

Alsin siRNA (h): sc-60158

BACKGROUND

Mutations in the ALS2 gene result in a number of juvenile recessive motor neuron diseases (MNDs), including juvenile primary lateral sclerosis (JPLS), a recessive form of amyotrophic lateral sclerosis (ALS2); infantile onset ascending hereditary spastic paralysis (IAHSP); and a form of complicated hereditary spastic paraplegia (cHSP). The ALS2 gene encodes the Alsin protein. Alsin acts as a guanine nucleotide exchange factor for Rab5, a modulator of the endocytic pathway. Alsin is a cytosolic protein that is associated with small, punctate membrane structures. Therefore, Alsin may mediate membrane transport events, potentially linking endocytic processes and actin cytoskeleton remodeling. The ALS2 C-terminal-like protein (ALS2CL) also modulates Rab 5 activity.

REFERENCES

1. Yang, Y., et al. 2001. The gene encoding alsin, a protein with three guanine nucleotide exchange factor domains, is mutated in a form of recessive amyotrophic lateral sclerosis. *Nat. Genet.* 29: 160-165.
2. Topp, J.D., et al. 2004. Alsin is a Rab5 and Rac1 guanine nucleotide exchange factor. *J. Biol. Chem.* 279: 24612-24623.
3. Hadano, S., et al. 2004. ALS2CL, the novel protein highly homologous to the carboxy-terminal half of ALS2, binds to Rab5 and modulates endosome dynamics. *FEBS Lett.* 575: 64-70.
4. Devon, R.S., et al. 2005. Cross-species characterization of the ALS2 gene and analysis of its pattern of expression in development and adulthood. *Neurobiol. Dis.* 18: 243-257.
5. Panzeri, C., et al. 2006. The first ALS2 missense mutation associated with JPLS reveals new aspects of alsin biological function. *Brain* 129: 1710-1719.
6. Matsuoka, M. and Nishimoto, I. 2006. Anti-ALS activity of Alsin, the product of the ALS2 gene, and activity-dependent neurotrophic factor. *Neurodegener. Dis.* 2: 135-138.
7. Jacquier, A., et al. 2006. Alsin/Rac1 signaling controls survival and growth of spinal motoneurons. *Ann. Neurol.* 60: 105-117.
8. Devon, R.S., et al. 2006. Als2-deficient mice exhibit disturbances in endosome trafficking associated with motor behavioral abnormalities. *Proc. Natl. Acad. Sci. USA* 103: 9595-9600.

CHROMOSOMAL LOCATION

Genetic locus: ALS2 (human) mapping to 2q33.1.

PRODUCT

Alsin 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 Alsin shRNA Plasmid (h): sc-60158-SH and Alsin shRNA (h) Lentiviral Particles: sc-60158-V as alternate gene silencing products.

For independent verification of Alsin (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-60158A, sc-60158B and sc-60158C.

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

Alsin siRNA (h) is recommended for the inhibition of Alsin 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

Alsin (2J10): sc-134256 is recommended as a control antibody for monitoring of Alsin 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 MarkerTM 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 Alsin gene expression knockdown using RT-PCR Primer: Alsin (h)-PR: sc-60158-PR (20 μ l, 510 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.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.