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N-type Ca⁺⁺ CP α 1B siRNA (h): sc-42698

BACKGROUND

N-type calcium channels are localized in high density presynaptic nerve terminals and are crucial elements in neuronal excitation-secretion coupling. Peripherally distributed N-type Ca⁺⁺ channel plays a key role in cardiovascular regulation through autonomic nervous system. The high-voltage activated Ca²⁺ channels that have been characterized biochemically are complexes of a pore-forming α -1 subunit; a transmembrane, disulfide-linked complex of α -2 and δ subunits; an intracellular β subunit; and in some cases, a transmembrane γ subunit. The α -1 subunit conducts N-type Ca²⁺ currents, which initiate rapid synaptic transmission. In addition to mediating Ca²⁺ entry to initiate transmitter release, N-type Ca²⁺ channels are thought to interact directly with proteins of the synaptic vesicle docking and fusion machinery. The synaptic protein interaction sites in the intracellular loop II-III of subunit α -1B of N-type Ca²⁺ channels bind to syntaxin, SNAP-25 and synaptotagmin.

REFERENCES

1. Catterall, W.A. 1999. Interactions of presynaptic Ca²⁺ channels and snare proteins in neurotransmitter release. *Ann. N.Y. Acad. Sci.* 868: 144-159.
2. Fossier, P., et al. 1999. Calcium transients and neurotransmitter release at an identified synapse. *Trends Neurosci.* 4: 161-166.
3. Uneyama, H., et al. 1999. Pharmacology of N-type Ca²⁺ channels distributed in cardiovascular system. *Int. J. Mol. Med.* 5: 455-466.
4. Catterall, W.A. 1999. Interactions of presynaptic Ca²⁺ channels and snare proteins in neurotransmitter release. *Ann. N.Y. Acad. Sci.* 868: 144-159.
5. Catterall, W.A. 2000. Structure and regulation of voltage-gated Ca²⁺ channels. *Annu. Rev. Cell Dev. Biol.* 16: 521-555.

CHROMOSOMAL LOCATION

Genetic locus: CACNA1B (human) mapping to 9q34.3.

PRODUCT

N-type Ca⁺⁺ CP α 1B 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 N-type Ca⁺⁺ CP α 1B shRNA Plasmid (h): sc-42698-SH and N-type Ca⁺⁺ CP α 1B shRNA (h) Lentiviral Particles: sc-42698-V as alternate gene silencing products.

For independent verification of N-type Ca⁺⁺ CP α 1B (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-42698A, sc-42698B and sc-42698C.

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.

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

N-type Ca⁺⁺ CP α 1B siRNA (h) is recommended for the inhibition of N-type Ca⁺⁺ CP α 1B 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

N-type Ca⁺⁺ CP α 1B (A-2): sc-377489 is recommended as a control antibody for monitoring of N-type Ca⁺⁺ CP α 1B 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 N-type Ca⁺⁺ CP α 1B gene expression knockdown using RT-PCR Primer: N-type Ca⁺⁺ CP α 1B (h)-PR: sc-42698-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.