



connexin 43 siRNA (r): sc-60008

BACKGROUND

The connexins are a group of gap junction proteins which form a hexamer to compose a connexon. Clusters of connexons form a gap junction through which low molecular weight proteins may diffuse from cell to cell. Several mammalian cells with malignant phenotypes exhibit decreased connexin expression and gap junction communication. In Src transformed cells, there is a decrease in gap junctional communication, which appears to be associated with tyrosine phosphorylation of connexin 43. Activated c-Src phosphorylates the C-terminal tail of connexin 43 on Tyr 265, resulting in a stable interaction between both proteins, which leads to inhibition of gap junctional communication. In addition to tyrosine phosphorylation, connexin 43 has also been shown to be phosphorylated on serine in the absence of Src kinases and on both serine and tyrosine in cells expressing Src kinases, such as c-Src and/or pp60v-Src. In human vascular endothelial cells, connexin 43 is post-translationally modified during mitosis. Mitosis-specific phosphorylation of connexin 43 correlates with the transient loss of gap junction intercellular communication and redistribution of connexin 43.

CHROMOSOMAL LOCATION

Genetic locus: Gja1 (rat) mapping to 20q11.

PRODUCT

connexin 43 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see connexin 43 shRNA Plasmid (r): sc-60008-SH and connexin 43 shRNA (r) Lentiviral Particles: sc-60008-V as alternate gene silencing products.

For independent verification of connexin 43 (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-60008A, sc-60008B and sc-60008C.

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

connexin 43 siRNA (r) is recommended for the inhibition of connexin 43 expression in rat cells.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.

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

connexin 43 (F-7): sc-271837 is recommended as a control antibody for monitoring of connexin 43 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 connexin 43 gene expression knockdown using RT-PCR Primer: connexin 43 (r)-PR: sc-60008-PR (20 μ l, 437 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Matsuyama, D. and Kawahara, K. 2009. Proliferation of neonatal cardiomyocytes by connexin 43 knockdown via synergistic inactivation of p38 MAPK and increased expression of FGF1. *Basic Res. Cardiol.* 104: 631-642.
2. Matsuyama, D. and Kawahara, K. 2011. Oxidative stress-induced formation of a positive-feedback loop for the sustained activation of p38 MAPK leading to the loss of cell division in cardiomyocytes soon after birth. *Basic Res. Cardiol.* 106: 815-828.
3. Tien, T., et al. 2013. Effects of high glucose-induced Cx43 downregulation on occludin and ZO-1 expression and tight junction barrier function in retinal endothelial cells. *Invest. Ophthalmol. Vis. Sci.* 54: 6518-6525.
4. Li, L., et al. 2017. VEGF promotes endothelial progenitor cell differentiation and vascular repair through connexin 43. *Stem Cell Res. Ther.* 8: 237.
5. Meinung, C.P., et al. 2024. OXTR-mediated signaling in astrocytes contributes to anxiolysis. *Mol. Psychiatry*. E-published.

RESEARCH USE

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