

CHMP6 siRNA (m): sc-60377

BACKGROUND

The charged multivesicular body proteins, commonly designated CHMPs, belong to the vacuolar sorting protein family and function as chromatin-modifying proteins. CHMP1-6 are all components of ESCRT (endosomal sorting complex required for transport) I, II or III complexes. These complexes are crucial for sorting endosomal articles into multivesicular bodies (MVBs), as well as required for the formation of these bodies. During HIV-1 infection, the virus uses the ESCRT-III complex to mediate budding and exocytosis of viral proteins. CHMP6, also known as VPS20, interacts with CHMP4 of the ESCRT-III complex. CHMP6 also interacts with SNF8, VPS25 and Vps36 of the ESCRT-II complex, where it regulates cargo sorting by acting as an acceptor for ESCRT-II on endosome membranes.

REFERENCES

1. von Schwedler, U.K., Stuchell, M., Müller, B., Ward, D.M., Chung, H.Y., Morita, E., Wang, H.E., Davis, T., He, G.P., Cimbara, D.M., Scott, A., Kräusslich, H.G., Kaplan, J., Morham, S.G. and Sundquist, W.I. 2003. The protein network of HIV budding. *Cell* 114: 701-713.
2. Yorikawa, C., Shibata, H., Waguri, S., Hatta, K., Horii, M., Katoh, K., Kobayashi, T., Uchiyama, Y. and Maki, M. 2005. Human CHMP6, a myristoylated ESCRT-III protein, interacts directly with an ESCRT-II component EAP20 and regulates endosomal cargo sorting. *Biochem. J.* 387: 17-26.
3. Langelier, C., von Schwedler, U.K., Fisher, R.D., De Domenico, I., White, P.L., Hill, C.P., Kaplan, J., Ward, D. and Sundquist, W.I. 2006. Human ESCRT-II complex and its role in human immunodeficiency virus type 1 release. *J. Virol.* 80: 9465-9480.

CHROMOSOMAL LOCATION

Genetic locus: Chmp6 (mouse) mapping to 11 E2.

PRODUCT

CHMP6 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see CHMP6 shRNA Plasmid (m): sc-60377-SH and CHMP6 shRNA (m) Lentiviral Particles: sc-60377-V as alternate gene silencing products.

For independent verification of CHMP6 (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-60377A, sc-60377B and sc-60377C.

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

CHMP6 siRNA (m) is recommended for the inhibition of CHMP6 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 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

CHMP6 (B-3): sc-398963 is recommended as a control antibody for monitoring of CHMP6 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 CHMP6 gene expression knockdown using RT-PCR Primer: CHMP6 (m)-PR: sc-60377-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.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.