

V-ATPase C2 siRNA (h): sc-63205

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. The V-ATPase is comprised of a peripheral V1 domain, which is responsible for ATP hydrolysis, and an integral V0 domain, which is responsible for proton translocation. 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, coupling ATP hydrolysis by the V1 domain to proton translocation by the V0 domain. V-ATPase C2, also known as ATP6V1C2, ATP6C2 or VMA5, is a member of the V-ATPase C subunit family and is specifically expressed in lung and kidney. The V-ATPase C subunit is required for the proper assembly of the catalytic portion of the V-ATPase enzyme and it may have a specific catalytic function.

REFERENCES

1. Smith, A.N., et al. 2002. Molecular cloning and characterization of novel tissue-specific isoforms of the human vacuolar H⁺-ATPase C, G and d subunits, and their evaluation in autosomal recessive distal renal tubular acidosis. *Gene* 297: 169-177.
2. Sun-Wada, G.H., et al. 2003. Mouse proton pump ATPase C subunit isoforms (C2- α and C2- β) specifically expressed in kidney and lung. *J. Biol. Chem.* 278: 44843-44851.
3. Sun-Wada, G.H., et al. 2003. Diversity of mouse proton-translocating ATPase: presence of multiple isoforms of the C, d and G subunits. *Gene* 302: 147-153.
4. Feng, N.H., et al. 2005. Differential expression of a V-type ATPase C subunit gene, Atp6v1c2, during culture of rat lung type II pneumocytes. *J. Biomed. Sci.* 12: 899-911.
5. Jouret, F., et al. 2005. Ubiquitous and kidney-specific subunits of vacuolar H⁺-ATPase are differentially expressed during nephrogenesis. *J. Am. Soc. Nephrol.* 16: 3235-3246.
6. Pietremont, C., et al. 2006. Distinct expression patterns of different subunit isoforms of the V-ATPase in the rat epididymis. *Biol. Reprod.* 74: 185-194.

CHROMOSOMAL LOCATION

Genetic locus: ATP6V1C2 (human) mapping to 2p25.1.

PRODUCT

V-ATPase C2 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 C2 shRNA Plasmid (h): sc-63205-SH and V-ATPase C2 shRNA (h) Lentiviral Particles: sc-63205-V as alternate gene silencing products.

For independent verification of V-ATPase C2 (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-63205A, sc-63205B and sc-63205C.

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 C2 siRNA (h) is recommended for the inhibition of V-ATPase C2 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 C2 (S-10): sc-81888 is recommended as a control antibody for monitoring of V-ATPase C2 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 C2 gene expression knockdown using RT-PCR Primer: V-ATPase C2 (h)-PR: sc-63205-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.