

NCS-1 siRNA (r): sc-270206

BACKGROUND

NCS-1 (for neuronal calcium sensor-1, also designated frequenin) belongs to a highly conserved family of EF-hand-containing Ca^{2+} -binding proteins expressed mainly in neurons. NCS-1 is localized to neuronal cell bodies and axons throughout the brain and spinal cord. It is also expressed in glial cells and in neuroendocrine bovine adrenal chromaffin and PC12 cells. NCS-1 is a regulatory protein involved in Ca^{2+} -dependent exocytosis of synaptic vesicles and dense core granules. NCS-1 also functions in the voltage-independent autocrine pathway that negatively regulates non-L-type Ca^{2+} channels.

REFERENCES

1. Pongs, O., et al. 1993. Frequenin—a novel calcium-binding protein that modulates synaptic efficacy in the *Drosophila* nervous system. *Neuron* 11: 15-28.
2. Cox, J.A., et al. 1994. Cation binding and conformational changes in VILIP and NCS-1, two neuron-specific calcium-binding proteins. *J. Biol. Chem.* 269: 32807-32813.
3. Olafsson, P., et al. 1997. The Ca^{2+} binding protein, frequenin is a nervous system-specific protein in mouse preferentially localized in neurites. *Brain Res. Mol. Brain Res.* 44: 73-82.
4. McFerran, B.W., et al. 1998. Neuronal Ca^{2+} sensor 1, the mammalian homologue of frequenin, is expressed in chromaffin and PC12 cells and regulates neurosecretion from dense-core granules. *J. Biol. Chem.* 273: 22768-22772.
5. Braunewell, K.H., et al. 1999. Intracellular neuronal calcium sensor proteins: a family of EF-hand calcium-binding proteins in search of a function. *Cell Tissue Res.* 295: 1-12.
6. Martone, M.E., et al. 1999. Cellular and subcellular distribution of the calcium-binding protein NCS-1 in the central nervous system of the rat. *Cell Tissue Res.* 295: 395-407.
7. Weiss, J.L., et al. 2000. NCS-1/frequenin functions in an autocrine pathway regulating Ca^{2+} channels in bovine adrenal chromaffin cells. *J. Biol. Chem.* 275: 40082-40087.

CHROMOSOMAL LOCATION

Genetic locus: Ncs1 (rat) mapping to 3p12.

PRODUCT

NCS-1 siRNA (r) 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 NCS-1 shRNA Plasmid (r): sc-270206-SH and NCS-1 shRNA (r) Lentiviral Particles: sc-270206-V as alternate gene silencing products.

For independent verification of NCS-1 (r) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-270206A, sc-270206B and sc-270206C.

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

NCS-1 siRNA (r) is recommended for the inhibition of NCS-1 expression in rat 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

NCS-1 (G-4): sc-376206 is recommended as a control antibody for monitoring of NCS-1 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 MarkerTM 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 NCS-1 gene expression knockdown using RT-PCR Primer: NCS-1 (r)-PR: sc-270206-PR (20 μl). Annealing temperature for the primers should be $55-60^{\circ}\text{C}$ and the extension temperature should be $68-72^{\circ}\text{C}$.

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.