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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) 

EGFL7 shRNA (h) Lentiviral Particles: sc-45471-V

BACKGROUND

Epidermal growth factor (EGF) repeat-containing proteins constitute an expanding family of proteins that are involved in several cellular activities, such as blood coagulation, fibrinolysis, cell adhesion and neural and vertebrate development. A human EGF repeat superfamily member that maps to human chromosome X, EGFL6 encodes a predicted signal peptide, suggesting that it is secreted. EGFL6 is expressed in brain and lung tumors and fetal tissues, but is generally absent from normal adult tissues. EGFL7 is a secreted protein that regulates vascular tubulogenesis *in vivo*. *In vitro*, EGFL7 inhibits platelet-derived growth factor induced smooth muscle cell migration and promotes adhesion of endothelial cells to the substrate. EGFL7 is expressed specifically by endothelial cells of the heart, lung and kidney.

REFERENCES

1. Soncin, F., et al. 2003. VE-statin, an endothelial repressor of smooth muscle cell migration. *EMBO J.* 22: 5700-5711.
2. Fitch, M.J., et al. 2004. EGFL7, a novel epidermal growth factor-domain gene expressed in endothelial cells. *Dev. Dyn.* 230: 316-324.
3. Parker, L.H., et al. 2004. The endothelial-cell-derived secreted factor EGFL7 regulates vascular tube formation. *Nature* 428: 754-758.
4. Campagnolo, L., et al. 2005. EGFL7 is a chemoattractant for endothelial cells and is up-regulated in angiogenesis and arterial injury. *Am. J. Pathol.* 167: 275-284.
5. Caetano, B., et al. 2005. Expression and purification of recombinant vascular endothelial-statin. *Protein Expr. Purif.* 46: 136-142.
6. Jiang W.D., et al. 2006. siRNA inhibits EGFL7 expression in human endothelial cell line HUVEC. *Zhonghua Xin Xue Guan Bing Za Zhi* 34: 643-646.

CHROMOSOMAL LOCATION

Genetic locus: EGFL7 (human) mapping to 9q34.3.

PRODUCT

EGFL7 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 EGFL7 siRNA (h): sc-45471 and EGFL7 shRNA Plasmid (h): sc-45471-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

EGFL7 shRNA (h) Lentiviral Particles is recommended for the inhibition of EGFL7 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

EGFL7 (B-1): sc-373898 is recommended as a control antibody for monitoring of EGFL7 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 EGFL7 gene expression knockdown using RT-PCR Primer: EGFL7 (h)-PR: sc-45471-PR (20 μ l, 540 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.