

ATAD3A siRNA (h): sc-88047

BACKGROUND

The AAA ATPase family of molecular chaperones are characterized by a highly conserved AAA motif. Composed of 200-250 residues, the AAA domain contains Walker homology sequences and imparts ATPase activity. Members of the AAA ATPase family act as DNA helicases as well as transcription factors and are thought to be involved in several cellular functions such as cell-cycle regulation, protein proteolysis, organelle biogenesis and vesicle-mediated protein transport. Mitochondrial membrane proteins ATAD3A and ATAD3B contribute to the stabilization of nucleoids which are large mitochondrial DNA (mtDNA)-protein complexes. ATAD3A/B may participate in the transformation pathway and the chemosensitivity of oligodendrogliomas. The genes encoding ATAD3A/B/C maps to human chromosome 1, which houses over 3,000 genes and is the largest human chromosome spanning about 260 million base pairs and making up 8% of the human genome.

REFERENCES

1. Patel, S., et al. 1998. The AAA team: related ATPases with diverse functions. *Trends Cell Biol.* 8: 65-71.
2. Neuwald, A.F., et al. 1999. AAA+: a class of chaperone-like ATPases associated with the assembly, operation, and disassembly of protein complexes. *Genome Res.* 9: 27-43.
3. Ogura, T., et al. 2001. AAA+ superfamily ATPases: common structure—diverse function. *Genes Cells* 6: 575-597.
4. Ye, Y., et al. 2001. The AAA ATPase Cdc48/p97 and its partners transport proteins from the ER into the cytosol. *Nature* 414: 652-656.
5. Iyer, L.M., et al. 2004. Evolutionary history and higher order classification of AAA+ ATPases. *J. Struct. Biol.* 146: 11-31.
6. Weise, A., et al. 2005. New insights into the evolution of chromosome 1. *Cytogenet. Genome Res.* 108: 217-222.
7. Kedzierska, S. 2006. Structure, function and mechanisms of action of ATPases from the AAA superfamily of proteins. *Postepy Biochem.* 52: 330-338.
8. He, J., et al. 2007. The AAA+ protein ATAD3 has displacement loop binding properties and is involved in mitochondrial nucleoid organization. *J. Cell Biol.* 176: 141-146.

CHROMOSOMAL LOCATION

Genetic locus: ATAD3A (human) mapping to 1p36.33.

PRODUCT

ATAD3A siRNA (h) is a pool of 2 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 ATAD3A shRNA Plasmid (h): sc-88047-SH and ATAD3A shRNA (h) Lentiviral Particles: sc-88047-V as alternate gene silencing products.

For independent verification of ATAD3A (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-88047A and sc-88047B.

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

ATAD3A siRNA (h) is recommended for the inhibition of ATAD3A 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

ATAD3A/B/C (A-4): sc-376185 is recommended as a control antibody for monitoring of ATAD3A 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 ATAD3A gene expression knockdown using RT-PCR Primer: ATAD3A (h)-PR: sc-88047-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.

PROTOCOLS

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