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SANTA CRUZ BIOTECHNOLOGY, INC.

mitoNEET siRNA (m): sc-149450

BACKGROUND

MitoNEET, also known as CISD1 (CDGSH iron sulfur domain 1) or ZCD1, is a 108 amino acid single-pass type II membrane protein that localizes to mitochondria and contains one CDGSH-type zinc-finger. Expressed at high levels in heart, liver and skeletal muscle, mitoNEET exists as a homodimer that can bind iron as a cofactor and plays an essential role in the regulation of electron transport capacity and oxidative phosphorylation. Additionally, mitoNEET is thought to be associated with CFTR (cystic fibrosis transmembrane conductance regulator) and may play a role in the pathogenesis of cystic fibrosis. MitoNEET expression is downregulated by glibenclamide (an anti-diabetic drug) and is upregulated by isoproterenol (a synthetic catecholamine that stimulates both β1-AR and β2-AR), suggesting that mitoNEET is under tight regulation by electron transport-associated molecules.

REFERENCES

1. Wiley, S.E., et al. 2007. The outer mitochondrial membrane protein mitoNEET contains a novel redox-active 2Fe-2S cluster. J. Biol. Chem. 282: 23745-23749.
2. Hou, X., et al. 2007. Crystallo-graphic studies of human mitoNEET. J. Biol. Chem. 282: 33242-33246.
3. Wiley, S.E., et al. 2007. MitoNEET is an iron-containing outer mitochondrial membrane protein that regulates oxidative capacity. Proc. Natl. Acad. Sci. USA 104: 5318-5323.
4. Paddock, M.L., et al. 2007. MitoNEET is a uniquely folded 2Fe-2S outer mitochondrial membrane protein stabilized by pioglitazone. Proc. Natl. Acad. Sci. USA 104: 14342-14347.
5. Lin, J., et al. 2007. Crystal structure of human mitoNEET reveals distinct groups of iron sulfur proteins. Proc. Natl. Acad. Sci. USA 104: 14640-14645.
6. Taminelli, G.L., et al. 2008. CISD1 codifies a mitochondrial protein upregulated by the CFTR channel. Biochem. Biophys. Res. Commun. 365: 856-862.

CHROMOSOMAL LOCATION

Genetic locus: Cisd1 (mouse) mapping to 10 B5.3.

PRODUCT

mitoNEET 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see mitoNEET shRNA Plasmid (m): sc-149450-SH and mitoNEET shRNA (m) Lentiviral Particles: sc-149450-V as alternate gene silencing products.

For independent verification of mitoNEET (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-149450A, sc-149450B and sc-149450C.

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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCL, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

mitoNEET siRNA (m) is recommended for the inhibition of mitoNEET 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 µM in 66 µl. 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

mitoNEET (AT1A8): sc-517413 is recommended as a control antibody for monitoring of mitoNEET 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-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 mitoNEET gene expression knockdown using RT-PCR Primer: mitoNEET (m)-PR: sc-149450-PR (20 µl). 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.