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P/Q-type Ca⁺⁺ CP α1A siRNA (h): sc-42700

BACKGROUND

Voltage-dependent Ca²⁺ channels mediate Ca²⁺ entry into excitable cells in response to membrane depolarization, and they are involved in a variety of Ca²⁺-dependent processes, including muscle contraction, hormone or neurotransmitter release and gene expression. Calcium channels are highly diverse, multimeric complexes composed of an α1 subunit, an intracellular β subunit, a disulfide linked α2/δ subunit and a transmembrane γ subunit. Ca²⁺ currents are characterized on the basis of their biophysical and pharmacologic properties and include L-, N-, T-, P-, Q- and R- types. P/Q-type Ca⁺⁺ channels are localized to presynaptic nerve terminals and are crucial elements in the coupling of neuronal excitation to secretion. P/Q-type Ca⁺⁺ currents initiate a rapid synaptic transmission that is regulated through G proteins, SNARE proteins and protein phosphorylation.

REFERENCES

1. Perez-Reyes, E. and Schneider, T. 1995. Molecular biology of calcium channels. *Kidney Int.* 48: 1111-1124.
2. Jodice, C., et al. 1997. Episodic ataxia type 2 (EA2) and spinocerebellar ataxia type 6 (SCA6) due to CAG repeat expansion in the CACNA1A gene on chromosome 19p. *Hum. Mol. Genet.* 6: 1973-1978.
3. Randall, A.D. 1998. The molecular basis of voltage-gated Ca²⁺ channel diversity: is it time for T? *J. Membr. Biol.* 161: 207-213.
4. Denier, C., et al. 1999. High prevalence of CACNA1A truncations and broader clinical spectrum in episodic ataxia type 2. *Neurology* 52: 1816-1821.
5. Jen, J., et al. 1999. A novel nonsense mutation in CACNA1A causes episodic ataxia and hemiplegia. *Neurology* 53: 34-37.
6. Duroc, A., et al. 1999. Recurrence of the T666M calcium channel CACNA1A gene mutation in familial hemiplegic migraine with progressive cerebellar ataxia. *Am. J. Hum. Genet.* 64: 89-98.

CHROMOSOMAL LOCATION

Genetic locus: CACNA1A (human) mapping to 19p13.2.

PRODUCT

P/Q-type Ca⁺⁺ CP α1A 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 P/Q-type Ca⁺⁺ CP α1A shRNA Plasmid (h): sc-42700-SH and P/Q-type Ca⁺⁺ CP α1A shRNA (h) Lentiviral Particles: sc-42700-V as alternate gene silencing products.

For independent verification of P/Q-type Ca⁺⁺ CP α1A (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-42700A, sc-42700B and sc-42700C.

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

P/Q-type Ca⁺⁺ CP α1A siRNA (h) is recommended for the inhibition of P/Q-type Ca⁺⁺ CP α1A 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 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

P/Q-type Ca⁺⁺ CP α1A (C-2): sc-390004 is recommended as a control antibody for monitoring of P/Q-type Ca⁺⁺ CP α1A 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_k BP-HRP: sc-516102 or m-IgG_k 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_k BP-FITC: sc-516140 or m-IgG_k 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 P/Q-type Ca⁺⁺ CP α1A gene expression knockdown using RT-PCR Primer: P/Q-type Ca⁺⁺ CP α1A (h)-PR: sc-42700-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.