SANTA CRUZ BIOTECHNOLOGY, INC.

VPS53 siRNA (h): sc-93834

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. VPS53 (vacuolar protein sorting 53), also known as PP13624 or HCCS1, is a 699 amino acid protein that localizes to both the Golgi apparatus and the endosome membrane and belongs to the VPS family. Expressed as multiple alternatively spliced isoforms, VPS53 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 VPS53 maps to human chromosome 17, which comprises over 2.5% of the human genome and encodes over 1,200 genes.

REFERENCES

1. Zhao, X., et al. 2003. The minimum LOH region defined on chromosome 17p13.3 in human hepatocellular carcinoma with gene content analysis. Cancer Lett. 190: 221-232.
2. Liewen, H., et al. 2005. Characterization of the human GARP (Golgi associated retrograde protein) complex. Exp. Cell Res. 306: 24-34.
3. Oka, T., et al. 2005. Multi-component protein complexes and Golgi membrane trafficking. J. Biochem. 137: 109-114.
4. Zhu, J.D., et al. 2006. Transcription of the putative tumor suppressor gene HCCS1 requires binding of ETS-2 to its consensus near the transcription start site. Cell Res. 16: 780-796.
5. Ko, J.K., et al. 2007. The tail-anchoring domain of Bfl1 and HCCS1 targets mitochondrial membrane permeability to induce apoptosis. J. Cell Sci. 120: 2912-2923.

CHROMOSOMAL LOCATION

Genetic locus: VPS53 (human) mapping to 17p13.3.

PRODUCT

VPS53 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 VPS53 shRNA Plasmid (h): sc-93834-SH and VPS53 shRNA (h) Lentiviral Particles: sc-93834-V as alternate gene silencing products.

For independent verification of VPS53 (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-93834A, sc-93834B and sc-93834C.

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.

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

VPS53 siRNA (h) is recommended for the inhibition of VPS53 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

VPS53 (H-4): sc-514920 is recommended as a control antibody for monitoring of VPS53 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 VPS53 gene expression knockdown using RT-PCR Primer: VPS53 (h)-PR: sc-93834-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.