SANTA CRUZ BIOTECHNOLOGY, INC.

NKHC1 siRNA (m): sc-36074

BACKGROUND

NKHC1 (neuronal kinesin heavy chain 1), also known as KIF5A; kinesin family member 5A, NKHC; kinesin heavy chain, neuron-specific, SPG10 and spastic paraplegia 10, is a neuronal-specific component of a multi-subunit "molecular motor" complex that mediates intracellular organelle transport. Mutations in the gene encoding NKHC1 cause autosomal dominant spastic paraplegia 10. NKHC1 has a pan-neuronal distribution in the nervous system. Rat tissue extracts by immunoblot of NKHC1 can produce a doublet only in brain and sciatic nerve tissue. NKHC1 is distributed throughout the central nervous system and is enriched in subsets of neurons. Within cultured hippocampal neurons, NKHC1 is concentrated in the perinuclear region of the cell body. Kinesin superfamily proteins like NKHC1 are the molecular motors conveying cargos along microtubules.

REFERENCES

1. Niclas, J., et al. 1994. Cloning and localization of a conventional kinesin motor expressed exclusively in neurons. Neuron 12: 1059-1072.
2. Rahman, A., et al. 1999. Defective kinesin heavy chain behavior in mouse kinesin light chain mutants. J. Cell Biol. 146: 1277-1288.
3. Kanai, Y., et al. 2000. KIF5C, a novel neuronal kinesin enriched in motor neurons. J. Neurosci. 20: 6374-6384.
4. Macioce, P., et al. 2003. β-dystrobrevin interacts directly with kinesin heavy chain in brain. J. Cell Sci. 116: 4847-4856.
5. Xia, C.H., et al. 2003. Abnormal neurofilament transport caused by targeted disruption of neuronal kinesin heavy chain KIF5A. J. Cell Biol. 161: 55-66.
6. Fichera, M., et al. 2004. Evidence of kinesin heavy chain (KIF5A) involvement in pure hereditary spastic paraplegia. Neurology 63: 1108-1110.
7. Sotelo-Silveira, J.R., et al. 2004. Myosin Va and kinesin II motor proteins are concentrated in ribosomal domains (periaxoplasmic ribosomal plaques) of myelinated axons. J. Neurobiol. 60: 187-196.
8. Ceccarini, M., et al. 2005. Molecular basis of dystrobrevin interaction with kinesin heavy chain: structural determinants of their binding. J. Mol. Biol. 354: 872-882.

CHROMOSOMAL LOCATION

Genetic locus: Kif5a (mouse) mapping to 10 D3.

PRODUCT

NKHC1 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see NKHC1 shRNA Plasmid (m): sc-36074-SH and NKHC1 shRNA (m) Lentiviral Particles: sc-36074-V as alternate gene silencing products.

For independent verification of NKHC1 (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-36074A, sc-36074B and sc-36074C.

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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

NKHC1 siRNA (m) is recommended for the inhibition of NKHC1 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 µM in 66 µl. 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

NKHC1 (C-11): sc-376452 is recommended as a control antibody for monitoring of NKHC1 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 NKHC1 gene expression knockdown using RT-PCR Primer: NKHC1 (m)-PR: sc-36074-PR (20 µl). 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.