

PACS-1a siRNA (h): sc-106348

BACKGROUND

Phosphofurin acidic cluster sorting protein-1 (PACS-1) is related to a family of cytosolic proteins, including HIV-1 Nef and β -arrestin, that direct the internalization of cell surface receptors through the association with the clathrin/AP-2 sorting machinery. Similarly, PACS-1 participates in the localization of membrane proteins to the secretory pathway membrane compartments. Within the secretory pathway, the *trans*-Golgi network (TGN)/endosomal system is essential for sorting and distributing soluble and membrane associated proteins, and for producing lysosomes for exocytosis. PACS-1 is expressed from two distinct reading frames, which generate both a larger form, designated PACS-1a, and a smaller protein that is designated PACS-1b. PACS-1 proteins preferentially bind to the endoprotease, furin, as well as to the mannose 6-phosphate receptor, where they then facilitate the trafficking and localization of these proteins to the TGN, in a phosphorylation dependent manner.

REFERENCES

1. Takahashi, S., et al. 1995. Localization of furin to the *trans*-Golgi network and recycling from the cell surface involves Ser and Tyr residues within the cytoplasmic domain. *J. Biol. Chem.* 270: 28397-28401.
2. Jones, B.G., et al. 1995. Intracellular trafficking of furin is modulated by the phosphorylation state of a casein kinase II site in its cytoplasmic tail. *EMBO J.* 14: 5869-5883.
3. Le Borgne, R., et al. 1997. Mannose 6-phosphate receptors regulate the formation of clathrin-coated vesicles in the TGN. *J. Cell Biol.* 137: 335-345.
4. Wan, L., et al. 1998. PACS-1 defines a novel gene family of cytosolic sorting proteins required for *trans*-Golgi network localization. *Cell* 94: 205-216.
5. Benmerah, A., et al. 1998. AP-2/Eps15 interaction is required for receptor-mediated endocytosis. *J. Cell Biol.* 140: 1055-1062.
6. Teuchert, M., et al. 1999. Sorting of furin at the *trans*-Golgi network. *J. Biol. Chem.* 274: 8199-8207.

CHROMOSOMAL LOCATION

Genetic locus: PACS1 (human) mapping to 11q13.1.

PRODUCT

PACS-1a 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 PACS-1a shRNA Plasmid (h): sc-106348-SH and PACS-1a shRNA (h) Lentiviral Particles: sc-106348-V as alternate gene silencing products.

For independent verification of PACS-1a (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-106348A, sc-106348B and sc-106348C.

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

PACS-1a siRNA (h) is recommended for the inhibition of PACS-1a 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

PACS-1a (4): sc-136344 is recommended as a control antibody for monitoring of PACS-1a 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 PACS-1a gene expression knockdown using RT-PCR Primer: PACS-1a (h)-PR: sc-106348-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Liu, H., et al. 2019. PACS1 is an HIV-1 cofactor that functions in Rev-mediated nuclear export of viral RNA. *Virology* 540: 88-96.

RESEARCH USE

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