SANTA CRUZ BIOTECHNOLOGY, INC.

V-ATPase A1 siRNA (h): sc-42686

BACKGROUND

The subunit of the vacuolar proton pump is a V-ATPase that has two different isoforms. The type I isoform contains an 18-base pair insert and is expressed in brain, whereas the truncated type II isoform is more widely expressed, including lung, kidney and spleen. The subunit of the vacuolar proton pump is located in clathrin-coated vesicles and is also found in osteoclasts. It consists of two fundamental domains, a hydrophilic amino-terminus, which has greater than 30% charged residues, and a hydrophobic carboxy terminus, which contains at least six transmembrane regions. The proton pump functions in coupling ATP hydrolysis by the cytoplasmic subunits to proton translocation by the intramembranous components of the pump. The inactivation of the osteoclast-specific vacuolar proton ATPase subunit is responsible for the lack of the enzyme in the apical membranes of osteoclast cells in osteosclerotic mutant mice, thus preventing the resorption function of these cells and leading to the osteopetrotic phenotype. The subunit, which co-localizes with the late endosomal marker Rab 7 on vacuolar membranes, is essential for vacuole formation by selective swelling of late endosomes.

REFERENCES

1. Perin, M.S., et al. 1991. Structure of the 116-kDa polypeptide of the clathrin-coated vesicle/synaptic vesicle proton pump. J. Biol. Chem. 266: 3877-3881.
2. Peng, S.B., et al. 1994. Alternative mRNA splicing generates tissue-specific isoforms of 116-kDa polypeptide of vacuolar proton pump. J. Biol. Chem. 269: 17262-17266.
3. Papini, E., et al. 1996. The vacuolar ATPase proton pump is present on intracellular vacuoles induced by *Helicobacter pylori*. J. Med. Microbiol. 45: 84-89.
4. Peng, S.B., et al. 1999. Identification and reconstitution of an isoform of the 116-kDa subunit of the vaculor proton translocating ATPase. J. Biol. Chem. 274: 2549-2555.
5. Scimeca, J.C., et al. 2000. The gene encoding the mouse homologue of the human osteoclast-specific 116-kDa V-ATPase subunit bears a deletion in osteosclerotic (oc/oc) mutants. Bone 26: 207-213.

CHROMOSOMAL LOCATION

Genetic locus: ATP6V0A1 (human) mapping to 17q21.2.

PRODUCT

V-ATPase A1 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see V-ATPase A1 shRNA Plasmid (h): sc-42686-SH and V-ATPase A1 shRNA (h) Lentiviral Particles: sc-42686-V as alternate gene silencing products.

For independent verification of V-ATPase A1 (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-42686A, sc-42686B and sc-42686C.

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 A1 siRNA (h) is recommended for the inhibition of V-ATPase A1 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 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 A1 (E-8): sc-374475 is recommended as a control antibody for monitoring of V-ATPase A1 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 A1 gene expression knockdown using RT-PCR Primer: V-ATPase A1 (h)-PR: sc-42686-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Li, M., et al. 2020. Characterization of Zika virus endocytic pathways in human glioblastoma cells. Front. Microbiol. 11: 242.

RESEARCH USE

For research use only, not for use in diagnostic procedures.