



Doublecortin siRNA (m): sc-35215

BACKGROUND

Lissencephaly (smooth brain) is an abnormality of brain development characterized by incomplete neuronal migration and a smooth cerebral surface, resulting in severe mental retardation. Genetic analysis identified two proteins that are mutated in some cases of lissencephaly, designated lissencephaly-1 protein (LIS1) and Doublecortin. LIS1 shows sequence homology to β -subunits of heterotrimeric G proteins. Doublecortin contains a consensus Abl phosphorylation site, and it has some sequence homology to a predicted kinase protein. Both proteins are highly expressed in developing brain, suggesting that they may be involved in a signal transduction pathway that is crucial to brain development.

REFERENCES

1. Reiner, O., et al. 1993. Isolation of a Miller-Dieker lissencephaly gene containing G protein β -subunit-like repeats. *Nature* 364: 717-721.
2. 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.
3. Albrecht, U., et al. 1996. Platelet-activating factor acetylhydrolase expression and activity suggest a link between neuronal migration and platelet-activating factor. *Dev. Biol.* 180: 579-593.
4. Walsh, C.A. 1998. LISsen up! *Nat. Genet.* 19: 307-308.
5. 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.
6. 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: Dcx (mouse) mapping to X F2.

PRODUCT

Doublecortin 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 Doublecortin shRNA Plasmid (m): sc-35215-SH and Doublecortin shRNA (m) Lentiviral Particles: sc-35215-V as alternate gene silencing products.

For independent verification of Doublecortin (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-35215A, sc-35215B and sc-35215C.

RESEARCH USE

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

PROTOCOLS

See our web site at 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 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

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

Doublecortin (E-6): sc-271390 is recommended as a control antibody for monitoring of Doublecortin 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 Doublecortin gene expression knockdown using RT-PCR Primer: Doublecortin (m)-PR: sc-35215-PR (20 μ l, 540 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.