SANTA CRUZ BIOTECHNOLOGY, INC.

SCAP siRNA (h): sc-36462

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The Power to Question

BACKGROUND

The transcription factors SREBPs (sterol regulatory element binding proteins) span the ER membrane, and in response to sterol depletion, the N-terminal domain of SREBPs are proteolytically activated, released from the membrane and then translocate to the nucleus where they induce the expression of genes regulating cholesterol metabolism. This proteolytic activation requires the sequential cleavage of SREBPs at Site-1, within the lumen of the ER, followed by cleavage at Site-2, within the first transmembrane domain. The cleavage at Site-1 separates the N-terminal and C-terminal domains of the protein and it requires the serine protease, S1P (Site-1 protease). Site-2 is subsequently processed by a putative zinc metalloprotease S2P, which releases the activated N-terminal domain for nuclear translocation. This proteolytic pathway is tightly regulated by sterol levels and is under the control of SCAP (SREBP cleavage-activating protein). SCAP, a sterol sensor, is latently bound to the C-terminal regulatory domains of the SREBPs, and it regulates cleavage of SREBPs at Site-1. Sterol levels influence the activity of SCAP, as SCAP is activated only in sterol-depleted cells, and it is inhibited by sterol accumulation.

REFERENCES

1. Hua, X., et al. 1996. Regulated cleavage of sterol regulatory element binding proteins requires sequences on both sides of the endoplasmic reticulum membrane. J. Biol. Chem. 271: 10379-10384.
2. Rawson, R.B., et al. 1997. Complementation cloning of S2P, a gene encoding a putative metalloprotease required for intramembrane cleavage of SREBPs. Mol. Cell 1: 47-57.
3. Sakai, J., et al. 1998. Molecular identification of the sterol-regulated luminal protease that cleaves SREBPs and controls lipid composition of animal cells. Mol. Cell 2: 505-514.
4. Sakai, J., et al. 1998. Cleavage of sterol regulatory element-binding proteins (SREBPs) at site-1 requires interaction with SREBP cleavage-activating protein. Evidence from *in vivo* competition studies. J. Biol. Chem. 273: 5785-5793.
5. Espenshade, P.J., et al. 1999. Autocatalytic processing of site-1 protease removes propeptide and permits cleavage of sterol regulatory element-binding proteins. J. Biol. Chem. 274: 22795-22804.
6. Brown, M.S. and Goldstein, J.L. 1999. A proteolytic pathway that controls the cholesterol content of membranes, cells, and blood. Proc. Natl. Acad. Sci. USA 96: 11041-11048.
7. Cheng, D., et al. 1999. Secreted site-1 protease cleaves peptides corresponding to luminal loop of sterol regulatory element-binding proteins. J. Biol. Chem. 274: 22805-22812.
8. Nohturfft, A., et al. 1999. Sterols regulate cycling of SREBP cleavage-activating protein (SCAP) between endoplasmic reticulum and Golgi. Proc. Natl. Acad. Sci. USA 96: 11235-11240.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.

CHROMOSOMAL LOCATION

Genetic locus: SCAP (human) mapping to 3p21.31.

PRODUCT

SCAP 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see SCAP shRNA Plasmid (h): sc-36462-SH and SCAP shRNA (h) Lentiviral Particles: sc-36462-V as alternate gene silencing products.

For independent verification of SCAP (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-36462A, sc-36462B and sc-36462C.

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

SCAP siRNA (h) is recommended for the inhibition of SCAP 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.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor SCAP gene expression knockdown using RT-PCR Primer: SCAP (h)-PR: sc-36462-PR (20 µl, 469 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Yu, Z., et al. 2022. GDNF regulates lipid metabolism and glioma growth through RET/ERK/HIF-1/SREBP-1. Int. J. Oncol. 61: 109.

RESEARCH USE

For research use only, not for use in diagnostic procedures.