

ATP6E siRNA (m): sc-36794

BACKGROUND

ATP6E, also known as V-ATPase E, is a vacuolar-type H⁺-ATPase (V-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. ATP6E controls acidification of the vacuolar system and provides the main proton-motive force.

REFERENCES

1. Baud, V., et al. 1994. The E subunit of vacuolar H⁺-ATPase localizes close to the centromere on human chromosome 22. *Hum. Mol. Genet.* 3: 335-339.
2. Oka, T., et al. 1997. Three vha genes encode proteolipids of *Caenorhabditis elegans* vacuolar-type ATPase. Gene structures and preferential expression in an H-shaped excretory cell and rectal cells. *J. Biol. Chem.* 272: 24387-24392.
3. Ludwig, J., et al. 1998. Identification and characterization of a novel 9.2-kDa membrane sector-associated protein of vacuolar proton-ATPase from chromaffin granules. *J. Biol. Chem.* 273: 10939-10947.
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: 8992). <http://www.ncbi.nlm.nih.gov/LocusLink/>

CHROMOSOMAL LOCATION

Genetic locus: Atp6v1e1 (mouse) mapping to 6 F1.

PRODUCT

ATP6E 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 ATP6E shRNA Plasmid (m): sc-36794-SH and ATP6E shRNA (m) Lentiviral Particles: sc-36794-V as alternate gene silencing products.

For independent verification of ATP6E (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-36794A, sc-36794B and sc-36794C.

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

ATP6E siRNA (m) is recommended for the inhibition of ATP6E 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

ATP6E (G-3): sc-514143 is recommended as a control antibody for monitoring of ATP6E 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 ATP6E gene expression knockdown using RT-PCR Primer: ATP6E (m)-PR: sc-36794-PR (20 μ l). 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.