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SANTA CRUZ BIOTECHNOLOGY, INC.

Gigaxonin siRNA (r): sc-156074

BACKGROUND

Gigaxonin, also refered to as giant axonal neuropathy, GAN1, or KLHL16, controls protein degradation and is essential for neuronal function and survival. Gigaxonin is a member of the cytoskeletal BTB/kelch repeat family and influences cytoskeletal organization and dynamics, playing a large role in neurofilament architecture. The amino terminal BTB domain of Gigaxonin binds to the ubiquitin-activating enzyme E1, while the carboxy-terminal kelch repeat domain interacts directly with the light chain of microtubule-associated protein 1B (MAP1B), and tags it for degredation. Overexpression of MAP1B may lead to neuronal cell death, whereas a reduction of MAP1B significantly improves the survival rate of neurons. Mutations in the Gigaxonin gene result in human giant axonal neuropathy (GAN), an autosomal recessive neurodegenerative disorder characterized by axonal degeneration caused by cytoskeletal abnormalities, including accumulated intermediate filaments.

REFERENCES

1. Ding, J., et al. 2002. Microtubule-associated protein 1B: a neuronal binding partner for Gigaxonin. J. Cell Biol. 158: 427-433.
2. Bomont, P., et al. 2003. Identification of seven novel mutations in the GAN gene. Hum. Mutat. 21: 446.
3. Bomont, P., et al. 2003. Intermediate filament aggregation in fibroblasts patients is aggravated in non-dividing cells and by microtubule destabilization. Hum. Mol. Genet. 12: 813-822.
4. Nakagawa, M., et al. 2003. Molecular mechanisms of hereditary neuropathy: genotype-phenotype correlation. Rinsho Byori 51: 536-543.
5. Cullen, V.C., et al. 2004. Gigaxonin is associated with the Golgi and dimerises via its BTB domain. Neuroreport 15: 873-876.
6. Bruno, C., et al. 2004. Clinical and molecular findings in patients with giant axonal neuropathy (GAN). Neurology 62: 13-16.
7. Allen, E., et al. 2005. Gigaxonin-controlled degradation of MAP1B light chain is critical to neuronal survival. Nature 438: 224-228.
8. Wang, W., et al. 2005. Gigaxonin interacts with Tubulin folding cofactor B and controls its degradation through the ubiquitin-proteasome pathway. Curr. Biol. 15: 2050-2055.

CHROMOSOMAL LOCATION

Genetic locus: Gan (rat) mapping to 19q12.

PRODUCT

Gigaxonin siRNA (r) 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 Gigaxonin shRNA Plasmid (r): sc-156074-SH and Gigaxonin shRNA (r) Lentiviral Particles: sc-156074-V as alternate gene silencing products.

For independent verification of Gigaxonin (r) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-156074A, sc-156074B and sc-156074C.

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

Gigaxonin siRNA (r) is recommended for the inhibition of Gigaxonin expression in rat 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

Gigaxonin (F-3): sc-376173 is recommended as a control antibody for monitoring of Gigaxonin 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 Gigaxonin gene expression knockdown using RT-PCR Primer: Gigaxonin (r)-PR: sc-156074-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.