

SREBP-1 shRNA Plasmid (m): sc-36558-SH

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 delineation of its target nucleotide sequence. *J. Biol. Chem.* 268: 14490-14496.

CHROMOSOMAL LOCATION

Genetic locus: Sreb1f1 (mouse) mapping to 11 B2.

PRODUCT

SREBP-1 shRNA Plasmid (m) is a pool of 3 target-specific lentiviral vector plasmids each encoding 19-25 nt (plus hairpin) shRNAs designed to knock down gene expression. Each vial contains 20 µg of lyophilized shRNA plasmid DNA. Suitable for up to 20 transfections. Also see SREBP-1 siRNA (m): sc-36558 and SREBP-1 shRNA (m) Lentiviral Particles: sc-36558-V as alternate gene silencing products.

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.

STORAGE AND RESUSPENSION

Store lyophilized shRNA plasmid DNA at 4° C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at 4° C for short term storage or -80° C for long term storage. Avoid repeated freeze thaw cycles.

Resuspend lyophilized shRNA plasmid DNA in 200 µl of the deionized water provided. Resuspension of the shRNA plasmid DNA in 200 µl of deionized water makes a 0.1 µg/µl solution in a 10 mM Tris, 1 mM EDTA buffered solution.

APPLICATIONS

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

SUPPORT REAGENTS

For optimal shRNA Plasmid transfection efficiency, Santa Cruz Biotechnology's shRNA Plasmid Transfection Reagent: sc-108061 (0.2 ml) and shRNA Plasmid Transfection Medium: sc-108062 (20 ml) are recommended. Control shRNAs are available as 20 µg lyophilized plasmid DNA. Each encodes a scrambled shRNA sequence that will not lead to the specific degradation of any known cellular mRNA. Control shRNA Plasmids include: sc-108060, sc-108065 and sc-108066.

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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

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 µl, 563 bp). 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 or our catalog for detailed protocols and support products.