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SANTA CRUZ BIOTECHNOLOGY, INC.

robo4 siRNA (m): sc-44501

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

1. Huminiecki, 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.
2. Park, K.W., et al. 2003. Robo4 is a vascular-specific receptor that inhibits endothelial migration. Dev. Biol. 261: 251-267.
3. 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.
4. Suchting, S., et al. 2005. Soluble Robo4 receptor inhibits *in vivo* angiogenesis and endothelial cell migration. FASEB J. 19: 121-123.
5. 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 (mouse) mapping to 9 A4.

PRODUCT

robo4 siRNA (m) is a pool of 3 target-specific 19-25 nt siRNAs designed to knock down gene expression. Each vial contains 3.3 nmol of lyophilized siRNA, sufficient for a 10 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see robo4 shRNA Plasmid (m): sc-44501-SH and robo4 shRNA (m) Lentiviral Particles: sc-44501-V as alternate gene silencing products.

For independent verification of robo4 (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-44501A, sc-44501B and sc-44501C.

STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at -20° C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at -20° C, avoid contact with RNAses and repeated freeze thaw cycles.

Resuspend lyophilized siRNA duplex in 330 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

robo4 siRNA (m) is recommended for the inhibition of robo4 expression in mouse cells.

SUPPORT REAGENTS

For optimal siRNA transfection efficiency, Santa Cruz Biotechnology's siRNA Transfection Reagent: sc-29528 (0.3 ml), siRNA Transfection Medium: sc-36868 (20 ml) and siRNA Dilution Buffer: sc-29527 (1.5 ml) are recommended. Control siRNAs or Fluorescein Conjugated Control siRNAs are available as 10 µM in 66 µl. Each contain a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA. Fluorescein Conjugated Control siRNAs include: sc-36869, sc-44239, sc-44240 and sc-44241. Control siRNAs include: sc-37007, sc-44230, sc-44231, sc-44232, sc-44233, sc-44234, sc-44235, sc-44236, sc-44237 and sc-44238.

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 reagents are recommended: 1) Western Blotting: use m-IgGκ BP-HRP: sc-516102 or m-IgGκ BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker™ Molecular Weight Standards: sc-2035, UltraCruz® Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgGκ BP-FITC: sc-516140 or m-IgGκ BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz® Mounting Medium: sc-24941 or UltraCruz® Hard-set Mounting Medium: sc-359850.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor robo4 gene expression knockdown using RT-PCR Primer: robo4 (m)-PR: sc-44501-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.