

# EHD1 siRNA (h): sc-40515

## BACKGROUND

The Eps15 homology (EH) domain-containing protein family consists of four members, designated EHD1, EHD2, EHD3 and EHD4. The EHD family members contain multiple conserved regions, which include an amino-terminal nucleotide-binding consensus site, a bipartite nuclear localization signal and a carboxy-terminal EH protein-binding domain with an EF-hand motif. EHD1 is ubiquitously expressed with increased expression in testis. EHD2, EHD3, and EHD4 have more specific expression, with EHD2 highly expressed in heart, EHD3 expressed in brain, kidney, liver, placenta, ovary and heart, and EHD4 expressed in heart, placenta and pancreas. The EHD proteins may participate in ligand-induced endocytosis, membrane fusion events and endosome recycling.

## REFERENCES

- Haider, N.B., et al. 1999. Evaluation and molecular characterization of EHD1, a candidate gene for Bardet-Biedl syndrome 1 (BBS1). *Gene* 240: 227-232.
- Mintz, L., et al. 1999. EHD1—an EH-domain-containing protein with a specific expression pattern. *Genomics* 59: 66-76.
- 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.
- 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.
- Caplan, S., et al. 2002. A tubular EHD1-containing compartment involved in the recycling of major histocompatibility complex class I molecules to the plasma membrane. *EMBO J.* 21: 2557-2567.
- 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.

## CHROMOSOMAL LOCATION

Genetic locus: EHD1 (human) mapping to 11q13.1.

## PRODUCT

EHD1 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  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see EHD1 shRNA Plasmid (h): sc-40515-SH and EHD1 shRNA (h) Lentiviral Particles: sc-40515-V as alternate gene silencing products.

For independent verification of EHD1 (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-40515A, sc-40515B and sc-40515C.

## PROTOCOLS

See our web site at [www.scbt.com](http://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  $\mu$ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330  $\mu$ l of RNase-free water makes a 10  $\mu$ M solution in a 10  $\mu$ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

## APPLICATIONS

EHD1 siRNA (h) is recommended for the inhibition of EHD1 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  $\mu$ M in 66  $\mu$ 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

EHD (E-8): sc-390513 is recommended as a control antibody for monitoring of EHD1 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 $\kappa$  BP-HRP: sc-516102 or m-IgG $\kappa$  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 $\kappa$  BP-FITC: sc-516140 or m-IgG $\kappa$  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 EHD1 gene expression knockdown using RT-PCR Primer: EHD1 (h)-PR: sc-40515-PR (20  $\mu$ l). 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.