

VPS4B siRNA (h): sc-41098

BACKGROUND

Class E vacuolar protein sorting (VPS) proteins are necessary for appropriate sorting of receptors in the yeast endocytic pathway. The yeast Vps4p is a member of the AAA protein family (ATPases associated with diverse cellular activities) and plays an important role in transporting proteins out of a pre-vacuolar endosomal compartment. In human, two non-allelic orthologous proteins (VPS4A and VPS4B) of yeast Vps4p are known and can form heteromeric complexes with each other. Both VPS4 (also known as SKD1 in mouse) proteins are class E VPSs and are involved in intracellular protein trafficking, similar to Vps4p in yeast. A human CHMP1 protein, which is implicated in multivesicular body formation, physically interacts with VPS4. HIV-1 uses cellular machinery to bud from infected cells and requires VPS4 and TSG101/VPS23 for this budding process. Dominant negative mutant of VPS4 inhibit vacuolar protein sorting and also arrest HIV-1 and MLV budding. Thus, retroviruses normally use the VPS pathway to form multivesicular bodies during the budding process.

REFERENCES

1. Bishop, N., et al. 2001. TSG101/mammalian VPS23 and mammalian VPS28 interact directly and are recruited to VPS4-induced endosomes. *J. Biol. Chem.* 276: 11735-11742.
2. Scheuring, S., et al. 2001. Mammalian cells express two VPS4 proteins both of which are involved in intracellular protein trafficking. *J. Mol. Biol.* 312: 469-480.
3. Howard, T.L., et al. 2001. CHMP1 functions as a member of a newly defined family of vesicle trafficking proteins. *J. Cell Sci.* 114: 2395-2404.
4. Perez, O.D., et al. 2001. Resistance is futile: assimilation of cellular machinery by HIV-1. *Immunity* 15: 687-690.
5. Garrus, J.E., et al. 2001. Tsg101 and the vacuolar protein sorting pathway are essential for HIV-1 budding. *Cell* 107: 55-65.

CHROMOSOMAL LOCATION

Genetic locus: VPS4B (human) mapping to 18q21.33.

PRODUCT

VPS4B 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see VPS4B shRNA Plasmid (h): sc-41098-SH and VPS4B shRNA (h) Lentiviral Particles: sc-41098-V as alternate gene silencing products.

For independent verification of VPS4B (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-41098A, sc-41098B and sc-41098C.

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 μ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μ l of RNase-free water makes a 10 μ M solution in a 10 μ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

VPS4B siRNA (h) is recommended for the inhibition of VPS4B 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 μ M in 66 μ 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

VPS4B (A-11): sc-377162 is recommended as a control antibody for monitoring of VPS4B 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 κ BP-HRP: sc-516102 or m-IgG κ 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 κ BP-FITC: sc-516140 or m-IgG κ 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 VPS4B gene expression knockdown using RT-PCR Primer: VPS4B (h)-PR: sc-41098-PR (20 μ 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.