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# Tim23 shRNA (h) Lentiviral Particles: sc-44155-V

## 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 preproteins 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-terminal 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 HSP 70 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. Bauer, M.F., et al. 1999. Genetic and structural characterization of the human mitochondrial inner membrane translocase. *J. Mol. Biol.* 289: 69-82.

## CHROMOSOMAL LOCATION

Genetic locus: TIMM23 (human) mapping to 10q11.23.

## PRODUCT

Tim23 shRNA (h) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200  $\mu$ l frozen stock containing  $1.0 \times 10^6$  infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see Tim23 siRNA (h): sc-44155 and Tim23 shRNA Plasmid (h): sc-44155-SH as alternate gene silencing products.

## STORAGE

Store lentiviral particles at  $-80^\circ$  C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at  $4^\circ$  C for up to one week. Avoid repeated freeze thaw cycles.

## PROTOCOLS

See our web site at [www.scbt.com](http://www.scbt.com) or our catalog for detailed protocols and support products.

## APPLICATIONS

Tim23 shRNA (h) Lentiviral Particles is recommended for the inhibition of Tim23 expression in human cells.

## SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200  $\mu$ l frozen viral stock containing  $1.0 \times 10^6$  infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

## GENE EXPRESSION MONITORING

Tim23 (T-20): sc-13297 is recommended as a control antibody for monitoring of Tim23 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 (secondary) reagents are recommended: 1) Western Blotting: use donkey anti-goat IgG-HRP: sc-2020 (dilution range: 1:2000-1:100,000) or Cruz Marker<sup>™</sup> compatible donkey anti-goat IgG-HRP: sc-2033 (dilution range: 1:2000-1:5000), Cruz Marker<sup>™</sup> Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use donkey anti-goat IgG-FITC: sc-2024 (dilution range: 1:100-1:400) or donkey anti-goat IgG-TR: sc-2783 (dilution range: 1:100-1:400) with UltraCruz<sup>™</sup> Mounting Medium: sc-24941.

## RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Tim23 gene expression knockdown using RT-PCR Primer: Tim23 (h)-PR: sc-44155-PR (20  $\mu$ l). Annealing temperature for the primers should be  $55-60^\circ$  C and the extension temperature should be  $68-72^\circ$  C.

## BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

## RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.