SANTA CRUZ BIOTECHNOLOGY, INC.

VPS4A siRNA (m): sc-108046

BACKGROUND

Class E vacuolar protein sorting (VPS) proteins are necessary for the appropriate sorting of receptors in the yeast endocytic pathway. 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 prevacuolar endosomal compartments. In humans, two non-allelic orthologous proteins, VPS4A and VPS4B, are known and can form heteromeric complexes with each other. Both VPS4A and VPS4B are class E VPSs and are involved in intracellular protein trafficking, similar to Vps4p in yeast. HIV-1 uses cellular machinery to bud from infected cells and requires the two human VPS4 proteins and tsg 101 (tumor susceptibility gene 101) for this budding process. Dominant negative mutants of VPS4 proteins 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. and Woodman, P. 2001. Tsg 101/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. and Nolan, G.P. 2001. Resistance is futile: assimilation of cellular machinery by HIV-1. Immunity 15: 687-690.
5. Garrus, J.E., et al. 2001. Tsg 101 and the vacuolar protein sorting pathway are essential for HIV-1 budding. Cell 107: 55-65.
6. Beyer, A., et al. 2003. Comparative sequence and expression analyses of four mammalian VPS4 genes. Gene 305: 47-59.
7. Chen, V.Y., et al. 2006. The role of the VPS4A-exosome pathway in the intrinsic egress route of a DNA-binding anticancer drug. Pharm. Res. 23: 1687-1695.
8. Stuchell-Brereton, M.D., et al. 2007. ESCRT-III recognition by VPS4 ATPases. Nature 449: 740-744.

CHROMOSOMAL LOCATION

Genetic locus: Vps4a (mouse) mapping to 8 D3.

PRODUCT

VPS4A 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 VPS4A shRNA Plasmid (m): sc-108046-SH and VPS4A shRNA (m) Lentiviral Particles: sc-108046-V as alternate gene silencing products.

For independent verification of VPS4A (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-108046A, sc-108046B and sc-108046C.

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

VPS4A siRNA (m) is recommended for the inhibition of VPS4A 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

VPS4A (A-11): sc-393428 is recommended as a control antibody for monitoring of VPS4A 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 VPS4A gene expression knockdown using RT-PCR Primer: VPS4A (m)-PR: sc-108046-PR (20 µl, 509 bp). 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.