SANTA CRUZ BIOTECHNOLOGY, INC.

MD-2 siRNA (m): sc-35890

BACKGROUND

Lipopolysaccharide (LPS) is the principal proinflammatory component of the Gram-negative bacterial envelope. The lipopolysaccharide (LPS) receptor is a multi-protein complex that consists of at least three proteins, CD14, TLR4 and MD-2. Each of these proteins are glycosylated. Specifically, MD-2 contains two N-linked glycosylation sites at positions Asn(26) and Asn(114). MD-2 is indispensable for TLR4-dependent LPS responses because cells expressing TLR4/MD-2, but not TLR4 alone, respond to LPS. Intestinal epithelial cells (IEC) express low levels of TLR4 and MD-2 and are LPS unresponsive. T cell-derived cytokines lead to increased expression of TLR4 and MD-2, and LPS-dependent pro-inflammatory cytokine secretion in IEC. The human MD2 gene maps to chromosome 8q13.3 and encodes a 162 amino acid protein with a predicted 16-amino acid signal peptide.

REFERENCES

1. Online Mendelian Inheritance in Man, OMIM™. 2000. Johns Hopkins University, Baltimore, MD. MIM Number: 605243. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/
2. Hajjar, A.M., et al. 2002. Human Toll-like receptor 4 recognizes host-specific LPS modifications. Nat. Immunol. 3: 354-359.
3. da Silva Correia, J. and Ulevitch, R.J. 2002. MD-2 and TLR4 N-linked glycosylations are important for a functional lipopolysaccharide receptor. J. Biol. Chem. 277: 1845-1854.
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. Abreu, M.T., et al. 2002. TLR4 and MD-2 expression are regulated by immune-mediated signals in human intestinal epithelial cells. J. Biol. Chem. 277: 20431-20437.
6. LocusLink Report (LocusID: 23643). http://www.ncbi.nlm.nih.gov/LocusLink/

CHROMOSOMAL LOCATION

Genetic locus: Ly96 (mouse) mapping to 1 A3.

PRODUCT

MD-2 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see MD-2 shRNA Plasmid (m): sc-35890-SH and MD-2 shRNA (m) Lentiviral Particles: sc-35890-V as alternate gene silencing products.

For independent verification of MD-2 (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-35890A, sc-35890B and sc-35890C.

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

MD-2 siRNA (m) is recommended for the inhibition of MD-2 expression in mouse 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.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor MD-2 gene expression knockdown using RT-PCR Primer: MD-2 (m)-PR: sc-35890-PR (20 µl, 420 bp). 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.