SANTA CRUZ BIOTECHNOLOGY, INC.

SKI-1 siRNA (h): sc-36496

BACKGROUND

Mammalian serine proteases function as proprotein convertases, processing inactive precursors to produce active peptides and proteins. SKI-1 (subtilisin/kexin-isozyme-1) is a type I membrane-bound Ca^{2+}-dependent serine proteinase. SKI-1 is related to bacterial subtilisin and yeast kexin. Subtilisin is an alkaline serine protease produced by *Bacillus subtilis* 168. Kexin is a prohormone-processing enzyme, which is encoded by the KEX2 gene of the yeast *Saccharomyces cerevisiae.* SKI-1 is present in most tissues and cells, and it is most concentrated in liver and thyroid tissues. SKI-1 has been shown to cleave the brain-derived neurotrophic factor (BDNF) precursor to produce the mature form of BDNF.

REFERENCES

1. Ikemura, H., et al. 1987. Requirement of prosequence for the production of active subtilisin E in *Escherichia coli.* J. Biol. Chem. 262: 7859-7864.
2. Van de Ven, W.J., et al. 1991. Furin: the prototype mammalian subtilisin-like proprotein-processing enzyme. Endoproteolytic cleavage at paired basic residues of proproteins of the eukaryotic secretory pathway. Enzyme 45: 257-270.
3. Seidah, N.G., et al. 1998. Precursor convertases: an evolutionary ancient, cell-specific, combinatorial mechanism yielding diverse bioactive peptides and proteins. Ann. N.Y. Acad. Sci. 839: 9-24.
4. Steiner, D.F. 1998. The proprotein convertases. Curr. Opin. Chem. Biol. 2: 31-39.
5. Seidah, N.G., et al. 1999. Mammalian subtilisin/kexin isozyme SKI-1: a widely expressed proprotein convertase with a unique cleavage specificity and cellular localization. Proc. Natl. Acad. Sci. USA 96: 1321-1326.

CHROMOSOMAL LOCATION

Genetic locus: MBTPS1 (human) mapping to 16q23.3.

PRODUCT

SKI-1 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 SKI-1 shRNA Plasmid (h): sc-36496-SH and SKI-1 shRNA (h) Lentiviral Particles: sc-36496-V as alternate gene silencing products.

For independent verification of SKI-1 (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-36496A, sc-36496B and sc-36496C.

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.

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

SKI-1 siRNA (h) is recommended for the inhibition of SKI-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

SKI-1 (A-11): sc-271916 is recommended as a control antibody for monitoring of SKI-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-IgGλ BP-HRP: sc-516132 or m-IgGλ BP-HRP (Cruz Marker): sc-516132-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-IgGλ BP-FITC: sc-516185 or m-IgGλ BP-PE: sc-516186 (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 SKI-1 gene expression knockdown using RT-PCR Primer: SKI-1 (h)-PR: sc-36496-PR (20 µl, 585 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.