

V-ATPase H siRNA (h): sc-36801

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 V₁ domain, which is responsible for ATP hydrolysis, and a integral V₀ domain, which is responsible for proton translocation, compose V-ATPase. Nine subunits (A-H) make up the V₁ domain and five subunits (a, d, c, c' and c'') make up the V₀ domain. Like F-ATPase, V-ATPase most likely operates through a rotary mechanism. The H subunit of V-ATPase, also designated SDF is comprised of two polypeptides derived from the same gene. This regulatory subunit plays a critical role in the functional coupling of ATP hydrolysis activity to proton transport in the V-ATPase pump.

REFERENCES

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2. Geyer, M., et al. 2002. Subunit H of the V-ATPase binds to the medium chain of adaptor protein complex 2 and connects Nef to the endocytic machinery. *J. Biol. Chem.* 277: 28521-28529.
3. Geyer, M., et al. 2002. Subunit H of the V-ATPase involved in endocytosis shows homology to β -adaptins. *Mol. Biol. Cell* 13: 2045-2056.
4. Morel, N. 2003. Neurotransmitter release: the dark side of the vacuolar H⁺-ATPase. *Biol. Cell* 95: 453-457.
5. Kawasaki-Nishi, S., et al. 2003. Proton translocation driven by ATP hydrolysis in V-ATPases. *FEBS Lett.* 545: 76-85.
6. Smith, A.N., et al. 2003. Revised nomenclature for mammalian vacuolar-type H⁺-ATPase subunit genes. *Mol. Cell* 12: 801-803.
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CHROMOSOMAL LOCATION

Genetic locus: ATP6V1H (human) mapping to 8q11.23.

PRODUCT

V-ATPase H 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 H shRNA Plasmid (h): sc-36801-SH and V-ATPase H shRNA (h) Lentiviral Particles: sc-36801-V as alternate gene silencing products.

For independent verification of V-ATPase H (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-36801A, sc-36801B and sc-36801C.

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 H siRNA (h) is recommended for the inhibition of V-ATPase H 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.

GENE EXPRESSION MONITORING

V-ATPase H (C-8): sc-166227 is recommended as a control antibody for monitoring of V-ATPase H 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-IgG κ BP-HRP: sc-516102 or m-IgG κ 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-IgG κ BP-FITC: sc-516140 or m-IgG κ 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 H gene expression knockdown using RT-PCR Primer: V-ATPase H (h)-PR: sc-36801-PR (20 μ l, 414 bp). 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.