SANTA CRUZ BIOTECHNOLOGY, INC.

HLA-DOα siRNA (h): sc-95165

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 and -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. HLA-DOα (HLA class II histocompatibility antigen, DO α chain) is a 250 amino acid single-pass membrane protein that forms a heterodimer with HLA-DOβ and through interaction with HLA-DM is an important modulator in the HLA class II restricted antigen presentation pathway.

REFERENCES

1. Trowsdale, J. and Kelly, A. 1985. The human HLA class II alpha chain gene DZ α is distinct from genes in the DP, DQ and DR subregions. EMBO J. 4: 2231-2237.
2. Jonsson, A.K. and Rask, L. 1989. Human class II DNA and DOB genes display low sequence variability. Immunogenetics 29: 411-413.
3. Young, J.A. and Trowsdale, J. 1990. The HLA-DNA (DZA) gene is correctly expressed as a 1.1 kb mature mRNA transcript. Immunogenetics 31: 386-388.
4. Naruse, T.K., et al. 1999. Limited polymorphism in the HLA-DOA gene. Tissue Antigens 53: 359-365.
5. van Lith, M., et al. 2002. Novel polymorphisms in HLA-DOA and HLA-DOB in B-cell malignancies. Immunogenetics 54: 591-595.
6. Fallas, J.L., et al. 2004. Ectopic expression of HLA-DO in mouse dendritic cells diminishes MHC class II antigen presentation. J. Immunol. 173: 1549-1560.
7. Moon, S.M., et al. 2005. Identification of four novel HLA-DOA alleles, DOA*010106, DOA*0102, DOA*0103, and DOA*0104N, by sequence-based typing*. Tissue Antigens 66: 242-245.

CHROMOSOMAL LOCATION

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

PRODUCT

HLA-DOα 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-DOα shRNA Plasmid (h): sc-95165-SH and HLA-DOα shRNA (h) Lentiviral Particles: sc-95165-V as alternate gene silencing products.

For independent verification of HLA-DOα (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-95165A, sc-95165B and sc-95165C.

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-DOα siRNA (h) is recommended for the inhibition of HLA-DOα 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-DOα (C-11): sc-515446 is recommended as a control antibody for monitoring of HLA-DOα 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-DOα gene expression knockdown using RT-PCR Primer: HLA-DOα (h)-PR: sc-95165-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.

PROTOCOLS

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