SANTA CRUZ BIOTECHNOLOGY, INC.

V-ATPase D1 siRNA (m): sc-63208

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 V_1 domain, which is responsible for ATP hydrolysis, and an integral V_0 domain, which is responsible for proton translocation, comprise the V-ATPase complex. 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. V-ATPase D1 (ATPase, H+ transporting, lysosomal, V_0 subunit D1), also known as ATP6V0D1, P39, VATX, VMA6, ATP6D or VPATPD, is the D subunit of the V_0 domain. Expressed ubiquitously, V-ATPase D1 acts in concert with other V_0 subunits to catalytically acidify a variety of intracellular compartments, thereby synthesizing ATP to be used for vacuolar transport.

REFERENCES

1. van Hille, B., Vanek, M., Richener, H., Green, J.R. and Bilbe, G. 1993. Cloning and tissue distribution of subunits C, D, and E of the human vacuolar H+-ATPase. Biochem. Biophys. Res. Commun. 197: 15-21.
2. Finbow, M.E. and Harrison, M.A. 1997. The vacuolar H+-ATPase: a universal proton pump of eukaryotes. Biochem. J. 324: 697-712.
3. Forgac, M. 1999. Structure and properties of the vacuolar (H+)-ATPases. J. Biol. Chem. 274: 12951-12954.
4. Agarwal, A.K. and White, P.C. 2000. Structure of the VPATPD gene encoding subunit D of the human vacuolar proton ATPase. Biochem. Biophys. Res. Commun. 279: 543-547.
5. Smith, A.N., Borthwick, K.J. and Karet, F.E. 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.
6. Nishi, T. and Forgac, M. 2002. The vacuolar (H+)-ATPases—nature's most versatile proton pumps. Nat. Rev. Mol. Cell Biol. 3: 94-103.

CHROMOSOMAL LOCATION

Genetic locus: Atp6v0d1 (mouse) mapping to 8 D3.

PRODUCT

V-ATPase D1 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 D1 shRNA Plasmid (m): sc-63208-SH and V-ATPase D1 shRNA (m) Lentiviral Particles: sc-63208-V as alternate gene silencing products.

For independent verification of V-ATPase D1 (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-63208A, sc-63208B and sc-63208C.

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 D1 siRNA (m) is recommended for the inhibition of V-ATPase D1 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.

GENE EXPRESSION MONITORING

V-ATPase D1 (D-4): sc-393322 is recommended as a control antibody for monitoring of V-ATPase D1 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 D1 gene expression knockdown using RT-PCR Primer: V-ATPase D1 (m)-PR: sc-63208-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.