SANTA CRUZ BIOTECHNOLOGY, INC.

# robo2 siRNA (m): sc-42255

## BACKGROUND

Specialized cells at the midline, which separates the left and right halves of the CNS, have a number of roles in directing growth cone behavior. In the vertebrate spinal cord, the insect ventral nerve cord and *C. elegans,* midline cells produce guidance cues such as Nectins and Slit, which act as attractants and repellents, respectively. These cells may act as gatekeepers to prevent axons from crossing the midline and to induce a switch in growth cone responsiveness to guidance cues beyond the gateway. One such gatekeeper, robo, is an axon guidance receptor that defines a novel subfamily of Ig superfamily proteins that are conserved from fruit flies to mammals. Robo acts as a receptor for the repellent Slit and functions in a cell-autonomous fashion. Non-crossing axons express high levels of robo, whereas crossing axons express low levels of robo before reaching the midline and high levels after they cross. Robo1 and robo2 are two human homologs of the *Drosophila* protein roundabout. Robo1 is also homologous to the *C. elegans* gene sax3, whereas robo2 is homologous to the zebrafish gene astray.

## REFERENCES


1. Kidd, T., et al. 1998. Roundabout controls axon crossing of the CNS midline and defines a novel subfamily of evolutionarily conserved guidance receptors. Cell 92: 205-215.
2. Zallen, J.A., et al. 1998. The conserved immuno-globulin superfamily member SAX-3/robo directs multiple aspects of axon guidance in *C. elegans.* Cell 92: 217-227.
3. van Vactor, D., et al. 1999. The middle and the end: Slit brings guidance and branching together in axon pathway selection. Neuron 22: 649-652.
4. Fricke, C., et al. 2001. Astray, a zebrafish roundabout homolog required for retinal axon guidance. Science 292: 507-510.
5. LocusLink Report (LocusID: 6091). http://www.ncbi.nlm.nih.gov/LocusLink/


## CHROMOSOMAL LOCATION

Genetic locus: Robo2 (mouse) mapping to 16 C3.1.

## PRODUCT

robo2 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 robo2 shRNA Plasmid (m): sc-42255-SH and robo2 shRNA (m) Lentiviral Particles: sc-42255-V as alternate gene silencing products.

For independent verification of robo2 (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-42255A, sc-42255B and sc-42255C.

## PROTOCOLS

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

## 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

robo2 siRNA (m) is recommended for the inhibition of robo2 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

robo2 (A-10): sc-376177 is recommended as a control antibody for monitoring of robo2 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 robo2 gene expression knockdown using RT-PCR Primer: robo2 (m)-PR: sc-42255-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.