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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) 



robo4 shRNA (h) Lentiviral Particles: sc-44500-V

BACKGROUND

Secreted leucine-rich repeat-containing proteins 1 through 3 (Slit1-3) are secreted glycoproteins that influence axonal guidance and mediate normal neural progression by acting as high-affinity signaling ligands for the repulsive guidance receptors, robo1 and robo2 (also designated roundabout 1 and 2). Interactions between the robo receptor and Slit ligand families of proteins initiate signaling cascades that repel axonal outgrowth. The arrangement of the extracellular domains of robo4 diverges significantly from that of all other robo family members. Robo4 is the only robo family member expressed in primary endothelial cells. It binds Slit and inhibits cellular migration in a heterologous expression system. Together, the robo proteins prescribe developmental paths during neural development.

REFERENCES

- Huminiacki, L., et al. 2002. Magic roundabout is a new member of the roundabout receptor family that is endothelial specific and expressed at sites of active angiogenesis. *Genomics* 79: 547-552.
- Park, K.W., et al. 2003. Robo4 is a vascular-specific receptor that inhibits endothelial migration. *Dev. Biol.* 261: 251-267.
- Middleton, R., et al. 2003. Improving the comparative map of porcine chromosome 9 with respect to human chromosomes 1, 7 and 11. *Cytogenet. Genome Res.* 102: 128-132.
- Suchting, S., et al. 2005. Soluble Robo4 receptor inhibits *in vivo* angiogenesis and endothelial cell migration. *FASEB J.* 19: 121-123.
- Seth, P., et al. 2005. Magic roundabout, a tumor endothelial marker: expression and signaling. *Biochem. Biophys. Res. Commun.* 332: 533-541.

CHROMOSOMAL LOCATION

Genetic locus: ROBO4 (human) mapping to 11q24.2.

PRODUCT

robo4 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 robo4 siRNA (h): sc-44500 and robo4 shRNA Plasmid (h): sc-44500-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

robo4 shRNA (h) Lentiviral Particles is recommended for the inhibition of robo4 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

robo4 (D-3): sc-166872 is recommended as a control antibody for monitoring of robo4 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 IgM-HRP: sc-2064 (dilution range: 1:500-1:5,000), TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgM-FITC: sc-2082 (dilution range: 1:100-1:400) or goat anti-mouse IgM-TR: sc-2983 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor robo4 gene expression knockdown using RT-PCR Primer: robo4 (h)-PR: sc-44500-PR (20 μ l, 418 bp). 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.