

ORP-8 siRNA (h): sc-96001

BACKGROUND

Members of the oxysterol-binding protein (OSBP) family function as intracellular lipid receptors. OSBPs are involved in lipid metabolism and signal transduction, as well as vesicle transport, and can translocate to the periphery of Golgi membranes when they are bound to oxysterols. ORPs (OSBP-related proteins) belong to a subfamily of OSBPs consisting of ORP-1 through ORP-11. The ORPs have a highly conserved OSBP-type sterol-binding region and a pleckstrin homology domain. They strongly bind to phosphatidic acid and weakly bind to phosphatidylinositol 3-phosphate. ORP-8 (oxysterol binding protein-like 8), also known as MST120, OSBP10, MSTP120 or OSBPL8, is an 889 amino acid widely expressed protein belonging to the OSBP family. Localized to the endoplasmic reticulum, ORP-8 suppresses ABC1 (ATP binding cassette transporter A1) expression and cholesterol efflux from macrophages. Existing as two isoforms due to alternative splicing events, ORP8 may modulate the development of atherosclerosis.

REFERENCES

1. Olkkonen, V.M., et al. 2006. The OSBP-related proteins (ORPs): global sterol sensors for co-ordination of cellular lipid metabolism, membrane trafficking and signalling processes? *Biochem. Soc. Trans.* 34: 389-391.
2. Raychaudhuri, S., et al. 2006. Nonvesicular sterol movement from plasma membrane to ER requires oxysterol-binding protein-related proteins and phosphoinositides. *J. Cell Biol.* 173: 107-119.
3. Yan, D., et al. 2007. Expression of human OSBP-related protein 1L in macrophages enhances atherosclerotic lesion development in LDL receptor-deficient mice. *Arterioscler. Thromb. Vasc. Biol.* 27: 1618-1624.
4. Suchanek, M., et al. 2007. The mammalian oxysterol-binding protein-related proteins (ORPs) bind 25-hydroxycholesterol in an evolutionarily conserved pocket. *Biochem. J.* 405: 473-480.
5. Fairn, G.D. and McMaster, C.R. 2008. Emerging roles of the oxysterol-binding protein family in metabolism, transport, and signaling. *Cell. Mol. Life Sci.* 65: 228-236.
6. Yan, D. and Olkkonen, V.M. 2008. Characteristics of oxysterol binding proteins. *Int. Rev. Cytol.* 265: 253-285.

CHROMOSOMAL LOCATION

Genetic locus: OSBPL8 (human) mapping to 12q21.2.

PRODUCT

ORP-8 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 ORP-8 shRNA Plasmid (h): sc-96001-SH and ORP-8 shRNA (h) Lentiviral Particles: sc-96001-V as alternate gene silencing products.

For independent verification of ORP-8 (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-96001A, sc-96001B and sc-96001C.

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

ORP-8 siRNA (h) is recommended for the inhibition of ORP-8 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

ORP-8 (PL-C26): sc-134409 is recommended as a control antibody for monitoring of ORP-8 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 ORP-8 gene expression knockdown using RT-PCR Primer: ORP-8 (h)-PR: sc-96001-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.