

KIF3A siRNA (h): sc-43374

BACKGROUND

The kinesins constitute a large family of microtubule-dependent motor proteins, which are responsible for the distribution of numerous organelles, vesicles and macromolecular complexes throughout the cell. Individual kinesin members play crucial roles in cell division, intracellular transport and membrane trafficking events including endocytosis and transcytosis. Members of the heterotrimeric kinesin II family of microtubule associated motors generally contain two different motor subunits from the KIF3 family, which includes KIF3A, B and C. KIF3 isoforms mediate anterograde transport of membrane bound organelles in neurons and melanosomes, transport between the endoplasmic reticulum and the Golgi, and transport of protein complexes within cilia and flagella required for their morphogenesis. KIF3A may influence neurogenesis at the level of embryonic cellular events, where the asymmetry of the genetic control circuit controlling left-right (L-R) axis determination is defined. Loss of KIF3A function in mice photoreceptors causes apoptotic cell death suggesting kinesin II mediated transport is required for proper cell fate.

REFERENCES

1. Hamm-Alvarez, S.F. 1998. Molecular motors and their role in membrane traffic. *Adv. Drug Deliv. Rev.* 29: 229-242.
2. Cole, D.G. 1999. Kinesin-II, the heteromeric kinesin. *Cell. Mol. Life Sci.* 56: 217-226.
3. Marszalek, J.R., et al. 1999. Situs inversus and embryonic ciliary morphogenesis defects in mouse mutants lacking the KIF3A subunit of kinesin-II. *Proc. Natl. Acad. Sci. USA* 96: 5043-5048.
4. Takeda, S., et al. 1999. Left-right asymmetry and kinesin superfamily protein KIF3A: new insights in determination of laterality and mesoderm induction by KIF3A^{-/-} mice analysis. *J. Cell Biol.* 145: 825-836.
5. Hirokawa, N. 2000. Stirring up development with the heterotrimeric kinesin KIF3. *Traffic* 1: 29-34.
6. Yang Z., et al. 2001. Molecular cloning and functional analysis of mouse C-terminal kinesin motor KIFC3. *Mol. Cell. Biol.* 21: 75-70.
7. Yang, Z., et al. 2001. Functional analysis of mouse kinesin motor KIF3C. *Mol. Cell. Biol.* 21: 5306-5311.

CHROMOSOMAL LOCATION

Genetic locus: KIF3A (human) mapping to 5q31.1.

PRODUCT

KIF3A siRNA (h) 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 KIF3A shRNA Plasmid (h): sc-43374-SH and KIF3A shRNA (h) Lentiviral Particles: sc-43374-V as alternate gene silencing products.

For independent verification of KIF3A (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-43374A, sc-43374B and sc-43374C.

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

KIF3A siRNA (h) is recommended for the inhibition of KIF3A expression in human 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

KIF3A (E-5): sc-376680 is recommended as a control antibody for monitoring of KIF3A 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 KIF3A gene expression knockdown using RT-PCR Primer: KIF3A (h)-PR: sc-43374-PR (20 μ l, 469 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

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.