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MASP-2 siRNA (h): sc-42903

BACKGROUND

Mannose (or mannan)-binding lectin (MBL), also known as serum mannose-binding protein (MBP), initiates the lectin branch of the innate immune response by binding to the surface of potentially pathogenic microorganisms and initiating complement fixation through an N-terminal collagen-like domain. MBL is a key component in immune response in that it can directly trigger neutralization of invading microorganisms by an Ab-independent mechanism. Mutations of human MBL are associated with immunodeficiency resulting from a reduction in the ability of the mutant MBL to initiate complement fixation. In human, three types of MBL-associated serine proteases, MASP-1, MASP-2 and MASP-3, and a truncated form of MASP-2 (small MBL-associated protein; sMAP or MAp19) complex with MBL to activate the lectin pathway of the complement system. Activated MASPs subsequently cleave and activate downstream components of the complement pathway. MASP-3 is an alternatively spliced product from the MASP-1 gene and may function to inhibit MASP-2 by competing for MBL binding and inhibiting the activation of MBL-associated MASP-2.

REFERENCES

1. Heise, C., et al. 2000. Impaired secretion of rat mannose-binding protein resulting from mutations in the collagen-like domain. *J. Immunol.* 165: 1403-1409.
2. Matsushita, M., et al. 2000. Proteolytic activities of two types of mannose-binding lectin-associated serine protease. *J. Immunol.* 165: 2637-2642.
3. Chen, C.B., et al. 2001. Stoichiometry of complexes between mannose-binding protein and its associated serine proteases: Defining functional units for complement activation. *J. Biol. Chem.* 276: 25894-25902.
4. Endo, M., et al. 2001. Regulation of *in situ* complement activation via the lectin pathway in patients with IgA nephropathy. *Clin. Nephrol.* 55: 185-191.
5. Thielens, N.M., et al. 2001. Interaction properties of human mannan-binding lectin (MBL)-associated serine proteases-1 and -2, MBL-associated protein 19, and MBL. *J. Immunol.* 166: 5068-5077.

CHROMOSOMAL LOCATION

Genetic locus: MASP2 (human) mapping to 1p36.22.

PRODUCT

MASP-2 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 MASP-2 shRNA Plasmid (h): sc-42903-SH and MASP-2 shRNA (h) Lentiviral Particles: sc-42903-V as alternate gene silencing products.

For independent verification of MASP-2 (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-42903A, sc-42903B and sc-42903C.

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 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

MASP-2 siRNA (h) is recommended for the inhibition of MASP-2 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

MASP-2 (B-10): sc-390200 is recommended as a control antibody for monitoring of MASP-2 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 MASP-2 gene expression knockdown using RT-PCR Primer: MASP-2 (h)-PR: sc-42903-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.