



connexin 32 siRNA (m): sc-43077

BACKGROUND

The connexin family of proteins form hexameric complexes called "connexons" that facilitate movement of low molecular weight proteins between cells via gap junctions. Connexin proteins share a common topology of four transmembrane α -helical domains, two extracellular loops, a cytoplasmic loop and cytoplasmic N- and C-termini. Many of the key functional differences arise from specific amino-acid substitutions in the most highly conserved domains, the transmembrane and extracellular regions. Each of the approximately 20 connexin isoforms produces channels with distinct permeabilities and electrical and chemical sensitivities; therefore, one connexin usually cannot fully substitute for another. Consequently, a wide variety of malignant phenotypes associate with decreased connexin expression and gap junction communication, dependent on the particular connexin that is affected. For instance, mutations in connexin 32 result in Charcot-Marie-Tooth disease, a demyelinating disease of the peripheral nervous system.

REFERENCES

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- Harris, A.L. 2001. Emerging issues of connexin channels: biophysics fills the gap. *Q. Rev. Biophys.* 34: 325-472.
- Menichella, D.M., et al. 2003. Connexins are critical for normal myelination in the CNS. *J. Neurosci.* 23: 5963-5973.
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- Yamamoto, T., et al. 2004. IL-1b regulates expression of Cx32, Occludin and claudin-2 of rat hepatocytes via distinct signal transduction pathways. *Exp. Cell Res.* 299: 427-441.
- Nakashima, Y., et al. 2004. Expression of gap junction protein connexin 32 in chronic hepatitis, liver cirrhosis and hepatocellular carcinoma. *J. Gastroenterol.* 39: 763-768.

CHROMOSOMAL LOCATION

Genetic locus: Gjb1 (mouse) mapping to X D.

PRODUCT

connexin 32 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 32 shRNA Plasmid (m): sc-43077-SH and connexin 32 shRNA (m) Lentiviral Particles: sc-43077-V as alternate gene silencing products.

For independent verification of connexin 32 (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-43077A, sc-43077B and sc-43077C.

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 32 siRNA (m) is recommended for the inhibition of connexin 32 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 32 (HAM8): sc-21794 is recommended as a control antibody for monitoring of connexin 32 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 connexin 32 gene expression knockdown using RT-PCR Primer: connexin 32 (m)-PR: sc-43077-PR (20 μ l). Annealing temperature for the primers should be 55-60 $^{\circ}$ C and the extension temperature should be 68-72 $^{\circ}$ 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.