

Aprataxin siRNA (h): sc-60196

BACKGROUND

Aprataxin is a nuclear protein, present in both the nucleoplasm and the nucleolus, which is a member of the histidine triad (HIT) superfamily. Aprataxin is involved in DNA single-strand break repair, mediating protein-protein interactions with molecules responding to DNA damage. Aprataxin contains three conserved domains: an N-terminal forkhead-associated (FHA) domain which mediates protein-protein interactions, a HIT domain that is similar to Hint, and a C-terminal zinc finger domain. Loss of function mutations in APTX, the gene encoding for Aprataxin, destabilize the Aprataxin protein and result in a rare neurological disorder known as ataxia-oculomotor apraxia, characterized by abnormal movements of the head and eyes. These mutations either target the HIT domain or truncate the protein N-terminal to a zinc finger.

REFERENCES

1. Gascon, G.G., et al. 1995. Ataxia-oculomotor apraxia syndrome. *J. Child Neurol.* 10: 118-122.
2. Online Mendelian Inheritance in Man, OMIM[™]. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 606350. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>
3. Gueven, N., et al. 2004. Aprataxin, a novel protein that protects against genotoxic stress. *Hum. Mol. Genet.* 13: 1081-1093.
4. Mosesso, P., et al. 2005. The novel human gene aprataxin is directly involved in DNA single-strand-break repair. *Cell. Mol. Life Sci.* 62: 485-491.
5. Criscuolo, C., et al. 2005. Very late onset in ataxia oculomotor apraxia type I. *Ann. Neurol.* 57: 777.
6. Ochsner, F., et al. 2005. Mutation of the aprataxin gene presenting with Charcot-Marie-Tooth-like neuropathy and cerebellar ataxia. *Rev. Neurol.* 161: 331-336.
7. Seidle, H.F., et al. 2005. Disease-associated mutations inactivate AMP-lysine hydrolase activity of Aprataxin. *J. Biol. Chem.* 280: 20927-20931.

CHROMOSOMAL LOCATION

Genetic locus: APTX (human) mapping to 9p21.1.

PRODUCT

Aprataxin 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 Aprataxin shRNA Plasmid (h): sc-60196-SH and Aprataxin shRNA (h) Lentiviral Particles: sc-60196-V as alternate gene silencing products.

For independent verification of Aprataxin (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-60196A, sc-60196B and sc-60196C.

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

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

Aprataxin (B-12): sc-374108 is recommended as a control antibody for monitoring of Aprataxin 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 Aprataxin gene expression knockdown using RT-PCR Primer: Aprataxin (h)-PR: sc-60196-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.