SANTA CRUZ BIOTECHNOLOGY, INC.

ATPAF1 siRNA (h): sc-78578

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The Power to Question

BACKGROUND

The mitochondrial ATP synthases transduce the energy contained in the membrane's electrochemical proton gradients into the energy required for synthesis of high-energy phosphate bonds. F1 is the hydrophilic domain of ATPase that has three identical alpha subunits, three identical β subunits and three additional subunits. Each ATPase contains three catalytic sites for synthesis, with one site located in each of the three β subunits. ATPAF1 (ATP synthase mitochondrial F1 complex assembly factor 1), also known as its yeast homolog Atp11p, is a 328 amino acid mitochondrial protein that is required for the assembly of F1-β and F1-α subunits into the mitochondrial ATPase. Both ATPAF1 and ATPAF2 are broadly conserved in eukaryotes and are widely expressed, suggesting that they are essential housekeeping proteins. Due to their influence on enzyme assembly, it has been suggested that evaluation of ATPAF1 and ATPAF2 may be of interest in patients with ATP synthase deficiencies in which the underlying biochemical defect is unknown.

REFERENCES

1. Wang, Z.G., et al. 1996. Identification of functional domains in Atp11p. Protein required for assembly of the mitochondrial F_1-ATPase in yeast. J. Biol. Chem. 271: 4887-4894.
2. Wang, Z.G., et al. 2000. The assembly factor Atp11p binds to the β-subunit of the mitochondrial F_1-ATPase. J. Biol. Chem. 275: 5767-5772.
3. Wang, Z.G., et al. 2001. Atp11p and Atp12p are assembly factors for the F_1-ATPase in human mitochondria. J. Biol. Chem. 276: 30773-30778.
4. Sheluho, D., et al. 2001. An accessible hydrophobic surface is a key element of the molecular chaperone action of Atp11p. J. Biol. Chem. 276: 39945-39949.
5. Ackerman, S.H. 2002. Atp11p and Atp12p are chaperones for F_1-ATPase biogenesis in mitochondria. Biochim. Biophys. Acta 1555: 101-105.
6. Online Mendelian Inheritance in Man, OMIM™. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 608917. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/

CHROMOSOMAL LOCATION

Genetic locus: ATPAF1 (human) mapping to 1p33.

PRODUCT

ATPAF1 siRNA (h) 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 ATPAF1 shRNA Plasmid (h): sc-78578-SH and ATPAF1 shRNA (h) Lentiviral Particles: sc-78578-V as alternate gene silencing products.

For independent verification of ATPAF1 (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-78578A, sc-78578B and sc-78578C.

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

ATPAF1 siRNA (h) is recommended for the inhibition of ATPAF1 expression in human 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

ATPAF1 (E-9): sc-393864 is recommended as a control antibody for monitoring of ATPAF1 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 ATPAF1 gene expression knockdown using RT-PCR Primer: ATPAF1 (h)-PR: sc-78578-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.

PROTOCOLS

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