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# mtTFA shRNA (m) Lentiviral Particles: sc-45912-V

## BACKGROUND

mtTFA (mitochondrial transcription factor A), also known as mtTF1, TFAM, TCF6 (for transcription factor 6-like1), TCF6L2 and tsHMG, is a nuclear-encoded gene product that is imported into the mitochondria. mtTFA is required for many aspects of mitochondrial biogenesis including the replication and transcription of mitochondrial DNA (mtDNA). In mouse, testis-specific mtTFA is missing the mitochondria targeting sequence and is present in the nucleus rather than the mitochondria. This form of mtTFA is located primarily in the nuclei of elongated spermatids and may be involved in the regulation of gene expression of the haploid male genome. During mouse and human spermatogenesis there is a reduction of mtTFA protein levels and a reduction in mtDNA copy number. These features may provide one of the mechanisms by which paternal mtDNA transmission is prevented. mtTFA has been associated with mitochondrial disorder in humans characterized by ocular myopathy, exercise intolerance and muscle wasting.

## REFERENCES

1. Boissonneault, G., et al. 1993. A testis-specific gene encoding a nuclear high-mobility-group box protein located in elongating spermatids. *Mol. Cell. Biol.* 13: 4323-4330.
2. Tiranti, V., et al. 1995. Chromosomal localization of mitochondrial transcription factor A (TCF6), single-stranded DNA-binding protein (SSBP), and endonuclease G (ENDOG), three human housekeeping genes involved in mitochondrial biogenesis. *Genomics* 25: 559-564.
3. Larsson, N., et al. 1996. A single mouse gene encodes the mitochondrial transcription factor A and a testis-specific nuclear HMG-box protein. *Nat. Genet.* 13: 296-302.
4. Siciliano, G., et al. 2000. Abnormal levels of human mitochondrial transcription factor A in skeletal muscle in mitochondrial encephalomyopathies. *Neurol. Sci.* 21: 985-987.
5. Tessa, A., et al. 2000. Abnormal H-Tfam in a patient harboring a single mtDNA deletion. *Funct. Neurol.* 15: 211-214.

## CHROMOSOMAL LOCATION

Genetic locus: Tfam (mouse) mapping to 10 B5.3.

## PRODUCT

mtTFA shRNA (m) 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 mtTFA siRNA (m): sc-45912 and mtTFA shRNA Plasmid (m): sc-45912-SH as alternate gene silencing products.

## STORAGE

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

## APPLICATIONS

mtTFA shRNA (m) Lentiviral Particles is recommended for the inhibition of mtTFA expression in mouse 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

mtTFA (F-6): sc-166965 is recommended as a control antibody for monitoring of mtTFA 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 goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker<sup>™</sup> compatible goat anti-mouse IgG-HRP: sc-2031 (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 goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (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 mtTFA gene expression knockdown using RT-PCR Primer: mtTFA (m)-PR: sc-45912-PR (20  $\mu$ l, 600 bp). Annealing temperature for the primers should be  $55-60^\circ\text{C}$  and the extension temperature should be  $68-72^\circ\text{C}$ .

## 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.

## PROTOCOLS

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