

Tom22 siRNA (h): sc-41265

BACKGROUND

The mitochondrial preprotein translocases of the outer membrane (Tom) is a multisubunit protein complex that facilitates the import of nucleus-encoded precursor proteins across the mitochondrial outer membrane. The Tom machinery consists of import receptors for the initial binding of cytosolically synthesized preproteins and a general import pore (GIP) for the membrane translocation of various preproteins into the mitochondria. The import receptors include Tom20 and Tom22, which form a heteromeric receptor complex that initiates the insertion of newly synthesized proteins into the outer membrane and then directs the precursor protein into the GIP. In yeast, Tom22 is the essential component of the import receptor complex, as it functions as a receptor for the preproteins and serves as a docking point for both Tom20 and the GIP. Tom22 directly associates with Tom40, the major component of the GIP, and forms a stable interaction between the two core complexes. This interaction facilitates the fluid movement of preproteins into the mitochondria. Structural features of Tom22 include an N-terminal negatively charged region exposed to the cytosol, a C-terminal innermembrane space region with little negative charge, and a putative transmembrane region. The gene encoding human Tom22 maps to chromosome 22q13.1.

REFERENCES

1. Rapaport, D., et al. 1997. Mitochondrial protein import. Tom40 plays a major role in targeting and translocation of preproteins by forming a specific binding site for the presequence. *J. Biol. Chem.* 272: 18725-18731.
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. Rapaport, D., et al. 1999. Biogenesis of Tom40, core component of the Tom complex of mitochondria. *J. Cell Biol.* 146: 321-331.
4. Ahting, U., et al. 1999. The Tom core complex: the general protein import pore of the outer membrane of mitochondria. *J. Cell Biol.* 147: 959-968.
5. van Wilpe, S., et al. 1999. Tom22 is a multifunctional organizer of the mitochondrial preprotein translocase. *Nature* 401: 485-489.
6. 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.

CHROMOSOMAL LOCATION

Genetic locus: TOMM22 (human) mapping to 22q13.1.

PRODUCT

Tom22 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 Tom22 shRNA Plasmid (h): sc-41265-SH and Tom22 shRNA (h) Lentiviral Particles: sc-41265-V as alternate gene silencing products.

For independent verification of Tom22 (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-41265A, sc-41265B and sc-41265C.

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

Tom22 siRNA (h) is recommended for the inhibition of Tom22 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

Tom22 (1C9-2): sc-58308 is recommended as a control antibody for monitoring of Tom22 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 Tom22 gene expression knockdown using RT-PCR Primer: Tom22 (h)-PR: sc-41265-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.