

VPS35 siRNA (h): sc-63218

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. VPS35 (vacuolar protein sorting 35), also known as MEM3, is the 796 amino acid human homolog of the *S. cerevisiae* Vps35 protein. Localized to the cytoplasm and to the peripheral membrane, VPS35 is an essential component of the retromer complex which is involved in retrieval of lysosomal enzyme receptors from endosomes to the *trans*-Golgi network. VPS35 is expressed ubiquitously with highest expression in heart, placenta, brain, testis, kidney, colon, ovary and spleen. In addition to its crucial role in the retromer complex, VPS35 is part of a subcomplex that is required to regulate transcytosis of the polymeric immunoglobulin receptor from the basolateral to the apical surface of epithelial cells and hepatocytes.

REFERENCES

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- Haft, C.R., et al. 2000. Human orthologs of yeast vacuolar protein sorting proteins VPS26, 29, and 35: assembly into multimeric complexes. *Mol. Biol. Cell* 11: 4105-4116.
- Edgar, A.J., et al. 2000. Human homologues of yeast vacuolar protein sorting 29 and 35. *Biochem. Biophys. Res. Commun.* 277: 622-630.
- Vergés, M., et al. 2004. The mammalian retromer regulates transcytosis of the polymeric immunoglobulin receptor. *Nat. Cell Biol.* 6: 763-769.
- Shi, H., et al. 2006. The retromer subunit VPS26 has an arrestin fold and binds VPS35 through its C-terminal domain. *Nat. Struct. Mol. Biol.* 13: 540-548.
- Damen, E., et al. 2006. The human VPS29 retromer component is a metallophosphoesterase for a cation-independent mannose 6-phosphate receptor substrate peptide. *Biochem. J.* 398: 399-409.

CHROMOSOMAL LOCATION

Genetic locus: VPS35 (human) mapping to 16q11.22.

PRODUCT

VPS35 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 VPS35 shRNA Plasmid (h): sc-63218-SH and VPS35 shRNA (h) Lentiviral Particles: sc-63218-V as alternate gene silencing products.

For independent verification of VPS35 (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-63218A, sc-63218B and sc-63218C.

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

VPS35 siRNA (h) is recommended for the inhibition of VPS35 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

VPS35 (C-20): sc-55803 is recommended as a control antibody for monitoring of VPS35 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 VPS35 gene expression knockdown using RT-PCR Primer: VPS35 (h)-PR: sc-63218-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.