

VPS11 siRNA (m): sc-76901

BACKGROUND

Vacuolar sorting proteins (VPSs) are required for proper trafficking of endocytic and biosynthetic proteins to the vacuole and play an important role in the budding process of cells. VPS11 (vacuolar protein sorting 11), also known as END1, PEP5, RNF108 or PP3476, localizes to the membrane of both the endosome and the lysosome and is the human homolog of yeast Vps11. Expressed ubiquitously with highest expression in heart, VPS11 is thought to play a role in vesicle-mediated protein trafficking, as well as fusion/docking reactions in late endosomes and lysosomes. VPS11 contains one clathrin repeat and one RING-type zinc finger and shares 24% amino acid identity with its yeast counterpart.

REFERENCES

1. Wurmser, A.E., et al. 2000. New component of the vacuolar class C-Vps complex couples nucleotide exchange on the Ypt7 GTPase to SNARE-dependent docking and fusion. *J. Cell Biol.* 151: 551-562.
2. Sato, T.K., et al. 2000. Class C VPS protein complex regulates vacuolar SNARE pairing and is required for vesicle docking/fusion. *Mol. Cell* 6: 661-671.
3. Kim, B.Y., et al. 2001. Molecular characterization of mammalian homologues of class C Vps proteins that interact with Syntaxin 7. *J. Biol. Chem.* 276: 29393-29402.
4. Peterson, M.R. and Emr, S.D. 2001. The class C VPS complex functions at multiple stages of the vacuolar transport pathway. *Traffic* 2: 476-486.
5. Huizing, M., et al. 2001. Molecular cloning and characterization of human VPS18, VPS11, VPS16 and VPS33. *Gene* 264: 241-247.
6. Kim, B.Y., et al. 2003. Identification of mouse VPS16 and biochemical characterization of mammalian class C VPS complex. *Biochem. Biophys. Res. Commun.* 311: 577-582.

CHROMOSOMAL LOCATION

Genetic locus: Vps11 (mouse) mapping to 9 A5.2.

PRODUCT

VPS11 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 VPS11 shRNA Plasmid (m): sc-76901-SH and VPS11 shRNA (m) Lentiviral Particles: sc-76901-V as alternate gene silencing products.

For independent verification of VPS11 (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-76901A, sc-76901B and sc-76901C.

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

VPS11 siRNA (m) is recommended for the inhibition of VPS11 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

VPS11 (C-12): sc-515094 is recommended as a control antibody for monitoring of VPS11 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 VPS11 gene expression knockdown using RT-PCR Primer: VPS11 (m)-PR: sc-76901-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.