

VAP-1 siRNA (h): sc-43197

BACKGROUND

Lymphocyte binding to vascular endothelium is a prerequisite for the movement of immune cells from the blood into lymphoid tissues and into sites of inflammation. Under inflammatory conditions, cell surface expression of VAP-1 (vascular adhesion protein-1) which is an endothelial sialoglycoprotein, is induced. VAP-1 is a type II transmembrane protein with a single transmembrane domain and N- and O-glycosylation sites in the extracellular domain. *In vivo*, VAP-1 exists predominantly as a homodimer and functions both as an enzyme (monoamine oxidase) and an adhesion molecule for lymphocytes. With the appropriate glycosylation and in the correct inflammatory setting, expression of VAP-1 on the luminal endothelial cell surface allows it to mediate lymphocyte adhesion and to function as an adhesion receptor involved in lymphocyte recirculation. VAP-1 is also expressed in all types of smooth muscle cells, except in cardiac and skeletal muscle cells. VAP-1 localized on smooth muscle cells does not support binding of lymphocytes, but it deaminates exogenous and endogenous primary amines. Soluble VAP-1 is found in circulation and its level is increased in patients who have inflammatory liver diseases.

REFERENCES

- Salminen, T.A., et al. 1998. Structural model of the catalytic domain of an enzyme with cell adhesion activity: human vascular adhesion protein-1 (HVAP-1) D4 domain is an amine oxidase. *Protein Eng.* 11: 1195-1204.
- Smith, D.J., et al. 1998. Cloning of vascular adhesion protein 1 reveals a novel multifunctional adhesion molecule. *J. Exp. Med.* 188: 17-27.
- Kurkijarvi, R., et al. 1998. Circulating form of human vascular adhesion protein-1 (VAP-1): increased serum levels in inflammatory liver diseases. *J. Immunol.* 161: 1549-1557.
- Slami, M., et al. 2000. Human vascular adhesion protein-1 (VAP-1) plays a critical role in lymphocyte-endothelial cell adhesion cascade under shear. *Circ. Res.* 86: 1245-1251.
- Tohka, S., et al. 2001. Vascular adhesion protein 1 (VAP-1) functions as a molecular brake during granulocyte rolling and mediates recruitment *in vivo*. *FASEB J.* 15: 373-382.

CHROMOSOMAL LOCATION

Genetic locus: AOC3 (human) mapping to 17q21.31.

PRODUCT

VAP-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 VAP-1 shRNA Plasmid (h): sc-43197-SH and VAP-1 shRNA (h) Lentiviral Particles: sc-43197-V as alternate gene silencing products.

For independent verification of VAP-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-43197A, sc-43197B and sc-43197C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

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

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

VAP-1 (A-8): sc-166713 is recommended as a control antibody for monitoring of VAP-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 VAP-1 gene expression knockdown using RT-PCR Primer: VAP-1 (h)-PR: sc-43197-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.