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Mfn2 shRNA (h) Lentiviral Particles: sc-43928-V

BACKGROUND

Mitofusin 1 (Mfn1) and mitofusin 2 (Mfn2) are homologs for the *Drosophila* protein fuzzy onion (Fzo). They are mitochondrial membrane proteins and are mediators of mitochondrial fusion. A GTPase domain is required for Mfn protein function but the molecular mechanisms of the GTPase-dependent reaction as well as the functional division of the two Mfn proteins are unknown. They are essential for embryonic development and may play a role in the pathobiology of obesity. Although the Mfn1 and Mfn2 genes are broadly expressed, they show different levels of expression in different tissues. Two Mfn1 transcripts are elevated in heart, while Mfn2 mRNA is abundantly expressed in heart and muscle tissue but present only at low levels in many other tissues. Mfn1 localizes to mitochondria and participates in at least two different high molecular weight protein complexes in a GTP-dependent manner. Purified recombinant Mfn1 exhibited approximately eightfold higher GTPase activity than Mfn2.

REFERENCES

1. Santel, A., et al. 2001. Control of mitochondrial morphology by a human mitofusin. *J. Cell Sci.* 114: 867-874.
2. Rojo, M., et al. 2002. Membrane topology and mitochondrial targeting of mitofusins, ubiquitous mammalian homologs of the transmembrane GTPase Fzo. *J. Cell Sci.* 115: 1663-1674.
3. Chen, H., et al. 2003. Mitofusins Mfn1 and Mfn2 coordinately regulate mitochondrial fusion and are essential for embryonic development. *J. Cell Biol.* 160: 189-200.
4. Bach, D., et al. 2003. Mitofusin-2 determines mitochondrial network architecture and mitochondrial metabolism. A novel regulatory mechanism altered in obesity. *J. Biol. Chem.* 278: 17190-17197.
5. Santel, A., et al. 2003. Mitofusin-1 protein is a generally expressed mediator of mitochondrial fusion in mammalian cells. *J. Cell Sci.* 116: 2763-2774.
6. Ishihara, N. et al. 2004. Mitofusin 1 and 2 play distinct roles in mitochondrial fusion reactions via GTPase activity. *J. Cell Sci.* 117: 6535-6546.

CHROMOSOMAL LOCATION

Genetic locus: MFN2 (human) mapping to 1p36.22.

STORAGE

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

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.

PRODUCT

Mfn2 shRNA (h) Lentiviral Particles are concentrated, transduction-ready viral particles containing a target-specific construct that encodes a 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 μl frozen stock containing 1.0×10^6 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 Mfn2 siRNA (h): sc-43928 and Mfn2 shRNA Plasmid (h): sc-43928-SH as alternate gene silencing products.

APPLICATIONS

Mfn2 shRNA (h) Lentiviral Particles is recommended for the inhibition of Mfn2 expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μl frozen viral stock containing 1.0×10^6 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

Mfn2 (H-68): sc-50331 is recommended as a control antibody for monitoring of Mfn2 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-rabbit IgG-HRP: sc-2004 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible goat anti-rabbit IgG-HRP: sc-2030 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-rabbit IgG-FITC: sc-2012 (dilution range: 1:100-1:400) or goat anti-rabbit IgG-TR: sc-2780 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Mfn2 gene expression knockdown using RT-PCR Primer: Mfn2 (h)-PR: sc-43928-PR (20 μl , 557 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.

PROTOCOLS

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