

VPS29 siRNA (h): sc-76903

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. VPS29 (vacuolar protein sorting 29 homolog), also known as DC7, DC15 or PEP11, is a 182 amino acid peripheral membrane protein that localizes to both the cytoplasm and to the endosomal membrane and exists as a component of a large multimeric retromer complex. VPS29 is expressed at high levels in heart, lung, placenta, spleen, peripheral blood leukocytes, thymus, colon skeletal muscle, kidney and brain, where it plays an important role in retrograde transport of proteins from endosomes to the *trans*-Golgi network. Multiple isoforms of VPS29 exist due to alternative splicing events.

REFERENCES

1. Damen, E., et al. 2006. The human Vps29 retromer component is a metallo-phosphoesterase for a cation-independent mannose 6-phosphate receptor substrate peptide. *Biochem. J.* 398: 399-409.
2. Shimada, T., et al. 2006. AtVPS29, a putative component of a retromer complex, is required for the efficient sorting of seed storage proteins. *Plant Cell Physiol.* 47: 1187-1194.
3. Vergés, M., et al. 2007. Phosphoinositide 3-kinase regulates the role of retromer in transcytosis of the polymeric immunoglobulin receptor. *Exp. Cell Res.* 313: 707-718.
4. Wassmer, T., et al. 2007. A loss-of-function screen reveals SNX5 and SNX6 as potential components of the mammalian retromer. *J. Cell Sci.* 120: 45-54.
5. Rojas, R., et al. 2007. Interchangeable but essential functions of SNX1 and SNX2 in the association of retromer with endosomes and the trafficking of mannose 6-phosphate receptors. *Mol. Cell. Biol.* 27: 1112-1124.
6. Hierro, A., et al. 2007. Functional architecture of the retromer cargo-recognition complex. *Nature* 449: 1063-1067.
7. Fuji, K., et al. 2007. *Arabidopsis* vacuolar sorting mutants (green fluorescent seed) can be identified efficiently by secretion of vacuole-targeted green fluorescent protein in their seeds. *Plant Cell* 19: 597-609.
8. Kim, E., et al. 2008. Identification of novel retromer complexes in the mouse testis. *Biochem. Biophys. Res. Commun.* 375: 16-21.

CHROMOSOMAL LOCATION

Genetic locus: VPS29 (human) mapping to 12q24.11.

PRODUCT

VPS29 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 VPS29 shRNA Plasmid (h): sc-76903-SH and VPS29 shRNA (h) Lentiviral Particles: sc-76903-V as alternate gene silencing products.

For independent verification of VPS29 (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-76903A, sc-76903B and sc-76903C.

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

VPS29 siRNA (h) is recommended for the inhibition of VPS29 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

VPS29 (D-1): sc-398874 is recommended as a control antibody for monitoring of VPS29 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 VPS29 gene expression knockdown using RT-PCR Primer: VPS29 (h)-PR: sc-76903-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.