

Tim8A siRNA (h): sc-41247

BACKGROUND

The majority of mitochondrial-directed proteins are encoded by the nuclear genome and are transported to the mitochondria via regulated processes involving the mitochondrial Tom and Tim proteins. The mitochondrial Tim protein family is comprised of a large group of evolutionarily conserved proteins that are found in most eukaryotes. Import of nuclear-encoded precursor proteins into and across the mitochondrial inner membrane is mediated by two distinct complexes, the Tim23 complex and the Tim22 complex, which differ in their substrate specificity. Defects in Tim proteins are implicated in several neuro-degenerative diseases, suggesting important roles for Tim proteins in development and health. Tim8A and Tim8B, which map to human chromosomes Xq22.1 and 11q23.1, respectively, are conserved proteins of the mitochondrial intermembrane space, which are organized in hetero-oligomeric complex with Tim13. Tim8A is highly expressed in fetal and adult brain. Tim8A is mutated in deafness dystonia syndrome, a novel type of disease that causes severe neurological defects, thought to be caused by a defective mitochondrial protein transport system.

REFERENCES

1. Jin, H., et al. 1999. The human family of deafness/dystonia peptide (DDP) related mitochondrial import proteins. *Genomics* 61: 259-267.
2. Bauer, M.F., et al. 1999. The mitochondrial Tim22 preprotein translocase is highly conserved throughout the eukaryotic kingdom. *FEBS Lett.* 464: 41-47.
3. Rassow, J., et al. 1999. The preprotein translocase of the mitochondrial inner membrane: function and evolution. *J. Mol. Biol.* 286: 105-120.
4. Koehler, C.M., et al. 1999. Human deafness dystonia syndrome is a mitochondrial disease. *Proc. Natl. Acad. Sci. USA* 96: 2141-2146.
5. Paschen, S.A., et al. 2000. The role of the Tim8-13 complex in the import of Tim23 into mitochondria. *EMBO J.* 19: 6392-6400.
6. Bauer, M.F. and Neupert, W. 2001. Import of proteins into mitochondria: a novel pathomechanism for progressive neurodegeneration. *J. Inher. Metab. Dis.* 24: 166-180.
7. LocusLink Report (LocusID 1678). <http://www.ncbi.nlm.nih.gov/LocusLink/>

CHROMOSOMAL LOCATION

Genetic locus: TIMM8A (human) mapping to Xq22.1.

PRODUCT

Tim8A 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 Tim8A shRNA Plasmid (h): sc-41247-SH and Tim8A shRNA (h) Lentiviral Particles: sc-41247-V as alternate gene silencing products.

For independent verification of Tim8A (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-41247A, sc-41247B and sc-41247C.

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

Tim8A siRNA (h) is recommended for the inhibition of Tim8A 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

Tim8A (T-40): sc-101282 is recommended as a control antibody for monitoring of Tim8A 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 Tim8A gene expression knockdown using RT-PCR Primer: Tim8A (h)-PR: sc-41247-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.