

V-ATPase A4 siRNA (h): sc-63203

BACKGROUND

Vacuolar-type H⁺-ATPase (V-ATPase) is a multisubunit enzyme responsible for the acidification of eukaryotic intracellular organelles. V-ATPases pump protons against an electrochemical gradient, while F-ATPases reverse the process, thereby synthesizing ATP. A peripheral V₁ domain, which is responsible for ATP hydrolysis, and an integral V₀ domain, which is responsible for proton translocation, comprise the V-ATPase complex. Nine subunits (A-H) make up the V₁ domain and five subunits (A, D, C, C' and C'') make up the V₀ domain. V-ATPase A4 (ATPase, H⁺ transporting, lysosomal V₀ subunit α 4), also known as ATP6N1B or ATP6N2, is an 840 amino acid multi-pass membrane protein that localizes to the apical cell membrane and exists as a subunit of the V₀ domain. Expressed in fetal and adult kidney, as well as in the inner ear, V-ATPase A4 is involved in the regulation of normal vectorial acid transport into the urine by the kidney. Defects in the gene encoding V-ATPase A4 are the cause of distal renal tubular acidosis with preserved hearing (RTADR), an autosomal recessive disorder that is characterized by metabolic acidosis accompanied by disturbances of potassium balance and urinary calcium solubility.

REFERENCES

1. Stevens, T.H. and Forgac, M. 1997. Structure, function and regulation of the vacuolar (H⁺)-ATPase. *Annu. Rev. Cell Dev. Biol.* 13: 779-808.
2. Karet, F.E., Finberg, K.E., Nayir, A., Bakaloglu, A., Ozen, S., Hulton, S.A., Sanjad, S.A., Al-Sabban, E.A., Medina, J.F. and Lifton, R.P. 1999. Localization of a gene for autosomal recessive distal renal tubular acidosis with normal hearing (rdRTA2) to 7q33-34. *Am. J. Hum. Genet.* 65: 1656-1665.
3. Wieczorek, H., Brown, D., Grinstein, S., Ehrenfeld, J. and Harvey, W.R. 1999. Animal plasma membrane energization by proton-motive V-ATPases. *Bioessays* 21: 637-648.
4. Forgac, M. 1999. Structure and properties of the vacuolar (H⁺)-ATPases. *J. Biol. Chem.* 274: 12951-12954.
5. Nelson, N. and Harvey, W.R. 1999. Vacuolar and plasma membrane proton-adenosinetriphosphatases. *Physiol. Rev.* 79: 361-385.
6. Brown, D. and Breton, S. 2000. H⁺-V-ATPase-dependent luminal acidification in the kidney collecting duct and the epididymis/vas deferens: vesicle recycling and transcytotic pathways. *J. Exp. Biol.* 203: 137-145.
7. Nishi, T. and Forgac, M. 2002. The vacuolar (H⁺)-ATPases—nature's most versatile proton pumps. *Nat. Rev. Mol. Cell Biol.* 3: 94-103.
8. Su, Y., Zhou, A., Al-Lamki, R.S. and Karet, F.E. 2003. The α -subunit of the V-type H⁺-ATPase interacts with phosphofructokinase-1 in humans. *J. Biol. Chem.* 278: 20013-20018.
9. Su, Y., Blake-Palmer, K.G., Sorrell, S., Javid, B., Bowers, K., Zhou, A., Chang, S.H., Qamar, S. and Karet, F.E. 2008. Human H⁺-ATPase α 4 subunit mutations causing renal tubular acidosis reveal a role for interaction with phosphofructokinase-1. *Am. J. Physiol. Renal Physiol.* 295: F950-F958.

CHROMOSOMAL LOCATION

Genetic locus: ATP6V0A4 (human) mapping to 7q34.

PRODUCT

V-ATPase A4 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see V-ATPase A4 shRNA Plasmid (h): sc-63203-SH and V-ATPase A4 shRNA (h) Lentiviral Particles: sc-63203-V as alternate gene silencing products.

For independent verification of V-ATPase A4 (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-63203A, sc-63203B and sc-63203C.

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 μ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μ l of RNase-free water makes a 10 μ M solution in a 10 μ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

V-ATPase A4 siRNA (h) is recommended for the inhibition of V-ATPase A4 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 μ M in 66 μ 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.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor V-ATPase A4 gene expression knockdown using RT-PCR Primer: V-ATPase A4 (h)-PR: sc-63203-PR (20 μ l). 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.