SANTA CRUZ BIOTECHNOLOGY, INC.

EHD3 siRNA (m): sc-40520

BACKGROUND

EHD proteins function in the exit of receptors and other membrane proteins from the endosomal recycling compartment. EHD3 is a protein with an Eps15 homology domain (EHD) that is predominantly expressed in human heart, brain, placenta, liver, kidney and ovary. In the cell, EHD3 localizes to endocytic vesicles and microtubule-dependent, membrane tubules where it may play a role in regulating endocytic tubular and vesicular structure microtubule-dependent movement. EHD3 binds to Rab 11-FIP2 via EH-NPF interactions and may function in early endosome to endocytic recycling compartment transport. Loss of EHD3 expression inhibits the delivery of internalized transferrin and early endosomal proteins to the endocytic recycling compartment.

REFERENCES

1. Pohl, U., et al. 2000. EHD2, EHD3 and EHD4 encode novel members of a highly conserved family of EH domain-containing proteins. Genomics 63: 255-262.
2. Galperin, E., et al. 2002. EHD3: a protein that resides in recycling tubular and vesicular membrane structures and interacts with EHD1. Traffic 3: 575-589.
3. 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.
4. Huang, Z., et al. 2004. Notch-induced E2A degradation requires ChIP and HSC 70 as novel facilitators of ubiquitination. Mol. Cell. Biol. 24: 8951-8962.
5. Braun, A., et al. 2005. EHD proteins associate with syndapin I and II and such interactions play a crucial role in endosomal recycling. Mol. Biol. Cell 16: 3642-3658.
6. Naslavsky, N., et al. 2005. Interactions between EHD proteins and Rab 11-FIP2: a role for EHD3 in early endosomal transport. Mol. Biol. Cell 17: 163-177.
7. Taniwaki, M., et al. 2006. Gene expression profiles of small-cell lung cancers: molecular signatures of lung cancer. Int. J. Oncol. 29: 567-575.

CHROMOSOMAL LOCATION

Genetic locus: Ehd3 (mouse) mapping to 17 E2.

PRODUCT

EHD3 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see EHD3 shRNA Plasmid (m): sc-40520-SH and EHD3 shRNA (m) Lentiviral Particles: sc-40520-V as alternate gene silencing products.

For independent verification of EHD3 (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-40520A, sc-40520B and sc-40520C.

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

EHD3 siRNA (m) is recommended for the inhibition of EHD3 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 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

EHD3 (RR-L): sc-100723 is recommended as a control antibody for monitoring of EHD3 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 EHD3 gene expression knockdown using RT-PCR Primer: EHD3 (m)-PR: sc-40520-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.