

SEMA6D siRNA (h): sc-63008

BACKGROUND

Semaphorins are a family of cell surface and secreted proteins that are conserved from insects to humans. Members of this family of proteins are approximately 750 amino acids in length (including signal sequences) and are defined by a conserved extracellular "semaphorin" domain of approximately 500 amino acids containing 14-16 cysteines, blocks of conserved sequences and no obvious repeats. Secreted and cell-bound semaphorins chemically attract and repel the growth of neural axons, guiding the development of intricate networks of neural tissue. SEMA6D (semaphorin-6D) is a 1,073 amino acid member of the semaphorin family. Localized to the cell membrane or the cytoplasm (depending on the isoform), SEMA6D is involved in remodeling and maintenance of neuronal connections and functions in growth cone collapsing activity. SEMA6D contains one PSI domain and one semaphorin domain and is thought to be a stop signal for dorsal root ganglion neurons once they reach their target areas. Seven isoforms exist due to alternative splicing events.

REFERENCES

1. Qu, X., et al. 2002. Identification, characterization, and functional study of the two novel human members of the semaphorin gene family. *J. Biol. Chem.* 277: 35574-35585.
2. Online Mendelian Inheritance in Man, OMIM™. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 609295. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>
3. Toyofuku, T., et al. 2004. Guidance of myocardial patterning in cardiac development by Sema6D reverse signalling. *Nat. Cell Biol.* 6: 1204-1211.
4. Comoglio, P.M., et al. 2004. Invasive growth: a two-way street for semaphorin signalling. *Nat. Cell Biol.* 6: 1155-1157.
5. Assou, S., et al. 2006. The human cumulus—oocyte complex gene-expression profile. *Hum. Reprod.* 21: 1705-1719.

CHROMOSOMAL LOCATION

Genetic locus: SEMA6D (human) mapping to 15q21.1.

PRODUCT

SEMA6D siRNA (h) 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see SEMA6D shRNA Plasmid (h): sc-63008-SH and SEMA6D shRNA (h) Lentiviral Particles: sc-63008-V as alternate gene silencing products.

For independent verification of SEMA6D (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-63008A, sc-63008B and sc-63008C.

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 μ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μ l of RNase-free water makes a 10 μ M solution in a 10 μ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

SEMA6D siRNA (h) is recommended for the inhibition of SEMA6D expression in human 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 μ M in 66 μ l. 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

SEMA6D (A-8): sc-393258 is recommended as a control antibody for monitoring of SEMA6D 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-IgG κ BP-HRP: sc-516102 or m-IgG κ 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-IgG κ BP-FITC: sc-516140 or m-IgG κ 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 SEMA6D gene expression knockdown using RT-PCR Primer: SEMA6D (h)-PR: sc-63008-PR (20 μ l). 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.