SANTA CRUZ BIOTECHNOLOGY, INC.

DENND4B shRNA Plasmid (h): sc-88449-SH

BACKGROUND

DENND4B (DENN/MADD domain containing 4B), also known as KIAA0476, is a 1,496 amino acid protein that contains a dDENN domain, a DENN domain, a uDENN domain, a MABP domain and two PPR (pentatricopeptide) repeats. The DENND4B protein is thought to target a tubular membrane compartment adjacent to the Golgi, where it activates Rab10. The gene encoding DENND4B 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.

REFERENCES

1. Watson, M.L., et al. 1990. Genomic organization of the selectin family of leukocyte adhesion molecules on human and mouse chromosome 1. J. Exp. Med. 172: 263-272.
2. Blackwood, D.H., et al. 2001. Schizophrenia and affective disorders-cosegregation with a translocation at chromosome 1q42 that directly disrupts brain-expressed genes: clinical and P300 findings in a family. Am. J. Hum. Genet. 69: 428-433.
3. Weise, A., et al. 2005. New insights into the evolution of chromosome 1. Cytogenet. Genome Res. 108: 217-222.
4. Gregory, S.G., et al. 2006. The DNA sequence and biological annotation of human chromosome 1. Nature 441: 315-321.
5. Hennah, W., et al. 2006. Genes and schizophrenia: beyond schizophrenia: the role of DISC1 in major mental illness. Schizophr. Bull. 32: 409-416.
6. Lans, H., et al. 2006. Cell biology: aging nucleus gets out of shape. Nature 440: 32-34.
7. McClintock, D., et al. 2006. Hutchinson-Gilford progeria mutant lamin A primarily targets human vascular cells as detected by an anti-lamin A G608G antibody. Proc. Natl. Acad. Sci. USA 103: 2154-2159.

CHROMOSOMAL LOCATION

Genetic locus: DENND4B (human) mapping to 1q21.3.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.

PROTOCOLS

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

PRODUCT

DENND4B shRNA Plasmid (h) is a pool of 3 target-specific lentiviral vector plasmids each encoding 19-25 nt (plus hairpin) shRNAs designed to knock down gene expression. Each plasmid contains a puromycin resistance gene for the selection of cells stably expressing shRNA. Each vial contains 20 µg of lyophilized shRNA plasmid DNA. Suitable for up to 20 transfections. Also see DENND4B siRNA (h): sc-88449 and DENND4B shRNA (h) Lentiviral Particles: sc-88449-V as alternate gene silencing products.

STORAGE AND RESUSPENSION

Store lyophilized shRNA plasmid DNA at 4° C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at 4° C for short term storage or -80° C for long term storage. Avoid repeated freeze thaw cycles.

Resuspend lyophilized shRNA plasmid DNA in 200 µl of the deionized water provided. Resuspension of the shRNA plasmid DNA in 200 µl of deionized water makes a 0.1 µg/µl solution in a 10 mM Tris, 1 mM EDTA buffered solution.

APPLICATIONS

DENND4B shRNA Plasmid (h) is recommended for the inhibition of DENND4B expression in human cells.

SUPPORT REAGENTS

For optimal shRNA Plasmid transfection efficiency, Santa Cruz Biotechnology's shRNA Plasmid Transfection Reagent: sc-108061 (0.2 ml) and shRNA Plasmid Transfection Medium: sc-108062 (20 ml) are recommended. Control shRNAs are available as 20 µg lyophilized plasmid DNA. Each encodes a scrambled shRNA sequence that will not lead to the specific degradation of any known cellular mRNA. Control shRNA Plasmids include: sc-108060, sc-108065 and sc-108066.

GENE EXPRESSION MONITORING

DENND4B (F-10): sc-514660 is recommended as a control antibody for monitoring of DENND4B 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 DENND4B gene expression knockdown using RT-PCR Primer: DENND4B (h)-PR: sc-88449-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.