

S2P siRNA (h): sc-41652

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 translocated 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 protease 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 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. 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.

CHROMOSOMAL LOCATION

Genetic locus: MBTPS2 (human) mapping to Xp22.12.

PRODUCT

S2P 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 S2P shRNA Plasmid (h): sc-41652-SH and S2P shRNA (h) Lentiviral Particles: sc-41652-V as alternate gene silencing products.

For independent verification of S2P (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-41652A, sc-41652B and sc-41652C.

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

S2P siRNA (h) is recommended for the inhibition of S2P 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

S2P (1A3): sc-293341 is recommended as a control antibody for monitoring of S2P 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 S2P gene expression knockdown using RT-PCR Primer: S2P (h)-PR: sc-41652-PR (20 μ l, 553 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.

PROTOCOLS

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