

VPS28 siRNA (m): sc-41101

BACKGROUND

Vacuolar protein sorting protein 28 (VPS28) is required for normal endocytic and biosynthetic trafficking to the vacuole. VPS28 mutants accumulate vacuolar endocytic and late Golgi markers in an aberrant endosome-like class E compartment. Class E compartments contain endocytosed markers, as well as precursors of vacuolar hydrolases and markers normally associated with the *trans* Golgi. VPS28 as well as other class E VPS proteins may facilitate the formation of transport intermediates required for efficient transport out of the prevacuolar endosome. Class E proteins appear to be important for sorting material bound for the vacuole away from proteins that cycle through the endocytic system. VPS28 of *Saccharomyces cerevisiae* and its human ortholog localize to the cytoplasm and can be found as subunits of a complex named ESCRT-1, endosomal sorting complex required for transport 1.

REFERENCES

1. Rieder, S.E., Banta, L.M., Kohrer, K., McCaffery, J.M. and Emr, S.D. 1996. Multilamellar endosome-like compartment accumulates in the yeast VPS28 vacuolar protein sorting mutant. *Mol. Biol. Cell* 7: 985-999.
2. Katzmann, D.J., Babst, M. and Emr, S.D. 2001. Ubiquitin-dependent sorting into the multivesicular body pathway requires the function of a conserved endosomal protein sorting complex, ESCRT-I. *Cell* 106: 145-155.
3. Bishop, N. and Woodman, P. 2001. TSG101/mammalian VPS23 and mammalian VPS28 interact directly and are recruited to VPS4-induced endosomes. *J. Biol. Chem.* 276: 11735-11742.
4. Hanson, P.K., Grant, A.M. and Nichols, J.W. 2002. NBD-labeled phosphatidylcholine enters the yeast vacuole via the pre-vacuolar compartment. *J. Cell Sci.* 115: 2725-2733.
5. SWISS-PROT/TrEMBL (Q02767). World Wide Web URL: <http://www.expasy.ch/sprot/sprot-top.html>

CHROMOSOMAL LOCATION

Genetic locus: Vps28 (mouse) mapping to 15 D3.

PRODUCT

VPS28 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 VPS28 shRNA Plasmid (m): sc-41101-SH and VPS28 shRNA (m) Lentiviral Particles: sc-41101-V as alternate gene silencing products.

For independent verification of VPS28 (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-41101A, sc-41101B and sc-41101C.

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

VPS28 siRNA (m) is recommended for the inhibition of VPS28 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

VPS28 (E-7): sc-166537 is recommended as a control antibody for monitoring of VPS28 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 VPS28 gene expression knockdown using RT-PCR Primer: VPS28 (m)-PR: sc-41101-PR (20 μ l, 585 bp). 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.