

ORP-1 siRNA (m): sc-62716

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 and consist of ORP-1 and ORP-2. 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. Three isoforms of ORP-1 are produced due to alternative splicing. Isoform ORP-1A is expressed only in retina, brain, pineal gland, fetal brain and cultured retinal pigment epithelial cells, whereas ORP-1B is expressed ubiquitously.

REFERENCES

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3. Lehto, M., et al. 2001. The OSBP-related protein family in humans. *J. Lipid Res.* 42: 1203-1213.
4. Jaworski, C.J., et al. 2001. A family of 12 human genes containing oxysterol-binding domains. *Genomics* 78: 185-196.
5. Online Mendelian Inheritance in Man, OMIM[™]. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 606730. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>
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7. Perry, R.J. and Ridgway, N.D. 2006. Oxysterol-binding protein and vesicle-associated membrane protein-associated protein are required for sterol-dependent activation of the ceramide transport protein. *Mol. Biol. Cell* 17: 2604-2616.

CHROMOSOMAL LOCATION

Genetic locus: Osbpl1a (mouse) mapping to 18 A1.

PRODUCT

ORP-1 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see ORP-1 shRNA Plasmid (m): sc-62716-SH and ORP-1 shRNA (m) Lentiviral Particles: sc-62716-V as alternate gene silencing products.

For independent verification of ORP-1 (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-62716A, sc-62716B and sc-62716C.

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-1 siRNA (m) is recommended for the inhibition of ORP-1 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 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-1 (B-3): sc-376602 is recommended as a control antibody for monitoring of ORP-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 ORP-1 gene expression knockdown using RT-PCR Primer: ORP-1 (m)-PR: sc-62716-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.