SANTA CRUZ BIOTECHNOLOGY, INC.

V-ATPase α1 siRNA (h): sc-63199

BACKGROUND

Vacuolar-type H^+-ATPase (V-ATPase) is a multisubunit enzyme responsible for acidification of eukaryotic intracellular organelles. V-ATPases pump protons against an electrochemical gradient, while F-ATPases reverse the process, thereby synthesizing ATP. The V-ATPase is comprised of a peripheral V1 domain, which is responsible for ATP hydrolysis, and an integral V0 domain, which is responsible for proton translocation. Nine subunits (A–H) make up the V1 domain and five subunits (a, d, c, c' and c") make up the V0 domain. Like F-ATPase, V-ATPase most likely operates through a rotary mechanism, coupling ATP hydrolysis by the V1 domain to proton translocation by the V0 domain. V-ATPase α1, also known as HO68, VA68, VPP2, Vma1 or ATP6V1A1, functions as the A subunit of the V1 domain. It is a 617 amino acid, ubiquitously expressed protein.

REFERENCES

1. van Hille, B., et al. 1993. Identification of two subunit A isoforms of the vacuolar H^+-ATPase in human osteoclastoma. J. Biol. Chem. 268: 7075-7080.
2. Nishi, T., et al. 2002. The vacuolar H^+-ATPases—nature's most versatile proton pumps. Nat. Rev. Mol. Cell Biol. 3: 94-103.
3. Online Mendelian Inheritance in Man, OMIM™. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 607027. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/
4. Morel, N. 2003. Neurotransmitter release: the dark side of the vacuolar-H^+ATPase. Biol. Cell 95: 453-457.
5. Kawasaki-Nishi, S., et al. 2003. Proton translocation driven by ATP hydrolysis in V-ATPases. FEBS Lett. 545: 76-85.
6. Sautin, Y.Y., et al. 2005. Phosphatidylinositol 3-kinase-mediated effects of glucose on vacuolar H^+-ATPase assembly, translocation, and acidification of intracellular compartments in renal epithelial cells. Mol. Cell. Biol. 25: 575-589.
7. Pietrement, C., et al. 2005. Distinct expression patterns of different subunit isoforms of the V-ATPase in the rat epididymis. Biol. Reprod. 74: 185-194.

CHROMOSOMAL LOCATION

Genetic locus: ATP6V1A (human) mapping to 3q13.2.

PRODUCT

V-ATPase α1 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 V-ATPase α1 shRNA Plasmid (h): sc-63199-SH and V-ATPase α1 shRNA (h) Lentiviral Particles: sc-63199-V as alternate gene silencing products.

For independent verification of V-ATPase α1 (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-63199A, sc-63199B and sc-63199C.

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

V-ATPase α1 siRNA (h) is recommended for the inhibition of V-ATPase α1 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

V-ATPase α1 (4F5): sc-293336 is recommended as a control antibody for monitoring of V-ATPase α1 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 V-ATPase α1 gene expression knockdown using RT-PCR Primer: V-ATPase α1 (h)-PR: sc-63199-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.