SANTA CRUZ BIOTECHNOLOGY, INC.

V-ATPase B1 siRNA (m): sc-36788

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. The V-ATPase V_1 B subunit exists as two isoforms. In the inner ear, the V-ATPase B1 isoform functions in proton secretion and is required to maintain proper endolymph pH and normal auditory function. The gene encoding the human V-ATPase B1 isoform maps to chromosome 2cen-q13. Mutations in this gene cause distal renal tubular acidosis associated with sensorineural deafness. The V-ATPase B2 isoform is expressed in kidney and is the only B isoform expressed in osteoclasts. The gene encoding the human V-ATPase B2 isoform maps to chromosome 8p21.3.

REFERENCES

1. Bernasconi, P., et al. 1990. An mRNA from human brain encodes an isoform of the B subunit of the vacuolar H^+-ATPase. J. Biol. Chem. 265: 17428-17431.
2. Ozcelik, T., et al. 1991. Chromosomal assignments of genes for vacuolar (endomembrane) proton pump subunits VPP1/Vpp-1 (116 kDa) and VPP3/Vpp-3 (58 kDa) in human and mouse. Cytogenet. Cell Genet. 58: 2008-2009.
3. Nelson, R.D., et al. 1992. Selectively amplified expression of an isoform of the vacuolar H^+-ATPase 56 kDa subunit in renal intercalated cells. Proc. Natl. Acad. Sci. USA 89: 3541-3545.
4. Lee, B.S., et al. 1996. Osteoclasts express the B2 isoform of vacuolar H^+-ATPase intracellularly and on their plasma membranes. Am. J. Physiol. 270: 382-388.
5. Karet, F.E., et al. 1999. Mutations in the gene encoding B1 subunit of H^+-ATPase cause renal tubular acidosis with sensorineural deafness. Nat. Genet. 21: 84-90.

CHROMOSOMAL LOCATION

Genetic locus: Atp6v1b1 (mouse) mapping to 6 C3.

PRODUCT

V-ATPase B1 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 B1 shRNA Plasmid (m): sc-36788-SH and V-ATPase B1 shRNA (m) Lentiviral Particles: sc-36788-V as alternate gene silencing products.

For independent verification of V-ATPase B1 (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-36788A, sc-36788B and sc-36788C.

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 B1 siRNA (m) is recommended for the inhibition of V-ATPase B1 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 B1/2 (D-4): sc-271832 is recommended as a control antibody for monitoring of V-ATPase B1 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 B1 gene expression knockdown using RT-PCR Primer: V-ATPase B1 (m)-PR: sc-36788-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.