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SANTA CRUZ BIOTECHNOLOGY, INC.

SREBP-1 siRNA (h2): sc-44327

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 10 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 leuicine 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 NH_2-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 mevatonate 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.
5. Wang, X., et al. 1993. Nuclear protein that binds sterol regulatory element of LDL 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 transcrption factor released by sterol-regulated proteolysis. Cell 77: 53-62.

CHROMOSOMAL LOCATION

Genetic locus: SREBF1 (human) mapping to 17p11.2.

PRODUCT

SREBP-1 siRNA (h2) 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see SREBP-1 shRNA Plasmid (h2): sc-44327-SH and SREBP-1 shRNA (h2) Lentiviral Particles: sc-44327-V as alternate gene silencing products.

For independent verification of SREBP-1 (h2) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3 nmol of lyophilized siRNA. These include: sc-44327A, sc-44327B and sc-44327C.

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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

SREBP-1 siRNA (h2) is recommended for the inhibition of SREBP-1 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 µM in 66 µl. 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

SREBP-1 (A-4): sc-365513 is recommended as a control antibody for monitoring of 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 gene expression knockdown using RT-PCR Primer: SREBP-1 (h2)-PR: sc-44327-PR (20 µl, 524 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.