

OSBP siRNA (h): sc-61264

BACKGROUND

The Oxysterol-binding protein (OSBP) family of proteins consist of OSBP (OSBP1) and OSBP2 (ORP-4), which share a high overall similarity. 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. The OSBP protein transports sterols from lysosomes to the nucleus, where sterols downregulate the genes for HMG synthetase, HMG-CoA reductase and the low density lipoprotein receptor (LDLR). OSBP localizes to the cytosol and is widely expressed, while OSBP2 is mainly detected in testis, retina and fetal liver. The extracellular signal-regulated kinase (ERK) signaling pathway is controlled by OSBP via its cholesterol-binding properties. OSBP binds with a high affinity to 25-hydroxy-cholesterol (25-HC), a suppressor of cholesterol synthesis gene transcription in cultured cells.

REFERENCES

1. Levanon, D., et al. 1990. cDNA cloning of human oxysterol-binding protein and localization of the gene to human chromosome 11 and mouse chromosome 19. *Genomics* 7: 65-74.
2. Online Mendelian Inheritance in Man, OMIM™. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 167040. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>
3. Im, Y.J., et al. 2005. Structural mechanism for sterol sensing and transport by OSBP-related proteins. *Nature* 437: 154-158.
4. Balla, A., et al. 2005. A plasma membrane pool of phosphatidylinositol 4-phosphate is generated by phosphatidylinositol 4-kinase type-III α : studies with the PH domains of the oxysterol binding protein and FAPP1. *Mol. Biol. Cell* 16: 1282-1295.
5. Nishimura, T., et al. 2005. Inhibition of cholesterol biosynthesis by 25-hydroxycholesterol is independent of OSBP. *Genes Cells* 10: 793-801.
6. Wang, P.Y., et al. 2005. OSBP is a cholesterol-regulated scaffolding protein in control of ERK 1/2 activation. *Science* 307: 1472-1476.

CHROMOSOMAL LOCATION

Genetic locus: OSBP (human) mapping to 11q12.1.

PRODUCT

OSBP 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 OSBP shRNA Plasmid (h): sc-61264-SH and OSBP shRNA (h) Lentiviral Particles: sc-61264-V as alternate gene silencing products.

For independent verification of OSBP (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-61264A, sc-61264B and sc-61264C.

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

OSBP siRNA (h) is recommended for the inhibition of OSBP 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

OSBP (A-5): sc-365771 is recommended as a control antibody for monitoring of OSBP 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 OSBP gene expression knockdown using RT-PCR Primer: OSBP (h)-PR: sc-61264-PR (20 μ l, 490 bp). 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.