

PN-1 siRNA (h): sc-45254

BACKGROUND

The serine protease inhibitors (serpins) compose a superfamily of proteins with a diverse set of functions, including the control of blood coagulation, complement activation, programmed cell death and development. Serpins are secreted glycoproteins that contain a stretch of peptide that mimics a true substrate for a corresponding serine protease. Protease nexin-1 (PN-1) is a serpin that inactivates several proteases, including thrombin, urokinase, plasminogen activators (PA) and plasmin. It is involved in tissue remodeling, cellular invasiveness, matrix degradation and tumor growth. PN-1 expression is abundant in the nervous system, where it inhibits thrombin, thereby playing a role in neural injury and repair processes. An imbalance between PN-1 and thrombin may be a contributing factor in the pathology of Alzheimer's disease.

REFERENCES

1. Mulligan, L.P., et al. 1991. Protease nexin-1 activity in cultured Schwann cells. *Neurosci. Lett.* 128: 42-46.
2. Vaughan, P.J., et al. 1994. Protease nexin-1, a potent thrombin inhibitor, is reduced around cerebral blood vessels in Alzheimer's disease. *Brain Res.* 668: 160-170.
3. Smith-Swintosky, V.L., et al. 1995. Protease nexin-1 and thrombin modulate neuronal Ca²⁺ homeostasis and sensitivity to glucose deprivation-induced injury. *J. Neurosci.* 15: 5840-5850.
4. Guttridge, D.C., et al. 1996. Characterization of the human protease nexin-1 promoter and its regulation by Sp1 through a G/C-rich activation domain. *J. Neurochem.* 67: 498-507.
5. Kim, N.K., et al. 2001. Increased expression and localization of a serine protease inhibitor, protease nexin-1 (PN-1), in the ovary and uterus during implantation in rat. *Thromb. Res.* 103: 135-142.
6. Giau, R., et al. 2005. Constitutive secretion of protease nexin-1 by glial cells and its regulation by G protein-coupled receptors. *J. Neurosci.* 25: 8995-9004.

CHROMOSOMAL LOCATION

Genetic locus: SERPINE2 (human) mapping to 2q36.1.

PRODUCT

PN-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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see PN-1 shRNA Plasmid (h): sc-45254-SH and PN-1 shRNA (h) Lentiviral Particles: sc-45254-V as alternate gene silencing products.

For independent verification of PN-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-45254A, sc-45254B and sc-45254C.

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 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

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

PN-1 (C-12): sc-365650 is recommended as a control antibody for monitoring of PN-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-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 PN-1 gene expression knockdown using RT-PCR Primer: PN-1 (h)-PR: sc-45254-PR (20 μ l). 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.