SANTA CRUZ BIOTECHNOLOGY, INC.

V-ATPase α1 siRNA (m): sc-63200

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.

CHROMOSOMAL LOCATION

Genetic locus: Atp6v1a (mouse) mapping to 16 B4.

PRODUCT

V-ATPase α1 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 V-ATPase α1 shRNA Plasmid (m): sc-63200-SH and V-ATPase α1 shRNA (m) Lentiviral Particles: sc-63200-V as alternate gene silencing products.

For independent verification of V-ATPase α1 (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-63200A, sc-63200B and sc-63200C.

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

V-ATPase α1 siRNA (m) is recommended for the inhibition of V-ATPase α1 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 60 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 (m)-PR: sc-63200-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.