SANTA CRUZ BIOTECHNOLOGY, INC.

VPS33B siRNA (m): sc-76906

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. VPS33B (vacuolar protein sorting 33 homolog B) is a 617 amino acid protein that localizes to the cytoplasmic side of the peripheral membrane, as well as to the late endosomal membrane, and belongs to the STXBP/SEC1 family. Expressed ubiquitously with highest expression in testis and lowest expression in lung, VPS33B is thought to play a role in vesicle-mediated protein trafficking to lysosomal compartments and may also be involved in membrane docking events at late endosomes. Defects in the gene encoding VPS33B are the cause of arthrogryposis-renal dysfunction-cholestasis syndrome (ARC), an autosomal recessive disorder that is characterized by renal tubular dysfunction, neurogenic arthrogryposis multiplex congenita and neonatal cholestasis with bile duct hypoplasia.

REFERENCES

1. Carim, L., et al. 2000. Cloning, mapping and expression analysis of VPS33B, the human orthologue of rat Vps33b. Cytogenet. Cell Genet. 89: 92-95.
2. Huizing, M., et al. 2001. Molecular cloning and characterization of human VPS18, VPS11, VPS16, and VPS33. Gene 264: 241-247.
3. Gissen, P., et al. 2004. Mutations in VPS33B, encoding a regulator of SNARE-dependent membrane fusion, cause arthrogryposis-renal dysfunction-cholestasis (ARC) syndrome. Nat. Genet. 36: 400-404.
4. Lo, B., et al. 2005. Requirement of VPS33B, a member of the SEC1/Munc18 protein family, in megakaryocyte and platelet α-granule biogenesis. Blood 106: 4159-4166.
5. Gissen, P., et al. 2005. Comparative evolutionary analysis of VPS33 homologues: genetic and functional insights. Hum. Mol. Genet. 14: 1261-1270.
6. Hershkovitz, D., et al. 2008. Defective lamellar granule secretion in arthrogryposis, renal dysfunction, and cholestasis syndrome caused by a mutation in VPS33B. Arch. Dermatol. 144: 334-340.
7. Bach, H., et al. 2008. *Mycobacterium tuberculosis* virulence is mediated by PtpA dephosphorylation of human vacuolar protein sorting 33B. Cell Host Microbe 3: 316-322.

CHROMOSOMAL LOCATION

Genetic locus: Vps33b (mouse) mapping to 7 D3.

PRODUCT

VPS33B 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see VPS33B shRNA Plasmid (m): sc-76906-SH and VPS33B shRNA (m) Lentiviral Particles: sc-76906-V as alternate gene silencing products.

For independent verification of VPS33B (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-76906A, sc-76906B and sc-76906C.

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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

VPS33B siRNA (m) is recommended for the inhibition of VPS33B 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 µM in 66 µl. 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

VPS33B (G-9): sc-398322 is recommended as a control antibody for monitoring of VPS33B 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-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 VPS33B gene expression knockdown using RT-PCR Primer: VPS33B (m)-PR: sc-76906-PR (20 µl). 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.