## SANTA CRUZ BIOTECHNOLOGY, INC.

# ATP5A siRNA (h): sc-60227



## BACKGROUND

Mitochondrial ATP synthases (ATPases) transduce the energy contained in membrane electrochemical proton gradients into the energy required for synthesis of high-energy phosphate bonds. ATPases contain two linked complexes: F<sub>1</sub>, the hydrophilic catalytic core; and F<sub>0</sub>, the membrane-embedded protein channel. F<sub>1</sub> consists of three  $\alpha$  chains and three  $\beta$  chains, which are weakly homologous, as well as one  $\gamma$  chain, one  $\delta$  chain and one  $\epsilon$  chain. F<sub>0</sub> consists of three subunits: a, b and c. The  $\alpha$  chain of F<sub>1</sub> is a regulatory subunit that contains 509 amino acids. Mitochondrial ATPase  $\alpha$  chain (ATP5A) localizes to the mitochondria and catalyzes ATP synthesis.

#### REFERENCES

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- 2. Kataoka, H. and Biswas, C. 1991. Nucleotide sequence of a cDNA for the  $\alpha$  subunit of human mitochondrial ATP synthase. Biochim. Biophys. Acta 1089: 393-395.
- 3. Shirakihara, Y., Leslie, A.G., Abrahams, J.P., Walker, J.E., Ueda, T., Sekimoto, Y., Kambara, M., Saika, K., Kagawa, Y. and Yoshida, M. 1997. The crystal structure of the nucleotide-free  $\alpha 3/\beta 3$  subcomplex of F<sub>1</sub>-ATPase from the thermophilic *Bacillus* PS3 is a symmetric trimer. Structure 5: 825-836.
- 4. Godbout, R., Pandita, A., Beatty, B., Bie, W. and Squire, J.A. 1997. Comparative genomic hybridization analysis of Y79 and FISH mapping indicate the amplified human mitochondrial ATP synthase  $\alpha$  subunit gene (ATP5A) maps to chromosome 18q12 $\rightarrow$ q21. Cytogenet. Cell Genet. 77: 253-256.
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#### CHROMOSOMAL LOCATION

Genetic locus: ATP5A1 (human) mapping to 18q21.1.

#### PRODUCT

ATP5A 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  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see ATP5A shRNA Plasmid (h): sc-60227-SH and ATP5A shRNA (h) Lentiviral Particles: sc-60227-V as alternate gene silencing products.

For independent verification of ATP5A (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-60227A, sc-60227B and sc-60227C.

## PROTOCOLS

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

#### STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at  $-20^{\circ}$  C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at  $-20^{\circ}$  C, avoid contact with RNAses and repeated freeze thaw cycles.

Resuspend lyophilized siRNA duplex in 330  $\mu$ l of the RNAse-free water provided. Resuspension of the siRNA duplex in 330  $\mu$ l of RNAse-free water makes a 10  $\mu$ M solution in a 10  $\mu$ M Tris-HCL, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

## **APPLICATIONS**

ATP5A siRNA (h) is recommended for the inhibition of ATP5A 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  $\mu$ M in 66  $\mu$ 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

ATP5A (51): sc-136178 is recommended as a control antibody for monitoring of ATP5A 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-lgG $\kappa$  BP-HRP: sc-516102 or m-lgG $\kappa$  BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker<sup>TM</sup> Molecular Weight Standards: sc-2035, UltraCruz<sup>®</sup> Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-lgG $\kappa$  BP-FITC: sc-516140 or m-lgG $\kappa$  BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz<sup>®</sup> Mounting Medium: sc-24941 or UltraCruz<sup>®</sup> Hard-set Mounting Medium: sc-359850.

## **RT-PCR REAGENTS**

Semi-quantitative RT-PCR may be performed to monitor ATP5A gene expression knockdown using RT-PCR Primer: ATP5A (h)-PR: sc-60227-PR (20  $\mu$ l, 560 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.