

Atlastin siRNA (m): sc-60226

BACKGROUND

Atlastins are Golgi-localized, integral membrane proteins that function as GTPases. The Atlastin proteins, also designated SPG3A and guanylate-binding protein 3, comprise a Dynamin superfamily that plays a role in axonal maintenance. Hereditary spastic paraplegia (HSP) is an inherited neurodegenerative disorder that is characterized by retrograde axonal degeneration. HSP primarily affects long corticospinal neurons and causes spastic lower extremity weakness. Spastin, a microtubule (MT)-severing AAA ATPase, is a binding partner of Atlastin that is involved in membrane dynamics. This Spastin/Atlastin binding may be involved in the biochemical pathway that leads to HSP development. Mutations in the Atlastin gene (SPG3A) account for approximately 10% of all autosomal dominant HSPs, while mutations in the Spastin gene (SPG4) account for almost 40%.

REFERENCES

1. Zhu, P.P., Patterson, A., Lavoie, B., Stadler, J., Shoeb, M., Patel, R. and Blackstone, C. 2003. Cellular localization, oligomerization and membrane association of the hereditary spastic paraplegia 3A (SPG3A) protein Atlastin. *J. Biol. Chem.* 278: 49063-49071.
2. Elliott, J.L. 2004. Beginning to understand hereditary spastic paraplegia Atlastin. *Arch. Neurol.* 61: 1842-1843.
3. Dürr, A., Camuzat, A., Colin, E., Tallaksen, C., Hannequin, D., Coutinho, P., Fontaine, B., Rossi, A., Gil, R., Rousselle, C., Ruberg, M., Stevanin, G. and Brice, A. 2004. Atlastin-1 mutations are frequent in young-onset autosomal dominant spastic paraplegia. *Arch. Neurol.* 61: 1867-1872.
4. Abel, A., Fonknechten, N., Hofer, A., Dürr, A., Cruaud, C., Voit, T., Weissenbach, J., Brice, A., Klümpe, S., Auburger, G. and Hazan, J. 2004. Early onset autosomal dominant spastic paraplegia caused by novel mutations in SPG3A. *Neurogenetics* 5: 239-243.
5. Hedera, P., Eldevik, O.P., Maly, P., Rainier, S. and Fink, J.K. 2005. Spinal cord magnetic resonance imaging in autosomal dominant hereditary spastic paraplegia. *Neuroradiology* 47: 730-734.

CHROMOSOMAL LOCATION

Genetic locus: Spg3a (mouse) mapping to 12 C2.

PRODUCT

Atlastin siRNA (m) 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 Atlastin shRNA Plasmid (m): sc-60226-SH and Atlastin shRNA (m) Lentiviral Particles: sc-60226-V as alternate gene silencing products.

For independent verification of Atlastin (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-60226A, sc-60226B and sc-60226C.

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

Atlastin siRNA (m) is recommended for the inhibition of Atlastin expression in mouse 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

Atlastin (E-9): sc-376619 is recommended as a control antibody for monitoring of Atlastin 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 Atlastin gene expression knockdown using RT-PCR Primer: Atlastin (m)-PR: sc-60226-PR (20 μ l). 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.