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SANTA CRUZ BIOTECHNOLOGY, INC.

HLA-DOβ siRNA (h): sc-42913

BACKGROUND

Peptide (antigen) binding to major histocompatibility complex (MHC) class II molecules destined for presentation to $CD4^+$ helper T cells is determined by two key events. These include the dissociation of class II-associated invariant chain peptides (CLIP) from an antigen binding groove in MHC II-Ig dimers and by the activity of MHC molecules HLA-DM and -DO. 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) 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.

REFERENCES

1. 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.
2. Siegmund, T., et al. 1999. HLA-DMα and HLA-DMβ alleles in German patients with type 1 diabetes mellitus. Tissue Antigens 54: 291-294.
3. Arndt, S.O., et al. 2000. Functional HLA-DM on the surface of B cells and immature dendritic cells. EMBO J. 19: 1241-1251.
4. Brunet, A., et al. 2000. Functional characterization of a lysosomal sorting motif in the cytoplasmic tail of HLA-DOβ. J. Biol. Chem. 275: 37062-37071.
5. Doebele, C.R., et al. 2000. Determination of the HLA-DM interaction site on HLA-DR molecules. Immunity 13: 517-527.
6. Louis-Plence, P., et al. 2000. The down-regulation of HLA-DM gene expression in rheumatoid arthritis is not related to their promoter polymorphism. J. Immunol. 165: 4861-4869.
7. Toussirot, E., et al. 2000. The association of HLA-DM genes with rheumatoid arthritis in eastern France. Hum. Immunol. 61: 303-308.

CHROMOSOMAL LOCATION

Genetic locus: HLA-DOB (human) mapping to 6p21.32.

PRODUCT

HLA-DOβ siRNA (h) 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see HLA-DOβ shRNA Plasmid (h): sc-42913-SH and HLA-DOβ shRNA (h) Lentiviral Particles: sc-42913-V as alternate gene silencing products.

For independent verification of HLA-DOβ (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-42913A, sc-42913B and sc-42913C.

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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCL, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

HLA-DOβ siRNA (h) is recommended for the inhibition of HLA-DOβ 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 µM in 66 µl. 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-DOβ (DOB.L1): sc-69739 is recommended as a control antibody for monitoring of HLA-DOβ 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-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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-DOβ gene expression knockdown using RT-PCR Primer: HLA-DOβ (h)-PR: sc-42913-PR (20 µl). 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.