SANTA CRUZ BIOTECHNOLOGY, INC.

VPS37 siRNA (m): sc-61797

BACKGROUND

Vacuolar sorting proteins (VPSs) are required for normal endocytic and biosynthetic trafficking to the vacuole. In addition, they play an important role in the budding process of cells. The charged multivesicular body (MVB) proteins, commonly designated CHMPs, belong to the vacuolar sorting protein family and function as chromatin-modifying proteins. In yeast, Vps23, Vps28, and Vps37 form endosomal sorting complex I required for transport (ESCRT-I), a complex whose assembly is directed by the C-terminal steadiness box of Vps23, the N-terminal half of Vps28, and the C-terminal half of Vps37. ESCRT-I recognizes ubiquitinated MVB protein cargo and functions to sort this cargo into into MVB vesicles. VPS37C represents the human homolog that functions in ESCRT-I.

REFERENCES

1. Katzmann, D.J., et al. 2001. Ubiquitin-dependent sorting into the function of a conserved endosomal protein sorting complex, ESCRT-I. Cell 106: 145-155.
2. Bache, K.G., et al. 2004. The growth-regulatory protein HCRP1/hVps37A is a subunit of mammalian ESCRT-I and mediates receptor down-regulation. Mol. Biol. Cell 15: 4337-4346.
3. Teo, H., et al. 2004. Structural insights into endosomal sorting complex required for transport (ESCRT-I) recognition of ubiquitinated proteins. J. Biol. Chem. 279: 28689-28696.
4. Stuchell, M.D., et al. 2004. The human endosomal sorting complex required for transport (ESCRT-I) and its role in HIV-1 budding. J. Biol. Chem. 279: 36059-36071.
5. Eastman, S.W., et al. 2005. Identification of human VPS37C, a component of endosomal sorting complex required for transport-I important for viral budding. J. Biol. Chem. 280: 628-636.
6. Kostelansky, M.S., et al. 2006. Structural and functional organization of the ESCRT-I trafficking complex. Cell 125: 113-126.
7. Pineda-Molina, E., et al. 2006. The crystal structure of the C-terminal domain of Vps28 reveals a conserved surface required for Vps20 recruitment. Traffic 7: 1007-1016.

CHROMOSOMAL LOCATION

Genetic locus: Vps37a (mouse) mapping to 8 A4.

PRODUCT

VPS37 siRNA (m) 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 VPS37 shRNA Plasmid (m): sc-61797-SH and VPS37 shRNA (m) Lentiviral Particles: sc-61797-V as alternate gene silencing products.

For independent verification of VPS37 (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-61797A, sc-61797B and sc-61797C.

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

VPS37 siRNA (m) is recommended for the inhibition of VPS37 expression in mouse 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

VPS37A (G-3): sc-376978 is recommended as a control antibody for monitoring of VPS37 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 VPS37 gene expression knockdown using RT-PCR Primer: VPS37 (m)-PR: sc-61797-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.