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DAPLE siRNA (m): sc-142874

BACKGROUND

DAPLE, also known as CCDC88C (coiled-coil domain containing 88C) or HkRP2 (hook-related protein 2), is a 2,028 amino acid protein that exists as a homo-oligomer and belongs to the CCDC88 family. Existing as three alternative isoforms, DAPLE is a negative regulator of the canonical Wnt signaling pathway where it inhibits β -catenin stabilization downstream from Dvl. DAPLE contains multiple leucine residues, interacts with Dvl-1 through its PDZ domain and is encoded by a gene mapping to human chromosome 14q32.12. Chromosome 14 houses over 700 genes, comprises nearly 3.5% of the human genome and encodes the Presenilin 1 (PSEN1) gene, which is one of the three key genes associated with the development of Alzheimer's disease (AD). The SERPINA1 gene is also located on chromosome 14 and, when defective, leads to the genetic disorder α 1-antitrypsin deficiency, which is characterized by severe lung complications and liver dysfunction.

REFERENCES

1. Oshita, A., et al. 2003. Identification and characterization of a novel Dvl-binding protein that suppresses Wnt signalling pathway. *Genes Cells* 8: 1005-1017.
2. Le-Niculescu, H., et al. 2005. Identification and characterization of GIV, a novel $G_{\alpha_{i/s}}$ -interacting protein found on COPI, endoplasmic reticulum-Golgi transport vesicles. *J. Biol. Chem.* 280: 22012-22020.
3. Kobayashi, H., et al. 2005. Novel DAPLE-like protein positively regulates both the Wnt/ β -catenin pathway and the Wnt/JNK pathway in *Xenopus*. *Mech. Dev.* 122: 1138-1153.
4. Simpson, F., et al. 2005. A novel hook-related protein family and the characterization of hook-related protein 1. *Traffic* 6: 442-458.
5. Enomoto, A., et al. 2006. Girdin, a novel Actin-binding protein, and its family of proteins possess versatile functions in the Akt and Wnt signaling pathways. *Ann. N.Y. Acad. Sci.* 1086: 169-184.
6. Topic, A., et al. 2009. α -1-antitrypsin phenotypes in adult liver disease patients. *Ups. J. Med. Sci.* 114: 228-234.

CHROMOSOMAL LOCATION

Genetic locus: Ccdc88c (mouse) mapping to 12 E.

PRODUCT

DAPLE 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see DAPLE shRNA Plasmid (m): sc-142874-SH and DAPLE shRNA (m) Lentiviral Particles: sc-142874-V as alternate gene silencing products.

For independent verification of DAPLE (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-142874A, sc-142874B and sc-142874C.

RESEARCH USE

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

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

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

DAPLE (F-3): sc-514550 is recommended as a control antibody for monitoring of DAPLE 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 DAPLE gene expression knockdown using RT-PCR Primer: DAPLE (m)-PR: sc-142874-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.