

# HLA-DRβ5 siRNA (h): sc-72095

## BACKGROUND

Major histocompatibility complex (MHC) class II molecules destined for presentation to CD4<sup>+</sup> 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- $\alpha/\beta$  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 ( $\beta$ -2-Microglobulin). Polymorphisms yield hundreds of HLA-B and -C alleles.

## REFERENCES

- Haynes, B.F., et al. 1982. Description of monoclonal antibody defining an HLA allotypic determinant that includes specificities within the B5 cross-reacting group. *Hum. Immunol.* 4: 273-285.
- 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  $\beta$ -2-microglobulin, immunoglobulin  $\kappa$  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.

## CHROMOSOMAL LOCATION

Genetic locus: HLA-DRB5 (human) mapping to 6p21.32.

## PRODUCT

HLA-DRβ5 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  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see HLA-DRβ5 shRNA Plasmid (h): sc-72095-SH and HLA-DRβ5 shRNA (h) Lentiviral Particles: sc-72095-V as alternate gene silencing products.

For independent verification of HLA-DRβ5 (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-72095A, sc-72095B and sc-72095C.

## PROTOCOLS

See our web site at [www.scbt.com](http://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  $\mu$ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330  $\mu$ l of RNase-free water makes a 10  $\mu$ M solution in a 10  $\mu$ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

## APPLICATIONS

HLA-DRβ5 siRNA (h) is recommended for the inhibition of HLA-DRβ5 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  $\mu$ M in 66  $\mu$ 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-DRβ5 (4D12): sc-53889 is recommended as a control antibody for monitoring of HLA-DRβ5 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 $\kappa$  BP-HRP: sc-516102 or m-IgG $\kappa$  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 $\kappa$  BP-FITC: sc-516140 or m-IgG $\kappa$  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-DRβ5 gene expression knockdown using RT-PCR Primer: HLA-DRβ5 (h)-PR: sc-72095-PR (20  $\mu$ 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.