

SREBP-1 shRNA (m) Lentiviral Particles: sc-36558-V

BACKGROUND

The low density lipoprotein (LDL) receptor mediates the endocytic uptake of cholesterol-carrying lipoproteins, thereby controlling cholesterol levels in cells and plasma. Transcription of the LDL receptor gene is controlled by a ten base pair sequence in the 5' flanking region, designated sterol regulatory element 1 (SRE-1). When cellular sterol stores are depleted, the element is activated, the gene is transcribed and the cellular uptake of LDL increases. A set of SRE-binding proteins (SREBPs) have been identified, including two basic helix-loop-helix leucine zipper (bHLH-Zip) transcription factors, designated SREBP-1 and SREBP-2. SREBP-1 (also designated ADD1, for adipocyte determination and differentiation factor) is synthesized as a precursor that is attached to the nuclear envelope and endoplasmic reticulum. In sterol-depleted cells, the membrane-bound precursor is cleaved to generate a soluble NH₂-terminal fragment that translocates to the nucleus to activate transcription. Sterols inhibit the cleavage of SREBP-1.

REFERENCES

1. Brown, M.S., et al. 1986. A receptor-mediated pathway for cholesterol homeostasis. *Science* 232: 34-47.
2. Smith, J.R., et al. 1990. Identification of nucleotides responsible for enhancer activity of sterol regulatory element in low density lipoprotein receptor gene. *J. Biol. Chem.* 265: 2306-2310.
3. Goldstein, J.L., et al. 1990. Regulation of the mevalonate pathway. *Nature* 343: 425-430.
4. Briggs, M.R., et al. 1993. Nuclear protein that binds sterol regulatory element of LDL receptor promoter. I. Identification of the protein and delimitation of its target nucleotide sequence. *J. Biol. Chem.* 268: 14490-14496.
5. Wang, X., et al. 1993. Nuclear protein that binds sterol regulatory element of low density lipoprotein receptor promoter. II. Purification and characterization. *J. Biol. Chem.* 268: 14497-14504.
6. Hua, X., et al. 1993. SREBP-2, a second basic-helix-loop-helix-leucine zipper protein that stimulates transcription by binding to a sterol regulatory element. *Proc. Natl. Acad. Sci. USA* 90: 11603-11607.
7. Wang, X., et al. 1994. SREBP-1, a membrane-bound transcription factor released by sterol-regulated proteolysis. *Cell* 77: 53-62.

CHROMOSOMAL LOCATION

Genetic locus: Sreb1 (mouse) mapping to 11 B2.

PRODUCT

SREBP-1 shRNA (m) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 μ l frozen stock containing 1.0 x 10⁶ infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see SREBP-1 siRNA (m): sc-36558 and SREBP-1 shRNA Plasmid (m): sc-36558-SH as alternate gene silencing products.

APPLICATIONS

SREBP-1 shRNA (m) Lentiviral Particles is recommended for the inhibition of SREBP-1 expression in mouse cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0 x 10⁶ infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

GENE EXPRESSION MONITORING

SREBP-1 (A-4): sc-365513 is recommended as a control antibody for monitoring of SREBP-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 SREBP-1 gene expression knockdown using RT-PCR Primer: SREBP-1 (m)-PR: sc-36558-PR (20 μ l, 563 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

STORAGE

Store lentiviral particles at -80° C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4° C for up to one week. Avoid repeated freeze thaw cycles.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.