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SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

[linkedin.com/company/szaboscandic](https://www.linkedin.com/company/szaboscandic) 

Perlecan shRNA (h) Lentiviral Particles: sc-44010-V

BACKGROUND

Perlecan is part of a large family of heparan sulfate proteoglycans (HSPGs). As key components of cell surfaces and extracellular matrices, HSPGs modulate growth factor activities and thereby influence cell growth and differentiation. Additionally, HSPGs play a critical role in regulating tumor cell metastasis by mediating cell adhesion and the activities of growth and angiogenic factors. Perlecan consists of five distinct structural domains that interact with a number of matrix molecules, cytokines and growth factors to influence cartilage development and neuromuscular junction activity. Antithrombin, a key regulator of blood coagulation proteases, and TGF β 1 act as inhibitors and stimulators of Perlecan expression, respectively, interactions which may provide avenues for therapeutic intervention in certain types of cancer.

REFERENCES

- Hassell, J., et al. 2002. Role of Perlecan in skeletal development and diseases. *Glycoconj. J.* 19: 263-267.
- Jiang, X. and Couchman, J.R. 2003. Perlecan and tumor angiogenesis. *J. Histochem. Cytochem.* 51: 1393-1410.
- Reiland, J., et al. 2004. Heparanase degrades Syndecan-1 and Perlecan Heparan sulfate: functional implications for tumor cell invasion. *J. Biol. Chem.* 279: 8047-8055.
- Zhang, W., et al. 2004. Antiangiogenic antithrombin downregulates the expression of the pro-angiogenic Heparan sulfate proteoglycan, Perlecan, in endothelial cells. *Blood* 103: 1185-1191.
- Casar, J.C., et al. 2004. Heparan sulfate proteoglycans are increased during skeletal muscle regeneration: requirement of Syndecan-3 for successful fiber formation. *J. Cell Sci.* 117: 73-84.

CHROMOSOMAL LOCATION

Genetic locus: HSPG2 (human) mapping to 1p36.12.

PRODUCT

Perlecan shRNA (h) 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 μ l frozen stock containing 1.0×10^6 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 Perlecan siRNA (h): sc-44010 and Perlecan shRNA Plasmid (h): sc-44010-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

Perlecan shRNA (h) Lentiviral Particles is recommended for the inhibition of Perlecan expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 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

Perlecan (E-6): sc-377219 is recommended as a control antibody for monitoring of Perlecan 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 goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker[™] compatible goat anti-mouse IgG-HRP: sc-2031 (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 goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz[™] Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Perlecan gene expression knockdown using RT-PCR Primer: Perlecan (h)-PR: sc-44010-PR (20 μ l, 466 bp). Annealing temperature for the primers should be $55-60^{\circ}$ C and the extension temperature should be $68-72^{\circ}$ 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.