

NG2 siRNA (h): sc-40771

BACKGROUND

NG2 (also known as melanoma-associated chondroitin sulfate proteoglycan 4, MCSP, MCSPG, MSK16 and MEL-CSPG) stabilizes cell-substratum interactions during early events of melanoma cell spreading on endothelial basement membranes. NG2 may facilitate primary melanoma progression by enhancing the activation of key signaling pathways important for tumor invasion and growth. Threonine 2256 phosphorylation of rat NG2 (Threonine 2252 phosphorylation of human NG2) leads to redistribution of NG2 on the surface of astrocytomas, polarization of the cell and a significant increase in cell motility. NG2 acts as a co-receptor for spreading and focal contact formation in association with $\alpha 4 \beta 1$ Integrin in malignant melanoma cells. NG2 is present on blood vessels throughout the rat embryo. Microvessels within the rat CNS express NG2 on endothelial cells, and outside the CNS, NG2 is present on smooth muscle cells. NG2 is a novel marker for epidermal stem cells that contributes to their patterned distribution by promoting stem cell clustering.

REFERENCES

1. Iida, J., et al. 1995. Spreading and focal contact formation of human melanoma cells in response to the stimulation of both melanoma-associated proteoglycan (NG2) and $\alpha 4 \beta 1$ Integrin. *Cancer Res.* 55: 2177-2185.
2. Grako, K.A., et al. 1995. Participation of the NG2 proteoglycan in rat aortic smooth muscle cell responses to platelet-derived growth factor. *Exp. Cell Res.* 221: 231-240.
3. Grako, K.A., et al. 1999. PDGF α -receptor is unresponsive to PDGF-AA in aortic smooth muscle cells from the NG2 knockout mouse. *J. Cell Sci.* 112: 905-915.
4. Makgiansar, I.T., et al. 2004. Phosphorylation of NG2 proteoglycan by protein kinase C- α regulates polarized membrane distribution and cell motility. *J. Biol. Chem.* 279: 55262-55270.
5. Pitera, J.E., et al. 2004. Dysmorphogenesis of kidney cortical peritubular capillaries in angiopoietin-2-deficient mice. *Am. J. Pathol.* 165: 1895-1906.
6. Aguirre, A.A., et al. 2004. NG2-expressing cells in the subventricular zone are type C-like cells and contribute to interneuron generation in the post-natal hippocampus. *J. Cell Biol.* 165: 575-589.

CHROMOSOMAL LOCATION

Genetic locus: CSPG4 (human) mapping to 15q24.2.

PRODUCT

NG2 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 NG2 shRNA Plasmid (h): sc-40771-SH and NG2 shRNA (h) Lentiviral Particles: sc-40771-V as alternate gene silencing products.

For independent verification of NG2 (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-40771A, sc-40771B and sc-40771C.

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

NG2 siRNA (h) is recommended for the inhibition of NG2 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

NG2 (LHM 2): sc-53389 is recommended as a control antibody for monitoring of NG2 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 NG2 gene expression knockdown using RT-PCR Primer: NG2 (h)-PR: sc-40771-PR (20 μ l, 574 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Joo, N.E., et al. 2014. Shedding of NG2 by MMP-13 attenuates anoikis. *DNA Cell Biol.* 33: 854-862.

RESEARCH USE

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