SANTA CRUZ BIOTECHNOLOGY, INC.

VPS54 siRNA (h): sc-94724

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. VPS54 (vacuolar protein sorting 54), also known as HCC8, SLP-8p or VPS54L, is a 977 amino acid protein that localizes to the Golgi apparatus and belongs to the VPS family. Expressed as multiple alternatively spliced isoforms, VPS54 functions as a component of the multi-protein Golgi-associated retrograde protein (GARP) complex and is thought to be involved in retrograde transport of early and late endosomes to the Golgi. The gene encoding VPS54 maps to human chromosome 2, which houses over 1,400 genes and comprises nearly 8% of the human genome. Harlequin icthyosis, a rare and morbid skin deformity, is associated with mutations in the ABCA12 gene, while the lipid metabolic disorder sitosterolemia is associated with defects in the ABCG5 and ABCG8 genes. Additionally, an extremely rare recessive genetic disorder, Alström syndrome, is caused by mutations in the ALMS1 gene, which maps to chromosome 2.

REFERENCES

1. Fuchs, S., et al. 2002. Comparative transcription map of the wobbler critical region on mouse chromosome 11 and the homologous region on human chromosome 2p13-14. BMC Genet. 3: 14.
2. Walter, L., et al. 2002. Identification, characterization and cytogenetic mapping of a yeast Vps54 homolog in rat and mouse. Gene 285: 213-220.
3. Liewen, H., et al. 2005. Characterization of the human GARP (Golgi associated retrograde protein) complex. Exp. Cell Res. 306: 24-34.
4. Oka, T. and Krieger, M. 2005. Multi-component protein complexes and Golgi membrane trafficking. J. Biochem. 137: 109-114.
5. Meisler, M.H., et al. 2008. Evaluation of the Golgi trafficking protein VPS54 (wobbler) as a candidate for ALS. Amyotroph. Lateral Scler. 9: 141-148.
6. Perez-Victoria, F.J., et al. 2008. Requirement of the human GARP complex for mannose 6-phosphate-receptor-dependent sorting of cathepsin D to lysosomes. Mol. Biol. Cell 19: 2350-2362.

CHROMOSOMAL LOCATION

Genetic locus: VPS54 (human) mapping to 2p14.

PRODUCT

VPS54 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 VPS54 shRNA Plasmid (h): sc-94724-SH and VPS54 shRNA (h) Lentiviral Particles: sc-94724-V as alternate gene silencing products.

For independent verification of VPS54 (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-94724A, sc-94724B and sc-94724C.

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

VPS54 siRNA (h) is recommended for the inhibition of VPS54 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

VPS54 (B-4): sc-398300 is recommended as a control antibody for monitoring of VPS54 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 VPS54 gene expression knockdown using RT-PCR Primer: VPS54 (h)-PR: sc-94724-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.