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mtTFA siRNA (m): sc-45912

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. Boissoneault, 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 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see mtTFA shRNA Plasmid (m): sc-45912-SH and mtTFA shRNA (m) Lentiviral Particles: sc-45912-V as alternate gene silencing products.

For independent verification of mtTFA (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-45912A, sc-45912B and sc-45912C.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.

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

mtTFA siRNA (m) is recommended for the inhibition of mtTFA 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 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

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 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 mtTFA gene expression knockdown using RT-PCR Primer: mtTFA (m)-PR: sc-45912-PR (20 μ l, 600 bp). 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.