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SANTA CRUZ BIOTECHNOLOGY, INC.

LIMP II siRNA (h): sc-41546

BACKGROUND

Lysosomes are intracytoplasmic organelles that are found within leukocytes (granulocytes, neutrophils, basophils and eosinophils) and function as storage granules for small particles. Lysosomes actively support subcellular protein degradation mechanisms through fusion with cellular organelles such as phagocytic vacuoles and the plasma membrane. Lysosome fusion to the plasma membrane, known as exocytosis, releases the contents of the vesicle into the extracellular environment. The lysosomal integral membrane proteins I-III, known as LIMP-I, LIMP-II and LIMP-III, localize from the *trans*-Golgi network to lysosomes via an AP-3-dependent pathway that may involve AP-1 and Clathrin. LIMP I-III are protein markers for the lysosome organelle. These markers are exceptionally useful for microscopy studies, cellular fractionation validation and studies pertaining to protein trafficking through the secretory pathway.

REFERENCES

1. Vega, M.A., et al. 1991. Targeting of lysosomal integral membrane protein LIMP II. The tyrosine-lacking carboxyl cytoplasmic tail of LIMP II is sufficient for direct targeting to lysosomes. J. Biol. Chem. 266: 16269-16272.
2. McIntyre, G.F., et al. 1993. The lysosomal proenzyme receptor that binds procathepsin L to microsomal membranes at pH 5 is a 43 kDa integral membrane protein. Proc. Natl. Acad. Sci. USA 90: 10588-10592.
3. Honing, S., et al. 1996. The tyrosine-based lysosomal targeting signal in LAMP-1 mediates sorting into Golgi-derived clathrin-coated vesicles. EMBO J. 15: 5230-5239.
4. Crombie, R., et al. 1998. Lysosomal integral membrane protein II binds Thrombospondin-1. Structure-function homology with the cell adhesion molecule CD36 defines a conserved recognition motif. J. Biol. Chem. 273: 4855-4863.
5. Le Borgne, R., et al. 1998. The mammalian AP-3 adaptor-like complex mediates the intracellular transport of lysosomal membrane glycoproteins. J. Biol. Chem. 273: 29451-29461.
6. Rohn, W.M., et al. 2000. Bi-directional trafficking between the *trans*-Golgi network and the endosomal/lysosomal system. J. Cell Sci. 113: 2093-2101.

CHROMOSOMAL LOCATION

Genetic locus: SCARB2 (human) mapping to 4q21.1.

PRODUCT

LIMP II 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 LIMP II shRNA Plasmid (h): sc-41546-SH and LIMP II shRNA (h) Lentiviral Particles: sc-41546-V as alternate gene silencing products.

For independent verification of LIMP II (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-41546A, sc-41546B and sc-41546C.

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

LIMP II siRNA (h) is recommended for the inhibition of LIMP II 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

LIMP II (D-3): sc-55570 is recommended as a control antibody for monitoring of LIMP II 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 LIMP II gene expression knockdown using RT-PCR Primer: LIMP II (h)-PR: sc-41546-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Lin, Y.W., et al. 2012. Human SCARB2-mediated entry and endocytosis of EV71. PLoS ONE 7: e30507.

RESEARCH USE

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