

VPS25 siRNA (m): sc-61795

BACKGROUND

Vacuolar sorting proteins (VPSs) are required for trafficking normal endocytic and biosynthetic proteins to the vacuole and also play an important role in the budding process of cells. VPS25 is a highly conserved and widely expressed eukaryotic protein, with single orthologs in chromalveolate, excavate, amoebozoan, plant, fungal and metazoan species. VPS25, along with Vps22p and Vps36p, functions as a subunit of ESCRT-II, an endosomal sorting complex required for protein transport. This complex is essential for the sorting of ubiquitinated biosynthetic and endosomal proteins into endosomes. ESCRT-II transiently associates with the endosomal membrane, thereby initiating the formation of ESCRT-III, a membrane-associated protein complex that functions immediately downstream of ESCRT-II during sorting of multivesicular body cargo.

REFERENCES

1. Hierro, A., et al. 2004. Structure of the ESCRT-II endosomal trafficking complex. *Nature* 431: 221-225.
2. Markovich, S., et al. 2004. Genomic approach to identification of mutations affecting caspofungin susceptibility in *Saccharomyces cerevisiae*. *Antimicrob. Agents Chemother.* 48: 3871-3876.
3. Slagsvold, T. and Stenmark, H. 2004. The structure of an endosomal protein sorter. *Dev. Cell* 7: 457-458.
4. Wernimont, A.K. and Weissenhorn, W. 2004. Crystal structure of subunit VPS25 of the endosomal trafficking complex ESCRT-II. *BMC Struct. Biol.* 4: 10.
5. Thompson, B.J., et al. 2005. Tumor suppressor properties of the ESCRT-II complex component VPS25 in *Drosophila*. *Dev. Cell* 9: 711-720.
6. Boysen, J.H. and Mitchell, A.P. 2006. Control of Bro1-domain protein Rim20 localization by external pH, ESCRT machinery, and the *Saccharomyces cerevisiae* Rim101 pathway. *Mol. Biol. Cell* 17: 1344-1353.
7. Herz, H.M., et al. 2006. VPS25 mosaics display non-autonomous cell survival and overgrowth, and autonomous apoptosis. *Development* 133: 1871-1880.

CHROMOSOMAL LOCATION

Genetic locus: Vps25 (mouse) mapping to 11 D.

PRODUCT

VPS25 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 VPS25 shRNA Plasmid (m): sc-61795-SH and VPS25 shRNA (m) Lentiviral Particles: sc-61795-V as alternate gene silencing products.

For independent verification of VPS25 (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-61795A, sc-61795B and sc-61795C.

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

VPS25 siRNA (m) is recommended for the inhibition of VPS25 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

VPS25 (B-4): sc-271648 is recommended as a control antibody for monitoring of VPS25 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 VPS25 gene expression knockdown using RT-PCR Primer: VPS25 (m)-PR: sc-61795-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.