

MD-1 siRNA (h): sc-40734

BACKGROUND

RP105 (CD180) was originally discovered as a mouse B cell surface molecule that transmits an activation signal. This signal leads to resistance against irradiation-induced apoptosis and massive B cell proliferation. RP105 is associated with another molecule, MD-1, which has an important role in the cell surface expression of RP105. MD-1, also known as Lymphocyte Antigen 68 and RP105 Associated Protein, associates with and regulates the cell surface expression of RP105. RP105/MD-1 constitutes an LPS-signaling complex on B cells and, like MD-2, enhances the LPS signaling via TLR4. MD-1 contains 162 amino acids and has a predicted 19-amino acid signal peptide and 2 N-glycosylation sites. MD1 is highly expressed in B cells, monocytes and tonsil and is localized on the surface of cells despite its lack of a trans-membrane region.

REFERENCES

1. Miura, Y., et al. 1998. RP105 is associated with MD-1 and transmits an activation signal in human B cells. *Blood* 92: 2815-2822.
2. Miyake, K., et al. 2000. Innate recognition of lipopolysaccharide by Toll-like receptor 4/MD-2 and RP105/MD-1. *J. Endotoxin Res.* 6: 389-391.
3. Online Mendelian Inheritance in Man, OMIM™. 2000. Johns Hopkins University, Baltimore, MD. MIM Number: 605241. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>
4. Nagai, Y., et al. 2002. Requirement for MD-1 in cell surface expression of RP105/CD180 and B cell responsiveness to lipopolysaccharide. *Blood* 99: 1699-1705.
5. Clark, D.A., et al. 2003. MD-1 is a critical part of the mechanism causing Th1-cytokine-triggered murine fetal loss syndrome. *Am. J. Reprod. Immunol.* 49: 297-307.
6. Hadidi, S. and Gorczynski, R.M. 2004. MD-1 expression regulates direct and indirect allorecognition. *Tissue Antigens* 63: 132-141.
7. Tsuneyoshi, N., et al. 2004. The functional and structural properties of MD-2 required for lipopolysaccharide binding are absent in MD-1. *J. Immunol.* 174: 340-344.

CHROMOSOMAL LOCATION

Genetic locus: LY86 (human) mapping to 6p25.1.

PRODUCT

MD-1 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see MD-1 shRNA Plasmid (h): sc-40734-SH and MD-1 shRNA (h) Lentiviral Particles: sc-40734-V as alternate gene silencing products.

For independent verification of MD-1 (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-40734A, sc-40734B and sc-40734C.

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

MD-1 siRNA (h) is recommended for the inhibition of MD-1 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

MD-1 (F-5): sc-390613 is recommended as a control antibody for monitoring of MD-1 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 MD-1 gene expression knockdown using RT-PCR Primer: MD-1 (h)-PR: sc-40734-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.