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SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

[linkedin.com/company/szaboscandic](https://www.linkedin.com/company/szaboscandic) 

MASP-1/3 siRNA (h): sc-45349

BACKGROUND

Mannose (or mannan)-binding lectin (MBL, also known as serum mannose-binding protein, or 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. It binds to sugars on the surface of bacterial, fungal and parasitic cells through C-terminal, Ca²⁺-dependent carbohydrate-recognition domains. 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, two types of MBL-associated serine proteases (MASP-1 and MASP-2) 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.

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. and Wallis, R. 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: MASP1 (human) mapping to 3q27.3.

PRODUCT

MASP-1/3 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-1/3 shRNA Plasmid (h): sc-45349-SH and MASP-1/3 shRNA (h) Lentiviral Particles: sc-45349-V as alternate gene silencing products.

For independent verification of MASP-1/3 (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-45349A, sc-45349B and sc-45349C.

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-1/3 siRNA (h) is recommended for the inhibition of MASP-1/3 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-1/3 (F-12): sc-166815 is recommended as a control antibody for monitoring of MASP-1/3 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-1/3 gene expression knockdown using RT-PCR Primer: MASP-1/3 (h)-PR: sc-45349-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.