

# V-ATPase D siRNA (m): sc-36792

## BACKGROUND

Vacuolar-type H<sup>+</sup>-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<sub>1</sub> domain, which is responsible for ATP hydrolysis, and an integral V<sub>0</sub> domain, which is responsible for proton translocation, compose V-ATPase. Nine subunits (A-H) make up the V<sub>1</sub> domain and five subunits (a, d, c, c' and c'') make up the V<sub>0</sub> domain. Like F-ATPase, V-ATPase most likely operates through a rotary mechanism. V-ATPase C is an auxiliary subunit with ubiquitous expression.

## REFERENCES

1. Nelson, H., et al. 1990. Molecular cloning of cDNA encoding the C subunit of H<sup>+</sup>-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<sup>+</sup>-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<sup>+</sup>-ATPases—nature's most versatile proton pumps. *Nat. Rev. Mol. Cell Biol.* 3: 94-103.
5. LocusLink Report (LocusID: 9114). <http://www.ncbi.nlm.nih.gov/LocusLink/>

## CHROMOSOMAL LOCATION

Genetic locus: Atp6v1d (mouse) mapping to 12 C3.

## PRODUCT

V-ATPase D 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  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see V-ATPase D shRNA Plasmid (m): sc-36792-SH and V-ATPase D shRNA (m) Lentiviral Particles: sc-36792-V as alternate gene silencing products.

For independent verification of V-ATPase D (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-36792A, sc-36792B and sc-36792C.

## 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  $\mu$ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330  $\mu$ l of RNase-free water makes a 10  $\mu$ M solution in a 10  $\mu$ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

## APPLICATIONS

V-ATPase D siRNA (m) is recommended for the inhibition of V-ATPase D 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  $\mu$ M in 66  $\mu$ 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 D (E-12): sc-390384 is recommended as a control antibody for monitoring of V-ATPase D 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 $\kappa$  BP-HRP: sc-516102 or m-IgG $\kappa$  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 $\kappa$  BP-FITC: sc-516140 or m-IgG $\kappa$  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 D gene expression knockdown using RT-PCR Primer: V-ATPase D (m)-PR: sc-36792-PR (20  $\mu$ 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](http://www.scbt.com) for detailed protocols and support products.