

VAP-B/C siRNA (h): sc-61770

BACKGROUND

SNAREs are compartmentally specific, integral membrane proteins that are involved in the fusion of membranes and the transport of intracellular proteins. SNAREs are expressed at high levels in all cell types. VAMP-associated proteins (VAPs) regulate the activity of SNAREs. VAP-B is a 243 amino acid protein, which consists of a conserved N-terminal domain, an α -helical coiled-coil domain and a C-terminal transmembrane domain. VAP-C is a 99 amino acid protein that is a splice variant of VAP-B and retains the N-terminal 70 residues, but lacks both the coiled-coil and the transmembrane domains. Mutations in this "VAP-B/C" gene may result in amyotrophic lateral sclerosis, pinal muscular atrophy, progressive bulbar palsy or primary lateral sclerosis. These are all motor neuron diseases which belong to a group of neurodegenerative disorders involving the upper and/or lower motor neurons.

REFERENCES

1. Skehel, P.A., et al. 1995. A VAMP-binding protein from *Aplysia* required for neurotransmitter release. *Science* 269: 1580-1583.
2. Ravichandran, V., et al. 1996. Identification of a novel syntaxin- and synaptobrevin/SNAP-23, expressed in non-neuronal tissues. *J. Biol. Chem.* 271: 13300-13303.
3. Nishimura, Y., et al. 1999. Molecular cloning and characterization of mammalian homologues of vesicle-associated membrane protein-associated (VAMP-associated) proteins. *Biochem. Biophys. Res. Commun.* 254: 21-26.
4. Zhou, Q.L., et al. 1999. Velvet antler polypeptides promoted proliferation of chondrocytes and osteoblast precursors and fracture healing. *Zhongguo Yao Li Xue Bao* 20: 279-282.

CHROMOSOMAL LOCATION

Genetic locus: VAPB (human) mapping to 20q13.32.

PRODUCT

VAP-B/C 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 VAP-B/C shRNA Plasmid (h): sc-61770-SH and VAP-B/C shRNA (h) Lentiviral Particles: sc-61770-V as alternate gene silencing products.

For independent verification of VAP-B/C (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-61770A, sc-61770B and sc-61770C.

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

VAP-B/C siRNA (h) is recommended for the inhibition of VAP-B/C 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

VAP-B/C (1A2): sc-293364 is recommended as a control antibody for monitoring of VAP-B/C 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 VAP-B/C gene expression knockdown using RT-PCR Primer: VAP-B/C (h)-PR: sc-61770-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Goonawardane, N., et al. 2017. Phosphorylation of serine 225 in hepatitis C virus NS5A regulates protein-protein interactions. *J. Virol.* 91 pii: e00805-17.

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.