SANTA CRUZ BIOTECHNOLOGY, INC.

V-ATPase D2 siRNA (m): sc-76886

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. A peripheral V_1 domain, which is responsible for ATP hydrolysis, and a integral V_0 domain, which is responsible for proton translocation, compose V-ATPase. Nine subunits (A-H) make up the V_1 domain and five subunits (a, d, c, c' and c") make up the V_0 domain. Like F-ATPase, V-ATPase most likely operates through a rotary mechanism. V-ATPase D2 is a 350 amino acid protein that is expressed in kidney, lung and osteoclast. V-ATPase D2 has been implicated as a regulator of urine acidification, osteoclast fusion and bone formation. Furthermore, V-ATPase D2 has been identified as a dendritic cell marker.

REFERENCES

1. Smith, A.N., et al. 2002. Molecular cloning and characterization of novel tissue-specific isoforms of the human vacuolar H^+-ATPase C, G and d subunits, and their evaluation in autosomal recessive distal renal tubular acidosis. Gene 297: 169-177.
2. Sun-Wada, G.H., et al. 2003. Diversity of mouse proton-translocating ATPase: presence of multiple isoforms of the C, d and G subunits. Gene 302: 147-153.
3. Smith, A.N., et al. 2005. Vacuolar H^+-ATPase d2 subunit: molecular characterization, developmental regulation, and localization to specialized proton pumps in kidney and bone. J. Am. Soc. Nephrol. 16: 1245-1256.
4. Pietrement, C., et al. 2006. Distinct expression patterns of different subunit isoforms of the V-ATPase in the rat epididymis. Biol. Reprod. 74: 185-194.
5. Sato, K., et al. 2006. Selective expression of vacuolar H^+-ATPase subunit d2 by particular subsets of dendritic cells among leukocytes. Mol. Immunol. 43: 1443-1453.
6. Lee, S.H., et al. 2006. V-ATPase V_0 subunit d2-deficient mice exhibit impaired osteoclast fusion and increased bone formation. Nat. Med. 12: 1403-1409.

CHROMOSOMAL LOCATION

Genetic locus: Atp6v0d2 (mouse) mapping to 4 A3.

PRODUCT

V-ATPase D2 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 D2 shRNA Plasmid (m): sc-76886-SH and V-ATPase D2 shRNA (m) Lentiviral Particles: sc-76886-V as alternate gene silencing products.

For independent verification of V-ATPase D2 (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-76886A, sc-76886B and sc-76886C.

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 D2 siRNA (m) is recommended for the inhibition of V-ATPase D2 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 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.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor V-ATPase D2 gene expression knockdown using RT-PCR Primer: V-ATPase D2 (m)-PR: sc-76886-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.