

DENND2D siRNA (m): sc-143000

BACKGROUND

DENND2D (DENN/MADD domain containing 2D) is a 471 amino acid protein that contains a dDENN domain, a DENN domain, and a uDENN domain and exists as two isoforms as a result of alternative splicing. The DENND2D protein is thought to target to actin filaments and control Rab9-dependent trafficking of mannose-6-phosphate receptor to lysosomes. The gene encoding DENND2D maps to human chromosome 1, the largest human chromosome which spans about 260 million base pairs and makes up 8% of the human genome. Other notable genes located on chromosome 1 include LMNA, which is associated with the rare aging disease Hutchinson-Gilford progeria, and the MUTYH gene, which is partially responsible for familial adenomatous polyposis. Stickler syndrome, Parkinsons, Gaucher disease and Usher syndrome.

REFERENCES

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3. Weise, A., Starke, H., Mrasek, K., Claussen, U. and Liehr, T. 2005. New insights into the evolution of chromosome 1. *Cytogenet. Genome Res.* 108: 217-222.
4. Gregory, S.G., Barlow, K.F., McLay, K.E., Kaul, R., Swarbreck, D., Dunham, A., Scott, C.E., Howe, K.L., Woodfine, K.C., Spencer, C.A., Jones, M.C., Gillson, C., Searle, S., Zhou, Y., Kokocinski, F., McDonald, L., et al. 2006. The DNA sequence and biological annotation of human chromosome 1. *Nature* 441: 315-321.

CHROMOSOMAL LOCATION

Genetic locus: Dennd2d (mouse) mapping to 3 F2.3.

PRODUCT

DENND2D 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 DENND2D shRNA Plasmid (m): sc-143000-SH and DENND2D shRNA (m) Lentiviral Particles: sc-143000-V as alternate gene silencing products.

For independent verification of DENND2D (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-143000A, sc-143000B and sc-143000C.

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

DENND2D siRNA (m) is recommended for the inhibition of DENND2D 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

DENND2D (H-6): sc-398374 is recommended as a control antibody for monitoring of DENND2D 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 DENND2D gene expression knockdown using RT-PCR Primer: DENND2D (m)-PR: sc-143000-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.