

V-ATPase C1 siRNA (m): sc-36790

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 an 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. V-ATPase C is an auxiliary subunit with ubiquitous expression. The gene encoding human V-ATPase C maps to chromosome 8q22.3. V-ATPase D is another auxiliary subunit.

REFERENCES

1. Nelson, H., et al. 1990. Molecular cloning of cDNA encoding the C subunit of H⁺-ATPase from bovine chromaffin granules. *J. Biol. Chem.* 265: 20390-20393.
2. van Hille, B., et al. 1993. Cloning and tissue distribution of subunits C, D, and E of the human vacuolar H⁺-ATPase. *Biochem. Biophys. Res. Commun.* 197: 15-21.
3. Hu, R.M., et al. 2000. Gene expression profiling in the human hypothalamus-pituitary-adrenal axis and full-length cDNA cloning. *Proc. Natl. Acad. Sci. USA* 97: 9543-9548.
4. Nishi, T., et al. 2002. The vacuolar H⁺-ATPases—nature's most versatile proton pumps. *Nat. Rev. Mol. Cell Biol.* 3: 94-103.
5. LocusLink Report (LocusID: 528). <http://www.ncbi.nlm.nih.gov/LocusLink/>

CHROMOSOMAL LOCATION

Genetic locus: Atp6v1c1 (mouse) mapping to 15 B3.1.

PRODUCT

V-ATPase C1 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see V-ATPase C1 shRNA Plasmid (m): sc-36790-SH and V-ATPase C1 shRNA (m) Lentiviral Particles: sc-36790-V as alternate gene silencing products.

For independent verification of V-ATPase C1 (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-36790A, sc-36790B and sc-36790C.

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.

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 C1 siRNA (m) is recommended for the inhibition of V-ATPase C1 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 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 C1 (G-5): sc-271077 is recommended as a control antibody for monitoring of V-ATPase C1 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 C1 gene expression knockdown using RT-PCR Primer: V-ATPase C1 (m)-PR: sc-36790-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.