

Tim50 siRNA (h): sc-63129

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 specificities. Defects in Tim proteins are implicated in several neurodegenerative diseases, suggesting important roles for Tim proteins in development and health. Tim50, also known as Tim50L or TIMM50, is ubiquitously expressed and functions as an important component of the Tim23 complex. Two isoforms of Tim50 are produced by alternative splicing. Isoform 1 localizes to the inner mitochondrial membrane, whereas isoform 2 localizes to nuclear speckles.

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. 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.

CHROMOSOMAL LOCATION

Genetic locus: TIMM50 (human) mapping to 19q13.2.

PRODUCT

Tim50 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 Tim50 shRNA Plasmid (h): sc-63129-SH and Tim50 shRNA (h) Lentiviral Particles: sc-63129-V as alternate gene silencing products.

For independent verification of Tim50 (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-63129A, sc-63129B and sc-63129C.

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

Tim50 siRNA (h) is recommended for the inhibition of Tim50 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

Tim50 (D-8): sc-515268 is recommended as a control antibody for monitoring of Tim50 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 Tim50 gene expression knockdown using RT-PCR Primer: Tim50 (h)-PR: sc-63129-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Zhang, X., et al. 2019. TIMM50 promotes tumor progression via ERK signaling and predicts poor prognosis of non-small cell lung cancer patients. *Mol. Carcinog.* 58: 767-776.
2. Metwally, E., et al. 2020. Tim50 facilitates calpain activation by anchoring it to calcium stores and increasing its sensitivity to calcium. *Cell Res.* E-published.

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.