SANTA CRUZ BIOTECHNOLOGY, INC.

connexin 46 siRNA (m): sc-43082

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, deletion of the gene encoding connexin 46, normally expressed in the lens, produces severe cataracts.

REFERENCES

1. von Maltzahn, J., et al. 2004. The novel mouse connexin 39 gene is expressed in developing striated muscle fibers. J. Cell Sci. 117: 5381-5392.
2. Xia, C.H., et al. 2006. Diverse gap junctions modulate distinct mechanisms for fiber cell formation during lens development and cataractogenesis. Development 133: 2033-2040.
3. Dunia, I., et al. 2006. Structural and immunocytochemical alterations in eye lens fiber cells from Cx46 and Cx50 knockout mice. Eur. J. Cell Biol. 85: 729-752.
4. Xia, C.H., et al. 2006. Absence of α3 (Cx46) and α8 (Cx50) connexins leads to cataracts by affecting lens inner fiber cells. Exp. Eye Res. 83: 688-696.
5. Xia, C.H., et al. 2006. Knock-in of α3 connexin prevents severe cataracts caused by an α8 point mutation. J. Cell Sci. 119: 2138-2144.
6. Tang, Y., et al. 2007. Age-related cataracts in α3Cx46-knockout mice are dependent on a calpain 3 isoform. Invest. Ophthalmol. Vis. Sci. 48: 2685-2694.

CHROMOSOMAL LOCATION

Genetic locus: GJA3 (mouse) mapping to 14 C3.

PRODUCT

connexin 46 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see connexin 46 shRNA Plasmid (m): sc-43082-SH and connexin 46 shRNA (m) Lentiviral Particles: sc-43082-V as alternate gene silencing products.

For independent verification of connexin 46 (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-43082A, sc-43082B and sc-43082C.

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

connexin 46 siRNA (m) is recommended for the inhibition of connexin 46 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 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

connexin 46 (C-3): sc-365394 is recommended as a control antibody for monitoring of connexin 46 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-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 46 gene expression knockdown using RT-PCR Primer: connexin 46 (m)-PR: sc-43082-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° 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.