SANTA CRUZ BIOTECHNOLOGY, INC.

Spastin siRNA (h): sc-61603

BACKGROUND

The AAA protein family members share an ATPase domain and have roles in various cellular processes including intracellular motility, membrane trafficking, proteolysis, protein folding and organelle biogenesis. Spastin, a member of the AAA protein family, is a 616 amino acid protein and is involved in the function or assembly of nuclear protein complexes. The Spastin protein is expressed ubiquitously and localizes to the nucleus and the cytoplasm, where it may also be involved in microtubule dynamics. Mutations in the Spastin gene (SPAST, SPG4) cause the most common form of spastic paraplegia 4, an autosomal dominant form of hereditary spastic paraplegia (HSP). HSPs comprise a group of inherited neurological disorders characterized by spastic lower extremity weakness due to a length-dependent, retrograde axonopathy of corticospinal motor neurons. SPAST-specific mutations account for approximately 40% of all autosomal dominant HSPs.

REFERENCES

1. Nielsen, J.E., et al. 2004. Hereditary spastic paraplegia with cerebellar ataxia: a complex phenotype associated with a new SPG4 gene mutation. Eur. J. Neurol. 11: 817-824.
2. Scheuer, K.H., et al. 2005. Reduced regional cerebral blood flow in SPG4-linked hereditary spastic paraplegia. J. Neurol. Sci. 235: 23-32.
3. Alber, B., et al. 2005. Spastin related hereditary spastic paraplegia with dysplastic corpus callosum. J. Neurol. Sci. 236: 9-12.
4. Claudiani, P., et al. 2005. Spastin subcellular localization is regulated through usage of different translation start sites and active export from the nucleus. Exp. Cell Res. 309: 358-369.

CHROMOSOMAL LOCATION

Genetic locus: SPAST (human) mapping to 2p22.3.

PRODUCT

Spastin 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 Spastin shRNA Plasmid (h): sc-61603-SH and Spastin shRNA (h) Lentiviral Particles: sc-61603-V as alternate gene silencing products.

For independent verification of Spastin (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-61603A, sc-61603B and sc-61603C.

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

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

Spastin (C-3): sc-374068 is recommended as a control antibody for monitoring of Spastin 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-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 Spastin gene expression knockdown using RT-PCR Primer: Spastin (h)-PR: sc-61603-PR (20 µl, 510 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Daftary, G.S., et al. 2011. A novel role for the AAA ATPase Spastin as a HOXA10 transcriptional corepressor in Ishikawa endometrial cells. Mol. Endocrinol. 25: 1539-1549.

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.