



connexin 43 siRNA (m): sc-35091

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.

REFERENCES

1. Manjunath, C.K., et al. 1987. Human cardiac gap junctions: isolation, ultrastructure and protein composition. *J. Mol. Cell. Cardiol.* 19: 131-134.
2. Dermietzel, R., et al. 1989. Differential expression of three gap junction proteins in developing and mature brain tissues. *Proc. Natl. Acad. Sci. USA* 86: 10148-10152.
3. Yamamoto, T., et al. 1990. LM and EM immunolocalization of the gap junctional protein connexin 43 in rat brain. *Brain Res.* 508: 313-319.

CHROMOSOMAL LOCATION

Genetic locus: Gja1 (mouse) mapping to 10 B4.

PRODUCT

connexin 43 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see connexin 43 shRNA Plasmid (m): sc-35091-SH and connexin 43 shRNA (m) Lentiviral Particles: sc-35091-V as alternate gene silencing products.

For independent verification of connexin 43 (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-35091A, sc-35091B and sc-35091C.

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 (m) is recommended for the inhibition of connexin 43 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 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).

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor connexin 43 gene expression knockdown using RT-PCR Primer: connexin 43 (m)-PR: sc-35091-PR (20 μ l, 445 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Squecco, R., et al. 2006. Sphingosine 1-phosphate induces myoblast differentiation through Cx43 protein expression: a role for a gap junction-dependent and -independent function. *Mol. Biol. Cell* 17: 4896-4910.
2. Bose, S., et al. 2010. Administration of connexin 43 siRNA abolishes secretory pulse synchronization in GnRH clonal cell populations. *Mol. Cell. Endocrinol.* 314: 75-83.
3. Shishido, S.N. and Nguyen, T.A. 2016. Induction of apoptosis by PQ1, a gap junction enhancer that upregulates connexin 43 and activates the MAPK signaling pathway in mammary carcinoma cells. *Int. J. Mol. Sci.* 17: 178.
4. Le, Q.A., et al. 2020. Distinct shear-induced Ca²⁺ signaling in the left and right atrial myocytes: role of P2 receptor context. *J. Mol. Cell. Cardiol.* 143: 38-50.

PROTOCOLS

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