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DCAMKL1 shRNA (m) Lentiviral Particles: sc-45619-V

BACKGROUND

Lissencephaly (smooth brain) is an abnormality of brain development characterized by incomplete neuronal migration and a smooth cerebral surface, manifesting as severe mental retardation. Genetic analysis has identified two proteins that are mutated in some cases of lissencephaly, designated lissencephaly-1 protein (LIS1) and doublecortin. LIS1 displays sequence homology to β -subunits of heterotrimeric G proteins, and doublecortin contains a consensus Abl phosphorylation site. In addition, the DCAMKL1 (doublecortin-like and CAM kinase-like 1) protein shows homology to doublecortin. All three proteins are highly expressed in developing brain and may function together to regulate microtubules involved in neuronal migration. The DCAMKL1 protein encodes a functional kinase that is capable of phosphorylating myelin basic protein and itself, but its kinase activity does not appear to affect its microtubule polymerization activity.

REFERENCES

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- Garcia-Higuera, I., et al. 1996. Folding of proteins with WD-repeats: comparison of six members of the WD-repeat superfamily to the G protein β subunit. *Biochemistry* 35: 13985-13994.
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- des Portes, V., et al. 1998. A novel CNS gene required for neuronal migration and involved in X-linked subcortical laminar heterotopia and lissencephaly syndrome. *Cell* 92: 51-61.
- Gleeson, J.G., et al. 1998. Doublecortin, a brain-specific gene mutated in human X-linked lissencephaly and double cortex syndrome, encodes a putative signaling protein. *Cell* 92: 63-72.

CHROMOSOMAL LOCATION

Genetic locus: Dclk1 (mouse) mapping to 3 C.

PRODUCT

DCAMKL1 shRNA (m) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 μ l frozen stock containing 1.0×10^6 infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see DCAMKL1 siRNA (m): sc-45619 and DCAMKL1 shRNA Plasmid (m): sc-45619-SH as alternate gene silencing products.

STORAGE

Store lentiviral particles at -80° C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4° C for up to one week. Avoid repeated freeze thaw cycles.

APPLICATIONS

DCAMKL1 shRNA (m) Lentiviral Particles is recommended for the inhibition of DCAMKL1 expression in mouse cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

GENE EXPRESSION MONITORING

DCAMKL1 (N-14): sc-46312 is recommended as a control antibody for monitoring of DCAMKL1 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 (secondary) reagents are recommended: 1) Western Blotting: use donkey anti-goat IgG-HRP: sc-2020 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible donkey anti-goat IgG-HRP: sc-2033 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use donkey anti-goat IgG-FITC: sc-2024 (dilution range: 1:100-1:400) or donkey anti-goat IgG-TR: sc-2783 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor DCAMKL1 gene expression knockdown using RT-PCR Primer: DCAMKL1 (m)-PR: sc-45619-PR (20 μ l). Annealing temperature for the primers should be $55-60^\circ$ C and the extension temperature should be $68-72^\circ$ C.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

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.

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