SANTA CRUZ BIOTECHNOLOGY, INC.

Tim44 siRNA (m): sc-41264

BACKGROUND

Translocation of nuclear encoded preproteins into the mitochondrial matrix requires the coordinated action of the translocases Tom and Tim, which are located in the outer mitochondrial membrane and the inner membrane, respectively. The mitochondrial preprotein translocases of the outer membrane (Tom) is a multi-subunit protein that contains at least eight proteins: four import receptor subunits (Tom70, Tom37, Tom22, and Tom20), three small proteins (Tom7, Tom6, and Tom5), and a structural component of the outer membrane channel (Tom40). The Tom machinery involves the import receptors, which initiate the binding of cytosolically synthesized preproteins to the outer membrane, and a general import pore (GIP), which promotes the translocation of various pre-proteins into the mitochondria. The TIM channel imports nuclear-encoded mitochondrial preproteins, and it involves three proteins, Tim17, Tim23 and Tim44, which are represented at equimolar ratios. Tim17 is expressed as two isoforms Tim17a and Tim17b, which differ only in their C termini sequences, and like Tim23, these proteins are ubiquitously expressed in fetal and adult tissues. Tim17 and Tim23 are integral membrane proteins that comprise the structural elements of the inner membrane channel by which the preproteins are transferred. The Tim44, on the other hand, is a largely hydrophilic protein that recruits the matrix located Hsp70 to the site where the preprotein emerges from the Tim channel.

REFERENCES

1. Neupert, W. 1997. Protein import into mitochondria. Annu. Rev. Biochem. 66: 863-917.
2. Yano, M., et al. 1998. Functional analysis of human mitochondrial receptor Tom20 for protein import into mitochondria. J. Biol. Chem. 273: 26844-26851.
3. Brix, J., et al. 1999. Distribution of binding sequences for the mitochondrial import receptors Tom20, Tom22 and Tom70 in a presequence-carrying preprotein and a non-cleavable preprotein. J. Biol. Chem. 274: 16522-16530.
4. Bauer, M.F., et al. 1999. Genetic and structural characterization of the human mitochondrial inner membrane translocase. J. Mol. Biol. 289: 69-82.
5. Schulke, N., et al. 1999. A multisubunit complex of outer and inner mitochondrial membrane protein translocases stabilized *in vivo* by translocation intermediates. J. Biol. Chem. 274: 22847-22854.

CHROMOSOMAL LOCATION

Genetic locus: Timm44 (mouse) mapping to 8 A1.1.

PRODUCT

Tim44 siRNA (m) 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 Tim44 shRNA Plasmid (m): sc-41264-SH and Tim44 shRNA (m) Lentiviral Particles: sc-41264-V as alternate gene silencing products.

For independent verification of Tim44 (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-41264A, sc-41264B and sc-41264C.

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

Tim44 siRNA (m) is recommended for the inhibition of Tim44 expression in mouse 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

Tim44 (A-9): sc-390755 is recommended as a control antibody for monitoring of Tim44 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 Tim44 gene expression knockdown using RT-PCR Primer: Tim44 (m)-PR: sc-41264-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.