SANTA CRUZ BIOTECHNOLOGY, INC.

EHD2 siRNA (h): sc-40517

BACKGROUND

Eps15 homology domain (EHD)-containing proteins function in the exit of receptors and other membrane proteins from the endosomal recycling compartment. EHD2 (EH-domain containing 2), also known as PAST2, is a 543 amino acid protein that contains one EF-hand domain and one EH domain. Expressed at high levels in heart and at lower levels in lung, placenta and skeletal muscle, EHD2 interacts with various proteins such as the glucose transporter Glut4 and the endocytotic-associated protein EHBP1. When EHD2 associates with Insulin-induced Glut4, it can recruit Glut4 to the plasma membrane, thereby allowing Glut4 to bind glucose and regulate blood sugar levels. Additionally, EHD2 interacts with EHBP1 and is thought to link EHBP1-associated endocytotic events with Actin cytoskeleton dynamics. Through its interactions with these two proteins, EHD2 is involved in both maintaining blood glucose levels and mediating Actin-associated endocytosis.

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. Park, S.Y., et al. 2004. EHD2 interacts with the Insulin-responsive glucose transporter (GLUT4) in rat adipocytes and may participate in Insulin-induced GLUT4 recruitment. Biochemistry 43: 7552-7562.
3. Guilherme, A., et al. 2004. EHD2 and the novel EH domain binding protein EHBP1 couple endocytosis to the Actin cytoskeleton. J. Biol. Chem. 279: 10593-10605.
4. 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.
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. Daumke, O., et al. 2007. Architectural and mechanistic insights into an EHD ATPase involved in membrane remodelling. Nature 449: 923-927.
7. George, M., et al. 2007. Shared as well as distinct roles of EHD proteins revealed by biochemical and functional comparisons in mammalian cells and C. elegans. BMC Cell Biol. 8: 3.

CHROMOSOMAL LOCATION

Genetic locus: EHD2 (human) mapping to 19q13.33.

PRODUCT

EHD2 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 EHD2 shRNA Plasmid (h): sc-40517-SH and EHD2 shRNA (h) Lentiviral Particles: sc-40517-V as alternate gene silencing products.

For independent verification of EHD2 (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-40517A, sc-40517B and sc-40517C.

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

EHD2 siRNA (h) is recommended for the inhibition of EHD2 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

EHD2 (G-3): sc-515458 is recommended as a control antibody for monitoring of EHD2 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 EHD2 gene expression knockdown using RT-PCR Primer: EHD2 (h)-PR: sc-40517-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.