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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) 



robo3 shRNA (h) Lentiviral Particles: sc-44498-V

BACKGROUND

Secreted leucine-rich repeat-containing proteins 1-3 (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, robo 1 and robo 2 (also designated Roundabout 1 and 2). Within the developing CNS of different vertebrate systems, Slit proteins are expressed in equivalent regions, suggesting there is a conservation of function for vertebrate homologs. Robo3 plays a crucial role in controlling axon guidance at the midline of the CNS. Two human robo3 isoforms, robo3A and robo3B, which differ by the insertion of 26 amino acids at the N-terminus, appear to be evolutionary conserved. Robo3 guides commissural axons by preventing premature sensitivity to Slit proteins thus inhibiting Slit signaling through robo1. Together, the robo proteins prescribe developmental paths during neural development.

REFERENCES

- Guthrie, S., et al. 2004. Axon guidance: mice and men need Rig and Robo. *Curr. Biol.* 14: R632-R634.
- Woods, C.G., et al. 2004. Neuroscience. Crossing the midline. *Science* 304: 1455-1456.
- Taylor, T.D., et al. 2004. Compartmentalization of visual centers in the *Drosophila* brain requires Slit and Robo proteins. *Development* 131: 5935-5945.
- Marillat, V., et al. 2004. The slit receptor Rig-1/Robo3 controls midline crossing by hindbrain precerebellar neurons and axons. *Neuron* 43: 69-79.
- Bosley, T.M., et al. 2005. Neurologic features of horizontal gaze palsy and progressive scoliosis with mutations in ROBO3. *Neurology* 64: 1196-1203.

CHROMOSOMAL LOCATION

Genetic locus: ROBO3 (human) mapping to 11q24.2.

PRODUCT

robo3 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 robo3 siRNA (h): sc-44498 and robo3 shRNA Plasmid (h): sc-44498-SH as alternate gene silencing products.

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.

APPLICATIONS

robo3 shRNA (h) Lentiviral Particles is recommended for the inhibition of robo3 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

robo3 (N-17): sc-46495 is recommended as a control antibody for monitoring of robo3 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 robo3 gene expression knockdown using RT-PCR Primer: robo3 (h)-PR: sc-44498-PR (20 μ l). 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.

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.