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connexin 30 siRNA (m): sc-43075

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 alpha-helical domains, two extracellular loops, a cytoplasmic loop and cytoplasmic N- and C-termini. Many of the key functional differences between connexins arise from specific amino-acid substitutions in the most highly conserved domains: the transmembrane and extracellular regions. Connexin 30, also known as GJB6 (Gap junction β 6), ED2, EDH, HED or DFNA3, is a 261 amino acid multi-pass membrane protein that localizes to the cell junction and belongs to the connexin family. Functioning as a hexamer with other connexin proteins, connexin 30 facilitates the diffusion of low molecular weight materials from one cell to another. Defects in the gene encoding connexin 30 are the cause of ectodermal dysplasia type 2 (ED2) and non-syndromic sensorineural deafness autosomal dominant type 3 (DFNA3), the former of which is characterized by abnormal development of ectodermal structures (such as skin and nails).

REFERENCES

1. Kelley, P.M., et al. 1999. Human connexin 30 (GJB6), a candidate gene for nonsyndromic hearing loss: molecular cloning, tissue-specific expression, and assignment to chromosome 13q12. *Genomics* 62: 172-176.
2. Smith, F.J., et al. 2002. A novel connexin 30 mutation in Clouston syndrome. *J. Invest. Dermatol.* 118: 530-532.
3. Del Castillo, I., et al. 2003. Prevalence and evolutionary origins of the del(GJB6-D13S1830) mutation in the DFNB1 locus in hearing-impaired subjects: a multicenter study. *Am. J. Hum. Genet.* 73: 1452-1458.
4. Essenfelder, G.M., et al. 2005. Gene structure and promoter analysis of the human GJB6 gene encoding connexin 30. *Gene* 350: 33-40.
5. Wilch, E., et al. 2006. Expression of GJB2 and GJB6 is reduced in a novel DFNB1 allele. *Am. J. Hum. Genet.* 79: 174-179.
6. Yum, S.W., et al. 2007. Human connexin 26 and connexin 30 form functional heteromeric and heterotypic channels. *Am. J. Physiol., Cell Physiol.* 293: C1032-C1048.

CHROMOSOMAL LOCATION

Genetic locus: Gjb6 (mouse) mapping to 14 C3.

PRODUCT

connexin 30 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 30 shRNA Plasmid (m): sc-43075-SH and connexin 30 shRNA (m) Lentiviral Particles: sc-43075-V as alternate gene silencing products.

For independent verification of connexin 30 (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-43075A, sc-43075B and sc-43075C.

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 30 siRNA (m) is recommended for the inhibition of connexin 30 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 30 (G-2): sc-514847 is recommended as a control antibody for monitoring of connexin 30 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 30 gene expression knockdown using RT-PCR Primer: connexin 30 (m)-PR: sc-43075-PR (20 μ l). 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.