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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) 

Podocalyxin-like 1 shRNA (m) Lentiviral Particles: sc-44765-V

BACKGROUND

Sialomucins are a family of cell adhesion molecules that mediate the interaction between leukocytes and endothelial cells during the inflammatory process. Podocalyxin-like protein 1 (PCLP1), a member of the sialomucin family, is a transmembrane glycoprotein and is structurally related to the L-Selectin ligand, CD34. Podocalyxin-like protein 1 encodes a 21 amino acid N-terminal signal peptide and a 26 amino acid transmembrane region. The extracellular domain contains sites for N- and O-linked glycosylation and the intracellular domain has several potential phosphorylated sites. Podocalyxin-like protein 1 is expressed on podocyte foot processes, where it maintains the glomerular filtration barrier. It is also expressed in endothelial cells as well as hemangioblasts, a precursor of hematopoietic stem cells (HSC). Subsequently, Podocalyxin-like protein 1 is thought to be an appropriate marker for hemangioblast detection.

REFERENCES

1. Lasky, L.A. 1994. Sialomucin ligands for selectins: a new family of cell adhesion molecules. Princess Takamatsu Symp. 24: 81-90.
2. Kershaw, D.B., et al. 1995. Molecular cloning, expression and characterization of podocalyxin-like protein 1 from rabbit as a transmembrane protein of glomerular podocytes and vascular endothelium. J. Biol. Chem. 270: 29439-29446.
3. Yang, D.H., et al. 1996. Glomerular epithelial protein 1 and podocalyxin-like protein 1 in inflammatory glomerular disease (crescentic nephritis) in rabbit and man. Lab. Invest. 74: 571-584.
4. Sassetti, C., et al. 1998. Identification of podocalyxin-like protein as a high endothelial venule ligand for L-selectin: parallels to CD34. J. Exp. Med. 187: 1965-1975.
5. Hara, T., et al. 1999. Identification of podocalyxin-like 1 as a novel cell surface marker for hemangioblasts in the murine aorta-gonad-mesonephros region. Immunity 11: 567-578.

CHROMOSOMAL LOCATION

Genetic locus: Podxl (mouse) mapping to 6 A3.3.

PRODUCT

Podocalyxin-like 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 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 Podocalyxin-like 1 siRNA (m): sc-44765 and Podocalyxin-like 1 shRNA Plasmid (m): sc-44765-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.

APPLICATIONS

Podocalyxin-like 1 shRNA (m) Lentiviral Particles is recommended for the inhibition of Podocalyxin-like 1 expression in mouse 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

Podocalyxin-like 1 (K-19): sc-10503 is recommended as a control antibody for monitoring of Podocalyxin-like 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 Podocalyxin-like 1 gene expression knockdown using RT-PCR Primer: Podocalyxin-like 1 (m)-PR: sc-44765-PR (20 μ l). Annealing temperature for the primers should be $55-60^\circ\text{C}$ and the extension temperature should be $68-72^\circ\text{C}$.

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.

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