SANTA CRUZ BIOTECHNOLOGY, INC.

EHD4 siRNA (h): sc-40521

BACKGROUND

Eps15 homology domain (EHD)-containing proteins function in the exit of receptors and other membrane proteins from the endosomal recycling compartment. EHD4 (Eps15 homology domain-containing protein 4), also known as PAST4 or Pincher, belongs to a subfamily of the EHD protein family that includes the closely related proteins EHD1, EHD2 and EHD3. EHD4 is predominantly expressed in pancreas and heart localizing to vesicular and tubular structures in the cell. It contains an EH domain as well as a calcium binding EF hand. EHD4 is believed to function in transport from the early endosome to the endocytic recycling compartment. In addition, EHD4 is capable of binding lipids via its EH domain. Loss of EHD4 can lead to retention of transferrin in peripheral compartments suggesting that EHD4 regulates the transport of transferrin out of the early endosome.

REFERENCES

1. Pohl, U., et al. 2000. EHD, EHD3, and EHD4 encode novel members of a highly conserved family of EH domain-containing proteins. Genomics 63: 255-262.
2. Kuo, H.J., et al. 2001. Characterization of EHD4, an EH domain-containing protein expressed in the extracellular matrix. J. Biol. Chem. 276: 43103-43110.
3. Online Mendelian Inheritance in Man, OMIM™. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 605892. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/
4. Guilherme, A., et al. 2004. Role of EHD1 and EHBP1 in perinuclear sorting and Insulin-regulated GLUT4 recycling in 3T3-L1 adipocytes. J. Biol. Chem. 279: 40062-40075.
5. Smith, C.A., et al. 2004. The cell fate determinant numb interacts with EHD/Rme-1 family proteins and has a role in endocytic recycling. Mol. Biol. Cell 15: 3698-3708.
6. Naslavsky, N., et al. 2005. C-terminal EH-domain-containing proteins: consensus for a role in endocytic trafficking, EH? J. Cell Sci. 118: 4093-4101.
7. Naslavsky, N., et al. 2007. EHD1 and Eps15 interact with phosphatidylinositols via their Eps15 homology domains. J. Biol. Chem. 282: 16612-16622.

CHROMOSOMAL LOCATION

Genetic locus: EHD4 (human) mapping to 15q15.1.

PRODUCT

EHD4 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 EHD4 shRNA Plasmid (h): sc-40521-SH and EHD4 shRNA (h) Lentiviral Particles: sc-40521-V as alternate gene silencing products.

For independent verification of EHD4 (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-40521A, sc-40521B and sc-40521C.

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

EHD4 siRNA (h) is recommended for the inhibition of EHD4 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

EHD4 (E-2): sc-376373 is recommended as a control antibody for monitoring of EHD4 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 EHD4 gene expression knockdown using RT-PCR Primer: EHD4 (h)-PR: sc-40521-PR (20 µl). 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.