

# VPS39 siRNA (h): sc-90114

## 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. VPS39 (vacuolar protein sorting 39), also known as VAM6 or TLP, is an 886 amino acid protein that localizes to the cytoplasm, as well as to the lysosomal and endosomal membrane, and contains one CNH domain. Expressed ubiquitously with highest expression in kidney, heart, lung, brain, placenta and skeletal muscle, VPS39 functions as a homooligomer that is thought to play a role in the clustering and fusion of endosomes and lysosomes. Multiple isoforms of VPS39 exist due to alternative splicing events. The gene encoding VPS39 maps to human chromosome 15, which houses over 700 genes and comprises nearly 3% of the human genome.

## REFERENCES

1. Chapman, R.E. 1994. Vacuolar sorting. Tracking down an elusive receptor. *Curr. Biol.* 4: 1019-1022.
2. Nagase, T., Ishikawa, K., Suyama, M., Kikuno, R., Miyajima, N., Tanaka, A., Kotani, H., Nomura, N. and Ohara, O. 1998. Prediction of the coding sequences of unidentified human genes. XI. The complete sequences of 100 new cDNA clones from brain which code for large proteins *in vitro*. *DNA Res.* 5: 277-286.
3. Hartley, J.L., Temple, G.F. and Brasch, M.A. 2000. DNA cloning using *in vitro* site-specific recombination. *Genome Res.* 10: 1788-1795.
4. Caplan, S., Hartnell, L.M., Aguilar, R.C., Naslavsky, N. and Bonifacio, J.S. 2001. Human VAM6P promotes lysosome clustering and fusion *in vivo*. *J. Cell Biol.* 154: 109-122.
5. Felici, A., Wurthner, J.U., Parks, W.T., Giam, L.R., Reiss, M., Karpova, T.S., McNally, J.G. and Roberts, A.B. 2003. TLP, a novel modulator of TGF $\beta$  signaling, has opposite effects on Smad2- and Smad3-dependent signaling. *EMBO J.* 22: 4465-4477.

## CHROMOSOMAL LOCATION

Genetic locus: VPS39 (human) mapping to 15q15.1.

## PRODUCT

VPS39 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  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see VPS39 shRNA Plasmid (h): sc-90114-SH and VPS39 shRNA (h) Lentiviral Particles: sc-90114-V as alternate gene silencing products.

For independent verification of VPS39 (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-90114A, sc-90114B and sc-90114C.

## PROTOCOLS

See our web site at [www.scbt.com](http://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  $\mu$ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330  $\mu$ l of RNase-free water makes a 10  $\mu$ M solution in a 10  $\mu$ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

## APPLICATIONS

VPS39 siRNA (h) is recommended for the inhibition of VPS39 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  $\mu$ M in 66  $\mu$ 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

VPS39 (C-5): sc-514762 is recommended as a control antibody for monitoring of VPS39 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 $\kappa$  BP-HRP: sc-516102 or m-IgG $\kappa$  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 $\kappa$  BP-FITC: sc-516140 or m-IgG $\kappa$  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 VPS39 gene expression knockdown using RT-PCR Primer: VPS39 (h)-PR: sc-90114-PR (20  $\mu$ 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.