SANTA CRUZ BIOTECHNOLOGY, INC.

SEMA6A siRNA (h): sc-63004

BACKGROUND

Semaphorins are a family of cell surface and secreted proteins involved in neural development that are conserved from insects to humans. Members of this family 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. The transmembrane semaphorins are characterized by an additional 80 amino acid transmembrane domain and an 80-110 amino acid cytoplasmic domain. SEMA6A, also known as SEMA VIA, is a single pass type-I transmembrane protein that exists as a homodimer or oligomer when active. It is expressed in undifferentiated embryonic stem cells, endodermal progenitors and adult brain. SEMA6A functions as a repellent for sympathetic ganglion axons and propagates this activity through its receptors, plexin-A2 and plexin-A4. SEMA6A may also inhibit growth factor- and tumor-induced angiogenesis.

REFERENCES

1. Zhou, L., et al. 1997. Cloning and expression of a novel murine semaphorin with structural similarity to insect semaphorin I. Mol. Cell. Neurosci. 9: 26-41.
2. Kikuchi, K., et al. 1999. Cloning and characterization of a novel class VI semaphorin, semaphorin Y. Mol. Cell. Neurosci. 13: 9-23.
3. Klostermann, A., et al. 2001. The orthologous human and murine semaphorin 6A-1 proteins (SEMA6A-1/Sema6A-1) bind to the enabled/vasodilator-stimulated phosphoprotein-like protein (EVL) via a novel carboxyl-terminal Zyxin-like domain. J. Biol. Chem. 275: 39647-39653.
4. Kerjan, G., et al. 2005. The transmembrane semaphorin SEMA6A controls cerebellar granule cell migration. Nat. Neurosci. 8: 1516-1524.
5. Dhanabal, M., et al. 2005. Recombinant semaphorin 6A-1 ectodomain inhibits *in vivo* growth factor and tumor cell line-induced angiogenesis. Cancer Biol. Ther. 4: 659-668.

CHROMOSOMAL LOCATION

Genetic locus: SEMA6A (human) mapping to 5q23.1.

PRODUCT

SEMA6A 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see SEMA6A shRNA Plasmid (h): sc-63004-SH and SEMA6A shRNA (h) Lentiviral Particles: sc-63004-V as alternate gene silencing products.

For independent verification of SEMA6A (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-63004A, sc-63004B and sc-63004C.

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

SEMA6A siRNA (h) is recommended for the inhibition of SEMA6A 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 µM in 60 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

SEMA6A (B-3): sc-398302 is recommended as a control antibody for monitoring of SEMA6A 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 SEMA6A gene expression knockdown using RT-PCR Primer: SEMA6A (h)-PR: sc-63004-PR (20 µl, 595 bp). 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.