-DMA and HLA-DMB alleles in German patients with type 1 diabetes mellitus. *Tissue Antigens* 54: 291-294.
- Arndt, S.O., et al. 2000. Functional HLA-DM on the surface of B cells and immature dendritic cells. *EMBO J.* 19: 1241-1251.
- Louis-Plence, P., et al. 2000. The downregulation of HLA-DM gene expression in rheumatoid arthritis is not related to their promoter polymorphism. *J. Immunol.* 16: 4861-4869.

CHROMOSOMAL LOCATION

Genetic locus: H2-Ea-ps (mouse) mapping to 17 B1.

PRODUCT

HLA-DR α siRNA (m) is a pool of 3 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-DR α shRNA Plasmid (m): sc-37114-SH and HLA-DR α shRNA (m) Lentiviral Particles: sc-37114-V as alternate gene silencing products.

For independent verification of HLA-DR α (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-37114A, sc-37114B and sc-37114C.

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-DR α siRNA (m) is recommended for the inhibition of HLA-DR α 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-DR α (G-7): sc-55593 is recommended as a control antibody for monitoring of HLA-DR α 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 MarkerTM 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-DR α gene expression knockdown using RT-PCR Primer: HLA-DR α (m)-PR: sc-37114-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

RESEARCH USE

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

PROTOCOLS

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