SANTA CRUZ BIOTECHNOLOGY, INC.

LIP5 siRNA (h): sc-95648

BACKGROUND

LIP5 (LYST-interacting protein 5), also known as VTA1 (Vps20-associated 1 homolog), DRG1 (dopamine-responsive protein DRG-1), SBP1 (SKD1 binding protein 1) or My012, is a 307 amino acid protein belonging to the VTA1 family. LIP5 plays a role in sorting membrane proteins, including lysosomal enzymes, lipids and stimulated growth factor receptors, for lysosomal degradation in a pathway known as the endosomal multivesicular bodies (MVB) pathway. Found in brain, liver, heart and kidney, LIP5 localizes to cytoplasm and both endosomal and peripheral membranes. RNAi studies demonstrate that LIP5 depletion decreases human immunodeficiency virus type 1 (HIV-1) budding, and LIP5 is known to interact with CHMP1B, CHMP2A, CHMP5, VPS4B, KIAA0174 (IST1) and possibly CHMP3.

REFERENCES

1. Shi, J., Cai, W., Chen, X., Ying, K., Zhang, K. and Xie, Y. 2001. Identification of Dopamine responsive mRNAs in glial cells by suppression subtractive hybridization. Brain Res. 910: 29-37.
2. Fujita, H., Umezuki, Y., Imamura, K., Ishikawa, D., Uchimura, S., Nara, A., Yoshimori, T., Hayashizaki, Y., Kawai, J., Ishidoh, K., Tanaka, Y. and Himeno, M. 2004. Mammalian class E Vps proteins, SBP1 and mVps2/CHMP2A, interact with and regulate the function of an AAA-ATPase SKD1/VPS4B. J. Cell Sci. 117: 2997-3009.
3. Ward, D.M., Vaughn, M.B., Shiflett, S.L., White, P.L., Pollock, A.L., Hill, J., Schnegelberger, R., Sundquist, W.I. and Kaplan, J. 2005. The role of LIP5 and CHMP5 in multivesicular body formation and HIV-1 budding in mammalian cells. J. Biol. Chem. 280: 10548-10555.
4. Welsch, S., Habermann, A., Jäger, S., Müller, B., Krijnse-Locker, J. and Kräusslich, H.G. 2006. Ultrastructural analysis of ESCRT proteins suggests a role for endosome-associated tubular-vesicular membranes in ESCRT function. Traffic 7: 1551-1566.
5. Online Mendelian Inheritance in Man, OMIM™. 2007. Johns Hopkins University, Baltimore, MD. MIM Number: 610902. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/

CHROMOSOMAL LOCATION

Genetic locus: VTA1 (human) mapping to 6q24.1.

PRODUCT

LIP5 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see LIP5 shRNA Plasmid (h): sc-95648-SH and LIP5 shRNA (h) Lentiviral Particles: sc-95648-V as alternate gene silencing products.

For independent verification of LIP5 (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-95648A, sc-95648B and sc-95648C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

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

LIP5 siRNA (h) is recommended for the inhibition of LIP5 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 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

LIP5 (D-6): sc-374012 is recommended as a control antibody for monitoring of LIP5 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 LIP5 gene expression knockdown using RT-PCR Primer: LIP5 (h)-PR: sc-95648-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

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