SANTA CRUZ BIOTECHNOLOGY, INC.

Paraplegin siRNA (h): sc-62755

BACKGROUND

Paraplegin, also known as SPG7 (spastic paraplegia protein 7), CAR, CMAR or PGN, is a 795 amino acid metalloprotease that is a member of the AAA protein family. Localized to the mitochrondrial membrane and expressed throughout the body, Paraplegin is a multi-pass membrane protein that is thought to be involved in signal transduction and chaperone-like activities in the mitochrondria. Defects in the gene encoding Paraplegin are the cause of spastic paraplegia type 7 (SPG7), a form of autosomal recessive hereditary spastic paraplegia (AR-HSP). HSPs are degenerative spinal cord disorders that are characterized by muscle spasms, stiffness in the legs and, in some cases, incontinence. Recent studies suggest that SPG7 may be a mitochondrial-based disease, as mutations in the Paraplegin gene lead to ragged-red fibers, oxidase-negative fibers and intense succinate dehydrogenase-stained areas of the mitochrondria. These mitochondrial dysfunctions lead to axonal degeneration and impaired axonal transport, thus causing the neurodegeneration seen in HSPs.

REFERENCES

1. Settasatian, C., et al. 1999. Genomic structure and expression analysis of the spastic paraplegia gene, SPG7. Hum. Genet. 105: 139-144.
2. Wilkinson, P.A., et al. 2004. A clinical, genetic and biochemical study of SPG7 mutations in hereditary spastic paraplegia. Brain 127: 973-980.
3. Lindholm, D., et al. 2004. Mitochondrial proteins in neuronal degeneration. Biochem. Biophys. Res. Commun. 321: 753-758.
4. Claypool, S.M. and Koehler, C.M. 2005. Hereditary spastic paraplegia: respiratory choke or unactivated substrate? Cell 123: 183-185.
5. Nolden, M., et al. 2005. The m-AAA protease defective in hereditary spastic paraplegia controls ribosome assembly in mitochondria. Cell 123: 277-289.
6. Pirozzi, M., et al. 2006. Intramuscular viral delivery of paraplegin rescues peripheral axonopathy in a model of hereditary spastic paraplegia. J. Clin. Invest. 116: 202-208.
7. Elleuch, N., et al. 2006. Mutation analysis of the paraplegin gene (SPG7) in patients with hereditary spastic paraplegia. Neurology 66: 654-659.
8. Warnecke, T., et al. 2007. A novel form of autosomal recessive hereditary spastic paraplegia caused by a new SPG7 mutation. Neurology 69: 368-375.

CHROMOSOMAL LOCATION

Genetic locus: SPG7 (human) mapping to 16q24.3.

PRODUCT

Paraplegin 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 Paraplegin shRNA Plasmid (h): sc-62755-SH and Paraplegin shRNA (h) Lentiviral Particles: sc-62755-V as alternate gene silencing products.

For independent verification of Paraplegin (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-62755A, sc-62755B and sc-62755C.

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

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

Paraplegin (C-5): sc-514393 is recommended as a control antibody for monitoring of Paraplegin 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 Paraplegin gene expression knockdown using RT-PCR Primer: Paraplegin (h)-PR: sc-62755-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.

PROTOCOLS

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