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PN-1 shRNA (m) Lentiviral Particles: sc-45255-V

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

- Mulligan, L.P., et al. 1991. Protease nexin-1 activity in cultured Schwann cells. *Neurosci. Lett.* 128: 42-46.
- 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.
- 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.
- 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.
- 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.

CHROMOSOMAL LOCATION

Genetic locus: Serpine2 (mouse) mapping to 1 C4.

PRODUCT

PN-1 shRNA (m) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 µl frozen stock containing 1.0 x 10⁶ infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see PN-1 siRNA (m): sc-45255 and PN-1 shRNA Plasmid (m): sc-45255-SH as alternate gene silencing products.

STORAGE

Store lentiviral particles at -80° C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4° C for up to one week. Avoid repeated freeze thaw cycles.

PROTOCOLS

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

APPLICATIONS

PN-1 shRNA (m) Lentiviral Particles is recommended for the inhibition of PN-1 expression in mouse cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 µl frozen viral stock containing 1.0 x 10⁶ infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

GENE EXPRESSION MONITORING

PN-1 (D-18): sc-32454 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 (secondary) reagents are recommended: 1) Western Blotting: use donkey anti-goat IgG-HRP: sc-2020 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible donkey anti-goat IgG-HRP: sc-2033 (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 donkey anti-goat IgG-FITC: sc-2024 (dilution range: 1:100-1:400) or donkey anti-goat IgG-TR: sc-2783 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor PN-1 gene expression knockdown using RT-PCR Primer: PN-1 (m)-PR: sc-45255-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.