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# p150 siRNA (h): sc-43986

## BACKGROUND

Accurate sorting and delivery of proteins to the proper organelles is essential for normal cellular functioning. The yeast Vps proteins are involved in sorting and delivering vacuolar proteins from the Golgi network, where they undergo posttranslational modification, to the vacuole. Vps34p, a key component of this protein trafficking system, shares homology with proteins in the PI 3-kinase family and is regulated by Vps15p. Vps15p is thought to recruit Vps34p to the membrane of the Golgi complex and to enhance Vps34p kinase activity. p150 is the human homolog of the yeast Vps15p and is ubiquitously expressed. p150, like Vps15p, is subject to posttranslational modification, including myristylation.

## REFERENCES

1. Rothman, J.H., Yamashiro, C.T., Kane, P.M. and Stevens, T.H. 1989. Protein targeting to the yeast vacuole. *Trends Biochem. Sci.* 14: 347-350.
2. Stack, J.H. and Emr, S.D. 1994. Vps34p required for yeast vacuolar protein sorting is a multiple specificity kinase that exhibits both protein kinase and phosphatidylinositol-specific PI 3-kinase activities. *J. Biol. Chem.* 269: 31552-31562.
3. Stack, J.H., Horazdovsky, B. and Emr, S.D. 1995. Receptor-mediated protein sorting to the vacuole in yeast: roles for a protein kinase, a lipid kinase and GTP-binding proteins. *Annu. Rev. Cell Dev. Biol.* 11: 1-33.
4. Stack, J.H., DeWald, D.B., Takegawa, K. and Emr, S.D. 1995. Vesicle-mediated protein transport: regulatory interactions between the Vps15 protein kinase and the Vps34 PtdIns 3-kinase essential for protein sorting to the vacuole in yeast. *J. Cell Biol.* 129: 321-334.
5. Volinia, S., Dhand, R., Vanhaesebroeck, B., MacDougall, L.K., Stein, R., Zvelebil, M.J., Domin, J., Panaretou, C. and Waterfield, M.D. 1995. A human phosphatidylinositol 3-kinase complex related to the yeast Vps34p-Vps15p protein sorting system. *EMBO J.* 14: 3339-3348.

## CHROMOSOMAL LOCATION

Genetic locus: PIK3R4 (human) mapping to 3q22.1.

## PRODUCT

p150 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 p150 shRNA Plasmid (h): sc-43986-SH and p150 shRNA (h) Lentiviral Particles: sc-43986-V as alternate gene silencing products.

For independent verification of p150 (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-43986A, sc-43986B and sc-43986C.

## 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

p150 siRNA (h) is recommended for the inhibition of p150 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

p150 (JK-13): sc-100798 is recommended as a control antibody for monitoring of p150 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 p150 gene expression knockdown using RT-PCR Primer: p150 (h)-PR: sc-43986-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.