SANTA CRUZ BIOTECHNOLOGY, INC.

VPS18 siRNA (h): sc-89965

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. VPS18 (vacuolar protein sorting 18), also known as PEP3 or hVPS18, is a 973 amino acid peripheral membrane protein that localizes to late endosomes and belongs to the VPS family. Expressed ubiquitously with highest expression in heart and lowest expression in lung, VPS18 is thought to play a role in membrane docking reactions of late endosomes and may also function in vesicle-mediated protein trafficking to lysosomal compartments. VPS18 contains one clathrin repeat and one RING-type zinc finger and exists in a large hetero-oligomeric complex with other VPS proteins, including VPS11 and VPS16. Two isoforms of VPS18 exist due to alternative splicing events.

REFERENCES

1. Huizing, M., et al. 2001. Molecular cloning and characterization of human VPS18, VPS 11, VPS16, and VPS33. Gene 264: 241-247.
2. Kim, B.Y., et al. 2001. Molecular characterization of mammalian homologues of class C Vps proteins that interact with syntaxin-7. J. Biol. Chem. 276: 29393-29402.
3. Geissenhöner, A., et al. 2001. Aspergillus nidulans DigA, a potential homolog of *Saccharomyces cerevisiae* Pep3 (Vps18), is required for nuclear migration, mitochondrial morphology and polarized growth. Mol. Genet. Genomics 266: 672-685.
4. Online Mendelian Inheritance in Man, OMIM™. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 608551. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/
5. Kim, B.Y., et al. 2003. Identification of mouse Vps16 and biochemical characterization of mammalian class C Vps complex. Biochem. Biophys. Res. Commun. 311: 577-582.
6. Yogosawa, S., et al. 2005. Ubiquitylation and degradation of serum-inducible kinase by hVPS18, a RING-H2 type ubiquitin ligase. J. Biol. Chem. 280: 41619-41627.

CHROMOSOMAL LOCATION

Genetic locus: VPS18 (human) mapping to 15q15.1.

PRODUCT

VPS18 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see VPS18 shRNA Plasmid (h): sc-89965-SH and VPS18 shRNA (h) Lentiviral Particles: sc-89965-V as alternate gene silencing products.

For independent verification of VPS18 (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-89965A, sc-89965B and sc-89965C.

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

VPS18 siRNA (h) is recommended for the inhibition of VPS18 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 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

VPS18 (237.1): sc-100890 is recommended as a control antibody for monitoring of VPS18 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 VPS18 gene expression knockdown using RT-PCR Primer: VPS18 (h)-PR: sc-89965-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.